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Preface 2015/2016

The goal remains the same – to make a textbook that is easily readable and can be used in the daily practice of HIV treatment. As in previous years, all the chapters of HIV 2015/2016 have been thoroughly revised.

Again, special emphasis was put on actuality. This is underlined by the fact that in the antiretroviral therapy chapter alone, more than 100 are dated in the years 2013 and 2014.

Previous issues of the progenitor "HIV Medicine" were available in several languages, such as Spanish, Romanian, Portuguese, Vietnamese and Persian. We are very proud that a Russian issue could be published in 2015.

Under certain conditions, the editors and the authors of this book may agree to remove the copyright on HIV 2015/2016 for all languages except English and German. You could therefore translate the content of this book into any language and publish it under your own name, without paying a license fee.

HIV 2015/2016 is also freely available on the Internet (www.hivbook.com), because we firmly believe that this is the way medical textbooks should be handled in the 21st century. Research, knowledge, and expertise in the field of HIV can be shared and accessible to those who are dedicated to the treatment and care of individuals affected by HIV.

Christian Hoffmann, Jürgen K. Rockstroh
Hamburg, Bonn – September 2015

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Contents

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Abbreviations

AIDS   Acquired Immunodeficiency Syndrome
AIN   Anal Intraepithelial Neoplasia
ART   Antiretroviral Therapy
AUC   Area under the curve
BAL   Bronchoalevolar Lavage
BGA   Blood Gas Analysis
BMI   Body Mass Index
CDC   Centers for Disease Control and Prevention
CHD   Coronoray Heart Disease
CMV   Cytomegalovirus
CNS   Central Nervous System
CROI   Conference on Retroviruses and Opportunistic Infections
CT   Computed Topography
CTL   Cytotoxic T Cells
CVC   Central Venous Catheter
DD   Differential diagnosis
DEXA   Dual Energy X-ray Absorptiometry
DNA   Deoxyribonucleic Acid
DOT   Directly Observed Therapy
EAP   Expanded Access Program
EBV   Epstein-Barr-Virus
ED   Erectile Dysfunction
ELISA   Enzyme Linked Immunosorbent Assay
ELISPOT   Enzyme Linked Immunosorbent Spot Assay
EMEA   European Medicines Agency
FDA   US Food and Drug Administration
FDC   Follicular Dendritic Cells
G-CSF   Granulocyte Colony-Stimulating Factor
GFR   Glomerular Filtration Rate
HAART   Highly Active Anti-Retroviral Therapy
HbsAG   Hepatitis B Surface Antigen
HBV   Hepatitis B Virus
HCC   Hepatocellular Carcinoma
HCV   Hepatitis C Virus
HDL   High-density Lipoprotein
HHV-8   Human Herpesvirus 8
HIV   Human Immunodeficiency Virus
HIVAN   HIV-Associated Nephropathy
HIVE   HIV-Encephalopathy
HLA   Human Leukocyte Antigen
HPV   Human Papillomavirus
HSR   Hypersensitivity Reaction
HSV   Herpes Simplex Virus
IC50   50% Inhibitory Concentration
INSTI   Integrase Strand Transfer Inhibitor

IRIS	Immune Reconstitution Inflammatory Syndrome
ITP	Idiopathic Thrombocytopenic Purpura
ITT Analysis	Intention-to-Treat Analysis
IU	International Unit
CHD	Coronary Heart Disease
KS	Kaposi's sarcoma
LDH	Lactate Dehydrogenase
LDL	Low-Density Lipoprotein
LGV	Lymphogranuloma venereum
LIP	Lymphocytic Interstitial Pneumonia
LTNP	Long-Term Non-Progressor
MAC	Mycobacterium Avium Complex
MCD	Multicentric Castleman's Disease
MDR	Multi-Drug Resistant
MHC	Major Histocompatibility Complex
MRT	Magnet Resonance Tomography
MSM	Men who have sex with men
NASBA	Nucleic Acid Sequence Based Amplification
NHL	Non-Hodgkin's Lymphoma
NK-Cells	Natural Killer Cells
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OHL	Oral Hairy Leukoplakia
OI	Opportunistic Infection
PBMC	Peripheral Mononuclear Cell
PCNSL	Primary Central Nervous System Lymphoma
PCP	Pneumocystis Pneumonia
PCR	Polymerase Chain Reaction
PEL	Primary Effusion Lymphoma
PEP	Post Exposure Prophylaxis
PI	Protease Inhibitor
PML	Progressive Multifocal Leukoencephalopathy
PNP	Polyneuropathy
PrEP	Pre-Exposure Prophylaxis
RNA	Ribonucleic Acid
SD	Sexual Dysfunction
SIV	Simian Immunodeficiency Virus
STD	Sexually Transmitted Diseases
STI	Structured Treatment Interruption
TAM	Thymidine Analogue Mutation
TCR	T Cell Receptor
TDM	Therapeutic Drug Monitoring
TSH	Thyroid-stimulating Hormone
VL	Viral Load
VZV	Varicella Zoster Virus

For abbreviations of antiretroviral agents, see ART chapter.

SECTION 1

The Basics

1. Introduction

JÜRGEN KURT ROCKSTROH

Acquired Immune Deficiency Syndrome (AIDS) was first described as a not needed clinical entity in 1981. Initial reports were based on an unusual increase in the incidence of Kaposi sarcoma (KS) and *Pneumocystis* pneumonia (PCP), diseases that were considered at that time to occur rarely. While both diseases are occasionally observed in different populations (e.g., KS in older men from the Mediterranean region or PCP in patients with leukemia after intensive chemotherapy), the occurrence of these diseases as indicators for severe immunodeficiency had not been observed before in otherwise healthy young individuals. Because the initially affected population were men who had sex with men (MSM) the disease as well as those with the disease were highly stigmatized. Though at first lifestyle and behavioral factors were hypothesized to be causally related, finally in 1983 the human immunodeficiency virus (HIV) was identified as the true cause of AIDS.

In 1987 the first antiretroviral agent, AZT (zidovudine, Retrovir®) was licensed for the treatment of HIV. Despite the failure of this therapeutic concept as monotherapy in achieving long-term suppression of HIV replication, symptoms and clinical manifestations of HIV infection were temporarily relieved with AZT (at 1500 mg/day) and the occurrence of AIDS was slightly delayed. What happened next is unprecedented in medicine to date – within a few years of its discovery, an inevitably deadly disease turned into a disease with durable and effective treatment options. The rapid introduction of additional antiretroviral drug classes and the concept of highly active antiretroviral therapy (HAART, an acronym that will be replaced in this book by ART) enabled a durable suppression of viral replication thereby preventing disease progression – as long as the antiretroviral drugs were tolerated and taken regularly. Long-term toxicities and the emergence of resistance led to a search for and identification of further promising drugs with other therapeutic mechanisms of action or better resistance profiles. In parallel, administration modalities and tolerability of antiretroviral drug regimens improved significantly. In 2015 several HIV therapies are available that only require an intake of 1–2 tablets a day mostly resulting from the introduction of fixed-dose combinations.

All these advances should not be confused with the fact that lifelong medical therapy will probably lead to substantial problems, especially in terms of adherence to therapy and possible long-term toxicities. With 20 years of experience so far, the latter aspect has thus far only been captured in part. Infection with HIV should still be avoided at all costs. Apart from further improvement of ART and development of new therapeutic concepts such as eradication, a main focus of our endeavors must be the prevention of HIV in order to contain the further spread of disease.

The HIV epidemic

In 1981 the first three clinical descriptions of AIDS were published in the Morbidity and Mortality Weekly Report and later the New England Journal of Medicine. These reports described an epidemic of community-acquired *Pneumocystis* pneumonia, in most cases combined with oral thrush in previously healthy homosexual men, as well as chronic ulcerating perianal herpes infections (Gottlieb 1981a, Gottlieb 1981b, Masur 1981, Siegal 1981).

A little later, in June 1982, a notice from the Centers for Disease Control and Prevention (CDC) on three PCP cases among hemophiliacs was issued (CDC 1982a). In the same year a case of cryptosporidiosis in a hemophiliac patient from

Pennsylvania (Eyster 1982) and an AIDS manifestation in an infant after a blood transfusion were reported (CDC 1982b). The occurrence of AIDS among hemophiliacs triggered a discussion of whether a viral infection could cause AIDS (Marx 1982). In particular, the similarity of populations at risk for AIDS and hepatitis B led to the hypothesis of a viral agent causing AIDS.

Studies on AIDS patients comprising different populations at risk quickly revealed common characteristics: compared to healthy controls, all AIDS cases had diminished counts of CD4-positive T lymphocytes. Conversely, a relative and absolute increase in CD8-positive T lymphocytes and a reduced mitogen-induced proliferative capacity of lymphocytes was observed (Gottlieb 1981, Masur 1981, Siegal 1981, Mildvan 1982, Stahl 1982). It became quickly clear, however, that the manifestation of AIDS was not a prerequisite for developing an immune deficiency. A defect of cellular immunity, associated with a generalized lymphadenopathy, had already been described very early in otherwise asymptomatic men who had sex with men (Kornfeld 1982, Stahl 1982). In January 1983 two cases of hemophiliacs with a lymphadenopathy syndrome were reported, both with significant dysfunction of cellular immunity (Ragni 1983). This led to the assumption that the lymphadenopathy syndrome and the observed cellular immune defects may have been precursors to AIDS and that a transmission of the AIDS causative agent via blood products was probable. Subsequently numerous studies on altered states of cellular immunity among hemophiliac patients were published. The main finding was a reduced CD4/CD8 ratio, the result of a relative and/or absolute decrease of CD4 lymphocyte counts together with elevated CD8 T cell counts. Only those patients who had been treated with small amounts of blood-clotting factors or where blood-clotting factors had been derived from small donor pools showed normal lymphocyte subpopulations (Luban 1983, Rasi 1984).

The altered immunological findings among hemophiliacs were discussed heatedly. In part they were attributed to a chronic antigen exposure due to the blood-clotting factor substitution. Other groups considered this hypothesis unlikely, given the fact that, prior to the advent of AIDS, no enhanced risk for infections was observed among hemophiliacs compared to other populations (except for viral infections, in particular hepatitis B and non-A-non-B-hepatitis via receipt of blood products). Overall, at that time no indication was seen to call into question the concept of blood-clotting substitution therapy among hemophiliacs (Anonymous 1983, Goldsmith 1983). As an alternative explanation of AIDS, particularly among the transmission group of men who have sex with men, coinfection with human cytomegalovirus, use of injection drugs, inhalation of amyl nitrate (poppers) and exposure to foreign proteins (spermatozoa) were discussed (Essex 1997).

In 1983 different working groups raised the hypothesis that a variant of the T-lymphotropic retrovirus (HTLV-I), which had been discovered in 1980 by Gallo and colleagues, could be the causative agent of AIDS (Essex 1983, Gallo 1983). Several arguments were in favor of this hypothesis. At that time HTLV-I was the only known virus with the potential to infect human CD4-positive T lymphocytes (Poiesz 1980). In addition, HTLV-I shared the same transmission routes with the potential AIDS agent, i.e., sexual contacts, blood-to-blood and perinatal transmission (Essex 1982). First experiments to isolate virus related to HTLV-I or -II were only partially successful. Though cross-reactive antibodies with HTLV-related genome sequences were found in a small subset of AIDS patients, the overall assay reactivity was weak and suggested a coinfection with HTLV. The observations led to the assumption of a genetically more distant virus, one with weaker assay reactivity, as a putative etiologic agent. Indeed only a short time later, HTLV-III, later renamed Human Immunodeficiency Virus type I (HIV-1), was discovered as the causative agent of AIDS (Barré-

Sinoussi 1983, Popovic 1984). In 2008 the French research group of Luc Montagnier and Francoise Barré-Sinoussi received the Nobel Prize in Medicine for their discovery of HIV-1.

Transmission routes

The main transmission routes of HIV are
1. unsafe sex with an HIV-infected partner
2. sharing injection paraphernalia with an HIV-infected partner
3. vertical transmission of HIV from the HIV+ mother to the newborn (before or at birth; or later, due to breastfeeding)

All other transmission routes, for the most part case reports, are notably rare. Among these are transmissions due to transfusion of blood or blood products in countries where blood donations are not routinely screened for HIV.

Extremely rare are transmissions due to contact with HIV+ blood through open wounds or mucosa, or transmission of HIV after a bite (Bartholomew 2008). Three cases were reported where mothers infected their newborns probably via pre-chewed food (Gaur 2008). These transmission routes however are of a casuistic nature. Large case registries, in particular from the CDC, which have investigated other transmission routes of HIV, clearly show that daily contacts of everyday life, such as the shared use of toilets or drinking from the same glass, cannot transmit HIV. Case registries in the health care setting, which analyze contact via saliva, urine, or infectious blood with intact skin, did not find a single transmission of HIV (Henderson 1990).

Potentially favorable factors and risks

Sex

The most important transmission route for HIV is sexual contact. The prerequisite for sexual transmission is direct exchange of infectious body secretions / fluids. The highest viral concentrations are found in blood and seminal fluid. A study investigating heterosexual transmission of HIV in female partners of HIV+ hemophiliacs in Bonn found an HIV seroconversion rate of 10% (Rockstroh 1995). The risk for sexual transmission was significantly higher if the HIV+ partner suffered from advanced immunodeficiency or an advanced clinical stage of HIV infection. It is important to note that a precise calculation of transmission risk of one individual exposure is not possible. Various environmental factors have an influence on the actual transmission risk, such as specific sexual practices, concurrent sexually transmitted diseases, skin lesions, circumcision and mucosal trauma, that are difficult to take into account. The average transmission risks according to different sexual practices are shown in Table 1.

The correlation of transmission risk with the level of HIV viremia has important epidemiological implications. In environments where body fluids like blood and seminal fluid are exchanged with many persons over days or weeks, the risk of meeting people who have been recently infected, and thus who are highly infectious, is high. Likewise, the probability of infecting someone else between the transmission event and the detection of HIV antibodies is high. The later stage of disease is also a highly infectious period, as HIV infection progresses and higher viral loads are again observed as one gets closer to falling below 200 CD4 T cells or AIDS. Sexually transmitted diseases and infections disrupt physiological skin and mucosal barriers and enhance the risk for HIV transmission. This is particularly true for endemic areas with a high prevalence of other sexually transmitted diseases. Primarily genital herpes lesions have been identified as a potential co-factor facilitating HIV transmission in endemic areas (Mahiane 2009).

Table 1: Likelihood for HIV transmission. (Modified from the guidelines of the German and Austrian AIDS Society; see also www.daignet.de)

Type of contact / partner	Probability of infection per contact
Unsafe receptive anal intercourse with HIV+ partner	0.82% (95% CI 0.24 – 2.76) Range 0.1 – 7.5%
Unsafe receptive anal intercourse with partner of unknown HIV serostatus	0.27% (95% CI 0.06 – 0.49)
Unsafe insertive anal intercourse with partner of unknown HIV serostatus	0.06% (95% CI 0.02 – 0.19)
Unsafe receptive vaginal intercourse	0.05 – 0.15%
Unsafe insertive vaginal intercourse	0.03 – 5.6%
Oral sex	No known probability, although case reports have been described, in particular after reception of seminal fluid into the mouth (Lifson 1990)

Note: 95% CI = Confidence Interval according to a large US HIV seroconverter study (Vittinghoff 1999)

The observation that the level of HIV RNA is obviously critical in the infectiousness of an HIV+ person initiated a discussion regarding the possibility of a seropositive person having "safe" unprotected sex. The Swiss Commission for AIDS ("Eidgenössische Kommission für AIDS-Fragen", EKAF) proposed to classify HIV+ persons who are on ART with a plasma HIV RNA below the level of detection for at least 6 months, if they are adherent to therapy, regularly come to medical examinations, and if they do not have any signs of other sexually transmitted diseases, as persons who most likely do not transmit HIV via sexual contact and therefore may have unprotected sex if they want (Vernazza 2008). The intention of the EKAF recommendation is to manage fears of HIV transmission and to enable a normal sex life, as far as possible, between persons with and without HIV. The EKAF recommendation is not agreed to by all HIV experts. A case report from Frankfurt raised questions (Stürmer 2008), where HIV transmission occurred though HIV viral load was not detectable and the HIV+ partner was on successful ART (see chapter 6.12 on Prevention). It is important to highlight though that large international studies in discordant couples with early ART initiation clearly demonstrate a dramatically reduced risk of HIV transmission to the seronegative partner in the setting of suppressed HIV viremia on HIV therapy (Cohen 2011). Ever since these results became available, immediate treatment of an HIV+ individual with a seronegative partner was possible according to most HIV treatment guidelines, with the accompanying liberty of condom-free sex.

Sharing injection paraphernalia

Sharing injection paraphernalia is the most important HIV transmission route for persons who use drugs intravenously. Due to the usually quite large amount of blood that is exchanged when sharing needles, the transmission risk is high. The aspiration of blood to control the correct intravenous position of the needle constitutes the reservoir for transmission. With the introduction of needle exchange programs, the installation of needle vendors, methadone substitution and multiple other preventive measures and social programs, HIV transmission rates have significantly decreased within intravenous drug users in Western Europe. In Eastern Europe, where intravenous drug use constitutes a criminal offence and clean needles are not provided, one sees an unyielding continual increase of HIV transmissions in this

population. One can only hope that the success of prevention efforts in Western Europe will lead to a more liberal management and implementation of prevention programs in Eastern Europe.

Vertical transmission

Without intervention up to 40% of newborns born to HIV-1-positive mothers are infected with HIV-1. The most important risk factor is viral load at the time of delivery. Since 1995 the mother-to-child transmission rate of HIV-1-infected mothers has been reduced to 1–2%. These low transmission rates were reached through the combination of antiretroviral therapy / prophylaxis for the pregnant woman, elective cesarian section prior to the start of labor (no longer necessary if the maternal HIV viral load is successfully controlled on ART and HIV RNA is persistently undetectable), antiretroviral post-exposition prophylaxis for the newborn and substitution for breast feeding. For details refer to the "HIV and Pregnancy" chapter as well as to the European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of HIV-infected adults (website http://www.europeanaidsclinicalsociety.org/).

Blood

The transmission of HIV via blood and blood products has been largely reduced on a global scale, though the risk is not completely eliminated. In Germany blood and blood products are considered safe. Since 1985 all blood donations are tested for HIV-1 via antibody tests, and since 1989 also against HIV-2. For a few years now blood donations are additionally tested via PCR to identify donors who may be in the window of seroconversion and where the HIV ELISA is still negative. Persons with so-called risk behavior, i.e., active injection drug users, sexually active men and women as well as immigrants from high-prevalence countries are excluded from blood donations.

Occupationally-acquired HIV infection

The overall risk for HIV infection after a needlestick injury is estimated to be around 0.3%. The risk for HIV transmission is significantly higher if the injury occurred using a hollow needle – e.g., during blood withdrawal – than with a surgeon's needle. For details on post-exposure prophylaxis (PEP) please refer to the respective chapter in this book. On the other hand, the risk of infecting a patient with HIV when the medical personnel is HIV+ is extremely low. In 1993 19,036 patients of 57 HIV+ physicians, dentists or medical students were screened for HIV infection (CDC 1993a). While 92 patients tested HIV-positive, none of the transmissions was related to the health practitioner.

Non-suitable transmission routes

In general, HIV-transmission due to day-to-day contact between family members is unlikely. It is important to avoid blood-to-blood contacts. Thus, razor blades or tooth brushes should not be commonly shared. In cases of cannula or needle usage, these should be safely deposited in appropriate sharps-containers and not be placed back into the plastic cover.

Insects

All studies that have investigated the possible transmission of HIV via insects have come to the same conclusion, that it is not possible. This holds true as well for studies performed in Africa with a high AIDS prevalence and large insect populations (Castro 1988).

The natural course of HIV infection

The natural course of HIV – in the absence of antiretroviral therapy – is shown in Figure 1. Shortly after infection a so-called acute retroviral syndrome is observed in some patients. This syndrome is characterized mainly by lymphadenopathy, fever, maculopapular rash, myalgia and usually does not last longer than four weeks (see chapter on *Acute HIV-1 Infection*).

The symptoms are unspecific and variable so that the diagnosis of HIV infection is rarely made without additional testing. A period of several years follows where most patients are clinically asymptomatic.

Thereafter symptoms or diseases may occur, classified according to the CDC as category B (Table 2). Among these, oral thrush, oral hairy leukoplakia and herpes zoster are particularly noteworthy, and HIV infection as an underlying diagnosis should always be taken into account. Diseases of category B are not AIDS-defining, however their occurrence is defined as symptomatic of HIV infection and suggests a disturbed cellular immune system.

Later in the course of HIV infection AIDS-defining illnesses occur, at a median of 8–10 years after infection. Without highly active antiretroviral therapy these illnesses eventually lead to death after a variable period of time.

The level of HIV RNA, which reaches extremely high values shortly after primary infection, usually decreases to less than 1% of the maximum value at the time of first HIV antibodies and remains relatively stable for a number of years. This level is called the viral set point. The level of the viral set point determines the speed of disease progression. While most patients with less than 1000 HIV RNA copies/ml are usually not affected by AIDS even 12 years after primary infection, more than 80% of patients have developed AIDS only 2 years after infection if the viral load remains at levels above 100,000 copies/ml (O'Brien 1996).
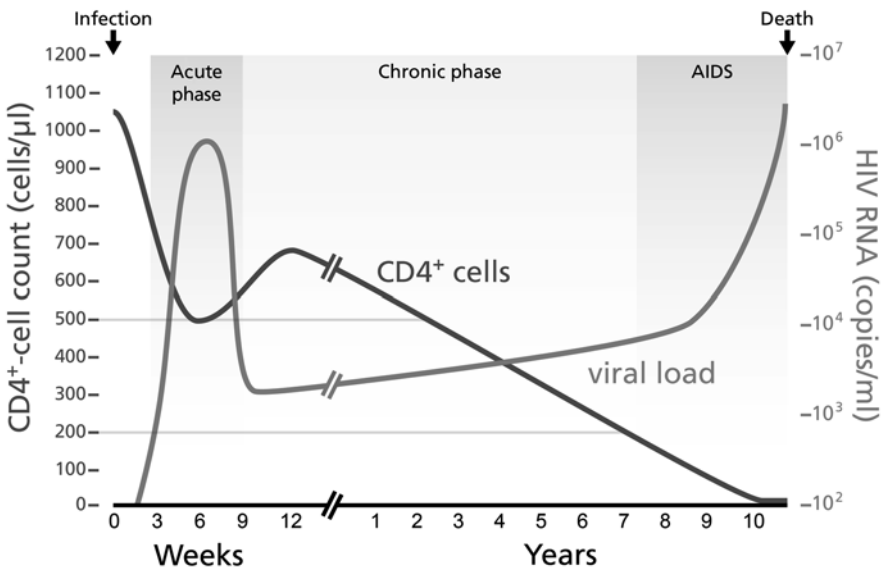
Figure 1: The natural course of HIV infection

Table 2: Clinical categories of HIV infection according to CDC Classification

Category A
Asymptomatic HIV infection
• Acute, symptomatic (primary) HIV infection
• Persistent generalized lymphadenopathy (LAS)

Category B
Symptoms or signs of diseases that do not fall into Category C but are associated with a disturbed cellular immunity. Among these are:
• Bacillary angiomatosis
• Infections of the pelvis, in particular complications of fallopian tube or ovarian abscesses
• Herpes zoster in the case of more than one dermatome or recurrence in the same dermatome.
• Idiopathic thrombocytopenic purpura
• Constitutional symptoms like fever or diarrhea lasting >1 month
• Listeriosis
• Oral hairy leukoplakia (OHL)
• Oropharyngeal candidiasis (oral thrush)
• Vulvovaginal candidiasis, either chronic (>1 month) or difficult to treat
• Cervical dysplasia or carcinoma *in situ*
• Peripheral neuropathy

Category C
AIDS-defining diseases
• Candidiasis of the bronchia, trachea, or lungs
• Esophageal candidiasis
• CMV infections (except liver, spleen and lymph nodes)
• CMV retinitis (with loss of vision)
• Encephalopathy, HIV-related
• Herpes simplex infections: chronic ulcer (>1 month); or bronchitis, pneumonia, esophagitis
• Histoplasmosis, disseminated or extrapulmonary
• Isosporiasis, chronic, intestinal, dura-tion >1 month
• Kaposi sarcoma
• Coccidioidomycosis, disseminated or extrapulmonary
• Cryptococcosis, extrapulmonary
• Cryptosporidiosis, chronic, intestinal, duration >1 month
• Lymphoma, Burkitt
• Lymphoma, immunoblastic
• Lymphoma, primary CNS
• *Mycobacterium avium complex* or *M. kansasii,* disseminated or extrapulmonary
• Mycobacterium, other or not identified species
• *Pneumocystis* pneumonia (PCP)
• Pneumonia, bacterial, recurrent (>2 within a year)
• Progressive multifocal leukoencephalopathy
• Salmonella Sepsis, recurring
• Tuberculosis
• Toxoplasmosis, cerebral
• Wasting Syndrome
• Cervix carcinoma, invasive

The higher the viral set point the faster the decrease of CD4 T cells. CD4 T cells usually drop considerably during acute primary infection. Subsequent CD4 counts recover after a few months to values within the normal range, though pre-infection values are rarely reached. Normal values for CD4 T cell counts vary from laboratory to laboratory, however these are usually in the range of absolute CD4-positive T lymphocytes in adults of 435–1600/µl or relative percentage between 31–60% of total lymphocytes. For children other values apply (see chapter 22).

During the progressive course of HIV infection a gradual decrease of CD4 T cells is observed. The risk for AIDS-defining illnesses increases with time when CD4 T cells decrease below 200. To ascertain the level of immunodeficiency the relative percentage of CD4 T cells should also be taken into account.

Under certain conditions (e.g., under myelosuppressive interferon therapy) low absolute CD4 T cell counts are observed in the context of leuko- and lymphopenia, while the immune status assessed by the relative CD4 T cell count remains normal. 200 CD4 T cells/µl correspond to approximately 15% of CD4 positive lymphocytes. Conversely, the absolute CD4 T cell count may suggest false high values, e.g., after a splenectomy.

Patients can be categorized depending on the speed of the CD4 T cell decrease (Stein 1997) to those with a high risk of disease progression (loss of more than 100 CD4 T cells/µl within 6 months), those with a moderate risk of disease progression (loss of 20–50 cells/µl per year) and those with a low risk of disease progression (loss of less than 20 cells/µl per year).

While the overall risk for AIDS increases if the CD4 T cell count drops below 200 cells/µl, considerable differences exist for the risk of individual AIDS manifestations (see chapter *AIDS*). As an example, opportunistic infections usually occur at far lower CD4 T cell counts than AIDS-associated malignancies (Schwartländer 1992). Apart from the level of HIV RNA and CD4 T cell count, the age of the patient is another important risk factor for progression to AIDS (Figure 2). A 55-year-old patient with a CD4 T cell count of 50 cells/µl and an HIV RNA of 300,000 copies/ml has an

Figure based on data from Philips et al. CASCADE Colaboration. AIDS 2004; 18 (1): 51-58.
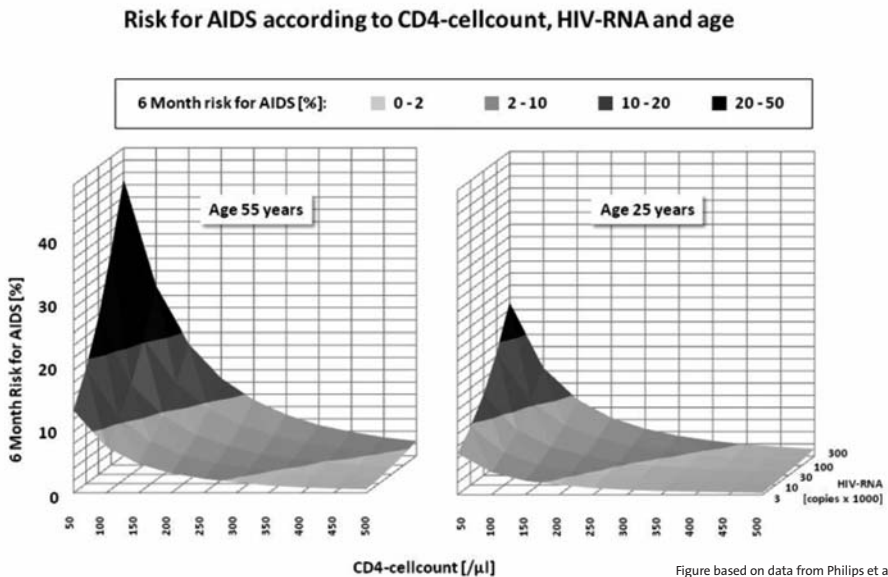
Figure 2: Risk for AIDS according to CD4-cellcount, HIV-RNA and age

almost twice as high risk of developing AIDS within six months as a 25-year-old patient. This explains why the latest antiretroviral treatment guidelines for HIV have included individual factors such as age and level of HIV viral load into their algorithms regarding when to start treatment.

In the pre-ART era the average time between the first manifestations of AIDS and death was 2–4 years. Without therapy probably more than 90% of all HIV+ patients die from AIDS. Today, the progression of HIV infection to AIDS can be halted with treatment. After reaching a maximal suppression of HIV RNA, CD4 T cell counts usually recover and patients regain an almost normal life expectancy.

The level of HIV RNA or the viral set point is dependent on a variety of host-specific factors such as HLA type, chemokine receptor mutations and other, as yet unidentified, factors. In addition, virus-related factors associated with HIV disease progression have to be taken into account.

It is important to visualize that the level of plasma viral load represents an equilibrium between new and dying HIV virions.

Disease progression

In order to classify the progression of HIV infection in most clinical settings, the 1993 CDC classification is still being used that takes the clinical presentation and CD4 T cell count into account (Table 3).

Table 3: Classification of HIV disease according to the CDC (1993)

Symptoms/ CD4 T cells	Asymptomatic or acute HIV disease	Symptomatic but not stage A or C	AIDS-defining illness*
>500/µl	A1	B1	C1
200–499/µl	A2	B2	C2
<200/µl	A3	B3	C3

* for AIDS-defining conditions please refer to Table 2

In 2008 a revised version of the CDC classification of HIV disease was presented. This revised version has been combined into a single case definition for adolescents ≥13 years and adults and is summarized in Table 4. The aim of the revised version was to introduce a simplified classification for continued epidemiological monitoring of HIV and AIDS, which reflected the improved diagnostics and treatment possibilities in HIV. In addition to the three stages listed below a fourth new stage (HIV infection, stage unknown) was introduced for patients in whom no CD4 T cell counts or patient history were available.

Table 4: Classification of HIV-disease according to the revised classification (2008)

Stage	AIDS-defining illness*	CD4 T cell count
1	None	>500/µl or ≥29%
2	None	200–499/µl or 14–28%
3 (AIDS)	Documented AIDS-defining illness	*or* <200/µl or <14%
unknown	No information available	No information available

* the AIDS-defining illnesses have remained unchanged and are listed in Table 2

As a general rule for the classification of a patient, the stage is always adapted according to progression of disease (e.g., someone who is previously asymptomatic, CD4 T cell count 530/µl is Category A; but if they develop oral thrush, their CD4 T cell count drops to 320/µl, they are Category B2). Reclassification upward upon improvement is not considered. If we take the same example as before and the patient has received fluconazole therapy and ART, and at present is asymptomatic and their CD4 T cells have returned to 550/µl, the CDC stage remains at B2. The case definitions of the revised 2008 CDC classification are intended for public health surveillance and not as a guide for clinical diagnosis. Whereas in Europe the term AIDS is only used in cases of clinically manifest AIDS, in the US a CD4 T cell count below 200 cells/µl is also considered AIDS.

Epidemiology

The Human Immunodeficiency Virus probably emerged in the 1920s or '30s when the Simian Immunodeficiency Virus (SIV) jumped host from the chimpanzee to the human in Western Africa (Worobey 2008). The oldest HIV-positive human blood sample was found in Kinshasa (Zaire, now the Democratic Republic of Congo) and dates back to 1959 (Zhu 1998). After the first description of AIDS in 1981, by now almost all countries in the world have been affected by HIV.

Table 5: AIDS epidemic according to UNAIDS, 2014 (www.unaids.org)

	HIV-infected adults and children	New infections 2013	Yearly deaths due to AIDS 2013
Sub-Saharan-Africa	24,700,000	1,500,000	1,100,000
Middle East and North Africa	230,000	25,000	15,000
Asia and the Pacific	4,800,000	350,000	250,000
Latin America	1,600,000	94,000	47,000
Caribbean	250,000	12,000	11,000
Eastern Europe and Central Asia	1,100,000	110,000	53,000
Western and Central Europe and North America	2,300,000	88,000	27,000
Global	35,000,000	2,100,000	1,500,000

The first to be infected are usually persons from so-called high-risk groups (intravenous drug users, professional sex workers, men who have sex with men) and subsequently other population groups are infected via unsafe sex. In industrialized countries homosexual sex is frequently the most common mode of transmission, whereas in countries of the former Soviet Union intravenous drug use (sharing injection paraphernalia) is the most common mode of transmission. In Africa most infections occur due to heterosexual intercourse.

The prevalence and subsequent implications on the epidemic are markedly different from country to country. Whereas HIV/AIDS constitutes a rather marginal health care problem in industrialized countries, in sub-Saharan Africa AIDS has become the most common cause of death: every 5th death in Africa is due to AIDS. The overall life expectancy has decreased in some African nations by more than 20 years. More than 10 million children have been orphaned. The economies of hard-hit nations have and are continuing to suffer from dramatic slumps. According to UNAIDS, in

2014 around 35 million people were infected with HIV/AIDS worldwide (of whom >50% were women) and 1.5 million [1.4 million–1.7 million] people died from AIDS in 2013 (see also Table 5). Overall AIDS-related deaths have fallen by 35% since the peak in 2005, demonstrating the success of a wider access to antiretroviral therapy particularly in sub-Saharan Africa. It also is encouraging that new HIV infections have fallen by 38% since 2001. Worldwide, 2.1 million [1.9 million–2.4 million] people were newly infected with HIV in 2013, down from 3.4 million [3.3 million– 3.6 million] in 2001. Worldwide, 240,000 [210 000–280 000] children became newly infected with HIV in 2013, down from 580,000 [530 000–640 000] in 2001. Most reassuringly, new HIV infections among children have declined by 58% since 2001. The most profoundly affected countries are in the regions of sub-Saharan Africa, where more than 24.7 million people are infected with HIV. The highest dynamic of spread and incidence rates are currently observed in countries of the former Soviet Union, in particular Estonia, Latvia, Russia and the Ukraine, as well as in South and South-East Asia.

In Germany in 2013, around 80,000 people were HIV-positive, among them 15,000 women (Table 6).

Table 6: Epidemiology of HIV/AIDS in Germany (modified according to www.rki.de)

Population	Total numbers (lower and upper estimate)
People with HIV/AIDS in 2013	80,000 (69,000–91,000)
Men	65,000 (56,000–75,000)
Women	15,000 (12,000–17,000)
Children	200
According to transmission group	
Men who have sex with men	53,500 (46,000–61,000)
Persons infected via heterosexual contacts	18,000 (15,000–21,000)
Including persons who got infected in Germany	10,000 (8,600–12,000)
Intravenous drug users	7,800 (6,000–9,500)
Hemophiliacs / received blood transfusions	450
Mother-to-child transmission	420

Summary

The first serological evidence for HIV infection was found in human sera from Zaire dating to 1959, Uganda dating back to1972 and Malawi to 1974 – evidence that HIV was circulating in Africa at those times. The first cases of AIDS were than described in the US in 1981. The discovery of HIV as the cause of AIDS was made in 1983. Since then HIV/AIDS has emerged as a worldwide epidemic which continues to spread today – 30 years later – with still more than 2 million new infections each year. In particular the high infection rates in Eastern Europe and Asia demonstrate the immense challenges that need to be met in current and future implementations of prevention measures. Even though the success of antiretroviral therapy in the treatment of HIV infection appears to normalize life expectancy for HIV+ patients, knowledge about the natural course of HIV infection remains important. Not only in order to make the correct decision on how to start ART in an individual patient, but also to correctly diagnose HIV in patients with first symptoms of HIV infection who have not previously shown AIDS manifestations, this knowledge is important. In light of the fact that in Europe about 50% of all HIV+ persons do not know their HIV status,

tremendous challenges remain in the area of early diagnosis of HIV infection. Joint efforts are being made (HIVeurope.eu) in order to diagnose HIV infection earlier and thus enable physicians and patients to start ART earlier, as well as to lower new infection rates by counseling patients on transmission modes and prevention.

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2. HIV Testing

CHRISTIAN NOAH

Early diagnosis of HIV infection is important: it allows the patient access to anti-retroviral therapy and it is crucial in order to avoid further transmission. Despite extensive testing possibilities and recommendations, HIV infection continues to be diagnosed at late stages. According to the 2014 report from the European Centre for Disease Prevention and Control (ECDC), 47% and 27% of HIV+ patients presented with a CD4 T cell count below 350/µl and 200/µl at the time of initial diagnosis. In Germany, the number of patients unaware of their positive HIV status is estimated at 14,000 (RKI 2014).

There are several indications and reasons for HIV testing. Every pregnant woman should be offered an HIV test to prevent mother-to-child transmission. HIV testing also plays an important security role in blood and organ donation. HIV testing is also indicated in case of symptoms compatible with an acute antiretroviral syndrome, in case of indicator diseases (oral thrush, OHL, etc) or an AIDS-defining illness, as well as after occupational or non-occupational exposure to HIV.

The basics of HIV diagnostics

The laboratory diagnosis of HIV infection is primarily based on a serologic screening test. A reactive result has to be confirmed by a confirmatory test. Due to its relatively high sensitivity, the 4th generation test ("Combo test") that simultaneously detects both HIV-specific antibodies and p24 antigen should be used (Breast 2000, Weber 2002, Sickinger, 2004, Skidmore 2009, Bentsen 2011). Any approved screening test detects all known HIV types (HIV-1 and -2), HIV groups and HIV subtypes.

There are numerous commercial systems available for screening. However, the basic technological principle is the same for all and is based on antigen-antibody binding. The prototype assay is the ELISA (enzyme linked immunosorbent assay). Its central element is a plastic plate with 96 wells (microtiter plate). The surface of each cavity is coupled with HIV antigens and HIV antibodies. When a patient's serum or plasma containing HIV antibodies is placed into one cavity, antibodies bind to the coupled antigen. An enzyme-labelled second antibody is then added, which recognizes and binds to human antibodies. Finally a substrate is added that is converted by the enzyme at the second antibody. The result is a color change, measured photometrically. The optical density correlates with the HIV antibody concentration in the sample of the patient – the higher the intensity, the more antibodies present in the sample. Based on this prototype several advances have improved the efficiency and effectiveness of the screening test (Perry 2008). Modern test systems are highly automated to achieve a very high degree of standardization and generate a result in less than an hour. In these systems, the solid phase consists of microparticles coupled with the virus antigens and antibodies. Accordingly, the method is referred to as a "microparticle enzyme immunoassay" (MEIA).

The measured value is usually an index without dimensions, calculated from the ratio of the measured value of the patient sample and the negative control (Sample/Control, S/Co). Values below 1 are considered negative, values above 1 as reactive. It should always be called "reactive" and not a "positive" result to document that this result needs to be confirmed by a second test.

With the screening test, sensitivity has the highest priority (this way, no infection should be missed), while a high specificity is preferred for the confirmatory test. Screening tests approved in Germany require a specificity of 99.5%. That means that

one in 200 HIV-negative samples could have a false-reactive test result. False-reactive results are caused for example by stimulation of the immune system (e.g., viral infections, pregnancy, vaccinations, autoimmune diseases). Thus, in certain patient groups (e.g., pregnant women, dialysis patients) an increased proportion of false reactive test results can occur.

To confirm a reactive screening test a Western Blot (immunoblot) analysis is typically carried out. Viral proteins (antigens) are separated by their molecular weight via electrophoresis and transferred to a membrane, which is then used as a test strip. An advance in terms of standardization is the so-called line blot produced by spraying recombinant HIV antigens directly onto a test membrane. The test strip is incubated with the serum or plasma. If HIV-specific antibodies are present, they bind to the antigen. Analogous to the ELISA the resulting antigen-antibody complex will become visible on the test strip using an enzyme-labeled second antibody and a corresponding substrate. According to the antibody specificities a corresponding band spectrum occurs on the test strip.

Ideally, the laboratory will use a Western Blot, which also can detect and differentiate antibodies against HIV-2. In some assays, a synthetic peptide is used for HIV-2 screening. In case of a reactive HIV-2 band, this result must be confirmed by an HIV-2-specific Western Blot. Generally, Western Blot analysis leads to definite discrimination between an HIV-1 or HIV-2 infection. However, due to the close relationship cross reactivity leading to antibody reactions against both virus types can occur. In those cases, type-specific PCR assays may help. The final laboratory report should indicate if a patient is infected with HIV-1 or HIV-2 since the virus type has implications with regard to the antiretroviral treatment. The various HIV proteins are assigned to three functional groups ("p" – protein, "gp" – glycoprotein. The numbers refer to the molecular weight):

Table 1: HIV antigens and functions

	Antigens		Function
	HIV-1	HIV-2	
Envelope proteins (env)	gp160	gp140	Precursor of envelope proteins
	gp120	gp125	Outer envelope protein
	gp41	gp36	Transmembrane protein
Polymerase proteins (pol)	p66	p68	Reverse Transcriptase, RNaseH
	p51	p53	Reverse Transcriptase
	p32	p34	Endonuclease, integrase
Core proteins (gag)	p55	p56	Precursor of core proteins
	p24	p26	Inner core protein
	p17	p16	Outer core protein

The formation of antibodies after infection follows a specific kinetic: while p24 and gp120 antibodies are detectable early, the p31 band usually occurs later in the course of infection (Fiebig 2003). With regard to the antibody specifics, the criteria for a positive result are not uniformly defined. In general, a Western Blot is considered positive when at least two or three bands are visible. For interpretation of a Western Blot the criteria specified by the manufacturer in the context of CE-marking are crucial. Furthermore, general guidelines exist. According to the German guidelines, based on the DIN 58969 Part 41 ("serodiagnosis of infectious diseases – immunoblot"), a test result is considered positive when antibodies to an env protein and also to a *gag* protein and/or a *pol* protein are detected. According to WHO criteria a Western Blot is positive when antibodies against at least 2 *env* proteins are

detectable. For example, a Western Blot with a gp120 and p24 band would be interpreted borderline according to the WHO and positive according to the German criteria. However, a weak band spectrum, especially if "early" antibodies were detected, may indicate an early phase of an HIV infection and further tests such as PCR should be carried out (see below).

Compared to a 4th generation screening test the p24 antigen is not included in the confirmatory test. In the case of "reactive screening test – negative confirmatory test", acute HIV infection cannot be excluded when HIV-specific antibodies are not yet formed although the p24 antigen is present. Such a result should be checked after 2-3 weeks. If a patient is concerned regarding an acute infection (acute retroviral syndrome, recent exchange of bodily fluids with an HIV+ person) the implementation of an HIV PCR is useful. The PCR is also recommended in case of a highly positive screening and negative confirmatory test result. It is recommended to consult the laboratory to discuss the adequate procedure.

To exclude sample confusion each first positive test result should be confirmed by examination of a second sample. If a patient is suspected to have an HIV infection, the result of viral load measurement can be used for confirmation (see chapter 6.11 on HIV monitoring). In this case, a second serological test is not necessary.

HIV PCR

In addition to the serological test systems, molecular methods for detection of HIV RNA (nucleic acid amplifications tests, NAT) are available. PCR is the NAT most frequently used for HIV RNA detection. Other techniques (b-DNA, NASBA) are less common. The quantitative detection of HIV RNA (a viral load determination) is one of the essential components of the monitoring of HIV infection (Wittek 2007, Thompson 2010). To increase the safety of blood products the HIV PCR is obligatory in the context of blood donation. Other indications for the use of the PCR are the exclusion of an HIV infection of newborns of HIV+ mothers (see below), the clarification of equivocal serological constellations or a suspected acute infection.

According to new recommendations, PCR analysis may be used for confirmation of a reactive screening test result instead of a Western Blot. For this purpose, a PCR test is considered positive in case of a viral load above 1000 copies/ml. If the viral load amounts to less than 1000 copies/ml or the PCR is negative subsequent Western Blot analysis is obligatory (DVV/GfV 2015). However, the HIV PCR is not recommended as a screening test. Since false negative results are possible it cannot replace the serological screening test.

Possible reasons for false negative results are as follows:
1. Commercially available HIV PCR tests usually do not cover HIV-2 (rare in Europe). Thus, an additional HIV-2 PCR must be carried out.
2. HIV is characterized by a high degree of genetic diversity. In case of infection with a new or previously unknown variant sensitivity of the PCR may decrease due to mutations affecting the primer or probe binding sites. Through a so-called "dual target" PCR the risk of false negative test results due to sequence variability may be reduced (Chudy 2012; see also chapter 6.11 on Monitoring). The "dual target" PCR is obligatory for screening blood donations.
3. A small number of HIV+ patients can suppress viral replication in the absence of ART ("elite controllers", prevalence less than 1%). Thus, despite serologically proven HIV infection a PCR test may be negative in those patients.
4. The aim of the antiretroviral treatment is the reduction of the viral load below the detection limit. As a consequence, the use of a PCR as a HIV screening test in a successfully treated patient would lead to a false-negative testing result.

For laboratories the use of the HIV PCR for primary diagnosis is challenging since commercially available test systems usually have not been validated by the manufacturers for this purpose. Thus, the laboratory is responsible for validation.

Rapid tests

Rapid HIV tests functionally correspond to a screening test, i.e., a reactive result must be confirmed by a Western Blot analysis. Rapid tests can be carried out quickly, easily and without any equipment expense and can therefore be used as so-called "point of care" tests. In addition to plasma and serum, full or capillary blood (from the fingertip or the ear lobe) is suitable as test material, so that no centrifuge is required. In some test systems urine or oral transudate (not saliva) may be used. However, rapid tests exhibit less sensitivity if specimens others than serum or plasma are used (Pavie 2010). Results are available within 15 to 30 minutes. Most frequently, rapid tests are based on immuno-chromatographic methods. Other techniques such as particle agglutination and immunofiltration are also used (Branson 2003, Greenwald 2006).

Rapid tests produced according to the European directive 98/79/EC on *in vitro* diagnostic medical devices (CE marking) are considered safe. These tests exhibit a high sensitivity and specificity in studies (Huppert 2010). However, apparently there are limitations regarding diagnosis of primary HIV infection: almost all currently available rapid tests only detect HIV antibodies but not p24 antigen, corresponding to the (outdated) 3rd generation HIV test. Since 2009 a certified 4th generation rapid test (Determine HIV-1/2 Ag/Ab Combo, Inverness Medical) is available which not only detects but can also differentiate HIV antibodies and p24 antigen. Although the superiority of this rapid test compared to the 3rd generation rapid test was illustrated (Chetty 2012), some studies indicate a lack of sensitivity in the context of acute HIV infections (Kilembe 2012, Brauer 2013). In a comparative study the test exhibited deficiencies regarding the recognition of primary HIV infections. About one third of the samples of patients with acute HIV infection tested falsely negative. Reactivity was delayed by one week compared to a reference test (Mohrmann 2009). Rapid tests should be used only for initial orientation. The results of the testing should be confirmed at the earliest opportunity in a routine laboratory with a standard HIV test. Rapid tests are particularly suitable for use in emergency situations where the test result has immediate consequences. These include emergency operations and needlestick injuries. Also in pregnant women with unknown HIV status at delivery a rapid test can be useful. However, the cooperating laboratory should be contacted to indicate the need for a rapid HIV result. When necessary, the result of a conventional HIV test can be available within one hour upon receipt of the sample. Rapid tests are also useful in countries with poor medical infrastructure (UNAIDS/WHO 2009) and in the context of low-threshold testing for individuals who would otherwise not be tested.

The diagnostic window

The "diagnostic gap" or "window" indicates the time period between transmission of a pathogen and the onset of biochemical measurable infection markers such as antibodies, antigen or nucleic acids (Busch 1997). At the earliest, HIV antibody production begins two weeks after transmission. HIV-specific antibodies can be detected after four weeks in 60–65%, after six weeks in 80%, after eight weeks in 90% and after twelve weeks in 95% of cases. A "seronegative" chronic HIV infection is an absolute rarity and irrelevant in practice (Spivak 2010). The p24 antigen is detectable

about five days before seroconversion (the first occurrence of specific antibodies). Therefore, 4th generation diagnostic tests can shorten the diagnostic gap by simultaneous detection of p24 antigen. The earliest lab marker is HIV RNA that is detectable approximately seven days before the p24 antigen (Fiebig 2003). In many cases HIV RNA can be detected by the second week after transmission. However, a negative result at this time point cannot exclude an infection.

A negative result in the HIV screening test precludes the existence of HIV antibodies and p24 antigen at the time of testing. A patient is considered HIV negative. The security of this result, however, depends particularly on the time interval from the possible transmission event. This has important consequences:

1. HIV testing immediately after a possible transmission is not meaningful. As no HIV antibodies are yet formed, an HIV test should be carried out at the earliest in the 3rd week after exposure. Exception: If it needs to be documented for legal reasons (e.g., needlestick injury) that at the time of transmission no existing HIV infection was present.

2. According to new testing guidelines (Gökengin 2014, DVV/GfV 2015) an HIV infection cannot be ruled out until 6 weeks after possible transmission with sufficient certainty when a 4th generation screening test was used. In case of a 3rd generation assay or a rapid screening test the diagnostic window amounts to 12 weeks. Even when using 4th generation tests in some circumstances a follow-up at 12 weeks after exposure is recommended, e.g., the simultaneous infection with another sexually transmitted pathogen or an impaired ability to develop antibodies. A further test beyond the diagnostic window is appropriate only in exceptional cases, for example, if there is suspicion of acute retroviral syndrome or if post-exposure prophylaxis was given.

3. A negative test result is dependable only in the case of no re-exposure within the past 6 or 12 weeks, respectively (from the time of the original exposure).

HIV diagnostics in newborns

In newborns of HIV+ mothers maternal antibodies may remain detectable until the age of 18 months. The antibodies are transplacentally transferred from the 32nd week of gestation although they do not have any protective effect. A positive HIV test result in the newborn indicates previous HIV exposure. However, a serological HIV test for the detection or exclusion of vertical transmission of HIV is not sufficient as a positive result will be expected in any case (Read 2007).

At least two negative PCR results are required to exclude HIV transmission. The first HIV PCR should be performed after the first month of life (sensitivity 96%, specificity 99%), then again because of the nearly 100% sensitivity and specificity after the third month. Vertical transmission can be ruled out, however, only if there was no renewed risk of transmission in the meantime through breastfeeding.

Even with negative PCR results, the disappearance of maternal antibodies should be documented at least once. In the case of positive results, these must be confirmed by examination of a second sample.

HIV diagnostics after occupational exposure

After a needlestick injury or other occupational exposure, HBV, HCV and HIV infection of the index patient should be excluded (of course, consent of the index patient is required). With regard to the potential necessary rapid start of post-exposure prophylaxis (PEP) a needle stick injury should always be considered an emergency. The earlier PEP is initiated (preferably within 24 hours), the better the chances of success.

If a rapid result of an HIV screening test is not available for logistical reasons, an HIV rapid test should be considered. To save time, PEP can be initiated immediately and terminated at any time in the case of a negative result.

If the index patient has no symptoms consistent with acute retroviral syndrome the negative result of the screening test excludes HIV infection with a high level of security. An HIV PCR test should be considered only if there is evidence of acute HIV infection of the index patient. Conversely, if the index patient is infected with HIV or if the HIV status is unknown, HIV screening should be performed in the exposed person. For legal reasons, the first HIV test should take place immediately after the needlestick injury to document that no HIV infection was present at the moment of the accident. Check-ups should be carried out at 6 weeks, at 3 and at 6 months. If the index patient is infected with HIV, testing at 12 months is recommended (Ridzon 1997, Ciesielski 1997).

What is relevant in practice?

• **The legal situation:** Although HIV infection has become manageable, the HIV test still retains a special status in laboratory diagnostics. Because of possible medical, social and legal consequences, an informed consent of the patient is required before performing an HIV test. Testing against the wishes of the patient is an invasion of privacy, potentially corresponding with legal consequences for the doctor. A written consent is not required, but the consent should be documented. In children or infants, the patient's parents or legal guardians must agree. With the aim to increase the readiness for testing and to enable early access to adequate antiretroviral therapy the CDC recommendations for HIV testing have been revised. These include a so-called "opt-out" screening concept: The patient is informed about the HIV test, but it will be performed provided the patient does not explicitly reject testing (Branson 2006).

• **Advice:** There should not be any HIV testing without counseling and education. The patient should be informed about the testing algorithm and the possibilities and limitations of HIV testing. Particularly, the limitations of the (frequently demanded) HIV PCR in primary diagnostics should be addressed: while a sensitive method for detection, it is only conditionally suitable for the rapid exclusion of HIV infection or transmission. Due to the distress caused to the patient, the high cost of the PCR as a counter argument against the method is a rare deterrent for the patient. During the consult, all the possibilities of the test result and in particular the "diagnostic window" should be noted. A desired HIV test could also be an occasion to discuss the risk of transmission in general (also for other sexually transmitted diseases) and appropriate prevention methods with the patient.

• **Reporting:** A negative test result can possibly be reported by telephone if the patient has been previously advised of its value. The diagnosis of HIV, however, has to be given in a personal counseling interview by a physician (or expert virologist) only (in many places, the result can be given by a registered nurse or counselor). The response of a patient cannot be assessed adequately when reporting is done by telephone. Sometimes patients can develop suicidal thoughts. Similarly, the negative result of a confirmatory test following a reactive screening test should be personally discussed with regard to the possibility of an acute infection. Patients should be directed to an HIV-focused practice. In addition, the patient should be advised of regional counseling and care centers. The result of a reactive HIV screening test should never be reported before the result of the confirmatory test is available.

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3. Pathogenesis of HIV-1 Infection

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(earlier versions by Andrea Rubbert, Georg Behrens and Mario Ostrowski)

Since the initial description of the Human Immunodeficiency Virus type I (HIV-1) in 1983 (Barré-Sinoussi 1983, Gallo 1983) and HIV-2 in 1986 (Clavel 1986), these two viruses have been identified as the primary cause of Acquired Immunodeficiency Syndrome (AIDS). As HIV-1 is the major cause of AIDS in the world today, our discussion will be primarily limited to HIV-1 infection. Worldwide, the number of HIV-1-infected persons exceeds 36 million (according to UNAIDS), the majority of whom live in developing countries in Sub-Saharan Africa, Asia and South America. Despite all the therapeutic advantages achieved over the last decades, including the evolution of "HAART", once an individual has become infected, eradication of the virus is not possible. In addition, new problems relating to the short- and long-term toxicity of drug treatments and the occurrence of resistance mutations in both circulating and transmitted viruses are emerging. In many countries in South East Asia and Africa, the incidence is failing to substantially decline. The prevalence of HIV-1 infection continues to increase and surpass that of Europe and North America. However, due to the high costs of drug regimens and the often precarious health-care infrastructure in these developing countries, the widespread use of ART is currently still partial at best. The further course of the HIV-1 pandemic, therefore, mainly depends on how and to what degree developing countries with a high HIV-1 prevalence are able to take advantage of the medical progress achieved overall, and whether an effective prophylactic vaccine will become available in the near future (see chapter on *Preventive HIV-1 Vaccine*).

An understanding of the immunopathogenesis of HIV-1 infection is a major prerequisite for rationally improving therapeutic strategies, developing immunotherapeutics and prophylactic vaccines. As in other virus infections, the individual course of HIV-1 infection depends on both host and viral factors.

The course of infection with HIV-1 in HIV-infected humans may vary dramatically, even when primary infection comes from the same source (Liu 1997). In some individuals with a long-term non-progressive HIV-1 infection (i.e., lack of decline in CD4 T cell counts, or chronic infection for at least 7 years without the development of AIDS), a defective virion has been identified (Kirchhoff 1995). Thus, infection with a defective virus, or one that has a poor capacity to replicate, may prolong the clinical course of HIV-1 infection. However, in most individuals, HIV-1 infection is characterized by a replication-competent virus with a high daily turnover of virions.

Host factors may also determine whether or not an HIV-1-infected individual rapidly develops clinically overt immunodeficiency, or whether this individual belongs to the group of long-term non-progressors that represents about 5% of all infected patients. The identification and characterization of host factors contributing to the course of HIV infection, including immunological defense mechanisms and genetic factors, will be crucial for our understanding of the immunopathogenesis of HIV infection and for the development of immunotherapeutic and prophylactic strategies.

The structure of HIV-1

HIV-1 is a retrovirus and belongs to the family of lentiviruses. Infections with lentiviruses typically show a chronic course of disease, a long period of clinical latency, persistent viral replication and involvement of the central nervous system. Visna in sheep, simian immunodeficiency virus (SIV) in monkeys, or feline immun-

odeficiency virus (FIV) in cats are typical examples of lentivirus infections in animals. Using electron microscopy, HIV-1 and HIV-2 resemble each other strikingly. However, they differ with regard to the molecular weight of their proteins, as well as having differences in their accessory genes. HIV-2 is genetically more closely related to SIV found in sooty mangabeys (SIV$_{sm}$) rather than HIV-1 and it is likely that it was introduced into the human population via monkeys. Both HIV-1 and HIV-2 replicate in CD4 T cells and are regarded as pathogenic in infected persons, although the immune deficiency may be less severe in HIV-2-infected individuals.

The morphologic structure of HIV-1

HIV-1 viral particles have a diameter of 120–150 nm and are surrounded by a lipoprotein membrane. Each viral particle contains up to 72 glycoprotein complexes, which are integrated into this lipid membrane, and are each composed of trimers of an external glycoprotein gp120 and a transmembrane spanning protein gp41. The bonding between gp120 and gp41 is only loose and therefore gp120 may be shed spontaneously within the local environment (also called "shedding"). Glycoprotein gp120 can be detected in the serum as well as within the lymphatic tissue of HIV-infected patients. During the process of budding, the virus may also incorporate different host proteins from the membrane of the host cell into its lipoprotein layer, such as HLA class I and II proteins, or adhesion proteins such as ICAM-1 that may facilitate adhesion to other target cells. The matrix protein p17 is anchored to the inside of the viral lipoprotein membrane. A capsid, composed of roughly 200 copies of the protein p24, encloses two copies of the HIV-1 RNA genome. The HIV-1 RNA is part of a protein-nucleic acid complex, which is composed of the nucleoprotein p7 and the reverse transcriptase p66 (RT). The viral particle contains major parts of the enzymatic equipment necessary for replication: a reverse transcriptase (RT), an integrase p32 and a protease p11 (Gelderbloom 1993) (Fig. 1).

The organization of the viral genome

Most retroviruses contain three genes: *gag*, *pol* and *env*: *gag* means "group-antigen", *pol* represents "polymerase" and *env* is for "envelope" (Wong-Staal 1991) (Fig. 2). The classical structural scheme of a retroviral genome is: 5'LTR-*gag-pol-env*-3'LTR. The LTR (long terminal repeat) regions represent the two end parts of the viral genome connected to the cellular DNA of the host cell after integration. These do not encode for viral proteins. This stable integration of the proviral DNA into the host genome leads to a permanent infection. The excision of the proviral DNA out of the human genome would lead to the cure of HIV infection. This was done by creating an enzyme (HIV-1 long terminal repeat site-specific recombinase), which excises the proviral DNA at the two LTR regions of the genome (Hauber 2013). The investigators were able to show that this enzyme can be expressed in HIV-infected cells and that it can excise the provirus precisely without harming the host DNA. The results were confirmed in humanized mouse models. For application in humans, the key question is how to introduce this enzyme into the infected cells.

The *gag* gene codes for the matrix, capsid and nucleocapsid and *env* for the glycoproteins of the viral memebrane; the *pol* gene codes for the reverse transcriptase and other enzymes. In addition, HIV-1 contains six genes (*vif*, *vpu*, *vpr*, *tat*, *rev* and *nef*) in its 9kB RNA that contribute to its genetic complexity. *Nef, vif, vpr* and *vpu* were classified as accessory genes in the past, as they are not absolutely required for replication *in vitro*. However, the regulation and function of these accessory genes and their proteins have been studied and characterized in more detail over the past few years. The accessory genes *nef*, *tat* and *rev* are all produced early in the viral replication cycle.
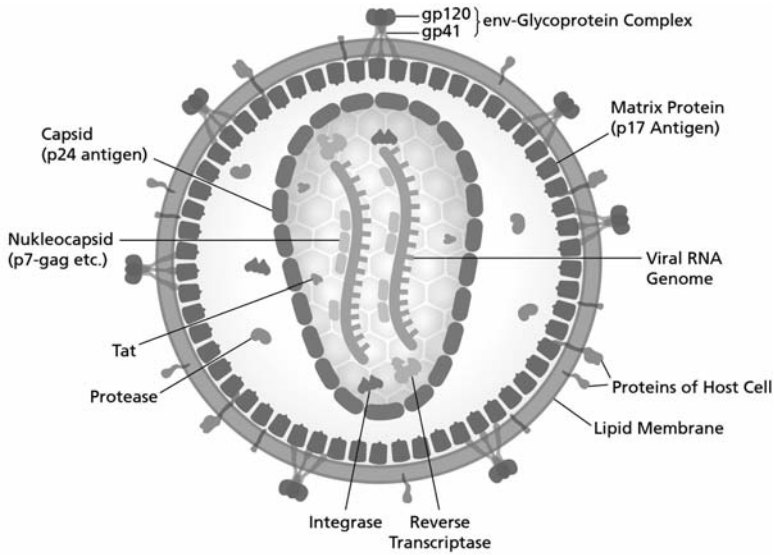
Figure 1: Structure of an HIV virion particle. For detailed explanations see text

Tat and *rev* are regulatory proteins that accumulate within the nucleus and bind to defined regions of the viral RNA: TAR (transactivation-response elements) found in the LTR; and RRE (*rev* response elements) found in the *env* gene, respectively. The *tat* protein is a potent transcriptional activator of the LTR promoter region and is essential for viral replication in almost all *in vitro* culture systems. Cyclin T1 is a necessary cellular cofactor for *tat* (Wei 1998). *Tat* and *rev* stimulate the transcription of proviral HIV-1 DNA into RNA, promote RNA elongation, enhance the transportation of HIV RNA from the nucleus to the cytoplasm and are essential for translation. *Rev* is also a nuclear export factor that is important for switching from the early expression of regulatory proteins to the structural proteins synthesized later on.

Nef has been shown to have a number of functions. It may induce down-regulation of CD4 and HLA class I molecules (Collins 1998) from the surface of HIV-1-infected cells, which may represent an important escape mechanism for the virus to evade an attack mediated by cytotoxic CD8 T cells and to avoid recognition by CD4 T cells. *Nef* may also interfere with T cell activation by binding to various proteins that are involved in intracellular signal transduction pathways (Overview in: Peter 1998). In SIV-infected rhesus macaques, an intact *nef* gene was essential for a high rate of virus production and the progression of disease. HIV-1, with deletions in *nef,* was identified in a cohort of Australian long-term non-progressors (Kirchhoff 1995). However, more recent reports indicate that some of these patients are now developing signs of disease progression including a decline of CD4 T cells. Thus, although deletions of the *nef* gene may slow viral replication, they cannot always prevent the eventual development of AIDS. *Nef* is very immunogenic which means that strong immune responses frequently exist towards this protein. These develop often during acute infection (Lichterfeld 2005).

Vpr seems to be essential for viral replication in non-dividing cells such as macrophages. *Vpr* may stimulate the HIV LTR in addition to a variety of cellular and viral promoters. More recently, *vpr* has been shown to be important for the transport of the viral pre-integration complex to the nucleus (Overview in: Miller 1997) and may arrest cells in the G2 phase of the cell cycle.
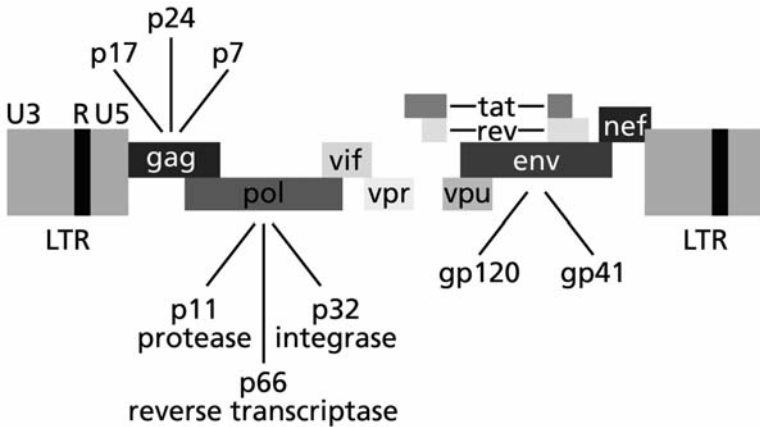
Figure 2: HIV and its genes. For detailed explanations see text

Vpu is important for the viral "budding" process, because mutations in *vpu* are associated with persistence of viral particles at the host cell surface. Membrane molecules such as tetherin (CD317) can bind *vpu*-deficient HIV-1 and prevent viral release. Thus, *vpu* can be considered as a viral escape mechanism in order to antagonise this effect (Neil 2009) and appears to be of great importance for the evolution of the pandemic virus (Sauter 2009). *Vpu* is also involved when CD4-gp160 complexes are degraded within the endoplasmic reticulum and therefore allows recycling of gp160 for the formation of new virions (Cullen 1998).

Vif is a viral protein that builds complexes with APOBEC3G (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G) and therefore inactivates this enzyme (Mariani 2003) (Fig. 3). APOBEC3G is a host restriction factor leading to the degradation of the viral DNA. It is therefore a mechanism of protection developed by higher organisms against viruses. It belongs to a family of intracellular enzymes that specifically deaminate cytosine to uracil in mRNA or single-strand DNA viruses. As a consequence, G to A mutations arise with stop codons. Often the DNA is degraded before that because uracil is changed by uracil-DNA glycosidases with the viral genome becoming the goal of specific endonucleases.

Of interest, the antiviral activity of APOBEC3G is highly conserved among various species, whereas the blockade of APOBEC3G by vif is highly specific for HIV. HIV-1 vif does not complex to murine or rhesus APOBEC3G. In the absence of vif, APOBEC3G is incorporated into newly formed viral particles and in subsequently infected target cells, synthesis of proviral DNA is blocked. In contrast, in the presence of vif, APOBEC3G is complexed, degraded and not incorporated in newly formed virions. APOBEC3G is expressed in lymphocytes and macrophages representing the primary target cells of HIV infection. In dendritic cells (DC), the activation status of the cells influences the amount of APOBEC3G. Upon DC maturation there is an increase of APOBEC3G expression (Pion 2006).

There are still a lot of open questions regarding the regulation of intracellular APOBEC3G. For example, whether there is a critical amount of intracellular APOBEC3G that restricts HIV infection in the presence of *vif,* or whether genetic polymorphisms of APOBEC3G exist that may potentially affect the course of disease, is not clear. Of note, specific inhibitors that block the interaction of *vif* and APOBEC3G or that interfere with the intracellular degradation of APOBEC3G could represent promising future treatments. In principle, blockade of cellular structures
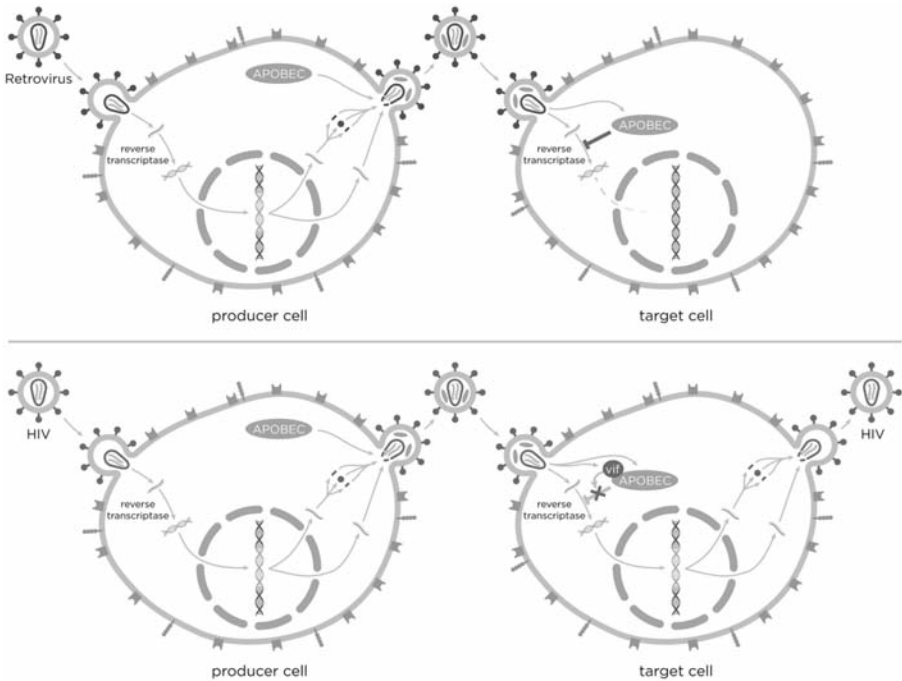
Figure 3: The protein APOBEC3G exerts innate immune activity against retroviruses by interfering with reverse transcription as well as integration of the viral DNA. The HIV-protein *vif* is able to sabotage this defense mechanism by binding to APOBEC3G, which prevents its incorporation in newly-formed viruses and by facilitating its proteosomal degradation

will likely be associated with a minimal risk that the development of resistance might compromise the efficacy of an antiviral agent. Therefore, targeting *vif* and APOBEC3G probably represents an interesting therapeutic track.

Vpx is a structural protein, only found in HIV-2 and SIV variants in primates (African green monkeys (SIVagm) and macaques (SIVmac)). *Vpx* was used to identify a novel viral restriction factor called SAMHD1 (sterile alpha motif and HD domain 1), for whom HIV-1 apparently does not have a counterstrategy. SAMHD1 plays a part in the pathogenesis of the genetically-determined encephalopathy Aicardi-Goutiéres syndrome. In addition, it is supposed to have a negatively regulating role in interferon responses. There is evidence that SAMHD1 inhibits HIV-1 replication through depletion of the intracellular pool of deoxynucleoside triphosphates. *Vpx* can counteract this effect by facilitating the proteosomal degradation of SAMHD1. Thus, SAMHD1 is an antiviral restriction factor, which inhibits the early steps in HIV-1 replication (Goldstone 2011, Lahouassa 2012).

The HIV replication cycle

HIV entry

CD4 as a primary receptor for HIV

CD4 is a 58 kDa monomeric glycoprotein that can be detected on the cell surface of about 60% of T lymphocytes, on T cell precursors within the bone marrow and thymus, and on monocytes and macrophages, eosinophils, dendritic cells and microglial cells of the central nervous system. CD4, as a primary and necessary recep-

tor for HIV-1, HIV-2 and SIV, was characterized in 1984 (Dalgleish 1984). Residues within the V2 region of CD4 (amino acids 40–55) are important for the binding of gp120 to CD4 and this region overlaps the part of the CD4 where its natural ligands, HLA class II molecules, bind.

CD4 attaches to the T cell receptor complex (TCR) on CD4 T cells and binds to HLA class II molecules on antigen-presenting cells. The binding of gp120 to CD4 is not only a crucial step for viral entry, but also interferes with intracellular signal transduction pathways and promotes apoptosis in CD4 T cells (Banda 1992).

Interestingly, monoclonal antibodies against CD4-induced conformational (CD4i) epitopes of gp120 bind well to the gp120 of CD4-independent viruses. This observation suggests that the gp120 of CD4-independent viruses already exposes the regions that are necessary for coreceptor recognition and binding and therefore binding to CD4 is not a prerequisite of entry for these viruses. CD4-independent viruses are easy to neutralize using the serum of HIV-infected patients, suggesting that the immune response selects against CD4-independent viruses (Edwards 2001).

Chemokine receptors as co-receptors for HIV entry

The expression of human CD4 receptors on the surface of a non-human cell line was not sufficient to allow entry of HIV. Therefore the existence of additional human co-receptors necessary for viral entry was postulated. CD8 T cells from HIV-infected patients are able to suppress viral replication in co-cultures with HIV-infected autologous or allogenic CD4 T cells, and this is independent of their cytotoxic activity (Levy 1996). Cocchi identified the chemokines MIP-1α, MIP-1β and Rantes in supernatants from CD8 T cells derived from HIV-infected patients, and was able to show that these chemokines were able to suppress replication in a dose-dependent manner of some, but not all, viral isolates tested (Cocchi 1995). MIP-1α, MIP-1β and Rantes are ligands for the chemokine receptor CCR5, and a few months later several groups were able to show that CCR5 is a necessary co-receptor for monocytotropic (M-tropic) HIV-1 isolates (Deng 1996, Doranz 1996, Dragic 1998). M-tropic HIV-1 isolates are classically those viruses that are most easily propagated in macrophage cultures, are unable to infect T cell lines (i.e., immortalized T cells), but are able to easily infect primary T cells from peripheral blood samples. Conversely, T cell-tropic HIV-1 isolates have classically been identified as being those that are easily propagated in T cell lines, and grow poorly in macrophages, but are also able to easily infect primary T cells from peripheral blood samples. It should be noted that both M-tropic and T-tropic HIV-1 variants can easily infect primary human non-immortalized T cells *in vitro*.

Approximately at the same time, the chemokine receptor CXCR4 (fusin) was described as being the co-receptor used by T cell-tropic (T-tropic) HIV isolates (Feng 1996). SDF-1 (stromal cell-derived factor 1) was identified as the natural ligand of CXCR4 and is able to inhibit the entry of T-tropic HIV-1 isolates into activated CD4 T cells. A schematic model is depicted in Fig. 4. T-tropic HIV-1 isolates mainly infect activated peripheral blood CD4 T cells and cell lines and use CXCR4 for entry into the CD4-positive target cell. M-tropic isolates are able to infect CD4 T cells, monocytes and macrophages, and depend on the use of CCR5 and CD4 for viral entry.

The interaction of gp120 and the cellular receptors is now understood in more detail. Gp120 primarily binds to certain epitopes of CD4. Binding to CD4 induces conformational changes in gp120 that promote a more efficient interaction of the V3 loop of gp120 with its respective co-receptor. Membrane fusion is dependent on gp120 co-receptor binding. Gp41, as the transmembrane part of the envelope glycoprotein gp160, is crucial for the fusion of the viral and host cell membrane. Similar to influenza hemagglutinin, it was postulated that consequent to the binding of gp120
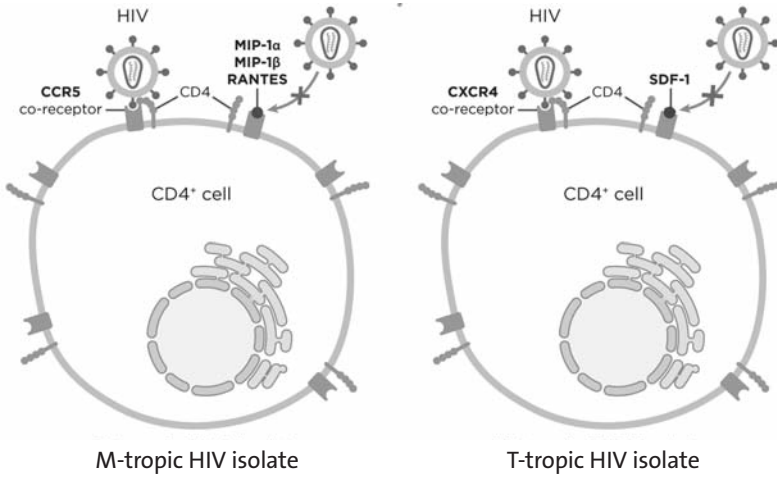
Figure 4: Inhibition of viral entry of CCR5-utilizing (monocytotropic) and CXCR4-utilizing (T cell tropic) HIV isolates by the natural ligands of the chemokine co-receptors CCR5 and CXCR4

to CD4, a conformational change is induced in gp41 that allows gp41 to insert its hydrophobic NH_2 terminal into the target cell membrane. Gp41 has been compared to a mouse trap and a crystallographic analysis of the ectodomain of gp41 seems to confirm that (Chan 1997). The identification of crucial amino acid sequences for this process was used to synthesize peptides that bind to gp41 within the domains, are critical for the induction of conformational changes, and may inhibit membrane fusion.

T-20 is the first of several peptides that bind to gp41 that was tested in clinical trials to suppress viral replication (see chapter on ART). T-20 is available as a therapeutic option for patients with advanced HIV. One disadvantage of T-20 is that it must be taken subcutaneously twice daily.

Despite a broad spectrum of potentially available co-receptors (e.g., CCR3, CCR2, CCR8, CCR9, STRL33), CCR5 and CXCR4 seem to represent the most relevant co-receptors for HIV-1 *in vivo*. The importance of CCR5 as the predominant co-receptor for M-tropic HIV isolates is underscored by another observation. The majority of individuals with a genetic defect of CCR5 are resistant to infection with HIV-1 (Liu 1996). *In vitro* experiments show that lymphocytes derived from these individuals are resistant to HIV-1 infection using M-tropic isolates but not to infection with T-tropic isolates. Lymphocytes from these individuals do not express CCR5 on their cell surface and genetically have a 32-basepair deletion of the CCR5 gene. Worldwide, a few patients have been identified that have acquired HIV-1 infection despite a homozygous deletion of the CCR5. As expected, all of them were infected with CXCR4-using HIV-1 isolates. In epidemiological studies, the allelic frequency of the CCR5 gene deletion is 10–20% among Caucasians, particularly amongst those of Northern European descent. The frequency of a homozygous individual is about 1% in Caucasians (Dean 1996). Studies conducted on African or Asian populations, however, do not find this 32-basepair deletion.

Individuals that are heterozygous for the 32-bp deletion of the CCR5 show a decreased expression of CCR5 on the cell surface and are more frequently encountered within cohorts of long-term non-progressors compared to patients who have a rapid progression of disease (Dean 1996). In addition, HIV-infected individuals who are heterozygous for the 32-bp deletion, have a slower progression to AIDS, a better

treatment response to ART, and lymphoma incidence is decreased. These data demonstrate that the density of CCR5 on the cell surface is not only a limiting factor for replication of HIV *in vitro* but *in vivo* as well.

Transmission of HIV-1 is caused by M-tropic viruses in most cases – even when T-tropic isolates predominate in the donor. In early HIV infection, mostly M-tropic virus isolates can be found. In patients who have a rapid progression of disease (rapid drop in CD4 T cell count), virus isolates that use CXCR4 as a predominant co-receptor tend to be frequently isolated from their cells in comparison to patients with a stable CD4 T cell count. The expression of co-receptors on CD4 lymphocytes depends on their activation level. CXCR4 is mainly expressed on naive T cells, whereas CCR5 is present on activated and effector/memory T cells.

The blockade of CCR5 therefore seems to represent a promising target for therapeutic intervention (see chapter on ART). Maraviroc is the first, FDA-approved CCR5 inhibitor and can be given to patients after a tropism check. CCR5 inhibitors have also been successfully given as microbicides in non-human primates and could represent an option for prevention of infection (Veazey 2005). *In vitro* studies as well as experiments using SCID mice, however, do suggest that blockade of CCR5-using isolates may alter their tropism towards increased usage of CXCR4 (De Clercq 2001). In this respect, the "second Berlin patient" is fascinating. This HIV-infected patient developed acute myeloic leukemia and needed bone morrow transplantation. He was transplanted from a donor who carried the homozygous delta32 mutation of the CCR5 receptor (Hütter 2009). After transplantation, antiretroviral treatment was stopped and HIV remained undetectable in this patient. Years later, a thorough search for HIV was conducted in the patient and none was found (Allers 2011).Therefore the patient is thought to be cured of HIV. This case has led to an intense search for other ways to delete the CCR5 receptor.

Although the therapeutic use of chemokine receptor blockers seems promising, a lot of questions still remain unanswered. Chemokine analogues such as AOP-Rantes theoretically also bind to other chemokine receptors. In knockout mice it was demonstrated that the absence of CXCR4 or SDF-1 is associated with severe defects in hematopoiesis and in cerebellar development (Zou 1997). Currently, it remains unclear whether the blockade of CXCR4 in postnatal or adult individuals may affect other organ systems.

Post-fusion events

Following membrane fusion the viral capsid uncoats into the cytoplasm of the target cell. Alternatively, receptor-mediated endocytosis and dynamin-dependent fusion with intracellular compartments (Miyauchi 2009) can lead to viral inoculation. HIV can enter into rhesus lymphocytes but replication is stopped before or during early reverse transcription. This intracellular blockade is mediated by a cellular factor, TRIM5α (tripartite motif 5α), a component of cytoplasmic bodies whose primary function is not yet understood. TRIM5α from various species exhibits differential inhibition on various retroviruses. For example, TRIM5α from rhesus macaques, TRIM5α$_{rh}$, more profoundly inhibits HIV replication than human TRIM5α, whereas SIV (simian immunodeficiency virus) which naturally infects Old World monkeys, is less susceptible to either form of TRIM5α, thus explaining in part the species specificity of HIV for human cells (Stremlau 2004). TRIM5α from human cells or non-human primates is able to inhibit replication of other lentiviruses and represents a novel cellular resistance factor whose definitive biological significance has yet to be fully characterized. TRIM5α serves as a mechanism for intracellular recognition and activation of the unspecific immune response (Pertel 2011), but it is unclear how exactly TRIM5α blocks reverse transcription. It has been hypothesized that TRIM5α
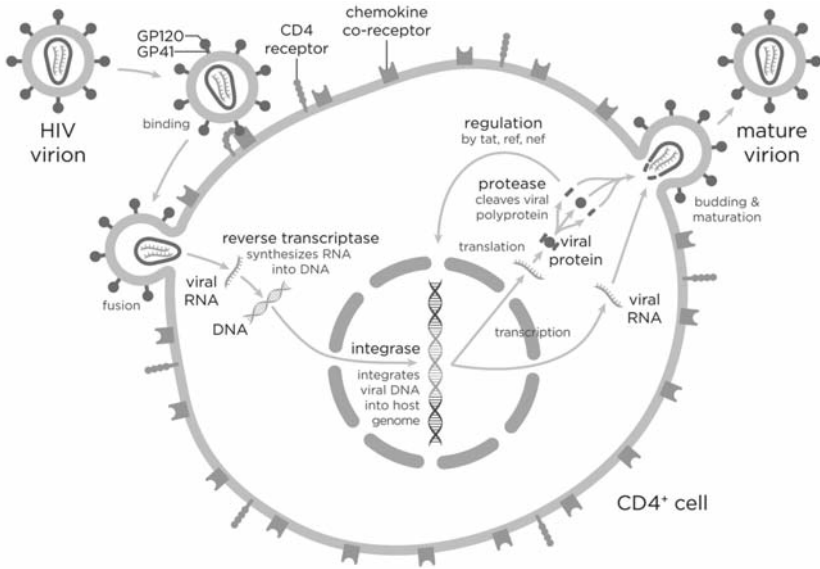
Figure 5: Life cycle of HIV within the host cell

interferes with the incoming virus capsid protein, targeting it for ubiquitination and proteolytic degradation.

HIV-1 entry into quiescent T cells is comparable to HIV-1 entry into activated T cells, but synthesis of HIV-1 DNA remains incomplete in quiescent cells (Zack 1990). The conversion of viral RNA into proviral DNA, mediated by the viral enzyme reverse transcriptase (RT), occurs in the cytoplasm of the target cell and is a crucial step within the viral replication cycle (Fig. 5). Blockade of the RT as therapeutic intervention has long been a therapeutic principle.

HIV-1 enters into quiescent T cells and reverse transcription may result in the accumulation of proviral, non-integrating HIV DNA. However, cellular activation is necessary for integration of the proviral HIV DNA into the host cell genome after transportation of the pre-integration complex into the nucleus. Cellular activation may occur *in vitro* after stimulation with antigens or mitogens. *In vivo,* activation of the immune system is observed after antigen contact or vaccination or during an opportunistic infection. In addition, evidence is emerging that HIV-1 gp120 itself may activate the infecting cell to enhance integration. Besides monocytes, macrophages and microglial cells, latently infected quiescent CD4 T cells that contain non-integrated proviral HIV DNA represent important long-lived cellular reservoirs of HIV (Chun 1997), and cellular microRNAs contribute to HIV-1 latency in resting primary CD4 T lymphocytes (Huang 2007). Since natural HIV-1 infection is characterized by continuing cycles of viral replication in activated CD4 T cells, viral latency in these resting CD4 T cells likely represents an accidental phenomenon and is not likely to be important in the pathogenesis of HIV. This small reservoir of latent provirus in quiescent CD4 T cells gains importance, however, in individuals treated with ART, since the antivirals do not affect non-replicating proviruses – the virus will persist in those cells and be replication-competent to start new rounds of infection if the drugs are stopped. It is the existence of this latent reservoir that has prevented ART from entirely eradicating the virus from infected individuals (Chun 2005).

For the integration of the proviral DNA into the host genome – the prerequisite for the synthesis of new virions (Zack 1990) – the viral enzyme integrase is needed. This enzyme which is highly conserved between different clinical isolates can be blocked by integrase inhibitors. Today there are three integrase inhibitors – raltegravir, elvitegravir and dolutegravir – approved (see chapter on ART).

Until recently it was not clear why HIV replicates poorly in quiescent CD4 T cells. The cellular protein Murr1 that plays a role in copper metabolism is able to inhibit HIV replication in unstimulated CD4 T cells. Murr1 was detected in primary resting CD4 T cells and interferes with activation of the transcription factor NFκB by inhibiting the degradation of IκBα. IκBα prevents NF-κB from migrating to the nucleus, especially after cytokine stimulation (e.g., TNFα). Because the HIV LTR region has multiple sites for NF-κB, preventing NF-κB migration to the nucleus should inhibit HIV replication. Inhibition of Murr-1 by siRNA is associated with HIV replication in quiescent CD4 T cells (Ganesh 2003). Persistence of HIV in quiescent CD4 T cells and other cellular reservoirs seems one of the main reasons why eradication of HIV is not feasible and why current therapies fail to achieve viral eradication (Dinoso 2009, Lewin 2011). A more detailed knowledge of how and when cellular reservoirs of HIV are established and how they may be targeted is of crucial importance for the development of strategies aiming at HIV eradication.

Cellular transcription factors such as NF-κB may also bind to the LTR regions. After stimulation with mitogens or cytokines NF-κB is translocated into the nucleus where it binds to the HIV LTR region, thereby initiating transcription of HIV genes. Transcription initially results in the early synthesis of regulatory HIV-1 proteins such as *tat* or *rev*. *Tat* binds to the TAR site (transactivation response element) at the beginning of the HIV-1 RNA in the nucleus and stimulates transcription and the formation of longer RNA transcripts. *Rev* activates the expression of structural and enzymatic genes and inhibits the production of regulatory proteins, therefore promoting the formation of mature viral particles.

The proteins coded for by *pol* and *gag* form the nucleus of the maturing HIV particle, while the gene products coded for by *env* form the gp120 spikes of the viral envelope. The gp120 spikes are synthesized as large gp160 precursor molecules and are cleaved by the HIV-1 protease into gp120 and gp41. The *gag* proteins are also derived from a large 53 kD precursor molecule, from which the HIV protease cleaves the p24, p17, p9 and p7 *gag* proteins. Cleavage of the precursor molecules by the HIV-1 protease is necessary for the generation of infectious viral particles, and therefore the viral protease represents another interesting target for therapeutic blockade. The inhibition of *gag* by application of siRNAs blocks viral replication effectively (Song 2005).

The formation of new viral particles is a stepwise process: a new virus core is formed by HIV-1 RNA, *gag* proteins and various *pol* enzymes and moves towards the cell surface. The large precursor molecules are cleaved by the HIV-1 protease, which results in the infectious viral particles budding through the host cell membrane. During the budding process, the virus lipid membranes may incorporate various host cell proteins and become enriched with certain phospholipids and cholesterol. In contrast to T cells, where budding occurs at the cell surface and virions are released into the extracellular space, the budding process in monocytes and macrophages results in the accumulation of virions within cellular vacuoles.

The replication of retroviruses is prone to error and is characterized by a high spontaneous mutation rate. On average, reverse transcription results in 1–10 errors per genome per round of replication. Mutations can lead to the formation of replication-incompetent viral species. Mutations that cause drug resistance may also accumulate, which, provided that there is selective pressure due to specific antiretroviral drugs and incomplete suppression of viral replication, may become

dominant. Selective pressure does not only result from antiretroviral drugs but also from immune responses (e.g., cytotoxic T cells or neutralizing antibodies).

In addition, viral replication is dynamic and turns over quickly, at an average rate of 10^9 new virus particles produced and subsequently cleared per day. Thus, within any individual, because of the extensive viral replication and mutation rates, there exists an accumulation of many closely-related virus variants within the population of viruses, referred to as a viral quasispecies.

HIV and the immune system

The human immune systems consists of many different components. The more research is done the more cell types and signaling pathways are described. It is a highly complex system and we are far from a complete understanding. Here we look at the most important elements of the immune system and their significance for the pathogenesis of HIV infection. Studies on immune responses in HIV infection are often performed in patient cohorts with different disease courses before starting antiretroviral treatment (ART). The most important definitions are:

• Progressors: Individuals who control HIV viremia poorly in the absence of ART. CD4 counts decline continuously and viral loads are medium to high.
• Controllers: Patients who control viremia spontaneously without ART with stable CD4 counts and low viral loads. However viral load definitions vary from study to study (e.g., <5000 or <2000 copies/ml).
• Elite controller (EC): Patients who spontaneously control HIV viremia to below detection levels without ART.

Innate immunity

The innate immune response is the first defense mechanism against microorganisms of our body. It is a genetic non-specific reaction towards foreign organisms. Evolutionarily it is old and can be found in all higher organisms.

Dendritic cells

Dendritic cells (DC) are derived from myeloid precursor cells of the bone marrow. However they are quite heterogeneous as far as localization, surface markers and function are concerned. The most important subtypes are myeloid DC and plasmacytoid DC (Miller 2013, Tsunetsugu-Yokota 2013). The most important function of DCs is their role as professional antigen-presenting cells. For this reason they express high levels of human leukocyte antigen (HLA) class II. DCs are represented in different tissues and organs, take up antigens and migrate to lymphatic tissues. Therefore they represent a key function for inducing the adaptive immune response.

HIV infection gets started for the most part in rectal or vaginal mucosa. As these mucus membranes are rich in DCs, it is assumed that DCs are the first target of HIV (Piguet 2007). However, HIV producing DCs can rarely be verified in the mucosa (Tsunetsugu-Yokota 2013). Still it is assumed that infected DCs migrate to the lymph nodes or other secondary lymph organs where CD4 T cells are infected with HIV. They play an important role in primary HIV infection. In the chronic phase of infection, memory T cells are an important reservoir for latent HIV (Pierson 2000). In these resting CD4 T cells HIV is integrated but does not replicate. Via the interaction between DCs and resting CD4 T cells, these CD4 T cells can be activated and HIV replication begins. DCs are therefore also key cells for activation of HIV from latent reservoirs.

HIV-1 itself directly and indirectly influences the function of DCs in order to inhibit the formation of an effective immune response as well as to force immune activa-

tion (Miller 2013). Myeloid DCs (mDC) are not able to recognize HIV adequately, leading to the failure of a complete maturation of these cells and consequently limiting their role in induction of innate and adaptive immune responses (Granelli-Piperno 2004, Sabado 2010, Miller 2012). Only partly mature mDC can lead to the formation of regulatory T cells (Treg) (Krathwohl 2006). In the chronic phase of HIV infection the function of mDC is clearly limited. The ability to produce IL-12 is a defect which leads to a restricted differentiation of naïve T cells to Th1 cells (Fan 2007, Miller 2012). Plasmacytoid DCs (pDC) are activated strongly by HIV-1 and they produce interferon-α as a response (Fonteneau 2004, Idoyaga 2011). *Ex vivo* studies show increased interferon-α levels by pDC in acute and chronic HIV-1 infection (O'Brien 2011). This is an important contribution to the well-known immune activation in HIV infection (see chapter on Immune Activation). In spite of this activation, the maturation of pDC is not complete which renders them less effective as antigen-presenting cells (Fonteneau 2004, O'Brien 2011). In addition, HIV-1 induces the production of indoleamine-2, 3-dioxigenase (IDO) in pDC which leads to further induction of Treg (Manches 2008). This limits HIV-specific immune responses although it can improve immune activation.

The effects of HIV-1 on DC are well described (Miller 2013). However even this short chapter the different and partly contrary roles that DC play in HIV infection are highlighted. This renders them an important component with regard to therapeutic and prophylactic vaccines.

Natural killer (NK) cells

NK cells are lymphocytes not considered T or B lymphocytes nor do they express antigen-specific receptors. They are important in the control of viruses and malignant tumors and belong to the innate immune system. NK cells express many different receptors, toll-like receptors (TLR) and killer immunoglobulin-like receptors (KIR) among them. KIR recognize HLA class I molecules on healthy cells which protect these cells against NK cell attack.

NK cells can eliminate HIV-infected cells rapidly either via direct cytolysis or via secretion of cytokines (Walker 2013). Population-wide genetic studies show an important influence of NK cells on disease progression (Jost 2013). Certain parts of the HLA class I alleles (mainly Bw4) lead to a slower disease progression (Flores-Villanueva 2001). This effect is even stronger when combined with KIR3DL1 (Martin 2007). In addition, single nucleotide polymorphisms (SNP) within the HLA-C allele have been identified which influence disease progression. The HLA-C molecule is the ligand for the KIR2D receptor and thereby influences the function of NK cells (Jost 2013). NK cells put selective pressure on the virus which can lead to escape mutations (Alter 2011). In HIV infection, different functional and phenotypical changes of NK cells have been found. Most of those are induced by a high viral load and consequently strong immune activation (Walker 2013). Interestingly, the antiviral activity of NK cells of elite controllers is relatively weak which implies that the contribution of NK cells to the control of viremia in these patients is rather low (O'Connell 2009).

γδ T cells

γδ T cells belong to the family of T cells, but they separate in the early phases of T cell development (before maturation in the thymus) (Fig. 6). Instead of the classic TCR-αβ these primitive thymocytes bear a TCR-γδ, representing a heterodimer of a γ and δ chain. In humans, there are only three different Vδ chains and seven Vγ chains to form a mature TCR. These receptors recognize a unique repertoire of non-peptidic antigens and do not need presentation by the classical HLA class I or II

molecules (Pauza 2011). These cells are therefore associated with the innate immune system.

Typically, the number of Vγ2Vδ2 TCR-bearing γδ T cells is reduced in early HIV infection. Both the extent of the loss of these cells and their loss of function correlate with disease progression (Wallace 1997). Surprisingly, these cells are maintained in the peripheral blood of EC in high numbers and comparable to healthy controls (Riedel 2009). It was also shown that Vγ2Vδ2 T cells were reduced in EC in the early disease phase. However, the cells were able to recover numerically with a normal function. This is a phenomenon that distinguishes EC of other people living with HIV. However, the authors tested only 21 individuals in this study. Therefore, it is worthwhile to pursue this in larger cohorts and to develop mechanisms that lead to the recovery of these cell types.

Adaptive immunity

In contrast to the innate immune system, the adaptive immune response cannot directly eliminate microorganisms. The adaptive immune system has to learn to recognize foreign pathogens und represents the second line of defense in our body. Parts of this system are also inherited (e.g., the HLA alleles). Others, the T cell receptors among them, are formed individually by rearrangement.

The HLA system

The system of human leukocyte antigens (HLA) comprises a group of membrane-bound receptors which present antigens for the TCR of the adaptive immune system. They are encoded in 40 genes on chromosome 6. The HLA genes that are immunologically important are divided into two classes, namely class I and II, which differ structurally and functionally (review: Klein 2000). The HLA class I alleles A, B and C are expressed on all somatic cells and present antigens for CD8 T cells. The complex HLA class II alleles are named with three letters: D for the class; M, O, P, Q or R for the family and A or B for the respective chain (α or β). An example for an HLA class II receptor is HLA-DRB1. HLA class II alleles are only expressed by certain immune cells, e.g., B lymphocytes, activated T lymphocytes, macrophages and dendritic cells. They present antigens for CD4 T cells. Via the presentation of short antigenic peptides, the "learning process" of the adaptive immune response is started.

A correlation between certain HLA class I alleles and control of HIV infection has been postulated in HIV research. Among those are the HLA class I alleles B*57, B*58:01 and B*27 (O'Brien 2001). Interestingly, a genome-wide analysis of HIV infected persons again resulted in the identification of just these HLA class I alleles as being associated with control of viremia (Pereyra 2010). Indirectly, this is proof of the significance of CD8 T cell responses for the control of HIV infection. It is striking that the allele B*57 on the one hand often leads to a good spontaneous control of HIV infection, but that it predestines to hypersensitivity reaction to abacavir on the other hand (Mallal 2002). There is also an association of HLA-B*35 and B*07 with rapid HIV disease progression (O'Brien 2001). Much less data exists concerning HLA class II alleles and their impact on HIV infection. However, variants have been described that have a favorable effect on the disease process, e.g., variants of the HLA-DRB1 (Ranasinghe 2013).

HLA class I alleles interact also with receptors of the innate immune system, for example, KIRs on NK cells, and this could have an impact on the control of HIV. But it has not yet been shown that controllers carry particularly favorable HLA-KIR combinations (O'Connell 2009). In addition HLA class I alleles bind to "leucocyte immunoglobulin-like receptors" (LILR), which are expressed on dendritic cells

(Huang 2009, Jones 2011). So far, however, it is not clear whether HLA LILR interactions affect HIV disease progression.

CD8 T cells

In the development of T lymphocytes, CD8 T cells separate from CD4 T cells in the thymus (Fig. 6). After a phase where the T lymphocytes are positive for both CD4 and CD8 receptors, one of the two receptors is down-regulated and either CD4 positive or CD8 positive T cells develop. The predominant task of CD8 cells is cytotoxicity, i.e., they eliminate virus-infected cells. Additionally, they secrete a number of cytokines and chemokines, including MIP-1β, interferon-γ, TNF-α and IL-2. They exert their function via their T cell receptor (TCR) recognizing the antigen in the HLA class I molecule. The T cell receptor of the CD8 cells (and also of CD4 cells) consists of an α- and a β-chain (as opposed to the γδ T cells). The α-chain recombines from 42 variable (V) segments and 61 "joining" (J) segments; the β-chain recombines from 47 V segments, 2 "diversity" (D) segments and 13 J segments. Since additional nucleotide additions or deletions occur at the junction of the two chains, a huge number ($\sim 10^{15}$) of diverse T cell receptors is guaranteed. However, only a fraction of them, about several thousand, meet a matching antigen during the lifetime of an individual (Arstila 1999).

HIV-specific CD8 cells were described early after the discovery of HIV. Back in 1987, two groups reported the discovery of cytotoxic T cells that eliminate HIV (Plata 1987, Walker 1987). Today we know that virus-specific CD8 cells are very important for the control of viremia.

This is due to, among other reasons, the strong association between certain HLA class I alleles and slow disease progression. In particular, for individuals with HLA-B*27 an epitope within *Gag* was defined which is responsible for viremic control. Mutations in this epitope are so devastating to the virus that it only leads to replication competent viruses if a compensatory mutation arises far from this epitope
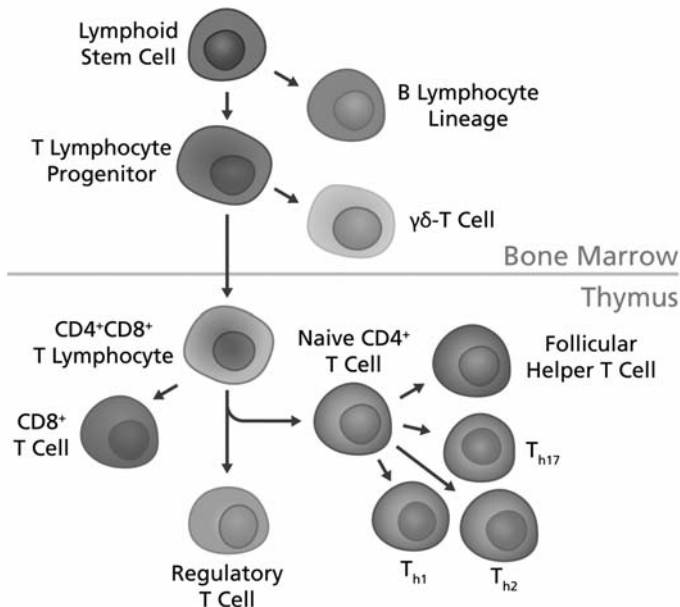
Figure 7: Development of B and T lymphocytes

(Goulder 1997, Schneidewind 2008). It appears to be different for patients with the HLA-B*57 allele: there are epitopes which rapidly escape after infection. This leads to a restriction of the replicative capacity of the virus and to the consequent control of viremia (Leslie 2004).

CD8 T cell responses exist in all HIV-infected patients and the variety of epitopes is immense (Addo 2003). From twins studies we know when the same virus hits the same immune system, the immune response is very similar (Draenert 2006). As a reaction to the immune pressure by a strong CD8 T cell response, viral variants with sequence changes arise, so-called escape mutations. This occurs most frequently in early HIV infection, which was shown nicely both in the monkey model and in humans (Allen 2000, O'Connor 2002, Allen 2005).

Also, in late disease stages, there are CD8 T cell responses, sometimes broad and strong (Draenert 2004). However, these usually do not induce escape mutations, which is an indirect indication that these responses are no longer effective (Draenert 2004). Consequently, the CD8 T cell response was examined not only quantitatively but also qualitatively. It turned out that "controllers" usually have a poly-functional CD8 T cell response, i.e., CD8 cells have many different effector functions (MIP-1β, interferon-γ, TNF-α, IL-2 and cytotoxicity). On the other hand "progressors" have CD8 cells that reply to an antigen stimulus with only one or two functions (Betts 2006). It has also been shown that effective CD8 cells of "controllers" proliferate well *ex vivo*, unlike those of "progressors" (Migueles 2002). This loss of effector functions is called immune exhaustion. It leads to an ineffective T cell response.

In recent years, causes of immune exhaustion have been studied. It was shown that inhibitory signaling pathways, especially programmed death-1 (PD-1), play a major role in the development of immune exhaustion (Day 2006, Petrovas 2006, Trautmann 2006). By blocking PD-1, proliferation of CD8 T cells increased significantly (Day 2006). Other inhibitory molecules are Tim-3 and CD244 (2B4) (Jones 2008, Pacheco 2013). In addition, new cell types that suppress immune responses play a role. Recently it was demonstrated that myeloid-derived suppressor cells (MDSC), which inhibit immune responses in solid tumors, occur in increased number of HIV-infected persons and restrict the function of CD8 cells *ex vivo* (Vollbrecht 2012, Qin 2013).

CD4 T cells

T lymphocytes that keep the CD4 receptor during T cell development, undergo further maturation in the thymus. They are divided into various sub-groups, which differ in their phenotype and function. In the thymus, a clearly separated subgroup splits off: the regulatory T cells (Fig. 6). What remains are the naïve CD4 T cells which can develop into four (and possibly more) cell lines, depending on the stimulus: Th1, Th2, Th17 and "follicular helper T cells" (TFH). The latter mediate B cell activation in the B cell follicles of the secondary lymphoid organs (Papp 2014). Best known are the true CD4 helper cells, namely Th1 and Th2 cells, which differ in their cytokine profile and also have different functions: Th1 cells mainly produce interferon γ, lymphotoxin α (LTα) and IL-2 and are considered crucial for fighting intracellular pathogens. Th2 cells on the other hand produce IL-4, IL-5, IL-9, IL-10, IL-13, IL-25 and amphiregulin. They aim at extracellular pathogens, including worms (Zhu 2008). CD4 T cells carry an αβ T cell receptor as described for the CD8 T cells which interacts distinctly from HLA class II molecules.

CD4 helper cells are important for the development of an effective CD8 T cell and B cell response. There are also directly acting antigen-specific CD4 cells. Since these cells are preferentially infected by HIV, it was not clear initially whether they function properly in HIV infection. Today we know that virus-specific CD4 T cell responses (Th1) lose effector functions early in HIV infection, including, e.g., IL-2 production

and polyfunctionality (Wahren 1987, Berzofsky 1988, Krowka 1989). The function of HIV-specific CD4 cells of elite controllers is significantly better than in patients with progressive disease (Betts 2001, Younes 2003, Harari 2004, Pereyra 2008). However, it has been shown that the differences in function are at least partly the consequence and not the cause of the low viral load of elite controllers (Harari 2004, Potter 2007, Tilton 2007).

For the CD4 T cell response, an important determinant is immune exhaustion as was already described for CD8 T cells. For CD4 T cells, the inhibitory molecule PD-1 also plays an important role (D'Souza 2007). Several inhibitory synergistic signaling pathways are responsible for the loss of function of CD4 T cells, in particular, CTLA-4 and TIM-3 (Jones 2008, Kassu 2010). Cytokine IL-10 has been identified as a crucial mediator (Clerici 1994, Brockman 2009). Blockade of the IL-10 receptor by an antibody resulted in improved proliferation of CD4 T cells as well as to an increased secretion of IL-2 and interferon-γ.

Regulatory T cells (Treg)

Regulatory T cells are CD4 and CD25-positive T cells which mature within the thymus (Fig. 6). They show a pronounced suppressive activity (suppressor cells), and inhibit the activation, proliferation, and function of a number of immune cells, including CD4 and CD8 T cells, NK cells, B cells and antigen-presenting cells such as dendritic cells and macrophages (Imamichi 2012, Josefowicz 2012). They protect the body against building harmful immune responses against self, foods or commensal (Josefowicz 2012), protecting against autoimmune diseases or allergies.

The role of Treg in HIV infection is an area of intense research, as well as debate. There are conflicting data on the benefits or harm of Treg in the pathogenesis of HIV infection. There are studies describing increasing, comparable or decreasing Treg numbers in HIV infection in comparison to healthy individuals (Seddiki 2008). These inconsistencies can be explained by different phenotypic markers or methods of counting (percentage or absolute numbers). In addition the stage of HIV infection and the compartment in which cells are measured, play a role. Recently, however, it was shown that the absolute number of Treg decreases over the course of HIV infection, while their percentage within CD4 T cells increases. The function of Treg seems to be unaffected in HIV infection (Angin 2012, Mendez-Lagares 2012, Simonetta 2012). At the moment, there is no data showing that Tregs suppress the immune response to HIV. Recently, HIV-specific Tregs were detected for the first time (Angin 2012). In conclusion, this is a cell type that needs to be studied more closely before conclusions about therapeutic options can be drawn.

Th17 cells

Th17 cells develop from naive CD4 cells in the thymus (Fig. 6). They were named after their IL-17 secretion which represents part of their function. They protect the body against a range of pathogens and can be found mainly in the mucosa and skin. They reach their destination via the homing receptor CCR6 (Elhed 2010).

The function of Th17 cells in HIV infection has been incompletely understood. Whether Th17 cells may have a direct antiviral effect is unclear (Brenchley 2008, Yue 2008). It may depend on the disease stage if HIV-specific Th17 cells can be found or not. More is known about their barrier function in the gut. The intestinal mucosa represents an important guard against invading pathogens. It is clear that Th17 cells are reduced in the colonic mucosa of HIV-infected people, which contributes to the weakening of the mucosal barrier (Brenchley 2008). This leads to an increased excretion of microbial products into the blood which increases immune activation. Therefore Th17 cells are causally involved in the pathologic immune activation in

HIV infection. However, it could also be shown that Th17 cells are reconstituted in the intestine after starting ART, although not to the same level as in healthy subjects (Macal 2008, Kim 2013). By administering IL-21, the protective function of the intestine could be recovered by increased Th17 cell numbers in SIV-infected rhesus macaques (Pallikkuth 2013).

Humoral immune response

New B cells are formed in the bone marrow throughout life. When mature B cells form, they leave the bone marrow and migrate to the secondary lymphoid organs (e.g., spleen, lymph nodes and Peyer's patches in the gut). After antigen challenge, a further maturation phase ensues, which leads to the formation of the humoral immune response. Newly derived plasma cells produce diverse antibodies. The recombination of the light or heavy chains of the immunoglobulins is very similar to the process of rearrangement of the T cell receptors. A distinction is made between neutralizing and non-neutralizing antibodies. Neutralization is considered the main mechanism to combat a pathogen and is mediated either by blocking a cellular receptor or by blocking the fusion of the virus (Corti 2013). Non-neutralizing antibodies help protect against pathogens, e.g., by recruiting effector cells or complement cells (Corti 2013). Natural HIV infection first induces non-neutralizing antibodies (nNAK) and neutralizing antibodies (NAK) which are specific for a certain viral strain. These can be detected soon after infection (via HIV testing). However, the virus is always one step ahead of these antibodies by developing escape mutations. This leads to the diversification of *Env* in early infection (Frost 2005). These antibodies barely contribute to the control of viremia.

In recent years, however, the discovery of broadly neutralizing antibodies (bNAK) led to new optimism. Up to 20% of all infected individuals build bNAK during the course of disease that can reach various strains of HIV effectively. However bNAK occur relatively late in the disease process, a minimum of two years after infection (Kwong 2013). The target of bNAK is the viral spike of the HIV-1 viral envelope, a heterodimer consisting of trimeric gp120 and the transmembrane glycoprotein gp41. Most bNAK bind to one of the following four binding sites (Kwong 2012):

- antibodies directed against the CD4 binding site that recognize the binding site of the CD4 receptor to gp120;
- antibodies directed against the variable region of V1 or V2 that often recognize glycopeptide epitopes near amino acid Asn160 on gp120;
- antibodies directed against V3 that recognize epitopes that contain the amino acid Asn332 within gp120; and
- antibodies directed against the membrane-proximal external region (MPER) that recognize a position of gp41 proximal to the transmembrane region.

bNAK are unique in that they do not arise from a primary antigen contact, but have to mature further through additional antigenic contacts (Kwong 2013). Interestingly, it is important that the antigen does not remain preserved continuously. Maturation of bNAK is achieved best by constantly changing *Env* sequences. It is this maturation process which is so difficult to induce by vaccination, which remains an important goal. In addition, the maturation process takes months to years (Gray 2011). The reasons for this are:

- first, HIV infection itself leads to an impaired immune response
- second, it may be due to *Env* because healthy adults who are vaccinated with an *Env* vaccination rarely produce bNAK
- third, most likely the co-evolution of virus and immune response contributes to the emergence of bNAK.

Interestingly, some bNAKs arise only due to a sequence representing an escape muta-

tion (Kwong 2013). Attempts to induce bNAK by vaccination were unsuccessful. This is certainly due to the fact that the right immunogen has not been found yet. However, research is very intense in this field (Kwong 2013).

In addition to the induction of bNAK by vaccination, bNAK can be passively transferred in order to control infection. This has been successfully done in humanized mice and in macaques (Moldt 2012, Horwitz 2013). Data in infected individuals are under way. Despite the euphoria surrounding bNAK, it should be noted that several studies in elite controllers detected bNAK to a lesser degree than in viremic progressors (Bailey 2006, Pereyra 2008, Lambotte 2009, Doria-Rose 2010). Other studies have also shown that a wider range of bNAK is typically associated with higher viral load and that this does not protect against disease progression (Deeks 2006, Sather 2009, Euler 2010).

Mucosal immunity

Since HIV infection is usually transmitted via the mucous membrane (mostly vaginally or rectally), the immune system of the mucous membranes needs to be mentioned. The gut-associated lymphoid tissue (GALT) is the largest immune organ of the body. Due to its high content of CD4 cells, the GALT is also the main target for HIV. The massive CD4 T cell depletion during early HIV infection leads to the microbial translocation which in turn leads to increased immune activation (Brenchley 2004+2006). The latter is pathognomonic of chronic HIV infection.

However, the T cell response of the mucosa was found to be a correlate for the control of viremia in recent years (Shacklett 2011). In the mucous membranes, virus-specific T cells among others can be found. HIV-specific CD8 T cells were detected in abundance in the rectal mucosa as demonstrated in chronically HIV-infected individuals (Shacklett 2003, Ibarrondo 2005). Here a correlation between viral load and polyfunctionality of the CD8 T cell responses could be demonstrated (Critchfield 2008). Elite controllers had significantly higher CD8 T cell responses with more effector functions in the rectal mucosa than progressors, whereas no difference in the CD8 T cell responses in peripheral blood was found (Ferre 2009). This shows that many controllers have strong, polyfunctional CD8 (and also CD4) T cell responses in the intestinal mucosa – a fact that is not reflected in the peripheral blood. Polyfunctional CD4 T cells also correlate with a high CD4 cell count and a good control of viremia, but the CD4 T cells were only non-specifically stimulated in the respective studies (Loke 2010).

NK cells were shown to be reduced in the intestine in chronic HIV infection. However, a subset of these cells remained stable in controllers. Interestingly, intestinal NK cells were significantly increased in patients who did not achieve a complete CD4 T cell recovery after the start of suppressive ART. In this situation, NK cells might expand in the gut in an effort to compensate for the CD4 cell loss (Sips 2012). In ART-naïve patients, it was demonstrated that pDCs accumulate in the terminal ileum and are accompanied by elevated levels of interferon-alpha. In that way pDC could contribute to the development of immune activation. Both parameters were normalized after the start of ART (Lehmann 2014).

Another aspect of mucosal immunity is the fact that the intestinal mucosa is an important reservoir of HIV. Two large studies have shown that in patients with effective ART and a viral load below 40 HIV RNA copies/ml HIV continue to be detectable in the intestinal mucosa (Chun 2008, Yukl 2010). So far, proviral DNA has not been studied in the gut in controllers or elite controllers. The fact that strong T cell responses can be detected in this compartment in patients with good control of HIV viremia is an indirect indication that antigen can still be found. T cell responses tend to grow weaker up to undetectable when the antigen disappears (Ferre 2009 + 2010).

Immune activation

Immune activation is defined as a sequence of signaling pathways with the production of various cytokines and chemokines that direct an orderly immune response. In most cases, it is switched off after elimination of the pathogen. For several years, it has been known that a persistent immune activation is one of the outstanding features of chronic progressive HIV infection and significantly contributes to the pathogenesis of the disease. In fact, the level of immune activation is the best prognostic marker for the disease progression regardless of viral load (Miedema 2013). Affected are the T lymphocytes that express the markers CD38 and HLA-DR when activated. In addition, an increased expression of pro-inflammatory cytokines, including Type I interferons (e.g., interferon-α), IL-6, TGF-β, IL-8, IL-1α and IL-1β, and inflammatory markers such as sCD14, CRP, cystatin C, and D-dimer (Deeks 2011). These markers are elevated not only in the blood. It was demonstrated that immune activation markers in blood and intestines were well correlated (Loke 2010).

Cause of the immune activation is – amongst other reasons – due to the pathology of HIV infection in the gut. The destruction and depletion of CD4 T cells in the intestine leads to an increased permeability of the gut for microbial products. Specifically, LPS was measured in increased levels (Brenchley 2004, Li 2005, Brenchley 2006). LPS activates the innate immune system via toll-like receptor 4 (TLR4) (Brenchley 2006, Gordon 2010). However, other microbial products such as flagellin, peptidoglycan and bacterial CpG-rich DNA domains contribute to immune activation via reactions with TLR-2, -5 and -9 (Brenchley 2006). More recently, it was shown that pDC in the intestines of HIV-infected individuals produce increased interferon-α leading to an increased immune activation in the gut (Lehmann 2014).

HIV itself has been identified as another cause of immune activation. Single-stranded HIV RNA can activate pDCs directly via TLR-7 and -8 leading to the production of interferon-α (Fonteneau 2004, Beignon 2005, Meier 2007). Additionally, NK cells can be activated by single-stranded HIV RNA and this process is dependent on the cell-cell contact of pDCs and monocytes (Alter 2007). Type I interferons are produced by pDCs in large quantities and are an essential link between the innate and acquired immune systems. In most cases pDCs become refractory to TLR stimulation, which stops interferon-α production. It has been shown, however, that HIV leads to the induction of only partially mature pDCs which are no longer refractory, but continuously produce interferon-α (O'Brien 2011).

Consequences of immune activation are an increasing loss of CD4 T cells and the destruction of the HIV-specific immune response as described above. However, the emergence of other diseases is also favored by this persistent immune activation. Particular examples are cardiovascular events, non-alcoholic hepatic steatosis, renal dysfunction, osteoporosis, insulin resistance, metabolic syndrome and neurocognitive disorders (Hsueh 2006 + 2009, Deeks 2011). While our knowledge is still deficient, some mediators of immune activation are known. This is important for new therapeutic strategies targeting immune activation (Miedema 2013).

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4. Preventive HIV-1 Vaccine

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Although great progress has been achieved in the field of treatment and prevention of HIV-1 infection, the HIV-1 pandemic will ultimately be controlled only by an effective HIV-1 vaccine. The following chapter will give a short overview of the current status of HIV-1 vaccine development.

Induction of neutralizing antibodies

Similar to successful vaccination strategies in other infections, initial HIV-1 vaccine research focussed on the development of vaccines with the capability of inducing neutralizing antibodies. A variety of studies examined the safety and efficacy of vaccines such as gp120, gp160, parts of gp160 and peptides from gp160 to induce antibodies against HIV-1 envelope proteins. These immunogens stimulated the production of specific antibodies that were able to neutralize HIV-1 strains *in vitro*, but they failed to induce broadly neutralizing antibodies in HIV-1 variants derived from patients (Mascola 1996).

Two gp120-based vaccines were tested in two large Phase III trials in healthy volunteers: a clade B gp120 from HIV-1 MN and a gp120 from the CRF01_AE HIV-1 isolate were used in the VAX 003 Study in Thailand (Pitisuttithum 2006), while clade B gp120 proteins from HIV-1 MN and HIV-1 GNE8 were tested in the VAX 004 Study in the USA and the Netherlands (Flynn 2005). Despite induction of antibodies against gp120, the incidence of new infections was not lowered in either trial. These studies and others demonstrate that it is difficult to neutralize the biological activity of the envelope molecule gp160 via antibodies. Prior to the binding of gp120 to the CD4 receptor, the conserved and functionally important epitopes are hidden in grooves of the gp120 molecules that are additionally masked by glycan shields and variable sequence loops (Kwong 2002). Therefore, it is difficult for antibodies to block the binding of gp120 to the CD4 molecule.

The binding of the gp120 trimer to CD4 induces a conformational change of the V3 loop that exposes a conserved high-affinity coreceptor binding site on the gp120 molecule. The subsequent binding to the coreceptors CCR5 or CXCR4 triggers structural modifications of the viral transmembrane molecule gp41 and starts the fusion of the virus with the host cell membrane. Antibodies against the V3 loop can neutralize the process, although these activated binding sites on the V3 loop are recognizable by antibodies only for a short period of time. Therefore, high antibody concentrations are required for an efficient neutralization. Another problem for antibody-mediated neutralization is the shielding of the V3 loop-coreceptor interaction site by the gp120 trimer, which also inhibits the binding of antibodies to the V3 loop (Labrijn 2003).

Approximately 30% of the HIV-1 infected patients generate neutralizing antibodies within two to three years after infection. However, in the majority of patients they are directed against the gp120 variable sequences. Due to the high sequence variability in gp120, HIV-1 can evade antibodies by a rapid generation of escape mutants. Thus, the majority of patients generate antibodies recognizing their own strain of HIV-1, but they neutralize HIV-1 variants from other patients poorly. There are only a few patients able to produce highly effective broadly cross-reacting neutralizing antibodies (bnAbs). These exceptional antibodies recognize the conserved binding site for CD4 in gp120 (antibodies: B12, PGT121, VRC01, VRC03, 3BNC117), a particular pattern of glycans in gp120 (2G12 antibody), the V1/V2 loops (PG9 and

PG16 antibodies), the V3/V4 loops,, a gp120-V2V3 conformational epitope and the membrane proximal external region (MPER) in gp41 (4E10 and 2F5 antibodies). The majority of the bnAbs show unusual characteristics such as a long complementarity-determining region 3 (CDR3) in the heavy-chain variable (VH) region, a large number of somatic mutations and polyreactivity with non-HIV-1 antigens (McMichael 2012). The requirement of affinity maturation of these antibodies and immune tolerance against polyreactive antibodies are probably important reasons that only approximately 20% of chronically infected patients are able to generate bnAbs and that these bnAbs emerge usually only after several years of infection.

Vaccination with a recombinant gp120 molecule is not able to induce antibodies against the V3 loop, as the V3 loop epitopes in the native gp120 molecule are not accessible to antibodies. To improve the induction of antibodies targeting the V3 loop, attempts are currently in progress to develop fusion molecules consisting of gp120 and CD4 that simulate the conformational changes in gp120 after binding to the CD4 molecule (Kwong 1998).

An innovative approach is the passive genetic immunization by the transfer of genes encoding highly active neutralizing antibodies or antibody-like immunoadhesins. In rhesus monkeys, the intramuscular injection of a recombinant adeno-associated virus (AAV) vector encoding such SIV-specific antibody genes could induce the *in vivo* production of SIV envelope-specific neutralizing antibody constructs that provided protection from intravenous challenge with SIV (Johnson 2009). Using a new self-complementary AAV (scAAV) vector for transfer of genes coding for neutralizing antibodies, protection from HIV-1 infection could be achieved in a humanized mouse model, too (Balazs 2012). This exciting new development stimulated a worldwide search for those few HIV-1 infected individuals who were able to generate unique highly active neutralizing antibodies which could be used for genetic immunization against HIV-1. The discovery of a panel of highly active broadly neutralizing antibodies provided the opportunity to test the activity of such broadly neutralizing antibodies *in vivo* in rhesus monkeys infected with SHIV, a chimeric SIV in which the SIV envelope has been replaced by an HIV-1 envelope. The infusion of a combination of monoclonal antibodies and even the sole application of the N332-dependent antibody PGT121 suppressed the SHIV plasma viremia below the limit of detection (Barouch 2013, Shingai 2013). A recent study in humans analyzed the antiviral activity and safety of the 3BNC117 antibody which is a potent CD4 binding site antibody cloned from a viremic controller. A single 30 mg/kg infusion of 3BNC117 was well tolerated and led to a reduction of HIV-1 viral load by 0.8 – 2.5 log copies/ml in HIV-1 infected viremic patients (Caskey 2015). Although resistance emerged in some patients, passive antibody transfer could be useful not only for treatment of HIV+ patients but also for prevention of mother-to-child transmission.

Induction of HIV-1-specific T cells

With all these hurdles regarding the induction of neutralizing antibody responses, the focus of vaccine development turned to vaccines that could elicit HIV-1-specific T cell responses. Cytotoxic T cells (CTL) play an important role in the control of HIV-1 in humans (Koup 1994, Harrer 1996b, Pantaleo 1997) and for the control of SIV in SIV models. Experimental depletion of CD8 T cells in SIV-infected monkeys abrogated immune control of SIV infection and was associated with a strong increase of viral replication (Schmitz 1999). In contrast to neutralizing antibodies, CTLs do not exert a sterilizing immunity as they can only recognize cells that are already infected.

However, the observation of HIV-1-specific CTLs in HIV-1 exposed but uninfected subjects raised the hope that a T cell-based HIV-1 vaccine could prevent an ongoing HIV-1 infection by containment and eradication of small foci of viral infection (Herr 1998, Rowland-Jones 1998). Even if a T cell-based vaccine could not prevent infection of the host, there is the chance that it could influence the course of infection by reducing the extent of viremia after infection, as seen in the SIV monkey models (Letvin 2006). The viral load four months after infection, also known as the viral setpoint, may be one of the most important prognostic parameters for the course of HIV-1 infection. A vaccine could provide a clinical benefit if it could reduce the viral setpoint by half a log (Johnston 2007). In addition, such a vaccine could possibly exert positive effects on the spread of the HIV epidemic, as a lower viremia probably diminishes the infectivity of the patients. The clinical evaluation of these vaccines that do not prevent infection, but rather influence the course of disease, is difficult to achieve as large numbers of patients must be followed for extended periods of time.

HIV-1 can evade CTL recognition via development of CTL escape mutants in T cell epitopes or in proteasome cleavage sites (Maurer 2008). At least in conserved proteins such as *gag* or protease CTL-mediated immune selection is a major driving force for the development of polymorphisms (Mueller 2007). Our observations in long-term non-progressors showed that the quality of the CTL response with recognition of conserved CTL epitopes is very important (Harrer 1996a, Wagner 1999). It is essential for an effective vaccine to contain enough highly conserved CTL epitopes for the individual HLA alleles.

CTLs can be induced only by vaccines that are able to load viral peptides on HLA class I molecules of dendritic cells which present these peptides to CTLs. Live attenuated viruses are effective against several infectious pathogens such as measles and they were protective against SIV in rhesus monkeys, but they are unlikely to be used in humans due to safety concerns. DNA vaccines alone are not very immunogenic in humans, but in DNA prime/vector boost strategies DNA priming could increase the immunogenicity of subsequent vaccinations with viral vectors. Lipopeptides allow the induction of CTL, but they can present only a limited repertoire of epitopes.

A new concept is the genetic immunization by transfer of genes encoding highly effective HIV-1-specific T cell receptors (TCR) into CD8+ cytotoxic T cells. In contrast to the transfer of antibody genes, transfer of TCR has to consider the HLA restriction of the targeted CTL epitope and the HLA-I type of the recipient. It has been shown in *in vitro* experiments that it is even possible to transfer two different exogenous HIV-1-specific TCRs into the same cell. If such techniques could be applied also *in vivo*, this could reduce the risk of selection of CTL escape mutations (Hofmann 2011).

Recombinant viral vectors

Recombinant vectors can achieve the induction of CTLs without the safety risks of attenuated live viruses. Several vectors have been tested in clinical studies: adenovirus 5 (Ad5) vectors, ALVAC canarypox viruses, MVA (Harrer 2005), NYVAC (Gomez 2007a+b), adenovirus-associated virus and fowlpox vectors.

A great disappointment was the termination of two placebo-controlled Phase IIb trials, the HVTN 502 study (STEP trial) (Buchbinder 2008) and the HVTN 503 study (Phambili Study) (Gray 2011). Both studies tested Merck's trivalent MRK Ad5 vaccine (V520), a mixture of Ad5 vectors expressing HIV-1 gag, pol and nef. The STEP trial started in December 2004 with 3,000 volunteers from North America, South America, the Caribbean, and Australia. The vaccine was immunogenic and induced HIV-1-

specific CD8 T cells in 73% and HIV-1-specific CD4 T cells in 41% of the vaccinees (McElrath 2008). Nevertheless, the study was terminated ahead of schedule in September 2007 because of lack of efficacy. The vaccine neither prevented HIV-1 infection nor did it lower the viral setpoint in those who were infected. In total, 83 volunteers became infected during the trial. As only one female was infected, the *post hoc* analyses were restricted to the 82 male newly-infected subjects. There was a non-significant trend towards a greater number of infections in the vaccine recipients (49 new infections in 914 subjects) versus the placebo recipients (33 new infections in 922 subjects). Interestingly, subjects with high pre-existing Ad5-specific neutralizing antibody titers (titer of >200) at enrolment showed a higher infection rate in those who got the vaccine (21 infections) *versus* those in the placebo arm (9 infections). In contrast there were no significant differences in subjects with absent or low Ad5-specific neutralizing antibody titers of ≤200 (28 infections in the vaccine arm, 24 infections in the placebo arm). Because of the potential risk of the MRK Ad5 vaccine in subjects with a strong immune response against adenovirus 5, the parallel Phambili trial in South Africa was terminated as well. In Phambili, the MRK Ad5 vaccine showed no efficacy, with 33 new HIV-1 infections (4.54 infections per 100 person-years) in patients receiving at least one vaccination versus 28 HIV-1 infections (3.70 infections per 100 person-years) in the placebo arm (non-significant difference) (Gray 2011).

The STEP trial raises important questions that can be answered only by further examination of infected subjects and transmitted viruses. The fact that the increased infection risk was only seen in subjects with high antibody titers against the Ad5 vector argues against a general risk of immunizing against HIV-1, but it demonstrates the important issue of pre-existing vector immunity. The optimal priming of the immune response by a vaccine seems to be a key element determining the success or failure of a vaccine. More basic research is needed for a better understanding of the mechanisms of HIV-1 immunological control. Because of the unfavourable effects of pre-existing immunity against the adenovirus 5 vector, other adenoviral vectors are currently developed from less frequent adenovirus serotypes. So far, two Phase 1 studies in healthy volunteers have demonstrated the immunogenicity of new HIV-1 vaccines based on the adenovirus serotypes AD26 (AD26.ENVA.01) and AD35 (AD35-GRIN/ENV) (Keefer MC 2010).

In contrast to the STEP trial, the RV144 study (Rerks-Ngarm 2009) involving more than 16,000 volunteers in Thailand showed a modest protective effect with a significant reduction of new HIV-1 infections by about 31%. The vaccine was Sanofi Pasteur's canarypox vector-based ALVAC HIV (vCP1521) expressing HIV-1 subtype B gag and protease and subtype E envelope in combination with AIDSVAX B/E gp120 proteins (MN rgp120/HIV-1 plus A244 rgp120/HIV-1). Among the 8,198 subjects receiving placebo, 74 new HIV-1 infections were observed during the three years follow-up compared to 51 infections among the other half of volunteers that had received four immunizations with the ALVAC HIV and two immunizations with AIDSVAX B/E gp120 glycoproteins within a six month period. The vaccine had no effect on viral set points and the clinical course of HIV-1 infection in the subjects infected (Rerks-Ngarm 2012). This was probably due to the fact that the vaccine induced only gp120-specific CD4 T cells (in 33% of the vaccinees), but almost no gag-specific CD4 T cells (in 1% of vaccines) and no HIV-1-specific CD8 T cells (measured by intracellular cytokine staining ICS). In contrast, almost every vaccinee developed high titer antibodies, although these antibodies only had a weak to moderate capacity to neutralize various HIV-1 strains. The mechanisms of the protective effect of the vaccine are still unresolved. It has been hypothesized that antibody-dependent cellular cytotoxicity (ADCC) may have played a role. Recent data indi-

cate a protective role of IgG antibodies to variable regions 1 and 2 (V1V2) of HIV-1 envelope proteins, whereas plasma IgA antibodies to gp120 were associated with higher rates of infections, presumably due to interference with epitope recognition by the protective IgG antibodies (Haynes 2012).

Another efficacy trial, the HVTN 505 study, started enrolment in 2009 (Hammer 2013). This study tested a prime-boost vaccination regimen. After three immunizations (week 0, 4, 8) with a DNA vaccine (6 plasmids: HIV-1 Clade B gag, pol, nef, and env of clades A, B and C), the subjects were vaccinated at week 24 with a mixture of four recombinant adenovirus 5 vectors (containing a gag-pol-fusion protein, and three env of clades A, B and C). Beyond 4 weeks after full immunization (week 28+), HIV-1 infections were observed in 27 of the 967 subjects in the vaccine arm (annual incidence: 2.8%) and in 21 of the 947 placebo recipients (annual incidence: 2.3%) but the difference was not significant. The vaccine had no influence on viral set points in the infected subjects although the vaccination had induced HIV-1-specific T cells and antibodies. However, the vaccine did not induce neutralizing antibodies and the IgG antibody response to the V1/V2 loop was much lower than in the RV144 study in which V1/V2-specific IgG antibodies were associated with a lower risk of HIV-1 infection.

A very interesting new approach is the use of a rhesus monkey cytomegalovirus (RhCMV) vector containing recombinant SIV genes. In rhesus monkeys, this vector induced a persistant and broad CTL response with induction of unusual non-canonical CD8 T cells restricted by HLA-II antigens which are not downregulated by the viral nef protein (Hansen 2013b). So far, it is unknown whether this non-canonical HLA-II – restricted CD8 T cells exist also in humans and whether they can be induced by vaccination.

A promising approach for the development of more effective HIV-1 vaccines is the therapeutic immunization of HIV-1-infected patients on ART who then undergo a treatment interruption (Harrer 2005). The analysis of a vaccine's ability to control HIV-1 replication during treatment interruption may be a good instrument in identifying vaccines that are also effective in prevention.

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5. Acute HIV-1 Infection

HENDRIK STREECK AND MARCUS ALTFELD

Introduction

Within days of HIV-1 acquisition, a transient symptomatic illness associated with high levels of HIV-1 replication and rapid loss of CD4 cells occurs. This highly dynamic phase is accompanied by clinical symptoms similar to mononucleosis. However, despite an estimate of 6,000 new HIV-1 transmissions per day (UNAIDS 2013 Global Report), the diagnosis is missed in the majority of cases. Most commonly other viral illnesses (i.e., flu) are often assumed to be the cause of the symptoms, and there are no HIV-1-specific antibodies detectable at this early stage of infection. The diagnosis requires a high degree of awareness and clinical knowledge based on clinical symptoms and history of exposure, in addition to specific laboratory tests (detection of HIV-1 RNA or p24 antigen and negative HIV-1 antibodies).

An accurate diagnosis of HIV-1 infection during this early stage of infection is particularly important as about 50% of new sexual transmissions are estimated to happen while a person is in this primary phase of infection (Brenner 2007). Indeed, phylogenetic analyses demonstrate a clustering of infections during primary HIV-1 infection, and the catalytic effect of acute HIV-1 infection on the HIV pandemic could be prevented or at least slowed by early diagnosis and immediate antiretroviral therapy intervention (see below). The potentially beneficial use of antiretroviral therapy as pre-exposure prophylaxis (PrEP) could change the face of acute HIV-1 infection in the future (see ART chapter). Recent studies conducted in South Africa, Europe and the US have demonstrated that the use of tenofovir or tenofovir gel might significantly protect from HIV infection (Cohen 2011, Karim 2011). It has been demonstrated that new HIV infections can be reduced by up to 86% (confidence interval 40–99%) in individuals at high risk (IperGAY study, www.ipergay.fr). While no resistant breakthroughs have been detected so far, it is currently unknown how much risk of increased viral resistance due to this monotherapeutic use of antiretroviral medication although it has not been seen to date except in people who were probably serconverting near the time of starting PrEP. Moreover, it is unknown whether other antiretrovial medications or longer-acting formulations may be better suited as PrEP.

Definition and classification

Acute HIV-1 infection (AHI) is defined by high levels of plasma HIV-1 RNA in the presence of a negative anti-HIV-1 ELISA and/or negative/indeterminate Western Blot (<3 bands positive) documenting the evolving humoral immune response; whereas early HIV-1 infection (EHI) includes anyone with documentation of being HIV-1 antibody negative in the preceding 6 months and is therefore broader than the definition of acute HIV-1 infection. Both are included in the term primary HIV-1 infection (PHI) (see Figure 1). A more detailed classification system of the early phases of HIV infection is now in use (Fiebig 2003), which has little relevance for clinical decisions but is important for scientific purposes. The definition used influences the methods needed to make the diagnosis and any considerations regarding the pathogenic implications of this stage of disease. Acute HIV-1 infection is often associated with an acute "retroviral syndrome" that usually includes fever with a variety of non-specific clinical and laboratory abnormalities. In contrast, subjects with early HIV-1 infection can be asymptomatic. The time from exposure to symptomatic disease is
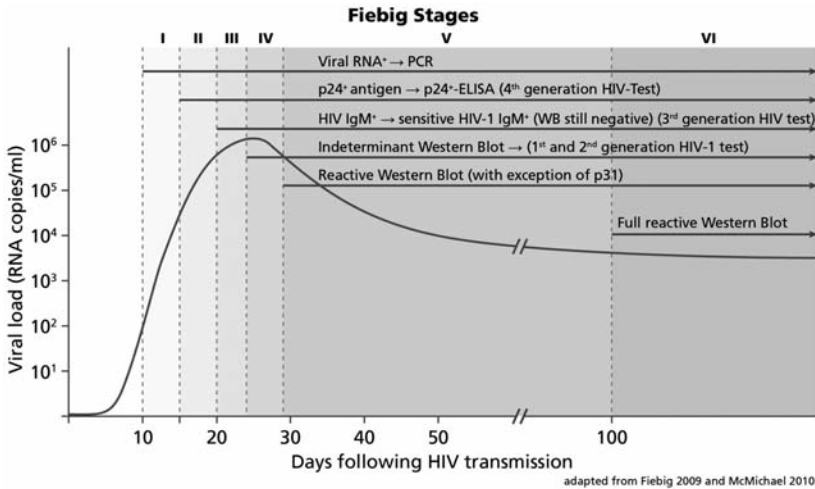
Fiebig Stages

Figure 1: Fiebig stages of acute HIV infection

typically 2 to 4 weeks, and the duration of illness is generally days to weeks. Identifying patients with this syndrome requires a thorough risk assessment, recognition of the variable clinical and laboratory manifestations, and understanding what tests need to be performed in order to make the diagnosis.

Signs and symptoms

After an incubation period ranging from a few days to a few weeks after exposure to HIV, infected individuals often present with an acute flu-like illness. Acute HIV-1 infection is a very heterogeneous syndrome and individuals presenting with more severe symptoms during acute infection and a longer duration of the acute infection syndrome tend to progress more rapidly to AIDS (Vanhems 1998, Pedersen 1989, Keet 1993). The clinical symptoms of acute HIV-1 infection were first described in 1985 as an illness resembling infectious mononucleosis (Cooper 1985). Several non-specific signs and symptoms have been reported in association with acute infection. Fever in the range of 38 to 40°C is almost always present; in addition, lymphadenopathy concomitant with the emergence of a specific immune response to HIV occurs. A generalized rash is also common in symptomatic acute HIV infection. The eruption typically occurs 48 to 72 hours after the onset of fever and persists for five to eight days. The upper thorax, collar region, and face are most affected with well-circumscribed, red colored macules or maculopapules. In addition, painful mucocutaneous oral, vaginal, anal or penal ulcerations are one of the most distinctive manifestations of the syndrome.

Further common symptoms (see Table 1) are arthralgia, pharyngitis, malaise, weight loss, aseptic meningitis and myalgia (Kahn 1998). Although none of these findings are specific, several features, combinations of symptoms and prolonged duration are suggestive of HIV. The highest sensitivity for a clinical diagnosis of acute HIV-1 infection are fever (80%) and malaise (68%), whereas weight loss (86%) and oral ulcers (85%) had the highest specificity (Hecht 2002). In this study, the symptoms of fever and rash (especially in combination), followed by oral ulcers and pharyngitis had the highest positive predictive value for diagnosis of acute HIV-1 infection. In another study, fever, rash, myalgia, arthralgia and night sweats were the best predictors of acute infection (Daar 2001).

Table 1: Main symptoms during acute HIV infection (Hecht 2002)

Symptom	Frequency	Odds Ratio (95% CI)
Fever	80%	5.2 (2.3–11.7)
Rash	51%	4.8 (2.4–9.8)
Oral ulcers	37%	3.1 (1.5–6.6)
Arthralgia	54%	2.6 (1.3–5.1)
Pharyngitis	44%	2.6 (1.3–5.1)
Loss of appetite	54%	2.5 (1.2–4.8)
Weight loss >2.5 kg	32%	2.8 (1.3–6.0)
Malaise	68%	2.2 (1.1–4.5)
Myalgia	49%	2.1 (1.1–4.2)
Fever and rash	46%	8.3 (3.6–19.3)

Diagnosis

Currently, four different HIV tests are commercially available, but they have limited sensitivity in detecting acute HIV-1 infection. In order to be able to correctly interpret a positive or negative result in the presence (or absence) of acute HIV infection symptoms and corresponding history, it is important to understand differences in the sensitivities of the available tests.

The 1st and 2nd generation EIA tests are able to detect HIV-1 infection with both high specificity and sensitivity, but only after HIV-1 seroconversion, as decent levels of anti-p24 IgG antibodies need to be present to give a positive result (see Figure 1). Recently, FDA approved the use of HIV rapid tests, that give results in 20 minutes or less. These are 2nd generation lateral-flow rapid tests and therefore not effective for detecting acute HIV infections.

3rd generation EIA tests can now detect IgM antibodies and therefore are able to detect a recent infection with HIV earlier than the 1st or 2nd generation tests (Hecht 2002). The recently developed 4th generation EIA test now combines the detection of p24 antigen and p24 antibodies and therefore is able to detect HIV infection prior to seroconversion (Ly 2007). However, although this test is able to detect HIV-1 infection much earlier than all previously developed tests, a second diagnostic false negative window can occur when equal levels of p24 antigen and anti-p24 antibody are present. The most substantiated diagnosis of acute HIV-1 infection is based on the detection of HIV-1 replication in the absence of HIV-1 antibodies (pre-seroconversion). The most sensitive test is therefore based on detection of plasma HIV-1 RNA. All assays for HIV-1 RNA that have been compared (branched chain DNA, PCR and GenProbe) have a sensitivity of 100%, but occasionally (in 2–5% of cases) can lead to false positive results (Hecht 2002). False positive results from these tests are usually below 2,000 copies HIV-1 RNA per ml, and therefore are far below the high titers of viral load normally seen during acute HIV-1 infection (in our own studies subjects average 13×10^6 copies HIV-1 RNA/ml with a range of $0.25–95.5 \times 10^6$ copies HIV-1 RNA/ml). Repetition of the assay for HIV-1 RNA from the same sample with the same test led to a negative result in all false positive cases. Measurement of HIV-1 RNA from duplicate samples therefore results in a sensitivity of 100% with 100% specificity. In contrast, detection of p24 antigen has a sensitivity of only 79% with a specificity of 99.5–99.96%. The diagnosis of acute infection must be subsequently confirmed with a positive HIV-1 antibody test (seroconversion) within the following weeks.

During acute HIV-1 infection, there is frequently a marked decrease of the CD4 cell count, which later increases again, but usually does not normalize to initial levels.
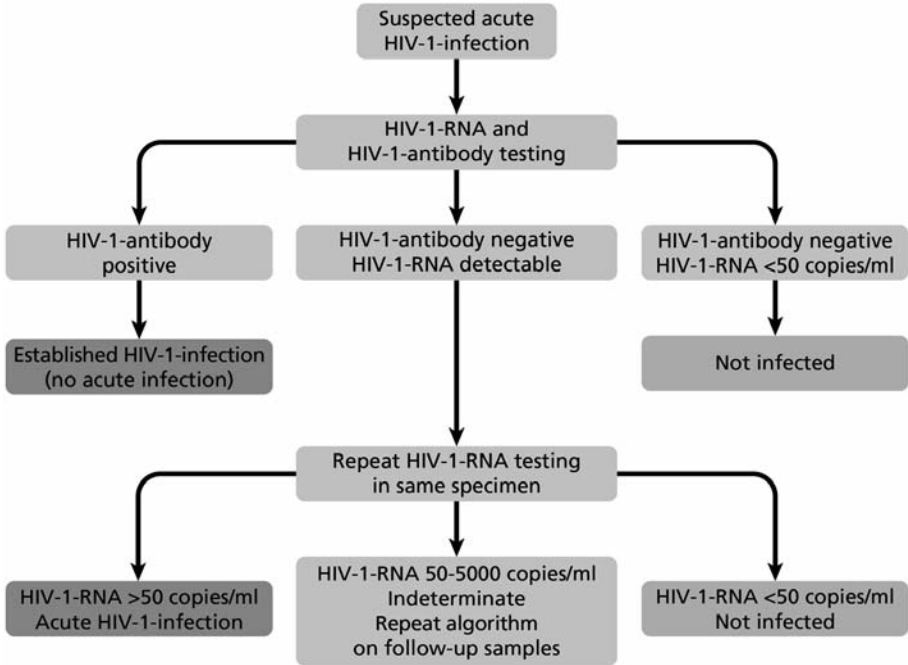
Figure 2: Algorithm for the diagnosis of acute HIV-1 infection

In contrast, the CD8 cell count rises initially, which may result in a CD4/CD8 ratio of <1. Infectious mononucleosis is the most important diagnosis to be aware of, but the differential diagnosis also includes cytomegalovirus, toxoplasmosis, rubella, syphilis, viral hepatitis, disseminated gonococcal infection, other viral infections and side effects of medications.

In summary, the most important step in the diagnosis of acute HIV-1 infection is to keep it in mind during diagnosis. The clinical hypothesis of acute infection requires performance of an HIV-1 antibody test and possibly repeated testing of HIV-1 viral load, as shown in the algorithm in Figure 1 (adapted from Hecht 2002).

Immunological and virological events during AHI

Transmission of HIV-1 generally results from viral exposure at mucosal surfaces followed by viral replication in submucosal and locoregional lymphoid tissues, and subsequently through overt systemic infection. Studies have estimated that most infections occur with a single virus (transmitted founder virus, TF) but in some instances it can occur with two or more viruses. There is the notion that an infection with more viruses is associated with higher viral loads. Moreover, recent studies have demonstrated that the TF is on average different compared to the majority of circulating viruses – higher env content, enhanced cell-free infectivity, improved dendritic cell interaction, and relative IFN-α resistance (Parrish 2013). The virus exponentially replicates in the absence of any detectable adaptive immune response, reaching levels of more than 100 million copies HIV-1 RNA/ml. It is during this initial cycle of viral replication that important pathogenic processes are thought to occur. These include the seeding of virus to a range of tissue reservoirs and the cellular

reservoir. Indeed, studies in rhesus macaques have demonstrated that the latent cellular reservoir is already established on day 3 and predominantly found in central memory and stem cell like memory CD4 T cells (Whitney 2014). Simultaneously to viral dissemination the destruction of CD4+ T lymphocytes, in particular within the lymphoid tissues of the gut occurs. Early on in infection, the very high levels of HIV-1 viremia are normally short-lived, indicating that the host is able to generate an immune response that can control viral replication. Over the following weeks, viremia declines by several orders of magnitude before reaching a viral setpoint. This setpoint following resolution of the acute infection is a strong predictor of long-term disease progression rates (Mellors 1995 & 2007). It is therefore of critical importance to characterize and understand the immune responses induced in the initial stages of HIV-1 infection as these first responses appear responsible for the initial control of viral replication.

In contrast to hepatitis B and C infection, acute phase HIV replication is associated with the activation of a dramatic cytokine cascade, with plasma levels of some of the most rapidly induced innate cytokines peaking 7 days after the first detection of plasma viremia and many other cytokines being upregulated as viral titers increase to their peak. Although some of the cytokines/chemokines produced in acute HIV infection may contribute to the control of viral replication, the exaggerated cytokine response likely also contributes to the early immunopathology of the infection and associated long-term consequences (Stacey 2009). Also, a specific activation and expansion of natural killer (NK) cells has been noted during the acute phase of infection (Alter 2007). Indeed, it has been shown that NK cells can recognize and kill HIV-infected cells (Alter 2011).

Several factors can influence viral replication during acute infection and the establishment of a viral setpoint. These include the fitness of the infecting virus, host genetic factors and host immune responses. While it has been shown that the transmitted/founder virus population has intact principal gene open reading frames and encodes replication-competent viruses (Salazar-Gonzalez 2009), the envelope (env) gene of elite controllers has been demonstrated to mediate less efficient entry than the envelope protein of chronic progressors (Troyer 2009). Interestingly, acute infection envs exhibit an intermediate phenotypic pattern not distinctly different from chronic progressor envs. These findings imply that lower env fitness may be established early and may directly contribute to viral suppression in elite controllers. Antibodies against HIV-1 with neutralizing capacities are rarely detectable during primary HIV-1 infection and are therefore less likely to be major contributors to the initial control of viral replication. However, broadly neutralizing antibodies develop over time in a rare subset of HIV-infected individuals and the expression of specific markers on CD4 T cells is modestly associated with the development of these responses (Mikell 2011). In addition, several studies have demonstrated a crucial role of HIV-1-specific cellular immune responses for the initial control of viral replication. A massive, oligoclonal expansion of CD8 T cell responses has been described during acute HIV-1 infection (Pantaleo 1994), and the appearance of HIV-1-specific CD8 T cells has been temporally associated with the initial decline of viremia (Koup 1994, Borrow 1994). These CD8 T cells have the ability to eliminate HIV-1-infected cells directly by MHC class I-restricted cytolysis or indirectly by producing cytokines, chemokines or other soluble factors, thus curtailing the generation of new viral progeny (Yang 1997). The biological relevance of HIV-1-specific cytotoxic T cells (CTL) in acute HIV-1 infection was highlighted in *in vivo* studies demonstrating a dramatic rise of SIV viremia and an accelerated clinical disease progression in macaques after the artificial depletion of CD8 T cells (Schmitz 1999, Jin 1999). Additional evidence for the antiviral pressure of HIV-1-specific CTLs during primary

HIV-1 infection was provided by the rapid selection of viral species with CTL epitope mutations that were detected within a few weeks of HIV-1 infection (Price 1997). A study assessing the impact of early HIV-1-specific CD8 T cell responses on the early viral set point in a cohort of over 420 subjects was able to demonstrate that the ability to mount a strong early CD8 T cell response during primary HIV-1 infection is moderately associated with a lower viral setpoint (Streeck 2009). Furthermore, the assessment of the CD8 T cell responses against autologous patient-virus-derived peptides in three subjects suggest that even more, yet undetectable, responses are present during the acute phase of the infection contributing up to 15% each to the initial control of viral replication (Goonetilleke 2009).

Many of the early immunodominant CD8 T cell responses have been shown to be restricted by HLA class I alleles, which have been previously associated with slower disease progression such as HLA-B57 or -B27. Moreover, these HLA-restricted responses preferentially target epitopes within a short highly conserved region of p24/Gag (Streeck 2007). This region encodes the HIV-1 capsid, which has been shown to be crucial for the stability of HIV-1 (Schneidewind 2007). The preservation of the early CD8 T cell responses has been associated with slower disease progression (Streeck 2009), which might be linked by the presence of HIV-1-specific CD4 T helper responses during the CTL priming process. During acute infection, the number of CD4 T cells decline, occasionally to levels that allow the development of opportunistic infections (Gupta 1993, Vento 1993). Even though the CD4 T cell count rebounds with the resolution of primary infection, it rarely returns to baseline levels in the absence of antiretroviral therapy. In addition to the decline in CD4 T cell counts, qualitative impairments of CD4 T cell function are perhaps the most characteristic abnormalities detected in HIV-1 infection. The impairment of HIV-1-specific CD4 T cell function occurs very early in acute infection (Rosenberg 1997, Lichterfeld 2004), potentially due to the preferential infection of virus-specific CD4 T cells by HIV (Douek 2002). This is followed by a functional impairment of CD4 T cell responses to other recall antigens, as well as a reduced responsiveness to novel antigens (Lange 2003). The impairment of HIV-1-specific CD4 T helper cell function in acute HIV-1 infection subsequently results in a functional impairment of HIV-1-specific CD8 T cells (Lichterfeld 2004). The antiviral contribution of CD4 T helper response against HIV-1 not been well studied. A recent study demonstrated that a specific CD4 T cell subset with cytolytic properties expands during acute infection only in those patients that can subsequently control viral replication (Soghoian 2012). Moreover, both the protein specificity (Schieffer 2014) and granzyme A levels in HIV-specific CD4 T cells can independently predict disease outcome. The relevance of this association is still under investigation.

However, CD4 T cells also contribute indirectly through the modulation of HIV-specific CD8 T cell responses (Chevalier 2011) or B cell responses to the control of viral replication (Lindqvist 2012). It has been demonstrated in the lymphocytic choriomeningitis virus (LCMV) mouse model that an efficacious CD8 T cell memory response is dependent on the presence of a CD4 T cell response (Janssen 2003, Williams 2006). However, the CD4 T cell signals involved in this interaction are not fully understood. Lack of CD4 T helper cells and chronic antigenic stimulation have been described to be the major cause of the functional deficits CD8 T cells undergo soon after the early phase of infection. It has been demonstrated that IL21-secreting HIV-specific CD4 T cells can preserve and maintain the effector function of HIV-specific CD8 T cells and indeed these responses are mainly found in HIV elite controllers (Chevalier 2011).

The hierarchical loss of CD8 T cell function has been linked to the expression of inhibitory molecules on the cell surface of HIV-1-specific CD8 T cells such as PD-1

and several others (Day 2006, Blackburn 2009). The identification of such receptors might help in the generation of potential immune therapeutics to boost HIV-1-specific CD8 T cell function.

In addition to host immune responses, host genetic factors play an important role in both susceptibility and resistance to HIV-1 infection and speed of disease progression following infection (see *Pathogenesis*). The most important of these is a deletion in the major co-receptor for entry of HIV-1 into CD4 T cells, a chemokine receptor called CCR5 (Samson 1996). Homozygotes for this 32 base pair deletion (CCR5delta32) do not express the receptor at the cell surface and can only be infected with HIV strains that are able to use other coreceptors such as CXCR4. Thus, although CCR5delta32 homozygotic individuals show a significant degree of resistance to HIV-1 infection (Samson 1996), a number of cases of infection with CXCR4-using HIV-1 strains have been described (O'Brien 1997, Biti 1997). Heterozygotes for this deletion exhibit significantly lower viral setpoints and slower progression to AIDS. In addition to mutations in the chemokine receptor genes, a number of HLA class I alleles, including HLA-B27 and -B57, have been described to be associated with both lower viral setpoints and slower disease progression (O'Brien 2001, Kaslow 1996). Studies demonstrate that individuals expressing HLA-B57 present significantly less frequently with symptomatic acute HIV-1 infection and exhibit a better control of viral replication following acute infection (Altfeld 2003). A number of further polymorphisms have been identified that have a potential impact on HIV-1 disease progression. Here especially, the axis between detrimental immune activation and beneficial immune responses is largely unknown and part of ongoing research. For example, it has been demonstrated that polymorphisms in the IL-10 promotor region directly inhibit HIV replication, but may also promote viral persistence through the inactivation of effector immune function (Naicker 2009). These data demonstrate that host genetic factors can influence the clinical manifestations of acute HIV-1 infection and can have an important impact on the subsequent viral setpoint and the speed of disease progression.

Treatment

The results of the START study in patients with chronic HIV infection clearly suggest that the initiation of antiretroviral therapy is beneficial for the patient and outweighs potential risks due to long-term toxicity of the medication. In addition, antiretroviral therapy during acute HIV infection may also be beneficial for the immune system of the patient and may lead to long-term control of viremia in the absence of antiretroviral therapy. Several studies have suggested that treatment of acute HIV-1 infection allows long-term viral suppression and might lead to a preservation and even increase of HIV-1-specific CD4 helper cell responses.

Pilot studies in patients who are treated during acute HIV-1 infection and subsequently start treatment interruptions show that the HIV-1-specific immune response can be boosted (Rosenberg 2000, Vogel 2006, Grijsen 2011), and that patients experience at least temporal control of viral replication. However, other studies were not able to confirm this theoretic benefit (Markowitz 1999, Streeck 2006). Viral load rebounded during longer follow-up, requiring the eventual initiation of therapy. Another study suggests that in comparison to untreated acutely infected patients, patients receiving ART during the acute phase of the infection are more likely to become post-treatment controllers (PTC) (Hocqueloux 2010), which appears to be independent from HLA class I allele expression in comparison to "regular" elite controllers (VISCONTI cohort, Saez-Cirion 2013). Indeed, the authors estimate that the probability of maintaining viral control in individuals treated during acute HIV infec-

tion followed by post-treatment interruption at 12 and 24 months was 15.3% [4.4–26.39]. This is about 10-fold higher in comparison to "elite controllers" (subjects who spontaneously control HIV replication in the absence of ART). This striking success of the VISCONTI cohort was not seen in a large randomized study (SPARTAC 2013) in patients with primary HIV infection. While the authors overall observed a delay in disease progression, it was not significant when the time on ART was removed.

Thus, while it is still unclear whether early initiation of ART has a substantial impact on disease outcome for the patient, studies suggest that early ART may reduce the overall viral reservoir (Ananworanich 2013) and may lead to an overall reduction of residual viral replication on ART (Yerly 2000, Ngo-Giang-Huong 2001). In addition, it has been speculated that the overall diversification of HIV is decreased (Delwart 2002) and that T, B and innate cell functions are preserved in treated individuals (Oxenius 2000, Alter 2005, Moir 2010). These may set the stage for interventions in the future, as the bar for a potential cure due to the lower viral reservoir is lowered. Taken together, while the data on significant beneficial effects of early initiation of ART during acute HIV infection is still not clear, early initiation of ART may reduce potential long-term harm of serious AIDS-related and serious non–AIDS-related events that are due to the lowering of the CD4 T cell count.

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Antiretroviral Therapy (ART)

6. ART 2015/2016

6.1. Perspective

CHRISTIAN HOFFMANN

The development of antiretroviral therapy has been one of the most dramatic evolutions in the history of medicine. Few other areas have been subject to such fast progress, along with some short-lived trends. Those who have experienced the rapid developments of the last few years have been through quite a ride.

The early years, from 1987–1990, brought great hope and the first modest advances with monotherapy (Volberding 1990, Fischl 1990). But when the results of the Concorde Study arrived (Concorde 1994) both patients and clinicians plunged into a depression that lasted several years. AZT (zidovudine) was introduced as a treatment in March 1987 with great expectations. Although quickly approved after rapid study, as monotherapy it was actually very limited. The same was true for the nucleoside analogs ddC (zalcitabine), ddI (didanosine) and d4T (stavudine), all introduced between 1991 and 1994. The lack of substantial treatment options led to a debate that lasted for several years about which nucleoside analog should be used, when, and at what dose. A typical question was, "Should the alarm clock be set during the night for a sixth dose of AZT?"

Patients infected during the early and mid-80s were dying, and quickly. Hospices were established as well as support groups and ambulatory nursing services. One became accustomed to AIDS and its resulting death toll. There was, however, definite progress in the field of opportunistic infections (OI) – cotrimoxazole, pentamidine, gancyclovir, foscarnet and fluconazole saved many patients' lives, at least in the short-term. But the general picture was still tainted by an overall lack of hope. Many remember the somber, still mood of the IXth World AIDS Conference in Berlin in June 1993. Between 1989 and 1994 not much improved.

Then in September 1995, the preliminary results of the European-Australian DELTA Study (Delta 1996) and the American ACTG 175 Study (Hammer 1996) attracted attention. It became apparent that two nucleoside analogs were more effective than monotherapy. Indeed, the differences made in the clinical endpoints (AIDS and death) were highly significant. Both studies demonstrated that it was of great importance to immediately start treatment with two nucleoside analogs, as opposed to using the drugs sequentially.

This turned out to be the beginning of many breakthroughs. The first studies with protease inhibitors (PIs), a new class of drugs, had been under way for several months. PIs had been designed in the lab using the knowledge of the molecular structure of HIV and protease, but their clinical value remained uncertain. Preliminary data, along with many rumors, were circulating. Great impatience pervaded the patients and clinician communities. By the fall of 1995, a fierce competition had started up between three companies: Abbott, Roche and MSD. The licensing studies for the three PIs, ritonavir, saquinavir and indinavir, were pursued with intense effort. The monitors of these studies lived for weeks at the participating clinical sites. Deep into the night, case report files were written up and thousands of queries were answered. These efforts led to fast track approval for all three PIs between December 1995 and March 1996 – first saquinavir, followed by ritonavir and indinavir – for the treatment of HIV.

Many clinicians (including this author) were not really aware of what was happening during these months. AIDS remained ever-present. Although the incidence of AIDS had dropped by half between 1992 and 1996, many were still dying. Doubts

remained. Hopes had already been raised too many times in the previous years by supposed miracles. Noone dared to proclaim a breakthrough.

In February 1996, during the 3rd Conference on Retroviruses and Opportunistic Infections (CROI) in Washington, many caught their breath as Bill Cameron reported the first data from the ABT-247 study during the late breaker session. The auditorium was absolutely silent. Riveted, listeners heard that the mere addition of ritonavir oral solution decreased the frequency of death and AIDS from 38% to 22% (Cameron 1998). These results were sensational in comparison to everything else that had been previously published.

The World AIDS Conference in Vancouver a few months later in June 1996, where the great potential of PIs became fully apparent, developed into a celebration. Even regular news channels reported in great depth on the new "AIDS cocktails". The strangely unscientific expression "highly active antiretroviral therapy" (HAART) began to spread irreversibly.

By this time, David Ho, Time magazine's "Man of the Year" in 1996, had shed light on the hitherto completely misunderstood kinetics of HIV with his breakthrough research (Ho 1995, Perelson 1996). A year earlier, Ho had already initiated the slogan "hit hard, hit early", and almost all clinicians were now taking him at his word. With the new knowledge of the incredibly high turnover of the virus and the relentless daily destruction of CD4 T cells, there was no longer any consideration of a latent phase – and no life without antiretroviral therapy. In many centers almost every patient was treated with ART. Within only three years, 1994-1997, the proportion of untreated patients in Europe decreased from 37% to barely 9%, whilst the proportion of patients on ART rose from 2% to 64% (Kirk 1998).

Things were looking good. By June 1996, a third drug class was introduced when the first non-nucleoside reverse transcriptase inhibitor, nevirapine, was licensed. One now had a great selection of medications at hand. Most patients seemed to tolerate the drugs. 20 or 30 pills a day? Not much of a problem, if it helped. And how it helped! The number of AIDS cases was drastically reduced. Within only four years, between 1994 and 1998, the incidence of AIDS in Europe was reduced from 30.7 to 2.5 per 100 patient years – i.e., to less than one tenth of what it was. Some of the most feared opportunistic infections now occurred only rarely (Mocroft 2000). HIV-specialized ophthalmologists began looking for new areas of work. The large OI trials, planned only a few months before, faltered due to a lack of patients. Hospices, which had been receiving substantial donations, shut down or changed their focus. The first patients began to leave the hospices and went back to work; ambulatory nursing services shut down. Patients with other diseases occupied AIDS wards.

However, in early 1997, some patients began to complain of an increasingly fat stomach, but was this not a good sign after years of wasting and supplementary nutrition? The lower viremia was thought to use up far less energy. It was assumed that, because patients were less depressed and generally healthier, they would eat more. At most, it was slightly disturbing that the patients retained thin faces. However, more and more patients also began to complain about the high pill burden. In June 1997, the FDA published the first warning about the development of diabetes mellitus associated with the use of PIs. In February 1998, CROI in Chicago finally brought home the realization among clinicians that PIs were perhaps not as selective as had long been believed. One poster after another, indeed whole walls of pictures, showed fat abdomens, buffalo humps, thin legs and faces. Lipodystrophy has become an ubiquitous term in HIV medicine today. However, our understanding of the reasons and mechanisms behind this phenomenon remains incomplete. Fortunately, lipodystrophy prevalence has decreased, with the introduction of new antiretroviral drug classes.

The dream of eradication (and cure), widely hoped for in the beginning, was eventually abandoned. Mathematical models were evidently not real life. In 1997, it was estimated that viral suppression with a maximum duration of three years was necessary; it was predicted that all infected cells would die in this time. Since then, the duration has constantly been adjusted upwards. Estimates evolved upwards to around 60 to 70 years (Silicano 2003). These numbers show one thing: HIV will not be cured with standard ART. More recent studies have come to the sobering conclusion that HIV remains detectable in latent infected cells, even after long-term suppression. And Timothy Brown, the only person up to now who has been cured from HIV infection (by an allogenous stem cell transplantation that transferred a rare genetic variation to his immune system) remains a singular case.

In fact, today's reality seemed impossible ten years ago: HIV infection is a chronic disease which, although incurable, is manageable lifelong with therapy, even in patients with resistant virus. CCR5 antagonists as well as integrase inhibitors have opened up new possibilities of treatment. It has become increasingly possible to lower viral loads to below detection in most patients. The pioneer drugs maraviroc and raltegravir have been shown to be extremely well-tolerated. These new drug classes will bring about fundamental changes to current ART. The dogma of always using two nucleoside analogs as the backbone of every therapy may start to change. Many of the currently widespread drugs will disappear over the next few years. The end of HIVID®, Agenerase®, Fortovase® or Viracept® is just the beginning. Veteran agents like AZT, d4T, ddI, nelfinavir or indinavir are not recommended by guidelines anymore although they served us in HIV management in the nineties. Will we be needing saquinavir, fosampranavir or even efavirenz and lopinavir as much as we do today five years from now?

A normal life expectancy seems realistic today with treatment. Therapy is likely to be permanent. This will pose a tremendous challenge for patients, physicians and for the pharmaceutical industry and payors. The comfortable situation at present does not mean one can relax. New drugs are urgently needed. There is uncertainty about whether our drugs can stand the test of time over decades. Effects on the heart, kidney, bones and other organs in an aging HIV population are difficult to foresee. If the cure is delayed, over the decades one will need a wider breadth and range of available drugs. It will not be easy for new drugs to be approved, as vicriviroc has shown. How do you show the advantages of a new drug over other successful therapies today? Approval for new drugs is becoming more strict and the market is tightening. Already one can observe the pharmaceutical industry's caution. The days may be over when an HIV drug got from the laboratory to the market within five years. Compared to the previous decade, the HIV ARV pipeline is now drying up. New strategies are needed.

At the same time, the simple question of "when to start" with ART has remained unanswered for a long time. Instead of David Ho's recommendation from the nineties "hit hard, hit early", we often heard "hit HIV hard, but only when necessary" (Harrington 2000) during the last decade. With the START study results appearing at the horizon, there is no doubt that this will change again. The pendulum will swing back. But do really all patients need a therapy? At any CD4 T cell count? What roles do the following play: viral load, CD4 T cell changes, CD4 percentages, age, gender, host elements and viral tropism? What about acutely infected patients? These strategically important questions will hopefully find some answers through detailed analysis of the START study that are underway now. Until then, this issue requires keen sensitivity.

HIV clinicians are well-advised to keep an open mind to new approaches. Those who do not make an effort to constantly expand their knowledge at conferences will not

be able to provide adequate treatment for their patients in a field that is still growing and learning and changing direction every two to three years. Those who adhere strictly to evidence-based medicine and only treat according to guidelines are quickly outdated. HIV medicine is ever-changing. Treatment guidelines remain just that, and are often out of date by the time of publication. There are no laws set in stone. However, those who confuse therapeutic freedom with random choices, and assume that data and results coming from basic research can be ignored are also missing the point. Individualized treatment is not random treatment. It cannot be stressed enough that clinicians are also responsible for the problem of poor adherence. Even if many experienced clinicians have come to disregard this, every patient has the right to know why they are taking the therapy they are on or, indeed, why certain therapies are not an option. The more they understand their therapies, the better the long-term results.

HIV remains a dangerous opponent. Patients and clinicians must tackle it together. The following chapters describe how this can be done.

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6.2. Overview of antiretroviral agents

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Preliminary remarks

As of now (March 2015) there are more than 30 individual or combination agents licensed for treatment of HIV infection. These drugs are from five different classes:

1. Nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs)

2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

3. Protease inhibitors (PIs)

4. Entry inhibitors (co-receptor antagonists and fusion inhibitors)

5. Integrase inhibitors (INSTIs)

In addition, several fixed-dose combinations (FDCs), among them four single-tablet regimens (STRs), and two pharmacoenhancers are available. As NRTIs and NNRTIs are blocking the same enzyme, there are now four targets for therapeutic interventions (Figure 2.1): The entry of HIV into the target cell (in theory, three steps can be distinguished) and the three enzymes reverse transcriptase, integrase and protease. For more details see also the Pathogenesis chapter.

In this chapter, individual agents listed by class are discussed with reference to their specific benefits and problems. Discussion on common primary therapy can be found in the chapter "What to start with?". Other chapters talk about adjusting ART, experimental agents and a possible cure.
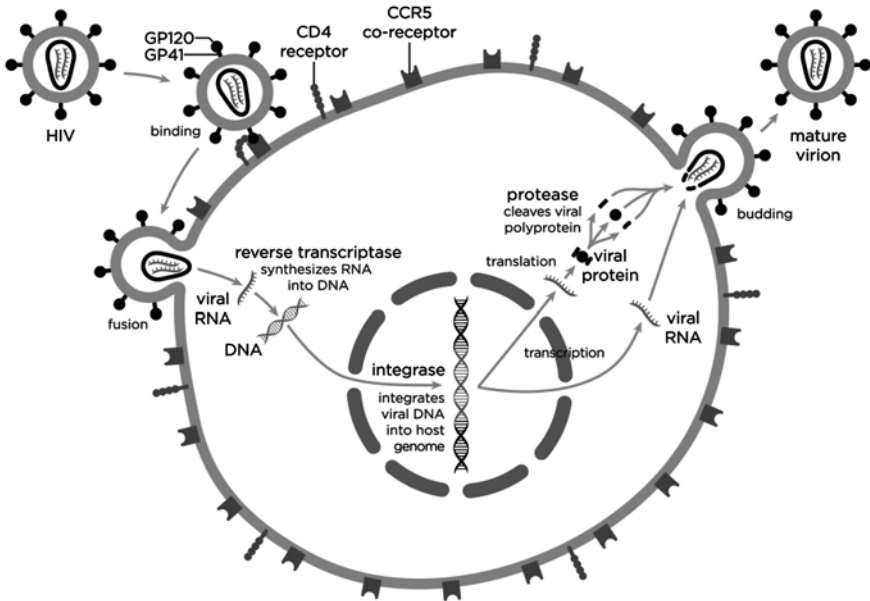
Figure 2.1. Replication cycle of HIV and the four targets for therapeutic intervention. Entry, reverse transcriptase, integrase, protease

Table 2.1: Overview of antiretroviral drugs approved in the US and/or Europe (March 2015, *not marketed in all countries)

Trade name	Abbrev.	Drug	Manufacturer
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTIs)			
Emtriva®	FTC	Emtricitabine	Gilead Sciences
Epivir®	3TC	Lamivudine	ViiV Healthcare, generics
Retrovir®	AZT	Zidovudine	ViiV Healthcare, generics
Videx®	ddI	Didanosine	Bristol Myers-Squibb, generics
Viread®	TDF	Tenofovir	Gilead Sciences
Zerit®	d4T	Stavudine	Bristol Myers-Squibb
Ziagen®	ABC	Abacavir	ViiV Healthcare
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)			
Edurant®	RPV	Rilpivirine	Janssen-Cilag
Intelence®	ETV	Etravirine	Janssen-Cilag
Rescriptor®*	DLV	Delavirdine	ViiV Healthcare
Sustiva®, Stocrin®	EFV	Efavirenz	BMS/MSD, generics
Viramune®	NVP	Nevirapine	Boehringer-Ingelheim, generics
Protease Inhibitors (PIs)			
Aptivus®	TPV	Tipranavir	Boehringer-Ingelheim
Crixivan®	IDV	Indinavir	MSD
Invirase®	SQV	Saquinavir	Roche, generics
Kaletra®	LPV	Lopinavir/Ritonavir	AbbVie
Prezista®	DRV	Darunavir	Janssen-Cilag
Reyataz®	ATV	Atazanavir	Bristol Myers-Squibb
Telzir®, Lexiva®	FPV	Fosamprenavir	ViiV Healthcare
Viracept®*	NFV	Nelfinavir	ViiV Healthcare
Entry Inhibitors			
Celsentri®, Selzentry®	MVC	Maraviroc	ViiV Healthcare
Fuzeon®	T-20	Enfuvirtide	Roche
Integrase Inhibitors			
Isentress®	RAL	Raltegravir	MSD
Tivicay®	DTG	Dolutegravir	ViiV Healthcare
Vitekta®*	EVG	Elvitegravir	Gilead Sciences
Combination Drugs			
Combivir®	CBV	AZT+3TC	ViiV Healthcare, generics
Dutrebis®*		RAL+3TC	MSD
Evotaz®*		ATV/c	BMS+Gilead Sciences
Kivexa®, Epzicom®	KVX	3TC+ABC	ViiV Healthcare
Rezolsta®, Prezcobix®*		DRV/c	Janssen-Cilag+Gilead Sciences
Trizivir®	TZV	AZT+3TC+ABC	ViiV Healthcare
Truvada®	TVD	TDF+FTC	Gilead Sciences
Single-Tablet Regimens (STR)			
Atripla®	ATP	TDF+FTC+EFV	Gilead+BMS+MSD
Complera®, Eviplera®	CPL	TDF+FTC+RPV	Gilead+Janssen-Cilag
Stribild®	STB	TDF+FTC+ELV/c	Gilead Sciences
Triumeq®		ABC+3TC+DTG	ViiV Healthcare
Pharmacoenhancers			
Norvir®	RTV	Ritonavir	AbbVie
Tybost®	COB	Cobicistat	Gilead Sciences

Brand names, indications

The Federal Drug Administration (FDA) in the US and the European Medicins Agency (EMA) do not always agree on the granting of brand names with the result that, in some cases, names differ from country to country. Sometimes a pharmaceutical company does not hold authorization for production worldwide. The NNRTI efavirenz, for example, is produced by BMS in Germany under the brand name Sustiva® and in Austria by MSD under the name of Stocrin®. Several agents such as AZT, 3TC, AZT+3TC but also efavirenz, nevirapine or saquinavir are available as generics. The situation will not improve when patents and rights for many agents, including blockbuster drugs such as tenofovir or darunavir will run out in the near future.

Moreover, definitions for indication areas vary widely. Some agents are specifically not licensed for primary (first line) therapy, such as entry inhibitors, the PI tipranavir and the NNRTI etravirine, as well as combination agents such as Atripla® in Europe. Other limits: The NNRTI rilpivirine is restricted to patients with a plasma viremia of less than 100,000 HIV RNA copies/ml. Before initiation of abacavir, HLA pretesting is necessary and the use of maraviroc requires a valid tropism test. Several drugs should be used in pregnant women and children. Complex dosing instructions have to be considered for some drugs, due to drug-drug interactions or due to to renal or hepatic insufficiency. More details can also be found in the chapter "Drugs" at the end of this book.

In the face of cost pressures suffered by health systems, it is advisable for clinicians to adhere to the specific indication areas of the individual agents. Due to such a wide range of choices, this is possible in most cases, although not in all. Clinicians should have good reason when using an agent outside the stated indication area. A thorough documentation should be kept in case of disagreement from payors.

Costs

Antiretroviral treatment is expensive. A health provider needs to be aware of drug costs. Even within drug classes, there are astonishing differences. For example, the PI indinavir (Crixivan®, hardly used today) is relatively cheap in most countries, while the PI tipranavir (Aptivus®) is more than three times the price. Even in recommended first-line therapies in guidelines there are great price variations: PIs are almost double the price of NNRTIs in many countries. A salvage therapy for a patient with multiresistant virus can amount to as much as € 30,000–50,000 and more per year. For pricing in low- or middle-income countries, please refer to the chapter *Global Access to HIV Treatment.*

It is difficult to comprehend the pricing policies of pharmaceutical companies. The reason why prices for directly competing agents (3TC and FTC) are almost exactly the same, whilst prices for other agents of the same drug class differ by 200–300%, cannot be explained by development costs alone. There is no doubt that ART is a money-maker and the market is full of competitors – monopolies and patents are being protected. Despite all the criticism and price discussions, two facts cannot be forgotten:

First, the high development costs for new medicines can rise to a billion dollars or more. Most agents never make it to the market. Even a licensed drug such as T-20 may never recoup its development costs. According to Roche, research and development alone chewed up 600 million dollars. To cover such production costs, thousands of patients worldwide would have to be treated with T-20 for several years – a very unrealistic scenario.

Second, there is hardly a more effective therapy than antiretroviral therapy. US estimations assume an expenditure of between $13,000 and $23,000 per additional QALY (quality-adjusted life year) (Freedberg 2001). Compared to many other therapies this is relatively cheap. ART reduces the cost of expensive treatment of opportunistic infections, inpatient and outpatient care. In one German study, between 1997 and 2001 total annual spending per patient decreased from € 35,865 to € 24,482 (Stoll 2002). Many patients return to work, resulting in an overall economic gain for society (Sendi 1999).

Nevertheless, ART *is* expensive. Therefore, it should be expected from patients to use up remaining packets of drugs, etc. if the reasons for a change in therapy are not urgent. Concerns of pill reduction or doubts about long-term toxicity should be part of an ongoing discussion with patients. All patients need to be made aware of the costs of medication so they can better understand the value of the therapy.

Initially, ART should be prescribed for a month. This way, mountains of unused pills will not be wasted if signs of intolerability or complicated adverse events occur. If response to ART is positive and its effects constant, prescriptions can then be done for a maximum period of three months. Many companies now offer three-month supply packages.

Nucleoside Analogs (NRTIs)

Mechanism of action

Nucleoside analogs ("nukes") are also referred to as nucleoside reverse transcriptase inhibitors (NRTIs). Their target is the HIV enzyme reverse transcriptase. Acting as alternative substrates, they compete with physiological nucleosides, differing from them only by a minor modification in the ribose molecule. The incorporation of nucleoside analogs induces the abortion of DNA synthesis because phosphodiester bridges can no longer be built to stabilize the double strand. Nucleoside analogs are pro-drugs. They are converted to the active metabolite only after endocytosis, whereby they are phosphorylated to the effective triphosphate derivatives.

Nucleoside analogs were the first antiretroviral agents on the market. AZT (zidovudine, Retrovir®) was approved for the treatment of HIV infection in 1987. Once-daily dosing is sufficient for many nukes. Overall tolerability is fairly good. However, frequent complaints during the first weeks are fatigue, headache and (mostly mild) gastrointestinal problems. The gastrointestinal complaints can be treated symptomatically (see chapter on *Management of Side Effects*).

Nucleoside analogs can cause a wide variety of long-term side effects, including myelotoxicity, lactate acidosis, polyneuropathy and pancreatitis. Many metabolic disorders, especially lipoatrophy, are also attributed to nucleoside analogs (Galli 2002, Mallal 2002). Long-term side effects that are possibly related to mitochondrial toxicity were first described in 1999 (Brinkmann 1999). Mitochondrial function requires nucleosides. The metabolism of these important organelles is disrupted by the incorporation of false nucleosides (the drugs) leading to mitochondrial degeneration. Clinical and scientific data indicate that there are considerable differences between individual drugs with regard to mitochondrial toxicity. Agents like d4T or ddI are more toxic than abacavir or 3TC and are therefore no longer used in HIV treatment today. Ddc has disappeared entirely.

However, it is also possible that beside mitochondrial damage other mechanisms contribute to toxicity. Recently it was shown that NRTIs (specifically tenofovir) but not NNRTIs can inhibit telomerase activity. Telomerase is a specialized reverse transcriptase responsible for the de novo synthesis of telomeric DNA repeats. Its inhibition by NRTIs may lead to accelerated shortening of telomere length in

activated PBMCs. A telomere is a region at each end of a chromatid, which protects the end of the chromosome from deterioration or from fusion with neighbouring chromosomes. Over time, due to each cell division, the telomere ends become slightly shorter. By inhibition of telomerase activity, NRTIs may thus contribute to accelerated aging in HIV+ patients (Hukezali 2012, Leeansyah 2013).

Nucleoside analogs are eliminated mainly by renal excretion and do not interact with drugs that are metabolized by hepatic enzymes. There is therefore little potential for interaction. However, ribavirin, used in the treatment of hepatitis C, can reduce intracellular phosphorylation of AZT or d4T (Piscitelli 2001). In patients with renal failure, the dosage of nucleoside analogs has to be adjusted. AZT and d4T are thymidine analogs, while FTC and 3TC are cytidine analogs. Combinations containing AZT plus d4T or FTC plus 3TC are pointless since these drugs compete for the same attachment pocket. There is a high degree of cross-resistance between NRTIs (see chapter on *Resistance*).

Individual agents

Abacavir (ABC, Ziagen®) is a guanosine analog. Monotherapy studies showed this drug to lower viral load by approximately 1.4 logs within 4 weeks, but that resistance develops rapidly (Harrigan 2000). Abacavir is phosphorylated intracellularly to carbovir triphosphate, which has a long half-life (Harris 2002). In 2004, following larger studies, abacavir was licensed for once-daily therapy (Moyle 2005, Sosa 2005).

ABC+3TC is comparable in efficacy to AZT+3TC (DeJesus 2004). In combination with AZT+3TC (Trizivir®, see section on Triple Nukes), abacavir was less effective than efavirenz (Gulick 2004) or indinavir (Staszewski 2001). In randomized studies, a switch from a successful PI- or NNRTI-containing therapy to abacavir plus two NRTIs proved relatively safe (Clumeck 2001, Katlama 2003, Martinez 2003, Bonjoch 2005). However, there is an increased risk of virological failure, especially in extensively pretreated patients (Opravil 2002, Martinez 2003, Bommenell 2011). In particular, with the combination ABC+TDF+3TC, resistance can develop rapidly (see section on Triple Nukes).

With respect to mitochondrial toxicity, abacavir seems to compare favorably to other NRTIs. In comparison with d4T, the risk of lipoatrophy is lower (Podzamczer 2006). Moreover, switching from d4T to abacavir led to improvements in subjects with existing lipodystrophy (Carr 2002, John 2003, Moyle 2003, McComsey 2005). Improvement was associated with an increase in mitochondrial DNA as shown in *in vitro* studies (Hoy 2004, Martin 2004, McComsey 2005).

One drawback to the use of abacavir is the risk of hypersensitivity reaction (HSR). HSR occurs in 4-6% of patients. On re-exposure after stopping ABC due to HSR, it can be fatal. Cases of severe HSR have been reported after only a single abacavir dose (De la Rosa 2004) or after treatment re-initiation despite prior tolerability (El-Sahly 2004). A genetic predisposition exists. 80% of cases of HSR occurs in patients with the HLA B*5701 allele (Mallal 2002, Hetherington 2002). The predictive value of the HLA test was proven in the large PREDICT trial (Mallal 2008), and the assay is now obligatory prior to starting abacavir. However, clinical HSR cases without the HLA B*5701 allele have been observed on very rare occasions.

Once the problem with HSR was largely resolved, abacavir came under pressure again in 2008. Cohort studies reported an association between recent use of abacavir and an increased risk of myocardial infarction (Sabin 2008, SMART 2008). Although this was not confirmed by two recent meta-analyses (Cruciani 2011, Ribaudo 2011), some experts still believe that alternative regimens should be considered for patients with underlying high cardiovascular disease risk (Behrens 2010, Sabin 2014).

Today, abacavir is mainly used in the combination tablet Kivexa® (US: Epzicom®) and in the single tablet regimen Triumeq® (see below).

AZT (zidovudine, Retrovir®) was the first antiretroviral agent in 1987 to make it to market. The earliest studies that tested AZT monotherapy were able to show a significant survival benefit – in very immunocompromised patients (Fischl 1987). In contrast, two other very large early studies, ACTG 016 and ACTG 019, were not able to demonstrate significant survival benefit in asymptomatic patients, although the risk for progression was significantly reduced in both (Fischl 1990, Volberding 1990). Even at that time, it was becoming apparent that the success of AZT monotherapy was likely to be limited. The Concorde Study brought AZT into disrepute by showing that there was no long-term benefit of AZT treatment. The higher doses (1500 mg/day) led to considerable myelotoxicity (Fischl 1990). Myelotoxicity should also not be underestimated with the current dosages of 500–600 mg/day; monitoring of the blood is obligatory. Long-term treatment almost always increases the mean corpuscular volume of erythrocytes, which is to some extent a measure of monitoring adherence. AZT is very effective in combination with other ARV drugs. In the nineties, the combination of AZT and 3TC was one of the most frequently used backbones in HIV therapy. AZT has been tested in numerous clinical studies and offers more experience than any other agent (over 20 years).

AZT came under heightened scrutiny when it performed worse than tenofovir in the Gilead 934 study. In this large-scale randomized study, ART-naïve patients were treated with efavirenz plus either AZT+3TC or TDF+FTC. In particular, severe anemia was more frequent on AZT, leading to withdrawal in 5.5% (Gallant 2006). After 144 weeks, fewer patients on AZT had a viral load of less then 400 copies/ml than on TDF (58% vs 71%). This difference was due in large part to the fact that more patients on AZT withdrew due to adverse events (11% vs 5%). Apart from myelotoxicity including anemia and neutropenia, side effects leading to discontinuation were mainly gastrointestinal complaints such as nausea, usually occurring within the first few weeks of treatment. Moreover, a significant reduction in fat tissue of the extremities while on AZT was observed (Arribas 2008). Many studies have confirmed an improvement of lipoatrophy after switching from AZT to other drugs (see below).

Consequently, in many guidelines AZT is no longer recommended. Another disadvantage is that AZT needs to be taken twice daily, thereby disqualifying it as being part of once-daily combinations. Thus, AZT currently remains a component of some salvage regimens that are used for resistant viruses. For example, a hypersensitivity to AZT is seen in viral isolates with mutations K65R or M184V. The good CNS penetration of AZT can be used in the setting of HIV-associated neurocognitive disorders (HAND, see there).

ddC (zalcitabine, HIVID®) was the third NRTI to reach the market in 1992. Limited efficacy, unfavorable pharmacokinetics and side effects led to its withdrawal from the market in June 2006 – a first in HIV therapy.

ddI (didanosine, Videx®) was, in 1991, the second NRTI to be licensed. Antiretroviral efficacy is comparable to AZT (Berenguer 2008). However, ddI is currently used only in very limited situations (Molina 2005) due to toxicity. Gastrointestinal complaints and polyneuropathy are the main side effects. Pancreatitis occurs in up to 10%, and can be fatal. This toxicity is probably dose-dependent. The cause for this is unclear, but could possibly be related to disorders of purine metabolism (Moyle 2004). Special caution should be given to combinations with ribavirin, d4T, hydroxyurea or tenofovir (Havlir 2001, Martinez 2004). The dosage needs to be adjusted according to the patient's weight. If body weight is less than 60 kg, the dose should be reduced from 400 mg to 250 mg. Of note, ddI has to always be taken on an empty stomach.

d4T (stavudine, Zerit®) was the second thymidine analog to be introduced after AZT. Although better tolerated (less gastrointestinal complaints) and just as effective as AZT, d4T is hardly ever used nowadays in western industrialized countries. This is mainly due to its long-term toxicities in comparison to other NRTIs, shown in large randomized studies (Gallant 2004, Saag 2004). Use of d4T is associated with lactic acidosis and Guillain-Barré-like syndromes (Mokrzycki 2000, Shah 2003), as well as for lipoatrophy (Mallal 2000, Mauss 2002). Numerous studies have now been published in which substitution of d4T by other NRTIs, particularly abacavir or tenofovir, had positive effects on lipoatrophy and other metabolic disorders (see chapter 6.7). In March 2011, a warning letter was distributed to physicians according that clarified that d4T is indicated only if there are no other options. Duration is limited to the shortest possible time and whenever possible patients should switch to alternatives. There is nothing else to be said.

3TC (lamivudine, Epivir®) was licensed in Europe in August 1996 as the fifth NRTI. It is a well-tolerated cytidine analog and part of various fixed-dose combinations, among them Combivir®, Kivexa® (US: Epzicom®) or Triumeq®. Its main disadvantage is its rapid development of resistance, and a single point mutation (M184V) is sufficient for compromising its effectiveness. Resistance is likely to develop after only a few weeks (Eron 1995). The full effect of 3TC only emerges in combination with other nucleoside analogs. Large studies such as NUCB 3002 or CAESAR showed a significant clinical benefit when 3TC was added to nucleoside therapy (Staszewski 1997). The M184V point mutation does have advantages: not only does it improve the susceptibility of certain AZT-resistant viruses in some patients but it also impairs viral fitness (Miller 2002). This was demonstrated in a study with monotherapy in patients with the M184V mutation: maintaining 3TC monotherapy was associated with a lower increase in viral load and slower CD4 T cell decline compared to completely stopping ART (see chapter 6.9). Keeping 3TC as part of a combination despite proven resistance is therefore sensible in order to conserve the M184V mutation and thus reduce the replicative capacity of HIV, especially when not all the other agents in the regimen are active. The antiviral efficacy of 3TC is the same as that for FTC (Rousseau 2003, Benson 2004). Once-daily dosing is possible although the half-life of 3TC is less than that of FTC (DeJesus 2004). 3TC has also efficacy against hepatitis B viruses. However, resistance mutations may occur rapidly. In HBV coinfected patients, 3TC should be combined with other HBV drugs.

FTC (emtricitabine, Emtriva®) is a cytidine analog. It is biochemically very similar to 3TC, but has a longer half-life. Once-daily dosing is possible, and the drug also has efficacy against HBV. Tolerability is good, while the potential for interactions is minimal (Frampton 2005). FTC seems to have a low affinity for the mitochondrial polymerase so the risk of mitochondrial toxicity is likely to be relatively low. FTC was as effective as 3TC both as monotherapy as well as in combination with AZT (Rousseau 2003, Benson 2004). However, as with 3TC, efficacy is limited by the M184V point mutation. The drug was licensed in 2003 when a randomized, double-blinded trial (FTC-301) showed that FTC was more effective and tolerable than d4T (Saag 2004). The combination of TDF+FTC was superior to AZT+3TC in the large GS-934 study, notably in terms of tolerability (Gallant 2006, Arribas 2008). Tolerability was probably in most part due to the second agent (AZT or d4T) and not FTC or 3TC. FTC is currently an important component in combination therapy as a fixed partner of tenofovir (Truvada®). The combination of FTC and tenofovir is found in three STRs, namely (Atripla®, Complera® and Stribild®). Like with 3TC, the individual agent (Emtriva®) does not play a role. Due to the fact that no clinical differences have yet been established between 3TC and FTC, the choice between the two is usually determined by its co-medication (abacavir, tenofovir, AZT).

TDF (tenofovir, Viread®) acts as a false building block similar to nucleoside analogs, targeting the enzyme reverse transcriptase. However, in addition to the pentose and nucleic base, it is monophosphorylated and therefore referred to as a nucleotide analog. A more accurate description of the agent is tenofovir DF (disoproxil fumarate), which refers to the phosphonate form from which the phosphonate component is only removed by a serum esterase, and which is activated intracellularly in two phosphorylation steps (Robbins 1998).

Tenofovir is available as a single agent, but is most often administered in fixed-dose combinations within Truvada®, Atripla®, Complera® and Stribild®. Tenofovir is well tolerated. Side effects in these studies were comparable to the placebo arms. The 903 trial showed at least equivalent potency with a significantly reduced incidence of polyneuropathy and lipid changes compared to d4T (Gallant 2004). It has been shown that phosphorylated tenofovir has a low affinity for mitochondrial poly-merase (Suo 1998). As a result of this convincing clinical data, the drug is still among the most widely used agents in antiretroviral therapies. In the 934 study, TDF+FTC were significantly better than AZT+3TC (Gallant 2006, Arribas 2008), particularly due to improved tolerability. Furthermore, tenofovir can help improve lipoatrophy and dyslipidemia (see chapter 6.7). Another advantage is its efficacy against the hepatitis B virus, which resulted in the licensing of this drug for HBV monoinfection. Other areas of use are in vertical prevention and pre-exposure prophylaxis (see appropriate chapters).

Some problems have come to light with the more extensive use of TDF. The combination with ddI should be avoided. An unfavorable interaction with atazanavir exists that calls for being boosted with ritonavir (Taburet 2004). Efficacy may also be limited in some triple nuke regimens (see section on Triple Nukes).

However, the main problem today with tenofovir is its potential risk of nephrotoxicity (see chapter on *HIV and Renal Function*). Nephrotoxicity is reflected by a mostly mild disturbance of renal function (Review: Hall 2011). Fortunately, severe dysfunctions are very rare (Gallant 2008, Scherzer 2012). In a Swiss cohort trial, 46 out of 2,592 patients (1.6%) had to discontinue tenofovir due to renal toxicity, on average within 442 days (Fux 2007). The risk of renal toxicity seems to be higher when tenofovir is combined with boosted PIs (Young 2012). Renal failure can also be observed in the setting of Fanconi syndrome, a defect of the proximal tubular transport (Schaaf 2003, Hall 2011). Patients with renal disease should either not be treated with tenofovir, or receive a lower dose (see chapter on *Drugs*). Elderly patients and patients with low body weight are particularly at risk (Crane 2006). However, it is so far impossible to predict who is at risk of developing renal dysfunction. According to current data, because it is taken by such a large number of patients, it is important to remain alert and to regularly check renal function of patients on tenofovir, especially of those on long-term therapy. Tenofovir is also associated with bone damage such as osteomalacia. There is no doubt, that during the next years, many patients will replace TDF by tenofovir alafenamide (TAF, see also next chapter). TAF is a novel prodrug of teno-fovir which has a different structure to TDF, reaching adequate tenofovir concen-trations in cells at a much lower dose, which has less potential to harm kidney and bone tissue. Gilead has applied for approval (or plans to do so) of different TAF-inclu-sive versions of Truvada®, Complera® and Stribild®.

The choice of nuke backbones

Until now, all classical ART regimens have contained two nucleoside or nucleotide analogs (the "nuke backbone"). This is mainly historical: nucleoside analogs were the first HIV drugs, and when PIs appeared years later, treatment with two nukes was standard. As knowledge has grown about the mitochondrial toxicity of some

NRTIs, this concept is now being questioned by an increasing number of experts (see section on Nuke-Sparing). However, data on combinations without NRTIs are still limited, and there are currently no recommendations for such strategies. The most frequently used backbones are TDF+FTC, and with some limitations, ABC+3TC. Both are available in fixed-dose combinations that can be taken once daily. AZT+3TC, the long-standing standard backbone in the nineties, is now considered an alternative.

Table 2.2: NRTI combinations

	3TC	ABC	ddI	d4T	FTC	TDF	AZT
3TC		+++	++	+	−	++	++
ABC	+++		0	0	0	0	+
ddI	+	0		−	0	−	0
d4T	+	0	−		0	0	−
FTC	−	0	0	0		+++	0
TDF	++	0	−	0	+++		0
AZT	++	+	0	−	0	0	

+++ preferred backbones, ++ recommended as alternative, + other alternative, 0 insufficient data, – should be avoided. d4T is only indicated "if other antiretroviral drugs can not be used" (see above)

TDF+FTC

There is convincing data for the combination of TDF plus FTC (or initially 3TC). In the Gilead 903 Study, the combination TDF+3TC was not only as virologically effective as d4T+3TC, but was also much better tolerated (Gallant 2004). Since the introduction of FTC and the fixed-dose combination tablets of Truvada®, Atripla®, and, more recently, Complera® and Stribild®, tenofovir is almost always co-administered with FTC, and TDF+FTC is the most frequently-used NRTI backbone. In the Gilead 934 Study (Gallant 2006), enrolling 509 ART-naïve patients, TDF+FTC was tested against AZT+3TC in an open-label design (all patients also received efavirenz). At 48 weeks, a larger proportion of patients in the TDF+FTC arm reached less than 50 copies/ml (80% versus 70%). This was even true for patients with a higher baseline viral load. The significant differences were primarily related to the poorer tolerability of Combivir®, which often resulted in the discontinuation of therapy (9% versus 4%). Virological failure and resistance mutations were approximately equal in both arms and were infrequent. After 144 weeks, lipoatrophy was less frequent in the TDF+FTC arm (Arribas 2008). In the near future, tenofovir alafenamide (TAF), a novel prodrug of tenofovir, will probably replace TDF in many patients. There is no doubt that TDF+FTC or TAF+FTC will remain the most frequently used backbone during the coming years.

ABC+3TC

Another frequent backbone is ABC+3TC, which is also available in a fixed-dose combination known either as Kivexa® or Epzicom®. The double-blind randomized CNA30024 Study showed the non-inferiority of ABC+3TC in comparison to Combivir® (DeJesus 2004). In the ABCDE Study, ABC+3TC had the same efficacy as d4T+3TC, but had less toxicity (Podzamczer 2006).

Over the last few years, ABC+3TC has been compared to TDF+FTC in several randomized studies of therapy-naïve patients (ASSERT, ACTG 5202, HEAT), as well as in treatment-experienced patients (BICOMBO, STEAL), see also the following Table.

As shown there, data is not consistent. ABC+3TC were equivalent to TDF+FTC in HEAT and STEAL. In contrast, ACTG 5202, ASSERT and BICOMBO showed some differences to the disadvantage of ABC+3TC. Efficacy of TDF+FTC seems to be better in highly viremic patients (Sax 2011) although a recent analysis has suggested that this was not due to a lower antiviral potency of ABC+3TC. Severe side effects are slightly more frequent under ABC+3TC. However, in studies like BICOMBO and ACTG 5202, HLA testing was not performed, which significantly reduces abacavir HSR and which is now routine testing. It must be stressed that overall, results of TDF+FTC and ABC+3TC do not vary greatly despite the very different settings. This applies also to the risk of lipoatrophy. At least two studies did not see significant differences between these two backbones (Curran 2011, McComsey 2011). In some randomized studies, lipid changes improved after switch from ABC+3TC to TDF+FTC (Behrens 2012, Campo 2013, Moyle 2015). In contrast, adverse events affecting bone density were more frequently seen with TDF+FTC (Haskelberg 2012, Rasmussen 2012, Negredo 2015).

Table 2.3: Randomized studies TDF+FTC (Truvada®, TVD) vs ABC+3TC (Kivexa®, KVX)

Study	Evaluating, 3rd agent	Major results
ART naïve patients		
HEAT (Smith 2009)	Double-blind (n=688) plus LPV/r	Non-inferiority of KVX shown, AE rates similar in both arms
ACTG 5202 (Sax 2011)	Double-blind (n=1858) plus EFV or ATV/r	TVD better with high VL, more AEs on KVX
Assert (Stellbrink 20010)	Open label (n=385) plus EFV	TVD virologically better. On KVX overall more AEs, but less AEs of bone and kidney
Pretreated patients		
STEAL (Martin 2009)	Open label (n=357) VL <50	Same efficacy, but more AEs on KVX (i.e., cardio-vascular, but less reduction of bone density)
BICOMBO (Martinez 2009)	Open label (n=333) VL <200 >6 months	Non-inferiority of KVX not shown, more AEs on KVX

VL=viral load in number of copies/ml, AE=Adverse Events

AZT+3TC

In the past, international guidelines recommended AZT+3TC as the standard back-bone for first-line therapy. There is more experience with this combination than with any other. The resistance profile is favorable: the M184V mutation that frequently develops during 3TC treatment increases sensitivity to AZT. AZT+3TC are usually given as Combivir®. Although the licensing study for Combivir® showed no difference in toxicity (Eron 2000), in our experience the 300 mg AZT dose in Combivir® is too high for some patients and can lead to anemia. In such cases, it is worth trying AZT+3TC as individual components, so that the dose of AZT can be reduced to 250 mg BID.

AZT+3TC has comparable efficacy to d4T+3TC or to AZT+FTC (Benson 2004). The ACTG 384 Study showed superiority of AZT+3TC over d4T+ddI (Robbins 2003, Shafer 2003). However, this notion did change over time: while early results suggested a lower rate of lipoatrophy (Molina 1999), the development of lipoatrophy with AZT+3TC occurred only slightly later than with d4T+ddI. AZT+3TC was shown to be less effective and less well-tolerated than TDF+FTC in the GS-934 study (Gallant 2006, Pozniak 2006). Another large ACTG study also showed that it was less well-

tolerated (Campbell 2011). Compared to ABC+3TC, immune reconstitution may be less impressive (DeJesus 2004). Facing these potential disadvantages and the fact that once daily dosing is not possible, most guidelines no longer recommend AZT+3TC as a preferred backbone in treatment-naïve patients. Since 2013, there are several generics available.

ddI+3TC (FTC)

In some treatment guidelines, this combination is listed as an alternative. Of note, data is limited. Some studies suggest a comparable efficacy (and better tolerability) versus AZT+3TC (Berenguer 2008). However, keeping in mind the long-term toxicity of ddI, we would only recommend ddI+3TC when there are significant reasons to not use TDF+FTC or ABC+3TC.

Poor and not-recommended backbones

It should be noted that the majority of the clinical trials cited above were conducted in treatment-naïve patients. In pretreated patients, other backbones may be necessary due to resistance or lack of tolerability. But the following backbones should be avoided whenever possible:

Guidelines explicitly recommend avoiding the previously very popular combination of **d4T+ddI** and of **d4T+3TC**. Mitochondrial toxicity is high, the use of d4T can no longer be justified.

Increased gastrointestinal side effects and the necessity of taking ddI on an empty stomach (AZT is better tolerated taken with a meal) speak against the combination **AZT+ddI**. Due to their divergent resistance pathways **AZT+TDF** is not recommended for primary therapy and should be restricted to treatment-experienced patients only. The combination **TDF+ddI** is relatively toxic and over the years many studies have shown less virologic and immunologic efficacy (see section on Inappropriate Initial Therapies). **TDF+ABC** are problematic due to rapid development of resistance. **AZT+d4T** and **FTC+3TC** are antagonistic (competitive, as noted above) and should not be employed.

Alternating backbones with regular changes from one backbone to another can not currently be recommended, although initial studies indicate that this strategy is at least not harmful (Molina 1999, Martinez-Picado 2003).

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Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Mechanism of action and efficacy

NNRTIs were first described in 1990. As with the nucleoside analogs, the target enzyme is reverse transcriptase. However, NNRTIs bind directly and non-competitively to the enzyme at a position near to but distinct from the substrate binding site for nucleosides. The resulting complex blocks the catalyst-activated binding site of the reverse transcriptase. This in turn can bind fewer nucleosides, slowing down polymerization significantly. In contrast to NRTIs, NNRTIs do not require activation within the cell.

Three first-generation NNRTIs – nevirapine, delavirdine and efavirenz – were introduced between 1996 and 1998. Although studies such as ACTG 241 or INCAS had already clearly demonstrated the superiority of triple therapy compared to double nukes (D'Aquila 1996, Raboud 1999, Conway 2000), the acceptance and use of NNRTIs was rather slow and did not receive the media attention given to the PIs.

This was due to the early observation that functional monotherapy with NNRTIs, i.e., the addition of an NNRTI to a failing NRTI regimen, showed practically no effect. There were also initial difficulties in dealing with the development of resistance: the risk of resistance is not only very high, but it can develop very rapidly. Once it occurs, it almost always indicates cross-resistance to other NNRTIs (Cozzi-Lepri 2012). Resistance has even been described in mothers who took a single dose of nevirapine as transmission prophylaxis. In large studies, the frequency of NNRTI mutations following a single perinatal nevirapine dose was between 14% and a worrying 65% (Cunningham 2002, Jourdain 2004, Johnson 2005), which can impair the success of later NNRTI therapies (Lockman 2010, Boltz 2012).

NNRTI resistance appears fast, possibly due to their long half-life (Muro 2005). This is why NNRTIs should always be stopped some days before the other drugs if a break in therapy is planned (see chapter on *Treatment Interruption*). If resistance develops, the drug should be stopped in order to avoid additional mutations which may compromise second generation or future NNRTIs. Moreover, the replication capacity of HIV is not reduced as much by NNRTI mutations as by some PI or NRTI mutations (Piketty 2004). In Europe, the prevalence of NNRTI resistance mutations in untreated patients is currently 2-3% (Vercauteren 2009). This is why a resistence test should be done before initiation of NNRTIs.

Despite the problems with resistance, both randomized and large cohort studies have demonstrated that NNRTIs are extremely effective when combined with nucleoside analogs. The immunologic and virologic potency of NNRTIs in treatment-naïve patients is at least equivalent to that of PIs (Torre 2001, Robbins 2003, MacArthur 2006, Riddler 2008, Daar 2011, DeJesus 2011, Soriano 2011). However, the efficacy of NNRTIs in treatment-experienced patients is probably weaker in comparison to PIs (Yazdanpanah 2004).

The simple dosing and overall tolerability have enabled nevirapine, efavirenz and rilpivirine to become important components of ART regimens, which are often ranked higher than those containing PIs. Over the last few years, many randomized studies have demonstrated that it is possible to switch from a PI to an NNRTI if good virological suppression has already been achieved. The efficacy was sometimes even better on NNRTIs than on the continued PI regimen (see chapter *When to Switch*).

All NNRTIs are metabolized by the cytochrome p450 system. Nevirapine is an inducer, whereas efavirenz is an inducer and an inhibitor of p450. In the combination of efavirenz plus lopinavir the effects are so strong that dose adjustment is necessary. So far, no study has provided definitive and convincing evidence that one NNRTI is more potent than another. Whereas delavirdine no longer has any significant role

(see below) and etravirine merely serves as a salvage drug, nevirapine and efavirenz have a similar standing in many countries (Mbuagbaw 2010). In the 2NN Study (The Double Non-Nucleoside Study), both agents were compared in a large-scale randomized study (Van Leth 2004). A total of 1,216 patients received a nuke backbone of d4T+3TC with either nevirapine 1 x 400 mg, nevirapine 2 x 200 mg, efavirenz 1 x 600 mg or efavirenz 1 x 800 mg plus nevirapine 1 x 400 mg. The only significant virological difference was an advantage of the efavirenz arm over the double NNRTI arm, mainly due to higher toxicity in the latter. In the nevirapine arm with 1 x 400 mg, severe hepatic side effects occurred more frequently than in the efavirenz arm; on the other hand, lipids were more favorably influenced in the nevirapine group. Sub-analyses of 2NN have shown that the hepatic toxicity associated with once-daily doses of nevirapine was observed in a single center in Thailand (Storfer 2005). In another randomized trial no increased risk for hepatotoxicity was observed in patients on once-daily nevirapine (Podzamczer 2008). In a subanalysis of the FIRST trial there were no differences with regard to efficacy between nevirapine and efavirenz (van den Berg 2008). In a small study more patients in ultrasensitive assays were below the detection level of 1 copy/ml with nevapirine than with efavirenz (Haïm-Boukobza 2011). In contrast, virological efficacy was lower with nevirapine in patients with TB (Bonnet 2013).

Since 2008, etravirine, a second-generation NNRTI can be an option for patients with NNRTI resistance mutations from nevirapine or efavirenz. Another second-generation NNRTI, rilpivirine, was approved in 2011. In large studies comparable efficacy of rilpivirine and efavirenz was shown, however, limited to patients with a baseline viremia of less than 100,000 HIV RNA copies/ml (see below).

Individual agents: Special features and problems

Delavirdine (DLV, Rescriptor®) was, in April 1997, the second NNRTI to be licensed by the FDA. Delavirdine is not licensed in Europe where, in 1999, an application for licensure was rejected due to insufficient efficacy data. Due to the pill burden and the required three times daily dosing, delavirdine is currently rarely prescribed.

Efavirenz (EFV, Sustiva®, Stocrin®, also in **Atripla®)** was the third NNRTI to be approved, and the first for which it could be shown that NNRTIs were at least as effective and maybe better than PIs in untreated or only slightly treatment-experienced patients. In particular, the 006 Study showed superiority of efavirenz over indinavir (Staszewski 1999). Since then efavirenz has often been compared to other drugs. In AI424-034 and ACTG 5202 it was at least as effective as atazanavir and atazanavir/r respectively (Squires 2004, Daar 2011). In ACTG 5142, efavirenz appeared to be superior to lopinavir/r although resistance mutations were more frequently observed in the efavirenz arm (Riddler 2008). Newer studies comparing efavirenz to rilpivirine or integrase inhibitors (see there) have shown inferiority to dolutegravir, mainly due to tolerability.

In many guidelines, efavirenz is still among the preferred drugs for treatment-naïve patients. However, there are some problems with its use, mainly CNS side effects. It is recommended to be taken in the evening before going to sleep. Patients should be warned about dizziness and numbness, vivid dreams or even nightmares. Potentially hazardous tasks such as driving or operating machinery are inadvisable. The side effects probably correlate with high plasma levels (Marzolini 2001). Black patients seem to have a genetic predisposition to the CNS effects (Haas 2004, Wyen 2008). The mechanism of CNS toxicity remains unclear. There is some evidence that metabolites may contribute to neurotoxicity (Tovar-y-Romo 2013). Efavirenz disrupts sleep architecture (Gallego 2004). In one study, after four weeks of treatment with efavirenz, 66% of patients complained of dizziness, 48% of abnormal dreams, 37%

of somnolence and 35% of insomnia (Fumaz 2002). Although these symptoms seem to resolve during the course of treatment, they may persist in about one fifth of patients (Lochet 2003). In such cases, efavirenz should be replaced if possible. A large randomized study has recently shown that 400 mg efavirenz have similar efficacy compared to 600 mg and are better tolerated (Encore 2014). However, this approach has not yet been validated in clinical routine.

Lipids are not as favorably affected as with nevirapine (Parienti 2007), etravirine (Fätkenheuer 2012) or rilpivirine (Behrens 2014). Gynecomastia is seen on efavirenz, which is not only a psychological burden, but can be physically painful as well (Rahim 2004). Efavirenz is teratogenic and contraindicated in pregnancy. Although, according to a newer meta-analysis, the teratogenic risk is relatively low (Ford 2011), efavirenz should be avoided in women of child-bearing age.

Etravirine (ETV, Intelence®) is a diarylpyrimidine (DAPY) analog developed by Janssen-Cilag. This second-generation NNRTI was approved in 2008 for antiretroviral treatment-experienced adult patients. Etravirine works well against wild-type viruses, as well as resistant mutants, among them the classical NNRTI mutations such as K103N (Andries 2004). The genetic resistance barrier is higher than that of other NNRTIs. This appears to be because by changing its confirmation etravirine can bind very flexibly to the HIV-1 reverse transcriptase (Vingerhoets 2005). Mutations at the enzyme binding site therefore hardly affect the binding and therefore the potency of this NNRTI (Das 2004). The reduction of etravirine activity by resistance mutations appears to occur slower in patients on a nevirapine-failing regimen compared to efavirenz (Cozzi-Lepri 2012).

In Phase I/II studies, etravirine lowered viral load by an average of 1.99 logs in treatment-naïve patients after only one week (Gruzdev 2003) and by 0.89 logs in the presence of NNRTI mutations (Gazzard 2003). In C233, a large Phase II trial on 199 patients with NNRTI and PI mutations, who had previously been intensively treated, the viral load was significantly lower than the placebo arm after 48 weeks (TMC125 Writing Group 2007). Another Phase II study (C227) brought a setback when etravirine was compared with an investigator-selected PI in NNRTI-resistant, PI-naïve patients. In an unplanned interim analysis, patients receiving etravirine demonstrated suboptimal virological responses relative to the control PI and trial enrolment was stopped prematurely (Ruxrungtham 2008). The sponsor argued that in this study baseline resistance was higher than expected. The formulation of etravirine used then also showed poor bioavailability, which has since been improved (Kakuda 2008). Up to now there is no evidence of a correlation between pharmacokinetic data and virological success (Kakuda 2010).

Two phase III studies, DUET-1 and -2, led to the approval of etravirine. In these, 1,203 patients on a failing ART regimen with resistance to currently available NNRTIs and at least three primary PI mutations were randomly assigned to receive either etravirine or placebo, each given twice daily with darunavir/r, investigator-selected NRTIs, and optional T-20 (Lazzarin 2007, Madruga 2007). After 96 weeks 57% of patients on etravirine achieved a viral load of less than 50 copies/ml compared to 36% on the placebo arm (Katlama 2010). However, the overall effect of etravirine decreased with an increasing number of NNRTI resistance mutations. As with all ARVs, etravirine needs active partner agents to develop full efficacy (Tambuyzer 2010, Trottier 2010).

In most cases, etravirine is well-tolerated (Cohen 2009). In the DUET trials, tolerability was comparable to placebo. Only the typical NNRTI rash was observed more frequently (19% versus 11%) although rash was mostly mild (Katlama 2009). In October 2009, FDA issued a warning on a limited number of cases of severe allergies

(toxic epidermal necrolysis, Lyell's syndrome, DRESS syndrome). A switch from efavirenz to etravirine can help reduce CNS side effects and improve lipid profiles (Gazzard 2011, Waters 2011, Faetkenheuer 2012). However, patients who are tolerating efavirenz will see no advantage in the switch (Ngyen 2011).

There does not appear to be any relevant interaction with methadone or with anti-retroviral agents, with one exception: the level of etravirine is lowered significantly when combined with tipranavir (Kakuda 2006). Etravirine, at a dose of 400 mg (2 x 200 mg tablets BID), should be taken with a meal as this increases absorption. Tablets can be dissolved in water. Once-daily dosing seems to be possible but has not been approved (Fätkenheuer 2012).

In conclusion, etravirine is an important and well-tolerated option for patients with NNRTI resistance. Current data suggest that etravirine should always be combined with a boosted PI, preferably darunavir/r.

Nevirapine (NVP, Viramune®) was the first licensed NNRTI in 1997. The combination of nevirapine with AZT+ddI is probably the oldest triple combination of all (D'Aquila 1996). In early randomized studies nevirapine performed comparably to indinavir (van Leeuwen 2003) and better than nelfinavir (Podzamczer 2002). Studies such as ARTEN or NEWART showed that the virological efficacy was comparable to boosted atazanavir (DeJesus 2011, Soriano 2011). However, some studies reported on a higher risk for virological failure with nevirapine compared to lopinavir. This was mainly observed in the setting of concurrent TB therapy or in women who had received single-dose nevirapine transmission prophylaxis (Boltz 2011, Swaminathan 2011, Clumeck 2012).

Nevirapine is usually well-tolerated, even in the long-term. It has a favorable impact on lipid changes compared to other drugs (Van der Valk 2001, Van Leth 2004). In the ARTEN trial, the lipid profile was even better than with atazanavir/r (Podzamczer 2011). In one small randomized trial, lipid profiles improved when efavirenz was replaced by nevirapine (Parienti 2007). Whether these positive effects will have clinical relevance and really help prevent cardiovascular events remains to be seen. Nevirapine causes elevation of liver enzymes in up to 20% of people, which may occasionally be severe. Lead-in dosing is always required. During the first eight weeks on nevirapine, biweekly monitoring of transaminases is recommended. A rash develops in 15-20% and leads to discontinuation in up to 7% (Miller 1997). Prophylactic administration of antihistamines or steroids does not prevent the rash (GESIDA 2004, Launay 2004). In the case of an isolated rash or isolated elevation of transaminases (up to five times the upper limit of normal), treatment can usually be continued but use caution if both occur simultaneously. It is recommended to stop treatment if a rash occurs together with even a slight elevation of transaminases (>2-fold ULN). It is important to note that hepatic toxicity may occur even after several months (Sulkowski 2002). Patients with chronic hepatitis are at higher risk, as are women with low body weight (Sulkowski 2000, Sanne 2005, Kappelhoff 2005).

An increased risk has also been reported for patients with good immune status. Women with CD4 T cells above 250/µl have a 12-fold elevated risk (11% versus 0.9%) of hepatic toxicity. In men there is an increased risk above 400 cells/µl (6.3% versus 1.2%). Although other studies failed to reveal an association between toxicity and immune status (Manfredi 2006, Wolf 2006, Chu 2010), it is recommended not to use nevirapine in treatment-naïve patients with higher CD4 T cell counts. In contrast, in ART-experienced patients switching to nevirapine, the risk is not elevated (Mocroft 2007, De Lazzari 2008, Wit 2008). Since 2010, the package information indicates that a switch to nevapirine is possible at a viral load of 50 copies/ml, regardless of CD4 T cell count.

There is some evidence for an association between hypersensitivity and specific alleles at the HLA-DRB1 (Martin 2005) and polymorphisms in the p-glycoprotein drug transporter MDR1 gene (Haas 2006, Ritchie 2006). However, there is currently no test available to predict hypersensitivity (Yuan 2011). Gamma-glutamyl transpeptidase (GGT) elevations are very common, which may subject patients to false appearances of excess alcohol consumption.

After several studies in both treatment-naïve and –experienced patients (Gathe 2011, Arasteh 2012), a nevapirine extended-release (NVP XR) formulation was approved in 2011, allowing once-daily dosing. The patients should know that the XR tablets are formulated in a non-digestible cellulose-based matrix, which may be seen in the feces. These softened tablet remnants may sometimes resemble whole tablets, but are inactive ingredients. Thus, there is no need to worry when patients observe tablets in their stool. The old 200 mg tablets are avaible as generics.

Rilpivirine (RPV, Edurant®, also in **Complera®** or **Eviplera®)** was approved in 2011. Like etravirine, it is also a DAPY NNRTI (Janssen 2005). It has a very long half-life of 40 hours. A Phase IIa study on therapy-naïve patients receiving monotherapy for 7 days decreased viral load by 1.2 logs but no dose-dependent effect between 25 and 150 mg was seen (Goebel 2005). The lowest dosage of 25 mg which is far lower than that of other NNRTIs was used in the development program.

In treatment naïve patients, rilpivirine has been tested in three large trials against efavirenz. In two Phase III trials (ECHO and THRIVE) on 1,368 patients a comparable effect with better tolerability was observed at 48-96 weeks (Cohen 2011, Molina 2011, Behrens 2014). Rilpivirine was associated with lower increases in lipid parameters and fewer dyslipidemia than efavirenz. Body fat distribution changes were similar. In the third large trial (STaR), the two single-tablet regimens Complera® (rilpivirine plus TDF+FTC) and Atripla® (efavirenz plus TDF+FTC) were studied. This randomized, open-label on 786 patients demonstrated non-inferior efficacy and improved tolerability compared to efavirenz. After 48 weeks, 89% versus 82% of the patients had reached an undetectable viral load, respectively. There were fewer discontinuations because of adverse events.

However, resistance as well as virological failure were observed more frequently with rilpivirine. In ECHO and THRIVE the rates were 9% vs 5%, in STaR 4% vs. 1%, respectively (Cohen 2012+2014). Resistance mutations were mainly seen in the NNRTI loci (mainly E138K or K101E) but also in the NNRTI region (Rimsky 2012). Compared to efavirenz, the risk of resistance-associated virologic failure was significantly elevated in highly viremic patients. Thus, the approval of rilpivirine is restricted to patients with a baseline viral load of less than 100,000 copies/ml.

Overall, rilpivirine is well tolerated. CNS side effects may occur but are less intensive than seen with efavirenz. The QT prolongation observed earlier (at a higher dose), seems to occur less frequently at 25 mg (Vanveggel 2009) and the teratogenic risk is small (Desmidt 2009). A parenteral nano-suspension is being investigated, in which ripilvirine levels are achieved via monthly injections, corresponding to a daily dose of 25 mg (Verloes 2008). Rilpivirine currently plays an important role in long-acting strategies.

In 2013, rilpivirine was also approved for treatment-experienced patients. In the SPIRIT trial, 476 patients with viral suppression have been randomized to remain on their PI-based regimen or to switch to rilpivirine. The switch was safe and improved lipid changes seen with PIs (Palella 2012).

In conclusion, rilpivirine has become an important option in antiretroviral therapy. A certain disadvantage in everyday practice is the requirement that the substance must be taken with food (a fatty meal of at least 500 kcal is necessary) to guarantee

sufficient resorption (Crauwels 2013). This can be a problem if patients have irregular daily habits.

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Protease Inhibitors (PIs)

Mechanism of action and efficacy

The HIV protease cuts the viral gag-pol polyprotein into functional subunits. If the protease is inhibited and proteolytic splicing prevented, non-infectious virus particles will result. With the knowledge of the molecular structure of the protease encoded by the virus, the first protease inhibitors were designed in the early nineties; these agents were modified in such a way that they fit exactly into the active site of the HIV protease (Youle 2007).

Since 1995, protease inhibitors have revolutionized the treatment of HIV infection. At least three large studies with clinical endpoints demonstrated the efficacy of indinavir, ritonavir and saquinavir (Hammer 1997, Cameron 1998, Stellbrink 2000). Although PIs were at first criticized for their high pill burden and side effects (see below), they remain an essential component of antiretroviral therapies. With growing knowledge of the mitochondrial toxicity of nucleoside analogs and through the

introduction of easier-to-take PIs, this class of drugs is currently experiencing a renaissance – today, even PI-only regimens are being investigated.

Boosted PI combinations are more effective than unboosted. There three widely used boosted PIs: atazanavir, darunavir and lopinavir. Current data suggest that the differences are not significant enough to completely rule out any of these agents. Besides gastrointestinal side effects and relatively high pill burden (no STRs available), all PIs used in long-term therapy show tolerability problems – to a greater or lesser extent, all are associated with lipodystrophy and dyslipidemia (Nolan 2003). Other problems include drug interactions, which can sometimes be substantial. Cardiac arrhythmias (Anson 2005) and sexual dysfunction have also been attributed to PIs (Schrooten 2001), although the data does not remain unchallenged (Lallemand 2002).

All PIs are inhibitors of the CYP3A4 system and interact with many other drugs (see chapter on *Drug Interactions*). Ritonavir is the strongest inhibitor, saquinavir probably the weakest. There is a high degree of cross-resistance between protease inhibitors, which was described even before PIs were put on the market (Condra 1995). With darunavir and tipranavir two second-generation PIs are available that are effective even in the presence of several resistance mutations (see below). All PIs must be boostered by the so called pharmacoenhancers, in order to achieve sufficient plasma levels.

Why boost PIs?

Ritonavir is a potent inhibitor of the isoenzyme 3A4, a subunit of the cytochrome P450 hepatic enzyme system. Inhibition of these gastrointestinal and hepatic enzymes allows the most important pharmacokinetic parameters of almost all PIs to be significantly increased or "boosted" (Kempf 1997): maximum concentration (Cmax), trough levels (Ctrough or Cmin) and half-life. The interaction between ritonavir and the other PIs simplifies daily regimens by reducing the frequency and number of pills to be taken every day, in many cases independent of food intake. Most PIs can now be used in once-daily regimens. There are two pharmacoenhancers available. Ritonavir (Norvir®) can be combined with all PIs. In 2014, cobicistat (Tybost®) was approved as a booster for atazanavir and darunavir.

Initially, cobicistat was developed for the integrase inhibitor elvitegravir in the fixed-dose combination Stribild® that came to market in 2013. PK studies, however, had shown that with cobicistat comparable levels of atazanavir and darunavir can be achieved (Elion 2011, Kakuda 2014). In a double-blind, randomized study on 692 ART-naive patients treated with TDF+FTC+atazanavir, efficacy and tolerability of cobicistat and ritonavir were comparable (Gallant 2013). Based on these data, cobicistat is now available as pharmacoenhancer for atazanavir and darunavir. More recently, the FDA and EMA have granted marketing approval to two fixed-dose combinations. Evotaz® is a combination of atazanavir and cobicistat, Prezcobix® or Rezolsta® contains cobicistat and darunavir. Cobicistat seems to be well-tolerated, although a slight increase of creatinine was noted. This may only be explained by a lessened tubular creatinine secretion and may not indicate an impairment of renal function (German 2013).

Boosting with ritonavir or cobicistat is usually indicated by addition of an "/r" or a "/c" after the drug name (see Table 2.4). Resistance is only rarely observed on boosted PIs, at least in ART–naïve patients, as the genetic barrier is high. This has been shown not only for lopinavir/r (Hammer 2006), but also for fosamprenavir/r (Eron 2006), atazanavir/r (Mallan 2008), saquinavir/r (Ananworanich 2006) and darunavir/r (Ortiz 2008). Many experts therefore recommend that in highly viremic patients, preferably PI/r-based regimens should be used.

Table 2.4: Current doses of protease inhibitors with ritonavir boosting

	Dose (mg)	Pills*/day	Comments
Atazanavir/r	1 x 300/100	1 x 2	No limitation
Atazanavir/c	1 x 300/150	1 x 1	No worldwide marketing
Darunavir/r	2 x 600/100	2 x 2	No limitation
Darunavir/c	1 x 800/150	1 x 1	No worldwide marketing
Darunavir/r	1 x 800/100	1 x 3	Only in patients with limited PI resistance
Fosamprenavir/r	2 x 700/100	2 x 2	Should be used instead of amprenavir
Fosamprenavir/r	1 x 1400/200	1 x 4	Approval only in US (PI-naïve patients)
Indinavir/r	2 x 800/100	2 x 3	No longer used (nephrolithiasis)
Lopinavir/r	2 x 400/100	2 x 2	The only fixed booster combination
Lopinavir/r	1 x 800/200	1 x 4	Only in patients with limited PI resistance
Saquinavir/r	2 x 1000/100	2 x 3	Officially approved for boosting
Tipranavir/r	2 x 500/200	2 x 4	Only approved in treatment-experienced pts

* Number of pills including the ritonavir dose

Boosting can be effective against resistant viral strains due to the elevated drug plasma levels (Condra 2000). However, at least one large randomized study evaluating TDM-guided dose escalation of boosted PIs in almost 200 patients with extensive resistance mutations failed to show a significant benefit with this strategy (Albrecht 2011). Boosting is also associated with risks. There is a high degree of variability in plasma levels among individuals. As well as trough levels, peak levels are also elevated, which may lead to more side effects. If in doubt (reduced efficacy, more side effects), plasma levels should be measured in cases of boosting, especially in patients with severe hepatic disease, because the extent of interaction cannot be predetermined for individual cases. Dose adjustment is often necessary.

Individual agents: Special features and problems

Amprenavir (APV, Agenerase®) was the fifth PI to enter the market in 2000. It was replaced by fosamprenavir in 2004 (Telzir® or Lexiva®, see below) and subsequently withdrawn from market.

Atazanavir (ATV, Reyataz®, also in Evotaz®) was licensed in 2004 as the first PI on the market for once daily administration. In treatment-naïve patients, atazanavir was compared to many other agents. Both boosted and unboosted atazanavir proved as effective as efavirenz (Squires 2004, Daar 2011) or nevirapine (Soriano 2011). The CASTLE study proved that virological efficacy of atazanavir/r was at least as good or even better with more favorable lipid profiles and better gastrointestinal tolerability than lopinavir/r (Molina 2008+2010). In the three-arm study ACTG 5257, however, atazanavir was inferior to raltegravir and darunavir in a tolerability endpoint. Atazanavir was also inferior to darunavir in the combined efficacy/safety endpoint (Lennox 2014).

Although several studies have shown no difference between boosted and unboosted atazanavir (Malan 2008, Squires 2012), boosting with ritonavir or cobicistat is recommended (Focà 2013). Unboosted atazanavir is slightly less effective than lopinavir in treatment-experienced patients (Cohen 2005). When boosted, it is comparable to lopinavir, at least when PI resistance is limited (Johnson 2006).

In comparison to lopinavir, atazanavir does not have such negative effects on lipid levels. However, it is not yet clear whether this is clinically relevant. Contrasting with earlier reports, recent data suggest that boosting atazanavir with ritonavir seems to have some negative effects on lipid levels (Review: Carey 2010). Moreover, current data suggest that there are no differences compared to darunavir/r (Aberg 2012,

Lennox 2014) and that lipid levels are even worse compared to nevirapine (Podzamczer 2011). There is at least one randomized study showing no beneficial effect on body changes after a switch from other boosted PIs to atazanavir/r (Moyle 2012). In a randomized trial on treatment-naïve patients, compared to the efavirenz group, subjects assigned to atazanavir/r had even a trend towards higher mean percentage increase in visceral fat (McComsey 2011).

More than half of patients on atazanavir experience elevated bilirubin levels, which can reach grade 3–4 in approximately one third of all cases (Squires 2004, Soriano 2008). Some patients even develop jaundice. In ACTG 5237, 8% of the patients discontinued atazanavir due to this adverse event (Lennox 2014). The mechanism resembles that of Gilbert's Syndrome; there is reduced conjugation in the liver. A genetic predisposition has been identified (Rotger 2005). Although the hyperbilirubinemia is understood to be harmless and only few cases of serious hepatic disorders have been published to date (Eholie 2004), liver function should be monitored. Treatment should be discontinued in cases of significantly elevated bilirubin (>5–6 times the upper limit of normal). Unfavorable interactions occur in combination with proton pump inhibitors (see chapter on *Drug Interactions*). Boosting is recommended, particularly for combinations including NNRTIs, tenofovir or raltegravir, which significantly lower atazanavir levels (Le Tiec 2005).

The primary resistance mutation for this drug is I50L, which does not impair sensitivity to other PIs (Colonno 2003). On the other hand, there are a number of cross-resistant mutations and susceptibility to many viral isolates with moderate PI resistance is reduced (Schnell 2003).

Darunavir (DRV, Prezista®, also in **Prezicom®** or **Rezolsta®)** is a nonpeptidic PI, developed by Janssen-Cilag. Due to its impressive potency in the presence of PI-resistant mutants (Koh 2003), darunavir was initially an important drug for therapy-experienced patients with limited options. In 2008 the license was extended to ART naïve patients. Two Phase II studies, POWER-I (US) and -2 (Europe) sped up the licensing in 2006/2007. Both trials included nearly 600 patients with extensive pretreatment (three classes and an average of 11 drugs) and high resistance (Clotet 2007). Despite considerable resistance at baseline, 46% in the 600/100 mg BID group achieved a viral load of less than 50 copies/ml at 48 weeks. This rate was significantly higher compared to the control PI (10%) and a success that had thus far not been seen in this patient group with such limited options. Encouraging results in salvage treatment were also reported from the DUET trials, in which darunavir was combined with etravirine (see above).

In patients with moderate pre-treatment, darunavir/r was superior to lopinavir/r. In the TITAN study with 595 (lopinavir-naïve) patients, mainly pretreated with PIs, at 48 weeks 71% showed a viral load of below 50 copies/ml compared to 60% on lopinavir (Madruga 2007). Superiority was observed in all patient groups. Virologic failure and resistance against associated agents were significantly less on darunavir. Of note, efficacy was not compromised by the occurrence of PI resistant mutations (De Meyer 2008+2009).

In 2008, the license was extended to treatment-naïve patients. The ARTEMIS trial demonstrated comparable efficacy of once-daily darunavir/r compared to lopinavir/r (Ortiz 2008, Mills 2009). Once-daily darunavir/r also showed potential in treatment-experienced patients with no darunavir resistance mutations (De Meyer 2008, Cahn 2011, Lathouwers 2013). More recently, darunavir was tested against integrase inhibitors. Although its high genetic barrier was confirmed in randomized trials such as FLAMINGO or ACTG 5237 (not a single resistance mutation detected), darunavir performed slightly worse than dolutegravir or raltegravir. This was mainly due to a lower tolerability which was driven by its gastrointestinal side effects (Clotet 2014,

Lennox 2014). However, these are moderate and less severe than with other PIs (Clotet 2007, Madruga 2007). Rash, occuring in up to 5%, is usually mild. Relevant interactions occur with lopinavir causing a decrease of plasma levels of darunavir. This combination must be avoided. The same applies for sildenafil and estrogen.

The potency of darunavir is, of course, not unlimited. 11 mutations associated with resistance were identified in the POWER studies. These mutations are usually located at codons 32, 47, 50 and 87 (De Meyer 2006). With accumulation of at least three mutations, susceptibility is reduced (Pozniak 2008). The in vitro susceptibility patterns of darunavir and fosamprenavir are very similar. However, predicted incidence of clinically meaningful cross-resistance is low, due to differences in clinical cut-offs, which are higher for darunavir (Parkin 2008). Thus, pretreatment with amprenavir or fosamprenavir does not appear to compromise efficacy. In view of the high resistance barrier, there are several trials currently testing darunavir as monotherapy (see below). In 2014, a single pill formulation that contains darunavir plus the pharmacoenhancer cobicistat was approved (US: Prezcobix®, EU: Rezolsta®). Other fixed-dose combination pills of darunavir/c (plus TAF+FTC or 3TC) are in progress.

Fosamprenavir (Telzir®, USA: Lexiva®) has better solubility and absorption than its original version, amprenavir. It was licensed in 2004. The recommended doses are either unboosted 1400 mg BID (not licensed in Europe!) or boosted with ritonavir as 700/100 mg BID or 1400/200 mg QD. Once-daily dosing is not recommended for treatment-experienced patients. A recent trial suggested that for once-daily dosing, 100 mg ritonavir is sufficient (Hicks 2009).

In treatment-naïve patients, fosamprenavir/r QD was as effective as atazanavir/r in the relatively small ALERT study (Smith 2006). No resistance was found with fosamprenavir/r even after 48 weeks (MacManus 2004). In the KLEAN study (Eron 2006), fosamprenavir/r twice daily in treatment-naïve patients provides similar antiviral efficacy as lopinavir/r, each in combination with ABC+3TC. Severe diarrhea and cholesterol elevations occurred at the same frequency. In treatment-experienced patients in the CONTEXT study, fosamprenavir was not quite as effective as lopinavir/r although the difference was not significant (Elston 2004).

Fosamprenavir currently does not play an important role in HIV medicine. There is no convincing argument for its use. One advantage of the drug is that there are no restrictions with respect to food intake. It is important to note that efavirenz can significantly lower plasma levels, as can nevirapine, although this does not occur when fosamprenavir is boosted (Elston 2004).

Indinavir (IDV, Crixivan®) was one of the first PIs, initially very successful in large studies (Gulick 1997, Hammer 1997). Its main problem is tolerability. Firstly, it causes nephrolithiasis in 5–25% (Meraviglia 2002) and thus requires good hydration (at least 1.5 liters daily). Unboosted indinavir must be taken three times daily on an empty stomach (Haas 2000). When boosted at 2 x 800/100 mg, tolerability is poor. Side effects resemble those of retinoid therapy: alopecia, dry skin and lips, and ingrown nails. Many patients also develop asymptomatic hyperbilirubinemia. Although it seems that the dose and toxicity can be reduced by TDM (Wasmuth 2007), indinavir does no longer play a role.

Lopinavir/r (LPV, Kaletra®) was licensed in April 2001 and is so far the only PI with a fixed boosting dose of ritonavir. This increases concentrations of lopinavir by more than 100-fold (Sham 1998). In 2006, the old Kaletra® capsules were replaced by tablets, allowing a pill reduction (Gathe 2008). Lopinavir is still the most frequently prescribed PI worldwide and has also been licensed as once-daily since 2009 after several studies showed efficacy and tolerability (Molina 2007, Gathe 2009, Gonzalez-Garcia 2010). However, other studies found a slightly reduced potency of QD dosing

(Ortiz 2008, Flexner 2010). Lopinavir QD is therefore only recommended if the number of PI resistance mutations is low.

In *treatment-naïve* patients, lopinavir/r was significantly superior to an unboosted regimen with nelfinavir (Walmsley 2002). It was regarded as the preferred PI for years. However, more recently, large randomized trials such as KLEAN, GEMINI, ARTEMIS and CASTLE have shown that there are no significant differences compared to boosted PIs such as fosamprenavir/r (Eron 2006), saquinavir/r (Walmsley 2009), or atazanavir/r (Molina 2008). In ACTG 5142, lopinavir/r was inferior to efavirenz (Riddler 2008), possibly due to lower tolerability.

In *treatment-experienced* patients, lopinavir/r showed slightly better results than boosted saquinavir (the old Fortovase® formulation) in an open-label randomized trial (MaxCmin2) on a heterogeneous population of treatment-experienced patients. This was particularly true for tolerability, but also with respect to treatment failure (Dragstedt 2005). On the other hand, in two smaller studies in PI-experienced patients, virologic efficacy of lopinavir/r was not significantly higher than that of boosted atazanavir (Johnson 2006) or fosamprenavir (Elston 2004). In comparison to darunavir, efficacy was even lower (Madruga 2007, De Meyer 2009).

Development of resistance in first-line is rare, but is theoretically possible (Kagan 2003, Conradie 2004, Friend 2004). Lopinavir/r has a high genetic barrier to resistance, and it is likely that at least 6-8 cumulative PI resistance mutations are necessary for treatment failure (Kempf 2002). That is why lopinavir is also considered for monotherapies (see below).

A significant concern with lopinavir are the gastrointestinal side effects (diarrhea, bloating) which are probably more frequent on a once-daily dosage (Johnson 2006). In addition, lipodystrophy and often considerable dyslipidemia, have been observed, probably more marked than with atazanavir (Molina 2008, Mallolas 2009), darunavir (Mills 2009) and saquinavir (Walmsley 2009), but not more so than with fosamprenavir (Eron 2006). A number of interactions should also be considered. The dose must be increased in combination with efavirenz and nevirapine, probably also with concurrent administration of fosamprenavir.

Nelfinavir (NFV, Viracept®) was the fourth PI on the market. Due to high pill burden, diarrhea and a lower antiviral potency, nelfinavir no longer plays much of a role in HIV treatment. In Europe, production has been discontinued.

Ritonavir (RTV, Norvir®) was the first PI for which efficacy was proven on the basis of clinical endpoints (Cameron 1998). However, ritonavir is now obsolete as a single PI, since tolerability is poor. As gastrointestinal complaints and perioral paresthesias can be very disturbing, ritonavir is now only given to boost other PIs. The "baby dose" used for this purpose (100 mg QD) is better-tolerated. Ritonavir inhibits its own metabolism via the cytochrome P450 pathway. The potent enzyme induction results in a high potential for interactions. Many drugs are contraindicated for concomitant administration with ritonavir. Metabolic disorders probably occur more frequently than with other PIs. Caution should be exercised in the presence of impaired liver function. It is no longer necessary to store ritonavir at cool temperatures thanks to the Meltrex formulation that came onto the market in 2010.

Saquinavir (**Invirase 500®**), previously **Invirase®, Fortovase®,** was the first HIV PI to be licensed in December 1995, and is still one of the few agents with efficacy based on clinical endpoints (Stellbrink 2000). Boosting with ritonavir raises the plasma level sufficiently, as does simultaneous food intake, so saquinavir should be taken with meals. The hard gel (Invirase®) and soft gel (Fortovase®) capsules were replaced in 2005 by Invirase 500® tablets, which significantly reduced the number of pills to six a day (including ritonavir boosting). The GEMINI trial compared ritonavir-

boosted Invirase 500® tablets to lopinavir/r in 330 ART-naïve patients who all received TDF+FTC. There were no significant differences with respect to efficacy at 48 weeks (Walmsley 2009). Some adverse effects such as lipid elevations were less pronounced with saquinavir, as was diarrhea. However, discontinuation rates due to adverse events were comparable between arms. During recent years, several warning letters were published, regarding QT prolongation and the need for ECG monitoring with saquinavir. Treatment naïve patients should be started on a reduced dose of 500 mg BID for the first seven days, before increasing to the standard dose of 1000 mg BID (always in conjunction with ritonavir 100 mg BID). In addition to baseline, the ECG should now be performed after approximately 10 days of treatment. Nobody wants this. Thus, it is difficult find any reason for starting saquinavir.

Tipranavir (TPV, Aptivus®) is the first non-peptidic PI licensed in Europe in July 2005 for treatment-experienced patients. As oral bioavailability is only moderate, double the standard ritonavir boosting (McCallister 2004) is necessary, so 2 x 200 mg (BID) has to be used. The plasma levels can also be increased by a high fat meal. Tipranavir shows good efficacy against PI-resistant viruses (Larder 2000). However, efficacy is not limitless – with a combination of the above mutations, sensitivity declines significantly (Baxter 2006).

RESIST-1 (USA) and RESIST-2 (Europe) were two Phase III studies on 1,483 intensively pretreated but viremic patients with at least one primary PI mutation. All patients received either tipranavir/r or a comparison PI/r, each combined with an optimized background therapy. After 48 weeks, virologic and immunologic response to tipranavir was better than with the comparison PI (Hicks 2006).

A significant problem with tipranavir, apart from dyslipidemia (grade 3-4 increase in triglycerides: 22% vs 13% for the comparison PI), is an increase in transaminases which is sometimes substantial (grade 3–4: 7% versus 1%) and requires careful monitoring. In treatment-naïve patients, tipranavir/r was less effective than lopinavir/r, mainly due to more adverse events leading to discontinuation (Cooper 2006). In addition, some unfavorable interactions also occur. Plasma levels of many PIs fall significantly, so that double PI therapy with tipranavir is not recommended. As the levels of AZT, abacavir and etravirine also drop, these combinations are not recommendable either. ddI has to be taken with a two-hour time delay.

Tipranavir remains an important option in extensively treated patients harboring PI-resistant viruses. A study that directly compared tipranavir/r to darunavir/r was halted due to slow accrual. Cross-trial comparisons between these drugs should be discouraged as patient populations in the RESIST (tipranavir/r) studies differed considerably from those of the POWER (darunavir/r) trials.

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Integrase inhibitors

Mode of action

Integrase, along with reverse transcriptase and protease, is one of the three key enzymes in the HIV replication cycle. It is involved in the integration of the viral DNA into the host genome and is essential for the replication of HIV (Nair 2002). It is of note that there is no integrase in human cells so selective inhibition of this enzyme that does not induce side effects seems possible.

There are at least four steps leading to the integration of viral DNA (Review: Lataillade 2006). All these steps may be theoretically inhibited by different integrase inhibitors. Briefly, these steps are:

1. **Binding** of the integrase enzyme to viral DNA within the cytoplasm. This results in a stable viral DNA-integrase binding complex (pre-integration complex, PIC). This step can be inhibited by binding inhibitors such as pyrano-dipyrimides.
2. **3' Processing**. The integrase removes a dinucleotide at each end of the viral DNA producing new 3' hydroxyl ends within the PIC. This step can be inhibited by 3' processing inhibitors such as diketo acids.
3. **Strand transfer**. After the transport of the PIC from the cytoplasm through a nuclear pore into the cell's nucleus, integrase binds to the host chromosomal DNA. By doing this, integrase mediates irreversible binding of viral and cellular DNA. This step can be inhibited by integrase strand transfer inhibitors (INSTIs). All available integrase inhibitors are INSTIs.
4. **Gap repair.** The combination of viral and cellular DNA is a gapped intermediate product. The gap repair is done by host cell DNA repair enzymes. Integrase seems not to be necessary in this last step, which can be inhibited by gap repair inhibitors such as methylxanthines.

For almost a decade, the development of integrase inhibitors was slow. This was largely because of a lack of good lead compounds and reliable *in vitro* screening assays that incorporate each of the integration steps (Lataillade 2006). Only after 2000 did development progress and the principle of strand transfer was elucidated (Hazuda 2000). Since 2005, numerous clinical studies have evaluated integrase inhibitors (mainly strand transfer inhibitors). In December 2007, raltegravir was licensed as the first integrase inhibitor for the treatment of HIV+ patients. Today, three integrase inhibitors are on the market, namely raltegravir, elvitegravir and dolutegravir. Given the good tolerability and the high potency of this drug class, INSTIs now play a major role in HIV medicine.

As with other antiretroviral drug classes, however, some questions remain unanswered. Although very well-tolerated during the first years of therapy, little is known about long-term toxicity of integrase inhibitors. There is no experience with long-term use beyond 5–10 years. Genetic resistance barriers, relatively low with raltegravir and elvitegravir, may also be an important issue. Increased viral rebound rates were observed with treatment-experienced patients on boosted PIs (viral load below the limit of detection) when switching to raltegravir, especially in those with pre-existing resistance (Eron 2009). There is also some evidence for cross-resistance. As soon as integrase inhibitor resistance develops, the agent should be stopped. This way, further resistance mutations (Wirden 2009) can be avoided, as well as unnecessary costs. Problems also exist with the measurement of plasma levels (Cattaneo 2012).

Individual agents

Dolutegravir (**DTG, Tivicay®**, also part of **Triumeq®**) is an integrase inhibitor that initially emerged via Shionogi cooperating with GSK and is now being developed by ViiV Healthcare. As a second-generation integrase inhibitor it shows improvements,

especially with regard to pharmacokinetics (once daily unboosted administration, independent from food intake) and resistance profile. In a Phase IIa study with 35 patients, a reduction of 2.5 logs was observed and 7/10 achieved a viral load below 50 copies/ml during 10 days monotherapy (Min 2011).

ART-naïve patients: after the encouraging results of SPRING-1, a Phase IIb study (Stellbrink 2012), dolutegravir was tested against its competitor drug, the first-in-class INSTI raltegravir. In SPRING-2, 822 patients received two NRTIs and either 50 mg QD or raltegravir 400 mg BID. At 96 weels, 81% versus 76% had achieved an undetectable viral load, respectively. Thus, non-inferiority to raltegravir was shown. In cases with virological failure, less resistance mutations were observed (Raffi 2013). In SINGLE, another double-blinded, randomized Phase III study on 833 previously untreated patients, the fixed-dose combination with dolutegravir and ABC+3TC (available as Triumeq®) was superior to Atripla®. However, a relatively large number of patients had discontinued Atripla® due to CNS toxicity (Walmsley 2013). Dolutegravir also performed very well in the FLAMINGO trial in which it was tested against darunavir (Clotet 2014). No resistance mutations were observed in cases of therapy failure.

Treatment-experienced patients: In SAILING, a randomised, double-blind, non-inferiority study in 715 patients with a detectable viral load and with resistance to two or more classes of antiretroviral drugs, 50 mg dolutegravir QD were well tolerated with greater virological effect compared with 400 mg raltegravir BID. At 24 weeks, 79% versus 70% of the patients had achieved an undetectable viral load. Of note, significantly fewer patients had virological failure with treatment-emergent integrase-inhibitor resistance than on raltegravir (Cahn 2013). Even in the setting of INSTI resistance mutations, dolutegravir retains its efficacy. Preliminary data from the VIKING study showed that a higher dosage (50 mg BID instead of 50 mg OD) may help overcome raltegravir resistance (Eron 2013, Castagna 2014). Tolerance was excellent and better than with efavirenz, showing only a slight increase in creatine levels, which seems not to be significant and is caused by inhibition of a renal transporter system. The resistance barrier is possibly higher than with other integrase inhibitors, probably due to prolonged binding with integrase complexes (Hightower 2011). Cross-resistance with other integrase inhibitors does not seem obligatory (Kobayashi 2011). An important resistance mutation appears at T124A, as well as mutations typical for raltegravir at codon 148. Efficacy seems to decline with Q148V and additional mutations (Canducci 2011, Garrido 2011, Castagna 2014).

There are no interactions with boosted PIs. However, etravirine reduces the levels of dolutegravir significantly (Song 2011). This also applies for antacids and it is recommended not to administer them simultaneously (Patel 2011). When rifampicin is given, a higher dose of dolutegravir seems necessary (Dooley 2012). Fortunately, there is no effect of food intake on resorption (Song 2012).

Since its approval in 2014, dolutegravir has rapidly gained an important role in HIV medicine. Good tolerability, high resistance barrier, once-daily dosing and the absence of any booster requirements are major advantages. The coformulation with ABC+3TC, the first STR without tenofovir, is also very attractive.

Elvitegravir (ELV, Vitekta®, also part of **Stribild®**) is an integrase strand transfer inhibitor developed by Gilead, with a biochemical similarity to chinolone antibiotics (Sato 2006). In a study with 40 patients (ART-naïve and pre-treated), viral load decreased by 2 logs at 10 days of monotherapy (DeJesus 2006). In pre-treated patients there was a good effect when compared to a boosted PI (Zolopa 2010). A disadvantage is that elvitegravir must be boosted (Kearney 2006), but on the other hand a single administration per day seems possible.

To avoid dependancy on ritonavir as a booster agent, Gilead has investigated combinations of elvitegravir with cobicistat, a new pharmacoenhancer (PKE). Stribild®, a fixed-dose combination of the four Gilead substances tenofovir, FTC, cobicistat and elvitegravir in a single tablet, showed good efficacy in a phase II trial on therapy-naïve patients (Cohen 2011). Two large phase III trials investigating QUAD on therapy-naïve patients led to the approval of Stribild®. In 236-0102, 700 patients received either Stribild®, or Atripla® (Sax 2012) and in 236-0103, 708 patients were treated with either Stribild® or TDF+FTC+atazanavir/r (DeJesus 2012). After 48 weeks, 88% under Stribild® (versus 84%) and 90% (versus 87%), respectively, achieved a viral load below 50 copies/ml. Both trials showed no difference in subgroups (sex, age, CD4 T-cell count, amount of viral load). Tolerance was good, except for more cases of nausea (21 versus 14%) under elvitegravir. In contrast, fewer cases of dizziness (7 versus 24%) and dyslipidemia were observed. The results were sustained over a period of 144 weeks (Clumeck 2014, Wohl 2014).

There are, however, some problems with nephrotoxicity. Cobicistat inhibits renal tubular secretion of creatinine and increases serum creatinine levels, resulting in a decrease in estimated glomerular filtration rate (GFR) without a true decline in GFR. Thus, it may difficult to distinguish between these effects and the "true" renal toxicity of tenofovir. In the phase III studies, the GFR declined by 13–14 mL/min. There are detailed recommendations for renal monitoring during therapy with Stribild®. In all patients, document estimated creatinine clearance (CrCl), urine glucose, and urine protein should be available at baseline. Stribild® should not be initiated or discontinued when estimated CrCl is <70 or <50 mL/min, respectively.

In 145, a large randomized double-blind Phase III trial on over 700 *pre-treated* patients with documented resistance showed similar effects with elvitegravir or raltegravir (Elion 2012). Consequently, Stribild® can also be used in treatment-experienced patients without known resistance mutations to INSTIs. The switch from PIs or NNRTIs in patients with sustained virological suppression (and very limited resistance) is also possible, as shown by two large randomized trials (Arribas 2014, Pozniak 2014). It remains to be seen if Stribild® is also potent in heavily pretreated patients. There seems to be at least two resistance pathways, located at the codons T66I or E92Q (Shimura 2008). Especially E92Q induces a high resistance. In the case of Y143, a raltegravir resistance, efficacy seems to persist (Métifiot 2011). Resistance mutations of elvitegravir and raltegravir overlap to a great extent (Garrido 2012). No virologic response was observed in a small clinical study with patients who switched from elvitegravir to raltegravir (DeJesus 2007).

Major interactions with elvitegravir are not expected, at least not with NRTIs, darunavir, tipranavir, fosamprenavir or etravirine. However, dose adjustments with lopinavir/r and atazanavir/r may be necessary. The dose of maraviroc must be halved (Ramanathan 2011). There are no clinically relevant interactions between boosted elvitegravir and H2-receptor antagonists or proton pump inhibitors. However, staggered antacid administration by 2 hours is recommended (Ramanathan 2013)

In September 2014, elvitegravir as single agent was approved for use with a protease inhibitor coadministered with ritonavir plus other antiretrovirals. The approval was based upon results from the Phase III Study 145 (see above).

Raltegravir (RAL, Isentress®) is a strand transfer inhibitor and was the first integrase inhibitor on the market (Hazuda 2000). Raltegravir has a wide range of efficacy for R5 and X4 tropic viruses, and inhibits HIV-2 replication. During a 10-day monotherapy, viral load declined by two logs (Markowitz 2006).

The encouraging results of an early Phase II study in *extensively pre-treated* patients (Grinsztejn 2007) were confirmed by two large Phase III studies which led to approval of raltegravir. In BENCHMRK-1 and -2, a total of 699 pretreated patients with triple-

class resistance were randomized to raltegravir 400 mg BID or placebo, each combined with an optimized background therapy (Cooper 2008, Steigbigl 2008). After 16 weeks, 79% (versus 43%) of patients showed a viral load below 400 copies/ml. Even in patients initially without an active substance in their background therapy in genotypic assays, the success rate reached 57% (versus 10%). The effects were sustained beyond 144 weeks (Eron 2010).

Raltegravir has also been effective in *treatment-naïve* patients. The encouraging data from an early Phase II study (Markowitz 2009) were confirmed by a large Phase III study in which 563 patients received either raltegravir or efavirenz (Lennox 2009): at week 48, rates of patients achieving undetectable plasma viremia (<50 copies/ml) were 86% and 82%, respectively. Tolerability was better and the effects were maintained over five years (Rockstroh 2013). In September 2009, raltegravir was approved for first-line therapy. In ACTG 5237, raltegravir was superior to the two boosted PIs atazanavir and darunavir (Landovitz 2014).

Tolerability of raltegravir has so far been excellent. In BENCHMRK, raltegravir side effects were comparable to placebo. Apart from some anecdotal reports of rhabdomyolysis, hepatitis, rash and insomnia (Gray 2009, Santos 2009, Dori 2010, Tsukada 2010), frequently appearing side effects with raltegravir have not been seen. Raltegravir seems to be safe, including in those with hepatitis coinfections (Rockstroh 2012). In patients with renal impairment, no dosage adjustment is required. Expected autoimmune diseases observed in animal testing have so far not been clinically confirmed (Beck-Engeser 2010). There is limited data for pediatric or pregnant patients (Taylor 2011). Due to its excellent tolerability, raltegravir is currently being evaluated in the setting of nuke-sparing strategies (see below).

The fact that viral load decreased more rapidly in the first weeks in patients taking raltegravir compared to those taking efavirenz led to some speculations about higher potency (Murray 2007). Several experimental studies looked at strategies aimed at achieving viral eradication with raltegravir intensification (see chapter on *Eradication*). However, some experts believe that the faster response on raltegravir-based regimens is not a matter of potency, but rather due to its unique effect of blocking integration of the HIV genome (Siliciano 2009).

What is known about resistance to raltegravir? There are at least two common resistance pathways, via mutations Q148K/R/H or N155H. Both mutations are localized within the catalytic core of the integrase (Grinsztejn 2007, Malet 2008). A third pathway seems to be Y143 (Delelis 2010). Resistance may occur quickly on a failing regimen (Grinsztejn 2007, Taiwo 2011). The resistance barrier of raltegravir seems not very high although it is higher than that for NNRTIs. A few days of monotherapy are not enough to select resistance mutations as is the case with nevirapine (Miller 2010). There is evidence for cross-resistance with elvitegravir (DeJesus 2007, Garrido 2012). Transmission of raltegravir-associated resistance mutations has been reported (Boyd 2011, Young 2011).

Switching to raltegravir is not always safe. This was shown by the randomized SWITCHMRK studies (Eron 2010) with more than 700 patients on a lopinavir/r-based ART with a viral load below 50 copies/ml for at least three months. Switching to raltegravir showed a better lipid profile, but did not demonstrate non-inferiority with respect to HIV RNA <50 copies/ml at week 24 versus remaining on lopinavir/r. In total, 6% of the patients showed a viral rebound. Again, these results provide evidence for a possibly lower resistance barrier of integrase inhibitors compared to boosted PIs. Even if the smaller Spanish SPIRAL study did not confirm these results (Martinez 2010), switching from boosted PIs to other agents should be considered with care. Switching from T-20 to raltegravir, however, is probably safe (De Castro 2009, Grant 2009, Gallien 2011).

The recommended dosage of raltegravir is 400 mg BID. Once daily dosing is less potent, as the QDMRK study has shown (Eron 2011). Currently, a new formulation of 1200 mg (two tablets with 600 mg) is evaluated for once-daily dosing.

Raltegravir is not an inducer or an inhibitor of the cytochrome P450 enzyme system. Clinically relevant interactions are not expected (Rizk 2014). Thus, raltegravir is an important option in patients with comedication of high risk for interactions, such as tuberculosis or cancer (Grinsztejn 2014). However, raltegravir plasma concentration increases with omeprazole coadministration in healthy subjects; this is likely secondary to an increase in bioavailability attributable to increased gastric pH – the clinical relevance remains questionable (Iwamoto 2009).

Taken together, there is no doubt that raltegravir has become an important option for patients harboring resistant viruses. Given its excellent efficacy and tolerability, application of raltegravir is also indicated for treatment-naïve patients. A disadvantage is that raltegravir must be taken twice daily, a new formulation is in development.

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Entry inhibitors

Mode of action

There are three crucial steps (Figure 2.2) for entry of HIV into the CD4 T cell:
1. Binding or attachment of HIV to the CD4 receptor (target of attachment inhibitors),
2. Binding to co-receptors (target of co-receptor antagonists),
3. Fusion of virus and cell (target of fusion inhibitors).
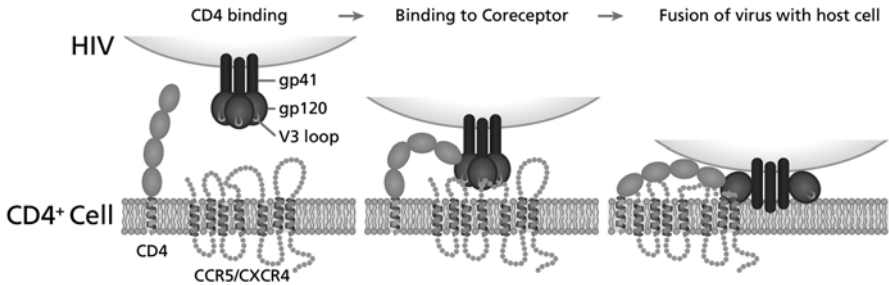
Figure 2.2: The three steps of HIV entry into the host cell

Every step of HIV entry can theoretically be inhibited. Step 1 is inhibited by attach-ment-inhibitors, step 2 by co-receptor antagonists and step 3 by fusion inhibitors. All three drug classes are currently called entry inhibitors. One important difference to other drug classes is that entry inhibitors do not inhibit HIV intracellularly. They interfere early in the replication cycle of HIV. It is speculated that this will lead to a better tolerability of this class.

In 2003 T-20 was licensed as the first entry inhibitor in Europe and the US. In 2007, maraviroc was the first CCR5 co-receptor antagonist and the first oral entry inhibitor. Numerous other drugs are in the pipeline, but will not be available soon. T-20 and maraviroc will be discussed in this section, for other entry inhibitors, including attachment inhibitors, refer to the next chapter, *ART 2017/2018*.

Co-receptor antagonists

Preface

In addition to CD4 receptors, HIV requires so-called co-receptors to enter the target cell. The two most important ones, CXCR4 and CCR5, were discovered in the mid-1990s (Alkhatib 1996, Deng 1996, Doranz 1996). These receptors, of which there are probably more than 200 in total, are named after the natural chemokines that usually bind to them. Their nomenclature is derived from the amino acid sequence. For CCR5 receptors these are the CC chemokine MIP and RANTES, for CXCR4 receptors it is the CXC chemokine SDF-1.

HIV variants use either the CCR5 or the CXCR4 receptors for entry into the target cell. HIV variants are termed R5-tropic if they use CCR5 as a co-receptor, whereas viruses with a preference for CXCR4 are termed X4-tropic viruses. R5 viruses pre-dominantly infect macrophages (formerly, M-tropic). X4 viruses mainly infect T cells (formerly, T-tropic). Dual-tropic viruses are able to use both receptors. There also exist mixed populations of R5 and X4 viruses. In most patients, R5 viruses are found in the early stages of infection. X4 viruses, which are probably able to infect a wider spectrum of cell types, usually occur in the later stages of disease. In addition, X4 viruses almost always occur in X4/R5-mixed populations and an exclusive X4 virus

population is very rare. The change in tropism is frequently associated with disease progression (Connor 1997, Scarlatti 1997).

It is still not completely clear why this happens after several years of infection, although the tropism shift only needs a few small mutations. However, it is possible that X4 viruses are significantly more virulent, but because of their low glycosylation, more immunogenic. X4 viruses are neutralized better by the immune system and it is therefore likely that they only become apparent in the presence of a significant immune deficiency.

In some individuals expression of CCR5 co-receptors on the cell surface is reduced. These individuals are usually healthy. The reduced expression of the receptor is usually due to a defective CCR5 allele that contains an internal 32-base pair deletion (Δ32 deletion). This deletion appears to protect homozygous individuals from sexual transmission of HIV-1. If infected, these individuals have a slower decrease in their CD4 T cell count and a longer AIDS-free survival than individuals with the wild type gene (Dean 1996, Liu 1996, Samson 1996). In healthy individuals, there is no strong evidence for any illness associated with the deletion. Thus, targeting the interaction between HIV-1 and the CCR-5 receptor appears to be an attractive therapeutic goal to prevent or slow disease progression.

In 2008 the case of a person (the "Berlin" patient) with acute myeloid leukemia and HIV-1 infection was published. This patient underwent stem cell transplantation from a donor who was homozygous for the CCR5 Δ32 deletion. The patient has remained without viral rebound for more than five years after transplantation and discontinuation of ART. This outcome demonstrates the critical role CCR5 plays in maintaining HIV-1 infection (Hütter 2009, Allers 2011).

In treatment-naïve patients, R5 strains are found in 80–90%, compared to only 50–55% in patients with antiretroviral exposure (Hoffmann 2007). The most important predictor of R5 tropism seems to be a higher CD4 T cell count in both naïve and antiretrovirally-pretreated patients. A low HIV plasma viremia seems to be associated with R5 tropism only in untreated patients (Moyle 2005, Brumme 2005). In contrast, X4 viruses are almost exclusively found in advanced stages of the disease. When the CD4 T cell count is >500/µl, they are only found in 6%; at <25 CD4 T cells/µl, in more than 50% of patients (Brumme 2005).

CCR5 antagonists probably need be given earlier in the course of disease. In the salvage situation, patients often harbor X4 viruses. The role of CCR5 antagonists might lie rather in the substitution of other antiretroviral agents in case of toxicity.

Testing for co-receptor usage (Tropism testing)

Since CCR5 blockers are effective only when a predominant R5 virus is present in the patient and co-receptor switch is not systematic, a baseline determination of the co-receptor usage of the virus is mandatory. Tropism testing prior to treatment avoids unnecessary costs and additional risks for the patient. Non-effectivity of CCR5 antagonists may cause regimen frailty and lead to resistance. This is why the development of CCR5 antagonists has brought along a completely new laboratory branch which focuses on predicting the co-receptors mainly or exclusively used by viral population (see the chapter on *Resistance*).

Several commercial assays have been developed to determine HIV tropism *phenotypically,* such as Trofile® (Monogram Biosciences). These assays are complex, time-consuming and require a viral load of at least 500–1,000 copies. A new version of the assay, Trofile-ES®, can detect smaller numbers of X4 virus (ES, enhanced sensitivity), resistant to CCR5 inhibitors, when they constitute a minor subpopulation. Several studies have illustrated the potential benefit of the use of the newer, more sensitive tests (Saag 2008, Su 2008).

Determing tropisms with *genotypic* testing is more easier, less time consuming and less expensive. Genotypic tropism testing has been validated by several studies and has now substituted the more complex and expensive phenotypic assay (Sierra 2007, Poveda 2009, Swenson 2011). Presently the focus of research is on the V3 loop of the envelope protein gp120, as this is the region where HIV binds to the co-receptor (Jensen 2003, Briz 2006). However, tropism does not only seem to be defined by the V3 loop sequence – viral isolates with identical V3 loops can differ in tropism (Huang 2006, Low 2007).

With genotypic testing, CCR5 antagonists may be suitable for many patients who have side effects on other agents, as long as the viral load is well suppressed. As mentioned above, phenotypic testing requires a viral load of at least 1000 copies/ml, whereas genotypic testing is possible with proviral DNA – even patients with an undetectable RNA in the plasma can be tested. Clinical studies have shown that this is possible and effective (Soulie 2009, Bellecave 2012). CCR5 antagonists may therefore be able to replace other drugs in the setting of a fully suppressed virus (i.e. in the context of side effects).

Tropism shift and other consequences

During treatment failure of antiretroviral regimens containing CCR5 antagonists, many patients often show a selection shift to X4 viruses. This shift is mainly due to selections from preexisting pools (Westba 2006). In a pilot study in which patients with X4/R5 mixed populations received maraviroc, CD4 T cells were higher in comparison to placebo (Saag 2009). An X4 shift (induced HIV progression) while on CCR5 antagonists therefore seems very unlikely.

What other consequences could a CCR5 blockade have? Although individuals with a Δ32 gene defect for the CCR5 receptor are usually healthy, there are worries about negative effects of blocking these receptors, i.e., this chemokine receptor must exist for some reason. Individuals with the Δ32 deletion have been examined in numerous studies to see if they suffer more frequently from illnesses compared to patients without this gene defect. An increased appearance of West Nile viral infection (Glass 2006) or FSME (Kindberg 2008) was greatly discussed, whereas the Δ32 deletion seems to be protective for rheumatism (Prahalad 2006). However, a recently published randomized trial did not show a beneficial effect of maraviroc in rheumatoid arthritis (Fleishaker 2012).
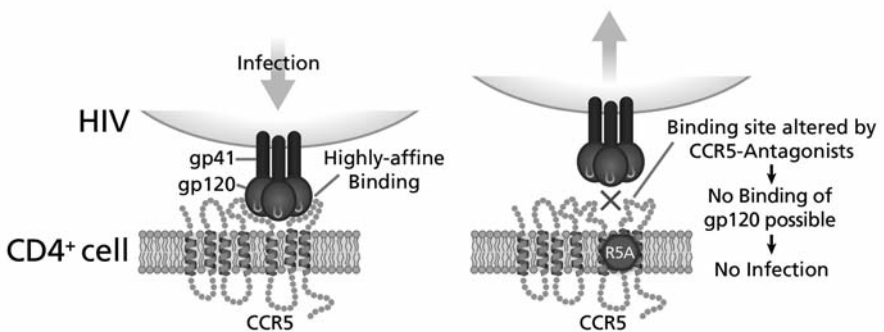
Figure 2.3: Mode of action of the allosteric CCR5 antagonists maraviroc. By binding to a hydrophobic cavity formed between transmembrane helices in CCR5 near the membrane surface, the receptor molecule undergoes conformational changes. This inhibits the binding of viral gp120 to the receptor. R5A = CCR5 antagonist

Presently the data is so heterogeneous that it is difficult to speak of a distinct asso-
ciation of the gene defect with certain illnesses. However, it is advisable to monitor
carefully, as experience with CCR5 antagonists has so far been limited. Moreover, in
theory, docking onto the receptor could cause an autoimmune reaction. However,
this has not occurred in testing with monkeys (Peters 2005). Negative effects towards
vaccinations have also bee discussed (Roukens 2009). An analysis of the complete
Phase I/II studies with maraviroc has shown no negative effects on immune func-
tion (Ayoub 2007). The initially disquieting report of malignancies in a study with
vicriviroc (Gulick 2007) has not been confirmed in any following studies.

Immune modulation with CCR5 antagonists?

Early observations led to the supposition that CCR5 antagonists may be able to serve
as immune modulators. Effects of an additional dosage in patients with poor immune
constitution have not shown the results hoped for in studies so far (Lanzafame 2009,
Stepanyuk 2009, Hunt 2011). A meta-analysis found no evidence for a beneficial
effect of maraviroc on immune reconstitution (Pichenot 2012). However, there are
indications of positive effects on immune activation (Funderberg 2009, Wilkin
2010+2011) and latent viral reservoir (Gutiérrez 2010). There is little experience
outside experimental studies and the results are not yet confirmed.

Individual agents (for unlicensed agents, see next chapter)

Maraviroc (MVC, Celsentri® or Selzentry®) from ViiV Healthcare was the first drug
in its class to be licensed for the treatment of HIV infection in September 2007.
Maraviroc allosterically binds to CCR5. This means that it does not bind directly to
the receptor but induces conformational changes within CCR5 that result in the
inhibition of its binding to viral gp120 (see Figure 2.3). During maraviroc monother-
apy, viral load declines by 1.6 logs after 10–15 days (Fätkenheuer 2005).
Two almost identical Phase III studies led to approval of the drug, MOTIVATE-1 (US,
Canada) and -2 (Europe, Australia, US). A total of 1,049 treatment-experienced
patients with R5 virus were enrolled (Gulick 2008, Fätkenheuer 2008). Patients had
been treated with or had resistance to three antiretroviral drug classes and had a
baseline viral load of more than 5,000 copies/ml. Patients were randomly assigned
to one of three antiretroviral regimens consisting of maraviroc once-daily, maraviroc
BID or placebo, each of which included OBT – agents such as darunavir, etravirine
or raltegravir were not allowed. At 48 weeks in both studies more patients in the
maraviroc arms were below 50 copies/ml (46% and 43% versus 17% with placebo).
A treatment benefit of maraviroc over placebo was also shown in patients with a
high viral load and multiple resistance mutations (Fätkenheuer 2008). Results
remained the same after 96 weeks (Hardy 2010). Tolerability of maraviroc was excel-
lent and did not differ from that of placebo. In addition, the shift to X4 viruses in
those with no virological success had no negative effects.
Maraviroc has also been tested in treatment-naïve patients (Cooper 2010, Sierra-
Madero 2010). In the MERIT study, a total of 721 patients randomly received
AZT+3TC plus either efavirenz or maraviroc BID (the arm with maraviroc QD was
prematurely closed due to lower efficacy). Virological failure was more frequent on
maraviroc (11.9% versus 4.2%). Although the CD4 T cell increases were significantly
more pronounced on maraviroc, the study failed to show non-inferiority of mar-
aviroc compared to efavirenz. Of note, there were significant differences seen between
study populations in the northern versus southern hemisphere in this worldwide
trial. Response rates proved almost equal in northern hemisphere countries, but not
as good south of the equator. In addition, a retrospective analysis revealed that at
least 4% of the patients in the maraviroc arm had experienced a tropism shift from

R5 to dual tropic virus between screening and baseline. In these patients with dual tropic virus, response rates were very poor. Would a better and more sensitive test have been able to demonstrate a more relevant difference between maraviroc and efavirenz? A retrospective analysis using the enhanced Trofile assay, in which no differences were observed, seems to back this argument (Cooper 2010, Swenson 2011, McGovern 2013). On the basis of this data the FDA extended the license for maraviroc to therapy-naïve patients in November 2009. However, the available data was not sufficient for EMA to permit such an extension in indication. Unfortunately, the experimental strategy of nuke sparing, i.e. maraviroc plus darunavir/r was also not as effective that standard therapy (Stellbrink 2014).

Maraviroc's tolerability is excellent over five years (Gulick 2014). In the MERIT study, the discontinuation rates due to adverse events were significantly lower than with efavirenz (4.2% vs 13.6%) and lipid profiles were better (MacInnes 2011). There seems to be no liver toxicity as seen with aplaviroc, a CCR5 antagonist whose development was halted in 2005, not even in those with existing liver damage (Abel 2009).

What about the efficacy of maraviroc in the presence of non-R5 viruses? In a double-blind randomized Phase II study on 113 patients the effect was, as expected, moderate. There was no antiviral effect compared to placebo. However, CD4 T cells improved significantly in those on maraviroc despite the lack of virologic efficacy (Saag 2009).

With regard to resistance, only limited data exist to date. Mutations in the gene regions coding for the V3 loop of the envelope protein gp120 may lead to complete resistance to maraviroc. This may occur by *de novo* acquisition of mutations allowing the virus to use the CXCR4 receptor or via "true" resistance. The latter may occur in viral isolates that remain R5 tropic. A shift to X4 tropism is not necessary as resistance may happen via an increased affinity of the viral envelope for unbound CCR5 molecules or through an ability of the viral envelope to use compound-occupied receptors for entry (Westby 2007, Lewis 2008). It seems that the resistance barrier for true maraviroc resistance in R5 viruses is high (Jubb 2009).

In practice it is important that the recommended dosage of maraviroc is adjusted depending on the concomitant therapy (Abel 2005). With boosted PIs (except for tipranavir) the usual dosage of 2x300 mg is halved, while with efavirenz (or other enzyme inducers such as rifampicin or carbamazepin) it is doubled. No adjustment is required with raltegravir (Andrews 2010).

Despite excellent tolerance, application of maraviroc still remains relatively limited, as obstacles such as requirement for tropism testing, restricted indication (in Europe) and slightly complicated dosage still stands in the way: Unfortunately, nuke-sparing strategies with maraviroc were not successful (see Nuke-Sparing).

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Fusion inhibitors

Fusion inhibitors prevent the final step of entry of HIV into the target cell. The fusion of virus and cell is complex and not completely understood. Simplified, it seems that binding to the CD4 and to the co-receptor induces conformational changes in the gp41, the transmembrane subunit of the viral envelope protein. In the course of these rearrangements, the N-terminal fusion peptide of gp41 translocates and inserts into the target cell membrane. A proposed extended conformation of the gp41 ectodomain, with its fusion peptide thus inserted and the transmembrane anchor still in the viral membrane, has been called the "pre-hairpin intermediate". This is the target of fusion inhibitors, including T-20 (Root 2001).

Individual agents

T-20 (Enfuvirtide, Fuzeon®) is the prototype of the fusion inhibitors. T-20 was licensed in Europe and the US in May 2003 for the treatment of HIV-1 infection in antiretroviral-experienced adults and children over 6 years of age. It is a relatively large peptide comprised of 36 amino acids, and therefore needs to be administered by subcutaneous injection. It binds to an intermediate structure of the HIV gp41 protein, which appears during fusion of HIV with the target cell.

Initially, HIV+ patients were given T-20 monotherapy intravenously. Antiviral activity was dose-dependent, and at the higher dose of 100 mg BID, the viral load was reduced by almost 2 logs (Kilby 1998+2002). In early studies of the subcutaneous application, an effect on viral load was still evident in one third of patients after 48 weeks.

Two Phase III studies led to the licensing of T-20. TORO 1 (T-20 versus Optimized Regimen Only) enrolled 491 extensively pretreated patients in North America and Brazil, most with multiresistant viruses. In TORO 2, 504 patients in Europe and Australia were enrolled. Patients in both studies on an optimized ART regimen either received 90 mg T-20 BID subcutaneously or none at all (Lalezari 2003, Lazzarin 2003). In TORO-1, the reduction in viral load was 0.94 logs better with T-20 than optimized therapy without T-20. In TORO-2 this difference was 0.78 logs (Nelson 2005). A strong impact on viral load was also seen with tipranavir, darunavir, maraviroc or raltegravir. In all large studies evaluating these agents (RESIST, POWER, MOTIVATE, BENCHMRK), the additional use of T-20 was of significant benefit. If at least two active substances are not available, the option of T-20 should be discussed with the patient. Small pilot studies such as INTENSE or INDEED suggest that T-20, given as "induction", i.e., in the first weeks of a new salvage therapy, lowers the viral load more rapidly (Reynes 2007, Clotet 2008).

The success of T-20 therapy should be monitored early on, particularly in view of the cost. Patients without a decrease in viral load of at least one log after 8-12 weeks will not benefit and can be spared the required twice-daily injections. It is also not recommended to inject the full daily dose of T-20 once a day: although 180 mg QD has the same bioequivalence (as measured by AUC) to the standard 90 mg BID, at least one study has shown a trend towards a smaller decrease in viral load with the QD dose that was clearly associated with lower trough levels (Thompson 2006).

One observation in the TORO studies was the increased frequency of lymphadenopathy and bacterial pneumonia in those on T-20 (6.7/100 versus 0.6/100 patient years) (Trottier 2005). Septicemia also occurred more often on T-20, but the difference was not significant. The reason for the increased rate of infections remains unclear, but binding of T-20 to granulocytes has been suspected. Substantial side effects remain constant (98% in the TORO studies), and over the course of therapy, severe local skin reactions occur at the injection site. These can be particularly painful and can result in interruption of therapy: 4.4% of cases in the TORO studies. In our experience of everyday clinical treatment, therapy is interrupted frequently due to these skin problems (see section on Side Effects). Unfortunately the development of a bio-injection system in which T-20 is pressed into the skin was halted.

Resistance mutations develop relatively rapidly on T-20, but seem to reduce viral fitness (Lu 2002, Menzo 2004). Receptor tropism of the virus seems to be not significantly affected. There are some changes to a short sequence on the gp41 gene, causing reduced susceptibility to T-20, which is due to simple point mutations (Mink 2005). In contrast, viruses resistant to NRTIs, NNRTIs and PIs are susceptible to T-20 (Greenberg 2003). As it is a relatively large peptide, it induces antibody production. This does not seem to impair efficacy (Walmsley 2003). More disturbing is the fact that in a large TDM study there was a very large interpatient variability and extremely low plasma levels were often found (Stocker 2006).

In summary, patients with a well-controlled viral load or who still have options with classical ART do not require T-20. For salvage therapy the drug seems to be very valuable in individual cases. However, T-20 probably has only a minor role to play in the future of HIV treatment. Many patients have already successfully replaced T-20 with newer oral antiretrovirals like raltegravir (DeCastro 2009, Grant 2009, Santos 2009, Talbot 2009, Gallien 2011).

Increasing efficacy of ART and/or emptying latent reservoirs with T-20, as first reports suggested (Lehrmann 2005, Molto 2006), seem unlikely now (Gandhi 2010, Morant-Joubert 2012). The price also remains significant – ART costs can skyrocket with the addition of T-20, the sponsor maintaining that it is one of the most complicated drugs it has ever manufactured.

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6.3. ART 2017/2018: The horizon and beyond

CHRISTIAN HOFFMANN

Almost all HIV+ patients can now be treated with a virologically successful regimen, even those with multiple resistance mutations. There are very few "untreatable" patients. However, despite this considerable progress, there is an urgent need for new drugs. This is not just true for patients with multiresistant viruses awaiting new treatment options. Significant problems related to long-term toxicity and adherence are anticipated for all therapies that will presumably need to span decades, as eradication of HIV is still out of reach for the majority of the patients. A recent study has demonstrated that the median time until exhaustion of currently available treatment options is 45 years. Of note, 10% of HIV+ patients are expected to exhaust all currently available ART options after just 26 years (Jansson 2013). As a result, new drugs are needed that are easier to take, with high genetic barriers to development of resistance, and above all less toxic. To reach the goal of eradication, new drugs need to be more potent than those available today. The following overview of agents that could make it to the clinic based on current data (mid-2015) does not claim to be complete.

New pharmacoenhancers (PKEs)

Many antiretroviral agents, among them almost all PIs, but also the integrase inhibotor elvitegravir, have to be boosted in order to enhance their pharmacokinetics. For more than a decade, ritonavir has been the only reliable option for boosting. In the meantime, cobicistat has been introduced to the market. The advantages of these new agents inhibiting the CYP3A system is that they have no antiviral effect and thus would not cause resistance.

SPI-452 is a PKE developed by Sequoia (Gulik 2009). In a first clinical study, different doses were given to 58 healthy volunteers. Tolerance was good. The levels of darunavir (37-fold) and atazanavir (13-fold) significantly increased. The booster effect lasted for a long time. Sequioa may continue research with SPI-452 as an individual agent and in fixed combinations. Their website recently closed down. Further development is questionable.

PF-03716539 is a PKE from Pfizer. Studies with healthy volunteers regarding its effects on midazolam, maraviroc and darunavir were concluded in 2009 according to clincaltrials.gov. The results have not yet been officially released. The product is not listed on either the Pfizer or the ViiV websites.

TMC-558445 manufactured by Tibotec pharmaceuticals was tested in a Phase I dose escalation study. Results are not available. Janssen, the current official name of the company, does not have PKEs on their short-list of priorities for the future.

Long-Acting Drugs, New formulations, Generics

Currently available drugs continue to be developed, the most important goals being a reduction in pill burden, easier dosing and fewer side effects. In addition, long-acting drugs are under development. These nanoformulated agents can be given much less frequently (i.e., an injection intramuscular every 1–3 months). They may be helpful in improving adherence but also in the setting of preexposure prophylaxis. In an early study, many patients indicated that they definitely or probably would try parenteral nanoformulated antiretroviral therapy (Williams 2013). Different techniques are in development (Guo 2014).

Cabotegravir (CAB, GSK-774) is developed as an injectable long-acting drug. PK studies evaluated a half-life of 21–50 days after single injections in healtly voluntees (Spreen 2013). In animals monthly injections were highly protective as PrEP (Andrews 2014, Radzio 2014). When given as a monotherapy in HIV+ patients, plasma viremia declined by 2.2–2.3 logs (Spreen 2013). The resistance barrier seems to be as high as that of dolutegravir. Given orally, cabotegravir is also very effective. In the LATTE-1 study, different dosages were evaluated in 243 ART-naive patients and compared with efavirenz (Margolis 2014). After an induction period during that cabotegravir and efavirenz were combined each with 2 NRTIs, patients remained on efavirenz or switched to 10–60 mg cabotegravir plus the NNRTI rilpivirine. At 48 weeks, the rates of patients with an undetectable viral load were 82% in the experimental arms with cabotegravir plus rilpivirine, compared to 71% with the standard regime of efavirenz plus 2 NRTIs. Only a few resistance mutations were observed. These early esults support the selected dose regimens for the ongoing LATTE-2 study with cabotegravir LA + rilpivirine LA as injectable two-drug maintenance therapy.

Rilpivirin LA – is a parenteral formulation enabling prolonged prolonged plasma and genital-tract exposure (Jackson 2013). In one study, a single injection yielded to measurable concentrations in plasma and genital fluids even after 84 days postdose (Else 2012, Jackson 2013) making this an attractive approach for PrEP. With monthly intramuscular injections, similar levels can be achived as with daily oral dosing of 25 mg. As mentioned above, rilpivirine is currently tested in combination with cabotegravir as injectable two-drug maintenance therapy. There are no relevant interactions (Ford 2014).

Atazanavir LA – was tested in mice. It had translational potential with sustained and targeted efficacy and with limited systemic toxicities. Folate coating of nano ART with atazanavir/r significantly enhanced cell uptake, retention and antiretroviral activities without altering cell viability (Dash 2012, Puligijja 2013).

Generics have been produced by companies from Africa, India, Brazil or Thailand (see Chapter on *Global Access*). In developing countries many new and previously unkown fixed drug combinations (FDC) are used. The most frequently used FDC is d4T+3TC+nevirapine that exists as Triomune (Cipla), GPO-vir (GPO), Triviro LNS (Ranbaxy) or Nevilast (Genixpharma). In most cases, bioequivalence has been demonstrated (Laurent 2004, Marier 2007). There are generics approved by FDA and WHO that are bioequivalent. Legally, a drug is not a generic if it is not bioequivalent to the original drug. FDCs also exist for AZT+3TC+nevirapine, called Duovir N (Cipla) or Zidovex-LN (Ranbaxy). Patent rights for generics have often been ignored, making these insignificant in industrial countries (see Chapter on *Global Access*).
However, as some patents have expired over the last years (and many will do so in the near future), even in Western countries many generics are already available for drugs such the NRTIs AZT, 3TC, AZT+3TC, the two NNRTIs efavirenz, nevirapine but also for the PI saquinavir.

Raltegravir 600 mg – with the sobering results of the QDMRK Study in which 800 mg QD had been less effective than 400 mg BID, MSD felt into lockdown. For more than two years, nothing happened. However, when it became apparent that raltegravir would be the only modern drug in HIV medicine that has to be given BID, the company finally decided to develop a new formulation. The 600 mg are now investigated (Krishna 2013). In June 2014, the ONCEMRK was initiated. In this double-blinded randomized trial, a total of 750 ART naive patients will receive raltegravir 1200 mg QD (two tablets) or 400 mg BID, all combined with TDF+FTC. Results will be available in early 2016.

Nelfinavir 625 mg – this formulation was approved in the US in April 2003. It reduces the nelfinavir dose to 2 tablets BID. One study has shown that this formulation is better tolerated, particularly with respect to gastrointestinal side effects – despite the fact that plasma levels are around 30% higher (Johnson 2003). In Europe, where nelfinavir has been produced and sold by Roche, the 625 mg tablet has never been made available.

Zerit PRC® (PRC = "prolonged release capsule" or XR = "extended release") is a capsulated once-daily d4T (Baril 2002). d4T XR was approved in Europe in 2002, but never made it to market – d4T is "gone". There are attempts underway to improve d4T through minor modifications to its molecular structure. **OBP-601** is a novel nucleoside analog with potent anti-HIV-1 activity and limited cellular toxicity with a unique *in vitro* resistance profile. BMS is apparently working on this substance under the name Festinavir (Haraguchi 2013).

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New nucleoside analogs

Since the development of dexelvucitabine came to a halt in 2006, hopes have been limited that there will be new nucleoside analogs on the market in the near future. Developing NRTIs with strong potency against NRTI-resistant viruses that at the same time show less mitochondrial toxicity appears to be difficult. It is unlikely that any of the following agents – except for Tenofovir-Alafenamide (TAF) – will make it to the market. Many of them have already disappeared.

Amdoxovir (DAPD) is a novel dioxolane guanosine NRTI that is converted *in vivo* to the highly efficient DXG. DAPD has good efficacy against viruses resistant to AZT/3TC and against hepatitis B virus (Corbett 2001). When patients showed changes of the ocular lenses during early clinical trials (Thompson 2003), development was halted in 2004 and Gilead withdrew its licensing agreement with two US universities. However, there is still hope for DAPD. Supported by RFS Pharma from Georgia (US), development is ongoing. In this program, DAPD is combined with AZT to use the distinct resistance profiles of both compounds. In the first double-blind, randomized study in 24 patients, the viral load declined by an impressive 1.97 logs after 10 days on 500 mg DAPD + 200 mg AZT BID. There are obviously synergistic effects (Murphy 2010) that cannot be explained by interactions alone (Hurwitz 2010). The question is how to avoid the toxicity of DAPD.

Apricitabine (ATC, AVX-754, formerly SPD-754) is a heterocyclic cytidine analog that was sold by Shire Biochem to Avexa in 2005. ATC chemically resembles 3TC but has *in vitro* activity against a broad spectrum of TAMs. Up to 5 nucleoside mutations do not significantly impair its activity (Gu 2006). However, susceptibility to ATC is reduced when the K65R is present (Frankel 2007). A first placebo-controlled

study in 63 HIV+ patients treated with ATC monotherapy showed decreases in viral load of 1.2-1.4 logs – good potency for an NRTI (Cahn 2006). In 50 patients harboring the M184V mutation there was a reduction of 0.7-0.9 logs after three weeks on ATC (Cahn 2010). ATC-specific resistance mutations were not observed and could not be selected in *in vitro* experiments (Oliveira 2009). Cephalgia and rhinitis are most frequent, otherwise tolerability of ATC seems to be good (Gaffney 2009). What about long-term toxicity? In monkeys, there were minor skin problems, usually hyperpigmentation, after 52 weeks of exposure. ATC was thus significantly less toxic than BCH-10652, which caused severe degenerative dermatopathy in all exposed monkeys (Locas 2004). 3TC and FTC significantly and competitively lower intracellular levels of ATC. Combination with other cytidine analogs is a problem. After negotiations with large pharmaceutical companies failed in May 2010, further development was discontinued and it is questionable if it will be resumed.

CMX 157 is also known as "HDP-Tenofovir" (HDP = hexadecyloxypropyl-ester). Like TAF it is prodrug of Tenofovir and probably less nephrotoxic. In vitro CMX 157 was effective against TDF-resistant mutations including K65R. A once weekly dosing seems to be possibe. In 2012 the compound was purchased by MSD.

Dioxolanthymidine (DOT) is a newer thymidine analog. Dioxolane appeared to be relatively good in preclinical trials (Chung 2005, Liang 2006). Presently, prodrugs are being tested, however, clinical studies have yet to be conducted (Liang 2009).

EFda or 4-ethynol-2-fluor-deoxyadenosine seems to be a very effective NRTI according to the results of monkey testing. The SIV viral load decreased after 7 days by 2–3 logs (Parniak 2009, Michailidis 2014). It is also being evaluated as a potential microbicide. There is an licensing agreement with MSD for the development of this novel drug with plans for full scale clinical development.

Elvucitabine (ACH-126,443) is a cytidine analog developed by Achillion Pharmaceuticals. It is an enantiomer of dexelvucitabine and is also effective against HBV. *In vitro* studies show potency even in the presence of numerous resistance mutations (Fabrycki 2003). It is also of interest as it seems to have an extremely long half-life of up to 150 hours – this may allow once-weekly dosing (Colucci 2005). A small double-blind study showed a reduction in viral load of between 0.7 and 0.8 logs after 28 days in HIV+ patients with the M184V mutation. However, this study had to be prematurely terminated, as 6/56 patients developed leucopenia or rash on a dose of 100 mg (Dunkle 2003). It seems that mitochondrial toxicity is lower than that of dexelvucitabine. On the other hand, this lower toxicity may also lower the efficiency of incorporation by drug-resistant versions of HIV-1 RT (Murakami 2004). Less toxicity at the expense of efficacy? In a smaller Phase II study in 77 therapy-naïve patients (with efavirenz and tenofovir), elvucitabine was comparable to 3TC at 96 weeks (DeJesus 2010). There appear to be problems with interactions with ritonavir, which may be due to ritonavir inhibiting an efflux gut transporter with activity present at various levels in subjects (Colucci 2009).

Fosalvudine is an NRTI from Heidelberg Pharma, a prodrug of the fluorothymidine alovudine. The active part is released only after enzymatic cleavage in the tissue. It is hoped that the toxicity commonly seen with fluorothymidines can thus be reduced. In a Phase II trial with 43 ART-naïve HIV+ patients, fosalvudine was well-tolerated and after 2 weeks of monotherapy with 5–40 mg, viral load decreased by up to 1 log (Cahn 2007). Trials with pretreated patients are being conducted in Russia as well as in Argentina, although nothing is listed on clinicaltrials.gov under "fosalvudine". Animal testing on rats, however, indicate high mitochondrial toxicity (Venhoff 2009). Further development is questionable.

Fozivudine is another NRTI developed by Heidelberg Pharma according to the "enhanced pro-drug-principle". In Phase I/II trials (Bogner 1997, Girard 2000), fozivudine was well-tolerated, but only moderately effective – after 4 weeks, viral load decreased by 0.7 logs at the highest doses (Girard 2000). According to the company's website, they are looking for partners to be able to conduct further trials. It has been silent for a while – no one seems to be interested in a new AZT.

Tenofovir alafenamide fumarate ("TAF", GS-7340) is a prodrug of tenofovir (TFV) that enables higher tenofovir concentrations in peripheral blood mononuclear cells. TAF is converted mostly intracellularly to TFV, resulting in intracellular concentrations of tenofovir diphosphate in PBMCs that are 5–7 fold higher and TFV plasma concentrations that are 90% lower.

TAF was evaluated in different doses versus tenofovir in 30 HIV+ patients. After 2 weeks viral load decreased to 1.71 logs versus 0.94 logs. In more recent trials even lower doses were looked at (Ruane 2012). After 10 days of 25 mg and 40 mg, respectively, viral load decreased by 1.46 and 1.73 logs. Tolerance was good. Thus, a highly promising tenofovir prodrug seems to be emerging here with improved efficacy and lower systemic exposure (Markowitz 2014). With the success of TDF and due to the fact that its patent will end by 2016, the company set up a broad development during recent years: In a Phase 2, randomized, double-blinded study the efficacy of the fixed-dose combination elvitegravir/c plus TAF+FTC was comparable to elvitegravir/c plus TDF+FTC. Patients on TAF experienced significantly smaller changes in estimated creatinine clearance, renal tubular proteinuria, and bone mineral density (Sax 2014). As TAF is not a substrate for tubular transport systems, no accumulation is expected. Even in the setting of severe renal insufficiency, there is no need for dose adjustment (Bam 2014). In a pair of two Phase III Studies (all patients received elvitegravir/c+FTC), non-inferiority of TAF versus TDF was demonstrated in 1,733 ART naïve patients (Wohl 2015). Again, patients on TAF experienced less changes in renal function and in bone mineral density (Sax 2015).

Based on these favorable findings, Gilead has submitted the TAF coformulation ("Stribild-TAF" or "E/C/F/TAF") for review in the U.S. and Europe. Decision is to be expected by the end of 2015. In December 2014, Gilead Sciences announced development and commercialization of a fixed-dose regimen containing Janssen's rilpivirine ("Complera-TAF" or "R/F/TAF"). TAF in combination with FTC ("F/TAF") but also stand-alone TAF (for hepatitis B) are also being developed. Furthermore, studies with TAF as a part of a PI fixed-dose combination (plus darunavir/c) are ongoing (Mills 2015).

Things will become even more complicated: as coadministration with boosted PIs increases TAF exposures by 2-fold, two different dosages are developed. The recommended dose in R/F/TAF and in F/TAF (when coadministered with NNRTIs or INSTIs) is 25 mg; if it is used as E/C/F/TAF or as F/TAF (in combination with a PI), the recommended dose is 10 mg. Confusion is suspected when more complex regimens or co-medication with a high potential for interactions are needed. It will be challenging to avoid over-dosing, and, more importantly, under-dosing of TAF.

Phosphazide (Nicavir) is a nucleoside analog that was developed (and is marketed) in Russia, which is very similar to AZT (Skoblov 2003). After 12 weeks of phosphazide monotherapy (400 mg), viral load in a small group of patients dropped by median 0.7 logs. Since phosphazide is a prodrug of AZT, it requires an additional activation step. The D67N mutation seems to reduce efficacy (Machado 1999). A small study has shown potency in combination with ddI and nevirapine (Kravtchenko 2000), another with ddI and saquinavir (Sitdykova 2003). It is still hard to see the advantage over AZT – although better tolerability was presumed, this has not been shown.

Racivir is a cytidine analog produced by Pharmasset. It is a mixture of FTC and its enantiomer, 3TC. Possibly, both enantiomers have different resistance profiles so that, theoretically, the development of resistance is impeded (Hurwitz 2005). It has shown good antiviral activity in combination with d4T and efavirenz after two weeks (Herzmann 2005). In a study in 42 patients harbouring the M184V mutation, viral load declined by 0.4 logs after 28 days (Cahn 2007). Pharmasset has been looking to out-license this compound, without success, since 2008.

Stampidine is a nucleoside analog developed by the Parker Hughes Institute. It resembles d4T and is apparently 100 times more potent than AZT *in vitro* (Uckun 2002). It also has activity against HIV mutants with up to 5 TAMs (Uckun 2006). It has been discussed also as a potential microbicide (D'Cruz 2004).

Out of sight, out of mind: the following NRTIs are no longer being pursued:
• Adefovir dipivoxil from Gilead, low activity against HIV, nephrotoxicity
• Dexelvucitabine (DFC or Reverset) from Incyte, pancreatitis
• dOTC from Biochem Pharma, toxicity in monkeys
• FddA (Lodenosine) from US Bioscience, severe liver/kidney damage
• KP-1461 from Koronis, lack of efficacy
• Lobucavir from BMS, carcinogenicity
• MIV-210 from Medivir/Tibotec, currently being developed for HBV
• MIV-310 (alovudine)) from Boehringer Ingelheim, disappointing Phase II study
• SPD-756 (BCH-13520) and SPD-761

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New NNRTIs

In 2008 and 2011, etravirine and rilpivirine were the first second-generation NNRTIs. Encouraged by this, some pharmaceutical companies have NNRTIs in their pipeline again. However, there were some drawbacks. In February 2013, the development of Lersivirine was stopped by ViiV Healthcare when it became evident that there was no advantage over the available NNRTIs. More recently, the delayed onset of seizures after fosdevirine exposure and persistence after discontinuation (which was without precedent in antiretroviral drug development) led to discontinuation of another promising NNRTI (Margolis 2014).

AIC 292 is a new NNRTI from the German company AiCuris, which gained much attention at its first presentation at ICAAC in 2013 (Wildum 2013). As a diarylpyrazole-carboxamide, it differs chemically from all other NNRTIs. AIC 292 is effective against NNRTI resistance mutations such as K103N, Y181C and G190A and even against L100I. In Phase I the compound was well tolerated at doses up to 1400 mg (n=16). There seems to be a low potential for interactions; half-life is 20 hours. A Phase II study is planned (Wildum 2013). It remains to be seen, if one of the big pharmaceutical players in HIV medicine will be interested.

Doravirine (MK-1439) is a NNRTI developed by MSD. Half-life is long enough to allow QD dosing. It is effective against wild type virus and against several NNRTI mutations such as Y181C (Côté 2014). The resistance profile is very similar to that of rilpivirine and etravirine. As all available NNRTIs is not effective against Y188L (Lai 2014). Safety and PK data were evaluated in healthy volunteers (Anderson 2013). In a first study on 18 ART-naive HIV+ patients, viral load felt by 1.3–1.4 logs during 7 days of monotherapy (Anderson 2013). In a Phase II study, in which different dosages from 25 to 200 mg doravirine were tested against efavirenz, 76% achieved an undetectable viral load, compared to 64% on efavirenz. There was no clear dose-effect relation (Morales-Ramirez 2014). For further development, MSD chose the 100 mg dosages. Several studies are ongoing.

RDEA806 is an NNRTI by Ardea Biosciences. The resistance barrier is very high and the potential for interactions low (Hamatake 2007). Monotherapy trials with HIV+ patients showed a reduction of over 1.8 logs at 7 days with excellent tolerability (Moyle 2010). The data seem promising enough to start Phase IIb trials. However, the company's website is strangely blank on the topic.

Out of sight, out of mind: the following NNRTIs are no longer being developed:
• Atevirdine – Upjohn focused their research on delavirdine (a good idea?)
• BIRL355BS from Boehringer Ingelheim, in 2007 due to toxicity/metabolites

- Calanolide A from Sarawak, poor efficacy
- Capravirine (AG1549) from Pfizer, limited activity
- DPC 083 (BMS-561390), poor PK/secure data
- DPC 961 due to suicidal thoughts in healthy volunteers; DPC 963
- Emivirine (EMV, MKC-442, coactinone) from Triangle, due to limited activity
- Fosdevirine (GSK 761, IDX-899) from ViiV Healthcare, seizures
- GW420867X from ViiV, too much of a me-too drug
- GW8248 and GW5624 from GSK, due to poor bioavailability
- HBY-097 from Hoechst-Bayer, due to unfavorable side effects
- Lersivirine from ViiV, nausea, no advantages (me-too drug)
- Loviride, Janssen pharmaceuticals, due to limited activity in the CAESAR study
- MIV-150 from Medivir, poor bioavailability, now b. developed as microbicide
- PNU 142721, Pharmacia & Upjohn, too similar to efavirenz (Me-too)
- TMC120 (Dapivirine) from Tibotec, poor oral bioavailability, now being studied as a microbicide

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New protease inhibitors (PIs)

Even among PIs, many agents have been lost along the way. Following the licensing of darunavir, not much can be expected from PIs in the near- to mid-term. This may also be due to the high bar for any new PI (Review: Pokorná 2009).

DG17 is a prodrug of DG35 and has been under clinical testing for some time. One study showed a clear boosting effect with ritonavir and significant pharmacoenhancement warranting further clinical development (Cherry 2008).

SM-309515 is a PI from Sumitomo Pharmaceuticals and has apparently entered Phase I studies. Earlier versions failed due to the short half-life, and attempts have been made to improve this (Mimoto 2008). The drug showed activity in the presence of some PI mutations. Ritonavir boosting is purportedly being tested in humans. No mention on their website or clinicaltrials.gov.

SPI-256 from Sequioa Pharmaceuticals is effective *in vitro* against PI-resistant isolates (Gulnik 2006). Healthy individuals have tolerated it well. There is no mention on clinicaltrials.gov and they do not seem to have a website.

TMC-310911 is a new PI from Tibotec, currently being examined with the booster-drug TMC-558445 in a Phase I study. *In vitro* data are encouraging (Dierynck 2012). The drug was well tolerated by healthy volunteers, showing a good dose-PK-relation (Hoetelmans 2014). In HIV+ patients, monotherapy (boostered by ritonavir) led to a decline in viral load by 1.5 logs after 14 days (Stellbrink 2014). It remains to be seen if this sufficient for further development.

Out of sight, out of mind, the following PIs are no longer being developed:
• AG-001859 from Pfizer
• Brecanavir from GSK, stopped in 2006 due to poor PK data
• DPC 684/681, narrow therapeutic range due to cardiotoxicity
• GS 9005, previously GS 4338, from Gilead
• JE-2147, AKA AG1776, KNI-764 from Pfizer, no news since 1999
• KNI-272, Kynostatin – due to poor PK data
• Mozenavir, DMP-450 from Gilead, a me-too drug, nothing new to offer
• PL-110 (MK8122) from Merck, allowed the out-license to expire
• RO033-4649 from Roche, probably too similar to saquinavir
• SC-52151 and SC-55389A, poor bioavailability
• TMC-126 from Tibotec, they concentrated on darunavir

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New integrase inhibitors

The integration of viral DNA, enabled by the HIV enzyme integrase into the host DNA, is a major step in the replication cycle of HIV. In 2007, raltegravir, the first integrase strand transfer inhibitor (INSTI) for treatment of HIV infection, was licensed, followed by the two INSTIs elvitegravir and dolutegravir (see Chapter 2).

LEDGINs (or ALLINIs) are a new class of integrase inhibitors. As allosteric inhibitors these compounds bind to the LEDGF/p75 binding pocket in integrase, thereby blocking the interaction with LEDGF/p75 and interfering not directly with the catalytic

activity of integrase. LEDGINs not only reduce the replication capacity of HIV particles produced in their presence. They also modulate impair the formation of regular cores during the maturation step, resulting in a decreased infectivity of the viral particles in the target cells. LEDGINs thus profile as unique antivirals with combined early (integration) and late (assembly) effects on the HIV replication cycle (Desimmie 2013, van Bel 2014). There is no doubt that LEDGINs are still early in development. A literature review, however, revealed that almost all major pharmaceutical companies active in the treatment of HIV/AIDS have taken a significant interest in this class. As a result, several of these inhibitors may soon enter clinical trials (Demeulemeester 2014).

BI 224436 acts through a mechanism that is distinct from that of INSTIs. Based on a promising biological and pharmacokinetic profile, BI 224436 was advanced into phase 1 clinical trials (Fenwick 2014). Results are pending.

Cabotegravir (GSK-774) is probably more than a backup for dolutegravir. It is now mainly tested as a long acting drug (see above).

GS-9224 is an analog of GS-9160, a previously reported investigational INSTI. GS-9224 was designed in an effort to optimize the pharmacokinetic profile of GS-9160 while retaining its antiviral potency (Jones 2014).

MK-2048 is a second-generation integrase inhibitor by MSD with presumably limited cross-resistance to raltegravir (Bar-Magen 2011, Van Weesenbeeck 2011). It also is being looked at for PrEP.

Out of sight, out of mind: integrase inhibitors no longer being studied:
- BMS-707035, probably no advantage over raltegravir
- GSK-364735, liver toxicity in monkeys, stopped in Phase I in 2007
- L-870810 (Merck), liver toxicity in dogs
- S-1360 (Shionogi/GSK), stopped in 2005 due to toxicity

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New entry inhibitors

As mentioned above, each of the three steps of HIV entry can theoretically be inhibited. Step 1 is inhibited by attachment-inhibitors, step 2 by co-receptor antagonists and step 3 by fusion inhibitors. All three drug classes are currently called entry inhibitors. Two entry inhibitors have already been licensed, namely the fusion inhibitor T-20 and and the co-receptor antagonist maraviroc (see Chapter 2). Even if the antiviral effects of the drugs are not overwhelming, the concept is intriguing and entry inhibitors could open up new possibilities for the treatment of HIV infec-

tion in the coming years. On the other hand, a lot of the data below does not go beyond basic science at this stage and many of the drugs discussed may eventually disappear.

New attachment inhibitors

Attachment of the viral glycoprotein gp120 to the CD4 receptor is the first step in the entry of HIV into the target cell. In theory, this step can be inhibited by at least two different mechanisms, namely blocking either gp120 or CD4. Both modes of action are currently under investigation. Consequently, attachment inhibitors are very heterogeneous and it is not possible to speak of a single drug class.

Since the beginning of the nineties, there have been a number of investigations into soluble CD4 molecules that prevent the attachment of HIV to the CD4 cell (Daar 1990, Schooley 1990). But, after disappointing results (probably due to the very short half-life of soluble CD4), this approach was abandoned for a time. With the growing knowledge of the mechanism of HIV entry, as well as following the success of T-20, the development of attachment inhibitors has been reinvigorated. However, most drugs are not yet very advanced, often have problematic PK data, and are therefore still in the proof-of-concept stage. There is some evidence for some polymorphisms in the gp120 gene associated with *in vitro* resistance to attachment inhibitors (Charpentier 2012).

Fostemsavir (BMS-663068) is an attachment inhibitor from BMS. It is a prodrug of **Temsavir (BMS-626529)**, with a broad range of efficacy against several HIV isolates (Nowicka-Sans 2011). It is the replacement for **BMS-488043,** stopped in 2004 after first clinical data were released (Hanna 2004). As a small molecule fostemsavir binds very specifically and reversibly to HIV gp120 and thereby prevents attachment of HIV to the CD4 cell. Thus, it does not bind to CD4 like ibalizumab (see below). This agent drew a lot of attention at CROI in 2011 (Nettles 2011). 50 treatment-naïve patients received different doses once or twice daily over 8 days. Viral load decreased by 1.2 and 1.8 logs – the maximum reduction in both arms was achieved a few days after treatment had concluded. Unfortunately, no dose-related dependence was observed and inter-individual bioavailability was high. Headaches (44%) and rash (16%, mostly mild) were most frequent. AI438011 is an ongoing Phase 2b, randomized trial investigating different doses (600–1200 mg QD or BID) of fostemsavir versus atazanavir/r (plus TDF and raltegravir) in 251 treatment-experienced patients. Through Week 48, fostemsavir showed similar efficacy to atazanavir/r. All fostemsavir doses were generally well tolerated with no dose response safety signals reported, thus supporting the continued development of fostemsavir (Lalezari 2014, Thompson 2015).

Resistance occurs quickly as the binding site of gp120 is one of the most variable gene regions of all (Madani 2010). Fortunately, no resistance to temsavir was selected on monotherapy with fostemsavir (Ray 2013). However, another study showed that some patients without previous treatment with attachment inhibitors developed resistance to temsavir due to subtype-related polymorphisms in the gp120 region (Charpentier 2012). Recently, the genotypic correlates of susceptibility to temsavir have been characterized (Zhou 2014).

Ibalizumab (formerly TNX-355 or HU5A8) is a monoclonal antibody that binds to the CD4 receptor preventing entry of HIV. The mechanism of action has not been clearly described. In contrast to other attachment inhibitors, ibalizumab does not seem to prevent binding of gp120 to CD4, but rather through conformational changes and thereby the binding of gp120 to CXCR4. Some experts describe it as a co-receptor antagonist. It is administered intravenously. Following the initial early

studies (Jacobsen 2004+2009, Kuritzkes 2004), data from a placebo-controlled Phase II trial were very encouraging (Norris 2006). In this study, extensively pretreated patients received ibalizumab as an infusion every two weeks for a year in two different doses (10 mg/kg or 15 mg/kg) or placebo in addition to an optimized ART regimen showed a long-lasting decrease in viral load of approximately one log after 48 weeks in both arms.

Following this, ibalizumab appears to be one of the more promising agents in HIV medicine. There seems to be an inverse correlation between the sensitivity for ibalizumab and soluble CD4, which does not work on its own, as shown above (Duensing 2006). Resistance causes a higher sensitivity towards soluble CD4 and the gp120 antibody VC01, which is why attempts were made to administer ibalizumab in a cocktail of CD4 and VC01 (Pace 2011). First data on resistance have been published (Toma 2011). However, one issue will be whether binding to CD4 will affect the functionality of the CD4 T cells. There have been no negative effects reported so far and it seems that the binding site for ibalizumab to CD4 receptors is localized differently from the molecules. The CD4 T cells may be able to function normally, even if ibalizumab occupies the HIV binding site.

Originally ibalizumab was being developed by Tanox Biosystems (Houston, USA) and later taken over by the biotechnology company Genentech in 2007. ACTG passed on sponsoring the Phase III trials. In mid-2007 Genentech sold the license for ibalizumab to TaiMed Biologics, a Taiwanese biotech company – they are presently planning Phase IIb trials in Europe and the USA. According to www.clinicaltrials.gov, however, the only study currently running is a trial on subcutaneous injections in the setting of pre-exposure prophylaxis.

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New co-receptor antagonists

In addition to CD4 receptors, HIV also requires so-called co-receptors to enter the target cell. The two most important ones, CXCR4 and CCR5, were discovered in the mid-90s (see Chapter 2). Both receptors can be blocked. In 2007, maraviroc was licensed as the first CCR5 antagonist. These small molecules are given orally and bind allosterically to the receptor. Besides these allosteric inhibitors there are monoclonal antibodies binding directly to the receptor. Below we will discuss those agents with published data.

CCR5 antagonists (small molecules)

Cenicrivoc (**TBR-652** or formerly **TAK-652**) is an orally-available CCR5 antagonist by the Japanese company Takeda that has now been bought by Tobira. Laboratory data demonstrated that several mutations in the V3 region (and in the *env* gene) must exist for complete resistance towards TAK-652. Tropism does not seem to change when resistance occurs (Baba 2007). Oral bioavailability is good and with a half-life of 35–40 hours a once-daily dosage is possible (Martin 2012). The oral availability is improved with food intake. TBR-652 also seems to be effective against CCR2, a receptor on monocytes, dendritic and memory T cells that may have anti-inflammatory properties as well. There are no concerns regarding its safety and the substance has shown good tolerability in healthy volunteers (Palleja 2009). In a first double-blind dose-ranging study of 10 days monotherapy in 54 patients, the viral load decreased by a maximum of 1.5–1.8 logs (Lalezari 2011, Marier 2011). Another Phase II study on 150 patients, in which different doses of cenicriviroc are compared with efavirenz (all patients received TDF+FTC), 73–76% and 71% of the patients achieved a viral load of less than 50 copies/ml, respectively. However, virological failure was more frequent with cenicriviroc (12–14% versus 4%). Tolerability was good (Gathe 2013).

PF-232798 is an orally available CCR5 antagonist by ViiV Healthcare. It has a long half-life and can probably be administered once daily. *In vitro* it reacts well to maraviroc resistance (Stupple 2011). In healthy volunteers, it was well tolerated (Dorr 2008).

SCH-532706 is a new CCR5 antagonist from Schering (now Merck). At first, there seems to be no advantage of this agent over vicriviroc. A total of 12 patients receiving 60 mg of SCH-532706 (with 100 mg ritonavir) showed a reduction of viral load of up to 1.6 logs at 15 days (Pett 2009). A once-daily administration seems possible. There may be a positive effect on immune activation (Pett 2010) – however, facing the experiences with vicriviroc, it seems unlikely that this agent will be further developed.

Vicriviroc (SCH-D) was a CCR5 antagonist from Schering-Plough. Clinical development of this promising substance was halted in July 2010 after a pooled analysis of two Phase III trials, VICTOR E3 and E4 (Gathe 2010). A total of 721 pretreated patients received 30 mg vicriviroc or placebo in an optimized therapy containing mainly darunavir/r and/or raltegravir. No difference was observed after 48 weeks (64% versus 62% below 50 copies/ml). Despite obvious differences in patients who only had two active drugs (70% versus 55%), Merck decided to stop development of vicriviroc. We mention this because it clearly shows the problems new agents will face in the future. With the improvement of therapies over time, it is becoming more and more difficult to show positive effects – background therapies have become "too" good.

Other innovative CCR5 blockers

HGS004 (CCR5mAb004) developed by Human Genome Sciences is a monoclonal antibody showing a high resistance barrier *in vitro* (Giguel 2006). The half-life is approximately 5–8 days, and 80% of the receptors are occupied over a period of up to 4 weeks after a single dose. In an initial trial, 54 ART-naïve patients received a single infusion between 0.4 and 40 mg/kg HGS004 or placebo (Lalezari 2008). More than half the patients in the higher dose arms showed a reduction of at least one log at 14 days. There are reports of synergistic effects with maraviroc (Latinovic 2011a). Possibly, the company has now turned their attention to HGS101, which is even more effective *in vitro* and in addition effective against maraviroc-resistant virus (Latinovic 2011b).

PRO 140 is a monoclonal antibody by the company Progenics, directed against human CCR5 receptors (Trkola 2001). It is not a chemokine derivative like maraviroc or vicriviroc and even seems to have a synergistic effect (Murga 2006). The resistance barrier is probably high (Jacobsen 2010). PRO 140 is administered intravenously. The normal function of CCR5 receptors should not be interfered with, at least not in the required dosages for inhibition of HIV replication (Gardner 2003). Healthy patients showed excellent tolerability to intravenous single administration of the drug and dose-dependent concentrations were measured (Olson 2005). Of note, CCR5 receptors were occupied for up to 60 days and more (Olson 2006). In a trial with 39 HIV+ patients treated with intravenous single doses of between 0.5 and 5.0 mg/kg, viral load decreased with the highest doses by 1.83 logs with a nadir at day 10 (Jacobson 2008). A higher dosage does not seem to achieve more (Jacobsen 2010). Comparable effects are reached with weekly subcutaneous administration (Jacobson 2010). PRO 140's tolerability seems excellent, and it has the possibility of being a weekly therapy (Tenorio 2011).

ESN-196 is a pilot agent developed by Euroscreen, which does not block the co-receptor, but is agonistic, like the chemokine RANTES, causing internalization of the receptor (Ferain 2011). This CCR5 agonist reduces the receptor density on the cell surface. Therefore, it is as effective as maraviroc *in vitro*. As an agent with an extended effect, it could become an alternative, if proven safe in clinical trials.

Aprepitant (Emend®) is approved as an antiemetic in patients receiving highly emetogenous chemotherapy. It apparently has an effect on R5-tropic viruses caused by a down-regulation of the CCR5 receptors. Lab data showed dose-dependent effects on HIV replication (Wang 2007, Manak 2010). In a first clinical trial in HIV+ patients, however, no effects on plasma viremia were found (Tebas 2011).

CXCR4 antagonists

In the early stages of infection, the R5 virus is found in most patients; X4 virus appears at later stages. X4 viruses are found in approximately 50% of cases in intensely pre-treated patients (Hoffmann 2007). This is why theoretically the blocking of CXCR4 receptors seems so attractive – those patients with limited options would benefit most. The combination with CCR5 antagonists seems to be an interesting option. However, the development of CXCR4 antagonists is less advanced than that of the CCR5 antagonists (Peled 2011). This is mainly because theoretically, less clinical consequences are feared with the CCR5 blockade – individuals with a CCR5 genetic defect are healthy; although, an inherent and mostly harmless defect with CXCR4 in humans, has not been seen. CXCR4 blockade had severe consequences in animal testing, for example in angiogenetic hematopoiesis or brain development (Tachibana 1998, Nagasawa 1998, Zou 1998).

Years of basic research will be necessary until large clinical studies can be attempted. Nevertheless, several chemically different substances are in preclinical testing (Jenkinson 2010, Miller 2010, Skerl 2010, Steen 2010, Thakkar 2010, auerbach 2012, Vinader 2013). Despite the hurdles, CXCR4 antagonists seem to be a promising class. Research has shown an interesting side effect: some agents are able to mobilize stem cells. This is why one of the pilot drugs, AMD 3100, presently under the name plexifor, is being further developed as a growth factor for leukocytes as well as a G-CSF alternative (Kean 2011, Ratajczak 2011). Such an effect, however, is obviously not desired in permanent HIV therapy. CXCR4 antagonists are also being discussed in lupus erythematodes therapy (Chong 2009).

AMD 11070 is a CXCR4 antagonist developed by AnorMED. Healthy volunteers showed good tolerability with AMD 070, but often developed leukocytosis (Stone 2004). The efficacy in HIV+ patients with dual-tropic viruses was validated in two pilot studies (Moyle 2007, Saag 2007). Viral load was lowered by at least one log in 7/15 patients on 10 days of monotherapy. However, in 2007, development was stopped because of liver toxicity. Binding to the X4 receptor is localized differently than the precursor agent AMD 3100, so there may be some scope for development of new, more potent and less toxic CXCR4 antagonists (Wong 2007) – at least a start was made with AMD 11070 and evidence of efficacy was found. Presently AMD 3465 seems to be another possibility (Bodart 2009).

KRH-3955 and **KRH-3140** are two CXCR4 antagonists that have proven effective in mouse models (Tanaka 2006). According to preclinical data, KRH-3955 seems especially promising (Murakami 2009) and bioavailability is good in dogs and monkeys (Nakasone 2013). Likewise, **POL3026** is still preclinical and may help inhibit selected X4 shifts while on CCR5 antagonists (Moncunill 2008).

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New fusion inhibitors

Since the first fusion inhibitor (FI) T-20, there has been little development in the field (Review: Berghout 2012). Subcutaneous injection required for many FIs is unappealing for patients and clinicians. It still needs to be demonstrated, whether small molecule FIs, i.e., that are orally bioavailable, are effective (Jiang 2004). Addition of a cholesterol group to an HIV-1 peptide fusion inhibitor may dramatically increase its antiviral potency (Ingallinella 2009).

Sifuvirtide is an FI developed in China. In animal testing with monkeys a longer half-life (39 hours) and a higher affinity towards gp41 rather than towards T-20 was observed (Dai 2005). Sifuvirtide was well-tolerated in healthy patients (He 2008) and interesting synergetic effects were reported with T-20 (Pan 2009). However, there seem to be some cross-resistances (Liu 2010, Yao 2012). This may not be the case for the newer FI **albuvirtide,** from China as well (Chong 2012).

SP01A from Samaritan Pharmaceuticals is especially interesting because its effects are different from other entry inhibitors. As a procaine hydrochloride, SP01A reduces the expression of the key enzyme HMG-CoA reductase, removes cholesterol from the cell membrane and seems to inhibit, not only *in vitro*, the fusion of virus and cell. The efficacy of this agent, which has been repeatedly tested in HIV+ patients for years, was shown in three Phase II trials. Results were moderate, showing that only 50% of patients have a reduction of viral load at the highest doses of 800 mg. After 10 days of monotherapy, viral load fell by 0.4 logs and after 28 days by 0.5 logs. These results were published in July 2007 on the company's website (www.samaritanpharma.com). No news since then.

TR-999 and **TR-1144** are two 2nd generation fusion inhibitors, developed by Trimeris in cooperation with Roche (Delmedico 2006). According to studies in monkeys, the potency, duration of action and pharmacokinetics of these peptides are much improved in comparison to T-20. Although administration is still by injection, it may be possible to limit this to once a week. They have only been involved in one clinical trial since 2007, with data due in 2008. They have stopped developing both compounds and were looking for buyers/investors.

Virip blocks entry of HIV-1 into the cell by interacting with the gp41 fusion peptides. It is also called an anchor inhibitor. Researchers from Ulm, Germany, discovered the peptide in hemofiltrate, the liquid that is filtered out of the blood of dialysis patients, when replacing it with electrolytic solution. Thus virip is a "natural" entry inhibitor whose antiretroviral activities can significantly be increased by slight modifications or replacement of certain amino acids (Munch 2007). Several modified agents are currently under investigations, such as **Virip-576** and **-353**. In a first study with HIV+ patients, continuous infusion with the highest dosage of Virip-576 led to a reduction of approximately 1 log at 10 days (Forssmann 2010). Tolerability was good and a subcutaneous application is presently being evaluated. However, potential resistance mutations have been shown (González-Ortega 2011).

Out of sight, out of mind – entry inhibitors not moving forward:
• AMD 3100 (CXCR4A) from AnorMed, due to cardiotoxicity
• Aplaviroc (CCR5A) from GSK, due to hepatotoxicity
• BMS 806, BMS-488043 two attachment inhibitors, due to poor PK data
• FP-21399 (FI) from Lexigen/Merck, due to low potency
• INCB9471 from Incyte
• PRO-542 from Progenics, to focus on PRO-140
• SCH-C/Ancriviroc (CCR5A) from Schering-Plough, due to cardiac arrhythmia
• T-1249 and T-649 (FIs) from Roche/Trimeris, due to little prospect of success
TAK-779, TAK-220 (CCR5A) from Takeda, replaced by TAK-652

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New maturation inhibitors

The so-called maturation inhibitors stop HIV replication in a very late phase of the HIV reproduction cycle, i.e., at the budding or maturation of new virions (review: Salzwedel 2007). As is the case for integrase inhibitors, 2005 can be called the introductory year: this was the first time an agent was shown to have an antiviral effect on HIV+ patients. Maturation inhibitors are, without a doubt, an interesting class of new drugs. Whether any of the agents will make it out of the clinic remains uncertain, as several problems have surfaced during the development of the prototype, bevirimat. However, these problems can be overcome with new agents (Urano 2014).

Bevirimat (MPC-4326, formerly PA-457) is a derivative of betulinic acid, which is isolated as triterpene carbonic acid from birch bark. It was produced by Panacos, which was sold to Myriad Pharmaceuticals. Bevirimat inhibits budding or maturation of new virions (Li 2003) by inhibiting the transition of the capsid precursor (p25) into the mature capsid protein (p24). This prevents the production of infectious viruses. Its long half-life allows once daily dosing (Martin 2007, Smith 2007). Tolerability of bevirimat in more than 650 patients has been good, including in the presence of atazanavir (Martin 2008).

Data from a placebo-controlled Phase IIa trial was published in autumn 2005, in which patients received an oral once-daily monotherapy of bevirimat for 10 days (Beatty 2005). In the highest dose group (200 mg) viral load decreased by 1.03 logs (median); in the 100 mg group it was just 0.48 logs. However, some patients showed no effect on the viral load, which can be ascribed to "natural" polymorphisms in the gag gene (van Baelen 2009, Lu 2011). Patients harbouring viruses with no gag polymorphisms (mutations) on the positions Q369, V370 or T371 before therapy responded better to bevirimat. In a more recent monotherapy study with 32 patients receiving higher dosing, a reduction of viral load of 0.54 and 0.7 logs respectively, was observed with 200 or 300 mg after 14 days. Without the polymorphisms, effects were greater than the one log level, those with the polymorphisms had a drop of only 0.2 logs (Bloch 2009). Only about 50-70% of all individuals tested, seemed not to have these gag polymorphisms. There appears to be no difference between treatment-naïve and pre-treated patients, nor is there any influence due to the degree of the underlying immunodeficiency (Margot 2009, Knapp 2009, Seclén 2010). However, there seems to be a strong correlation with PI resistance (Verheyen 2010, Fun 2011).

This shows clearly the need for tests on these gag polymorphisms before starting therapy with bevirimat and possibly with other maturation inhibitors – not unlike the tropism test with CCR5 antagonists. In June 2010, Myriad announced that they will not continue to develop bevirimat. However, newer in vitro data on maturation inhibitor show that this potential drug class remains a focus for ongoing research. Second-generation maturation inhibitors may overcome the problem of the gag polymorphisms (Urano 2014).

BMS-955176 looks much more promising than bevirimat, with retained activity even against viruses with baseline gag polymorphisms. In a Phase IIa, randomized, trial, activity and safety of 10 days of monotherapy with BMS-955176 (dosages 5-120 mg OD) was evaluated in 40 HIV+ patients. There was an increase in maximum median response over the range of 5–40 mg, which plateaued at 1.64 logs at doses of 40–120 mg. Maximum median declines in HIV-1 RNA were similar for the 40–120 mg once-daily dose groups regardless of baseline gag polymorphisms such as V362, Q369, V370, and T371 (Hwang 2015).

BIT-225 from the Australian company Biotron is a specific HIV replication inhibitor in macrophages, but not in T cells (Khoury 2007). It works with a different mechanism than the Vpu ion channel inhibitor and inhibits the release of viral particles from macrophages. BIT-225 could play a role in eradication from latent cell reservoirs. According to Biotron, a successful Phase I study ended in September 2007, showing no relevant toxicity with 40 healthy volunteers receiving doses of 35–400 mg and providing acceptable PK data.

Vivecon (MPC–9055) is, like bevirimat, a maturation inhibitor by Myriad Pharmaceuticals in Salt Lake City, US. The agent demonstrated good tolerance and acceptable pharmacokinetics with 55 healthy volunteers (Beelen 2009). After the end of bevirimat, development of MPC-9055 seems unlikely.

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Immunotherapy

In recent years, in addition to ART, immunomodulatory treatment strategies have been investigated. Although repeatedly discussed as an alternative or supplement, these therapies lack proof of clinical benefit. An important example is the failure of the two large IL-2 studies (see below). Some approaches are nevertheless addressed here briefly (in alphabetic order).

Corticosteroids do not stand the test of controlled studies. In a placebo-controlled study with 0.5 mg prednisone/kg over 8 weeks, there were no effects on CD4 T cells or viral load (McComsey 2001). In ACTG 349, 24 patients were treated with 40 mg prednisone daily or not in a double-blind randomized design (Wallis 2003). After 8 weeks, there was a trend towards higher levels of CD4 T cells in the prednisone arm, but there were no effects on activation markers or apoptosis. Two patients on prednisone developed necrosis of the femoral head. This study should caution anyone before considering steroids for immunological reasons.

Cyclosporin A (Sandimmune®) – Immune activation may lead to increased HIV replication, and a treatment hypothesis has been to suppress the immune system in an attempt to slow down viral replication. This is the rationale behind studies investigating the use of cyclosporin A, a drug normally used for prophylaxis of transplant rejection after allogenic organ transplantation. However, results of clinical trials have been disappointing. Cyclosporin A had no effect on CD4 or CD8 T cell count, nor on expression of activation markers (Calabrese 2002, Lederman 2006). This was not only the case in chronically but also in acutely infected patients (Miro 2009, Markowitz 2010). Cyclosporin A therefore probably has a limited future in HIV therapy.

G-CSF (granulocyte colony-stimulating factor) is available as filgastrim (Neupogen®), lenogastrim (Granocyte®) and most recently as less expensive biosimilars (in Europe). It is also licensed for permanent neutropenia in advanced HIV infection to avoid bacterial infection. In a randomized study with 258 HIV-infected patients with CD4 T cells under 200/µl, the rate of severe neutropenia was 2% versus 22% in the control group after 24 weeks (Kuritzkes 1998). Incidence of bacterial infection was reduced by 31% and the number of inpatient days dropped by 45%. No effects on viral load

were seen. Patients with CMV retinitis showed a large survival benefit on G-CSF (Davidson 2002). Although severe neutropenia has become rare on ART, G-CSF can be useful, especially in chemotherapy, with interferon or other myelo-suppressive drugs such as valgancyclovir.

GM-CSF (granulocyte macrophage colony-stimulating factor) is available as molgramostim (Leucomax®) or sargramostim (Prokine®). Three double-blind, randomized studies showed a slight effect on viral load (Angel 2000, Skowron 1999, Brites 2000). However, in one study in patients with uncontrolled infection, there was a slight increase of viremia (Jacobsen 2003). GM-CSF seems to prevent significant loss of CD4 T cells during treatment interruptions (Fagard 2003). Given the side effects and significant cost of GM-CSF, it cannot be recommended outside clinical studies. GM-CSF is not licensed in Europe.

Hydroxyurea (HU, Litalir®) is an old chemotherapeutic agent with relatively low toxicity still being used today in hematology (mostly in chronic myelogenous leukemia). It inhibits DNA synthesis via the ribonucleotide reductase, and leads to an intracellular shortage of deoxynucleotide triphosphates. A synergistic effect on HIV replication in combination with ddI was demonstrated in 1994 (Lori 1994). A Swiss study, in which 144 patients were treated with hydroxyurea (HU) or placebo plus d4T+ddI, attracted attention in 1998 (Rutschmann 1998). After 12 weeks, 54% (versus 28% in the placebo group) demonstrated a viral load below 200 copies/ml. Was this the discovery of a new cheaper option for HIV treatment? Hydroxyurea became even more fashionable after publication of the first "Berlin Patient", a patient who had been treated with hydroxyurea in addition to indinavir and ddI during acute infection, had stopped all therapy after a few months and subsequently showed no detectable plasma viremia (Lisziewicz 1999). Was this unexpected outcome due to hydroxyurea? Several small studies from the US and Argentina seemed to confirm these positive results. Many treating physicians added the drug to ART and many started to dream of a cheap combination of ddI+HU for Africa. These initial hopes subsided rapidly. In particular, the combination of HU with ddI and d4T turned out to be particularly toxic: severe polyneuropathy (Moore 2000) and fatal pancreatitis were reported (Havlir 2001). Three randomized studies failed to show any effect, except for high rates of toxicity (Blanckenberg 2004, Stebbing 2004, Swindels 2005). Even in patients with acute HIV infection there was no effect. Thus, more Berlin patients could not be "reproduced" (Zala 2002). Hydroxyurea should not be used in antiretroviral therapy.

Interferons have an antiretroviral effect that has been known for years (Mildvan 1996). The antiviral effect of 3 million IU daily or with pegylated interferon weekly is about 0.5-1 log (Haas 2000, Hatzakis 2001, Asmuth 2010). Higher dosing may increase this effect (Hatzakis 2001). We have seen patients coinfected with HIV/HCV, who achieved an undetectable HIV RNA during hepatitis C therapy with interferon and ribavirin only. However, an in-depth investigation of the antiviral activity of interferon was not conducted, because of the subcutaneous delivery route and its not insignificant side effects. Recently, interferons seem to be experiencing a comeback, as they may acheive importance as an immune modulator and play a role in eradication (Papasavvas 2012, Mexas 2012). In one trial, 9 out of 20 patients who received pegylated interferon and interrupted ART, showed viral load below 400 copies/ml after 12 weeks of IFN monotherapy (Azzoni 2013).

Interleukin-2 (IL-2, aldesleukin, Proleukin®) is a cytokine produced by activated T cells that induces proliferation and cytokine production in T cells, B cells and NK cells. It has been employed in oncology for years and is now usually administered

subcutaneously. The most important effect of IL-2 in HIV medicine is the increase in CD4 and CD8 T cells, which may be quite impressive in individual cases. CD45RO memory cells initially increase, followed by naïve CD45RA T cells (Chun 1999, Carcelain 2003). This effect is mainly due to a reduced T cell turnover (Kovacz 2005, Sereti 2005, Vento 2006).

The question of whether the CD4 T cells generated by IL-2 would lead to clinical benefit, was answered by two large randomized studies, ESPRIT and SILCAAT, in 2009 (Abrams 2009). In the ESPRIT study, 4,131 patients with at least 300 CD4 T cells/µl were treated with and without IL-2 in addition to ART. SILCAAT had a similar concept, but enrolled 1695 patients with 50-299 CD4 T cells/µl. The results were very disappointing. Although supplementation of ART with IL-2 resulted in a statistically significant increase in CD4 T cell count (ESPRIT: +160, SILCAAT: +59 CD4 T cells/µl), it did not lead to a clinical benefit. Despite improved CD4 T cells with IL-2, patients did not develop less opportunistic infections and mortality was not reduced. Moreover, serious adverse events (including fever, malaise, injection site reactions and deep-vein thrombosis) were more likely to occur among patients receiving IL-2 in the ESPRIT study. Another randomized study (STALWART) provided similar results (Tavel 2011). Conclusion: IL-2 as a supplementary therapy in HIV+ patients is no longer viable.

Interleukin-7 may be more promising. This cytokine plays a fundamental role in T cell homeostasis and is implicated in thymopoiesis and in peripheral expansion and survival of T lymphocytes (Review: Chahroudi 2010). Two small randomized placebo-controlled pilot trials with 6 and 16 HIV+ patients, respectively, demonstrated a good increase of CD4 T cells with different subcutaneous doses. The tolerability was good and side effects typical for interleukin-2 were not observed (Levy 2009, Sereti 2009). Another small studies showed promising results (Lévy 2013). If these results are confirmed, interleukin-7 may become an option for patients whose immune constitution remains poor despite good viral load suppression on ART.

Interleukin-12 stimulates T lymphocytes and NK cells to generate a Th1-type immune response. In a randomized Phase I study with rhIL-12 100 ng/kg 2 x week, the drug was well tolerated but had no effect on lymphocyte subpopulations, antigen-specific immune response or viral load (Jacobson 2002). Further development has not happened. The same would appear to be true for **interleukin-10** (Angel 2000) or **interleukin-15** (Ahmad 2005). In the age of highly effective antiretroviral therapies, such experimental therapies have to meet ever-increasing standards.

Murabutide is a synthetic muramyldipeptide with a variety of effects on the immune system. It can raise unspecific resistance to infection, induce anti-inflammatory cytokines and growth factors, and strengthen the antiviral effects of cytokines such as IL-2 or interferon. In HIV+ patients, a team in France has used it mainly as an immune modulator, although only in small studies, and at best, with moderate results (Bahr 2003).

Mycophenol (Cellcept®) has a theoretical concept similar to that of hydroxyurea and cyclosporin A. Mycophenol inhibits inosine monophosphate (IMP) dehydrogenase and is normally used for prophylaxis of acute transplant rejection in patients with allogenic kidney, heart or liver transplants, as well as for some autoimmune diseases. Inhibition of lymphocyte proliferation and the subsequent reduction of target cells should theoretically inhibit replication of HIV. Initial reports seem to demonstrate an effect on viral load, at least in some patients (Margolis 2002, Press 2002). Whether this will be confirmed by randomized trials seems uncertain. More current data suggest that this is unlikely (Sankatsing 2004, Margolis 2006).

Neutralizing antibodies: First generation monoclonal antibodies were clinically ineffective. However, single-cell-based antibody cloning methods have recently uncovered a new generation of far more potent broadly neutralizing antibodies (BNAbs) to HIV that have shown prophylactic and therapeutic activities in animal models. In a first-in-man dose escalation Phase I trial, **3BNC117**, a potent human CD4 binding site antibody, was well tolerated. A single 30 mg/kg infusion reduced the viral load by 0.8-2.5 logs and viraemia remained significantly reduced for 28 days. Emergence of resistant viral strains was variable, with some individuals remaining sensitive to 3BNC117 for a period of 28 days. It is clear that with these results, immunotherapy will be explored as a new modality for HIV-1 prevention and therapy (Caskey 2015).

Remune®, the prototype of therapeutic vaccination, has gone from disaster to disaster. Developed by a team headed by the since-deceased Jonas Salk, Remune® was a therapeutic vaccine comprised of an envelope-depleted (gp120) virus which, although indeed immunogenic, does not seem to provide any clinical benefit (i.e., prolongation of life or delay of disease progression). A large trial was interrupted prematurely in May 1999. More than 2500 patients had taken part for a mean of 89 weeks in this study, which was designed to evaluate the addition of Remune® to ART. As well as the lack of clinical benefit, advantages with respect to CD4 T cell counts or viral loads could not be shown (Kahn 2000).

THC, cannabinoids have no anti-HIV effect. A controlled, randomized study, in which patients could either smoke marijuana or receive THC (dronabinol, Marinol®) or placebo in addition to ART, showed no effects on lymphocyte subpopulations, lymphocyte function or viral load after three weeks (Bredt 2002). THC, which is metabolized via the cytochrome P450 system, had no detrimental effects on PI plasma levels (Abrams 2003). One randomized study showed that smoking cannabis was well-tolerated and effectively relieved chronic neuropathic pain from HIV-associated sensory neuropathy. The findings were comparable to oral drugs used for chronic neuropathic pain (Abrams 2007).

Vitamins: It remains a matter of debate whether the addition of micronutrient supplements to ART may provide clinical benefits. Vitamins may even be harmful. In a large, double blinded, randomized study in Africa, 3.418 HIV+ patients received high-dose vs standard-dose multivitamin supplementation (vitamin B complex, vitamin C, and vitamin E) for 24 months (Isanaka 2012). High-dose multivitamin supplements did not result in a decrease in HIV disease progression or death. The study was stopped early in March 2009 because of increased ALT levels in patients receiving the high-dose multivitamin supplement. However, in another randomized study in Botswana on 878 patients, 24 months with a single supplement containing multivitamins and selenium was safe and significantly reduced the risk of immune decline and morbidity (Baum 2013).

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6.4. Goals and principles of therapy

CHRISTIAN HOFFMANN

With current antiretroviral therapies, eradication of HIV is not possible. The ultimate goal in HIV medicine – a cure – is not a realistic scenario in the immediate future, although more and more effort and time is being spent in the cure arena. Thus, the current (and realistic) goal of ART in 2015/16 is to prolong the patient's life and maintain the best possible quality of health and life. Patients and physicians most likely have to deal with lifelong treatment. Lifelong, meaning decades, as many epidemiological studies suggest a normal life expectancy for HIV+ patients (Obel 2011, Nakagawa 2012). The goals and principles of lifelong treatment will be discussed in the first part of this. The treatment goal "HIV cure" will be discussed separately in the second part.

6.4.1. Success and failure of lifelong treatment

Both success and failure of treatment can be evaluated using the same criteria – virologic, immunologic or clinical. The first indicator, virologic, is the change in viral load. This is followed, often a little later, by immunologic markers (rise or fall in CD4 T cell count). Clinical outcome usually only becomes apparent much later – first the lab values deteriorate, then the patient; or vice-versa, as lab values get better, the patient generally follows. The clinical success of ART for asymptomatic patients is often not perceived, although the risk of opportunistic infections is reduced to half after only three months on ART (Ledergerber 1999) – the individual may not realize what was avoided by starting therapy.

Virological treatment success and failure

On ART, viral load declines in at least two phases (see Monitoring). An initial, very rapid decrease in the first few weeks is followed by a longer phase, in which plasma viremia declines slowly. Virological treatment success is usually understood as being the reduction of viral load to below the level of detection (usually 50 copies/ml). This should be reached after 3–4 months; in cases of very high baseline viral load it may take longer. However, a viral load above the level of detection after six months of treatment almost always needs to be evaluated. The same is true if a rebound in viral load is confirmed.

The more rapid and greater the decrease in viral load, the longer the therapeutic effect (Kempf 1998, Powderly 1999). In the early INCAS Trial, the relative risk of treatment failure in patients who had reached a viral load below 20 copies/ml was 20 times lower than in those who never reached 400 copies/ml (Raboud 1998).

Virologic treatment failure can be recognized quite early. In practice, viral load should be monitored after four weeks on ART. This is useful not only to the patient for reasons of well-being ("less virus, more CD4 cells"). But it is also an important indication for the continued success of treatment. If the viral load is not below 5,000 copies after four weeks of ART, later treatment failure is likely (Maggiolo 2000). If the viral load is not below 500 copies/ml or at least one log below baseline, the likelihood of having a viral load of 500 copies/ml at week 24 is only 9% (Demeter 2001). In ACTG 5202, a less robust week 4 virologic response was associated with higher risk for subsequent virologic failure (Gant 2013). According to another prospective study, virological response can be predicted even after 7 days (Haubrich 2011). However, viral load testing after such short periods of ART in previously untreated patients is not clinical routine.

The cut-off point of 20 or 50 copies/ml is somewhat arbitrary. It is based on the currently available viral load assays. Whether 60 copies/ml are indeed worse than 30 copies/ml and indicate a lower success of treatment has yet to be proven. In the case of a persistent low level viremia (LLV) between 20 and 50 copies/ml, the risk of virological failure seems not to be increased (Charpentier 2012). There are, however, other studies suggesting an association between the level of viremia and virological failure, even at very low levels (Maggiolo 2012, Pugliuese 2013). Thus, the significance of LLV is still a matter of a debate. At such low levels, methodological inaccuracies must also be taken into account. A single detectable viral load "blip" to low levels (~1000 copies/ml) is often irrelevant (see below). Blips need to be distinguished from low, repetitive, measurable plasma viremia (50–400 copies/ml), in which the risk of resistance has been shown to be higher (Gunthard 1998, Nettles 2004, Taiwo 2012). If immune activation and inflammatory parameters are increased in these patients is still controversially discussed (Eastburn 2011, Taiwo 2012, Reus 2013).

A viral load "below the level of detection" of 50 copies/ml means just that – no more, no less. A total of 50 copies/ml indicate that 5 liters of blood contain 250,000 virions; in addition, even more actively replicating viruses are present in the lymphatic organs. Thus, theoretically, a measurable viremia, even at very low levels, may possibly translate to a higher risk of resistance in the long-term. Perhaps there is indeed a relevant difference between 50 and 10 copies/ml with regard to the risk of developing resistance. We just do not know yet.

Risk factors for virological failure are pre-treatment with antiretroviral agents (existing resistance mutations) and low adherence. Whether the baseline CD4 T cell counts or the baseline plasma viremia play a role in treatment-naïve patients has not been conclusively proven (see chapter on When to Start ART). It seems that many other risk factors associated with virological failure or response are not known. A new area in this setting is pharmacogenetic research focusing on how genes influence an individual response to drugs. Investigators have begun to identify associations among human genetic variants, predisposition to HIV drug toxicities, and likelihood of virologic response. These include HLA typing and enzyme polymorphisms (Haas 2006). Pharmacogenomic testing will ultimately benefit persons living with HIV through better individualized treatment.

More good news for today is that morbidity and mortality may be lowered significantly even if the viral load is not decreased to below the level of detection (Grabar 2000, Deeks 2002). Patients often remain immunologically stable for a long time, even with insufficient viral suppression. A large cohort study has shown that CD4 T cells do not drop as long as the viral load remains below 10,000 copies/ml or at least 1.5 logs below the individual set point (Lederberger 2004). However, with the new drug classes much more is possible now than in the 90s. Thus, plasma viremia should be reduced to below the detection limit in all patients.

How long does virological treatment success last?

Little is known about how long treatments remain effective. The belief that treatment success is limited to only a few years is widespread. It originated during the early years of ART. Many patients at the time were inadequately pretreated with mono- or dual-therapy, and had thus developed extensive resistance. In such patients, the effect of treatment was often limited, as even a single point mutation was often enough to topple a whole regimen. Today, especially in therapy-naïve patients without pre-existing mutations, the risk of treatment failure is much less.

After almost 20 years of using combination ART, a very high number of patients still have viral loads below the level of detection. This is particularly true for patients who were adequately treated from the start (starting with triple therapy and/or rapid

switching of several drugs upon failure). One of the few trials with a longer follow-up period studied 336 antiretroviral-naïve patients who had reached a viral load below 50 copies/ml within 24 weeks (Phillips 2001). After 3.3 years, the risk of viral rebound seemed at first glance to be relatively high at 25.3%. More detailed analysis showed that a large proportion of the patients experiencing viral rebound had actually interrupted ART. True virological failure was only seen in 14 patients, which corresponds to a risk of 5.2% after 3.3 years. Most importantly, the risk of virological failure decreased significantly with time.

This is supported by cohort studies showing that the rates of virological failure due to resistance have markedly declined in recent years (Lohse 2005, Lampe 2006). Antiretroviral therapies and treating physicians are getting better and better. As demonstrated by a large cohort study in Europe in 1995–96, 58% achieved HIV-1 RNA of 500 copies/ml or less by 6 months, compared with 83% in 2002–03 (May 2006). Nowadays, most patients have a constant viral load below 50 copies/ml. In many centers today, at least 90% of patients on ART have an undetectable plasma viremia. The cohort in Bonn is a good example. In 2007, only 57 out of 560 (10%) patients on ART showed detectable viremia. In 32 of these patients, adherence problems were a major cause and only 9% had a multiresistant virus (Klein 2009). These studies clearly show that, providing treatment is not interrupted, viral load can remain below the level of detection for many years, probably decades.

Blips – do they mean virological failure?

Blips are understood to be transient and relatively small increases in viral load, where the viral load before and after the blip was below 50 copies/ml. At least three measurements of viral load are therefore required to be able to identify a blip. Blips are a frequent phenomenon of patients on ART and are observed in 20–40% (Sungkanuparph 2005). Blips often worry both patients and clinicians: Is this the beginning of treatment failure? Although a few studies indicate that this is not the case in the medium-term (Havlir 2001, Mira 2002, Sungkanuparph 2005), little is known about the causes of blips. For example, there has been no consistent data about association between compliance and blip frequency. While some studies did not find any association (Di Mascio 2003, Miller 2004), others did (Podsadecki 2007). It is also possible that blips are the result of immunological mechanisms. The earlier patients are treated in the course of infection, i.e., the higher the CD4 T cell count at therapy initiation, the more seldom blips seem to occur (Di Mascio 2003+2004, Sungkanuparph 2005). There does not appear to be any association with particular antiretroviral combinations – in a large cohort study (Sungkanuparph 2005), the frequency of blips on an NNRTI regimen was 34% and 33% on a PI regimen, even the size of the blips were equivalent (median 140 and 144 copies/ml, respectively). In both groups, the risk of virological failure at 2 years was 8%. One important observation of this trial was that blips did not increase the risk of treatment failure, not even on NNRTIs, anticipated due to the rapid development of resistance to NNRTIs. Another team has since confirmed these results (Martinez 2005).

But what do blips actually mean? At the beginning of 2005, a study team led by Bob Siliciano set out to investigate this. In a labor-intensive study (Nettles 2005), 10 stalwart patients who had had a viral load of less than 50 copies/ml for at least six months, had blood samples taken every 2-3 days over a period of 3–4 months. The obvious result: the more you look, the more you find. During the observation time, at least one transient increase in the viral load was measurable above 50 copies/ml in nine of the ten patients. Each blip was moderate, with a median value of 79 copies/ml, ranging from 51 to 201 copies/ml. The blips were not associated with either specific clinical data, low plasma levels, or resistance. This observation led the

authors to believe that blips (with low, measurable values) mainly represent biological or statistical exceptions and are not involved in treatment failure. In an estimated steady state level of viral load at around 20 copies/ml, the values are distributed randomly. However, 96% of the randomly distributed measurements ("random noise") are less than 200 copies/ml. In other words: "Random noise" above 200 copies/ml is unlikely.

Many factors may be responsible for intermittent viremia. It should always be kept in mind that a long sample processing time may lead to apparent low-level viremia (Portman 2012). Sporadic immune activation during concomitant infections may elevate the level of chronically infected cells and replenish viral reservoirs, including the latent reservoir, providing a mechanism for recurrent viral blips and low levels of viremia while on ART (Jones 2007). In one large retrospective analysis, 26% of blips were caused by intercurrent infections (Easterbrook 2002). For example, syphilis can cause a significant increase in viral load and reduction of CD4 T cells (Buchacz 2004). Viral load can also increase temporarily after immunizations (Kolber 2002). Based on available data, blips do not necessitate an immediate change of ART. However, caution should be applied for higher blips (>200–500 copies/ml).

It should be stressed that blips need to be distinguished from low, repetitive, measurable plasma viremias ("low level viremia", LLV), in which the risk of resistance has been shown to be much higher (Gunthard 1998, Nettles 2004, Taiwo 2012). However, every blip should raise the opportunity to talk to the patient about compliance. It cannot be discussed often enough. Does the patient take his or her drugs regularly or are doses occasionally missed? Are the dosing directions (on an empty stomach or with a meal) followed correctly? All these points should be considered before changing therapy prematurely. Each new therapy can cause new problems. Therefore, any suspected increase in the viral load should be controlled within a short interval (two weeks), especially if it is relatively small, before the treatment is changed.

Immunological treatment failure and success

Immunological treatment success is generally defined as an increase in the CD4 T cell count. A more precise definition for immunological treatment success does not currently exist. Depending on the study, increases of 50, 100 or 200 CD4 T cells/µl or increases to above 200 or 500 CD4 T cells/µl are evaluated as a success. Failure is usually described as a lack of increase or reduction of CD4 T cell count in patients receiving ART.

It is difficult to individually predict the immunologic success of therapy for patients on ART, as it varies significantly from one person to another. As with the decrease in viral load, the increase in CD4 T cell count also seems to have two phases. After a first, usually rapid increase over the first three to four months, further increases are considerably less pronounced. In a prospective study involving some 1000 patients, the CD4 T cell counts increased during the first three months by a median of 21.2 cells/µl per month; in the following months the increase was only 5.5 cells/µl (Le Moing 2002). In EuroSIDA, the greatest mean increase in CD4 count of 100 cells/µl per year was seen in the year after starting ART. Significant, but lower, increases, around 50 CD4 T cells/µl per years, were seen even at 5 years after starting ART in patients whose current CD4 T cell count was less than 500 cells/µl (Mocroft 2007). Of course, this might also depend where you start. If you start relatively late in the disease, CD4 T cell recovery will be more blighted than if you start closer to transmission.

It is still under debate whether the immune system is restored continuously after a long period of viral load suppression or whether a plateau is reached after three to

four years beyond which there is little or no expected improvement (Smith 2004, Mocroft 2007, Lok 2010). In our experience, both are possible. There are patients showing immunological improvement even 6-8 years after initiation and there are patients in which CD4 T cells remain stable at a low level. The lower the CD4 count at baseline, the less likely it is to normalize completely (Kaufmann 2005, Robbins 2009). The immune system often does not recover completely. In the Swiss Cohort, only 39% of 2,235 patients who had begun ART in 1996-97 reached a CD4 T cell count above 500/µl (Kaufmann 2003). However, it appears that the increase within the first 3–6 months provides certain clues as to how well the immune system will be restored (Kaufmann 2005). Negative consequences of a low CD4 T cell count at the time of ART initiation are often present for a long time. In one study, 25% of patients who started an ART at lower levels of CD4 T cell count did not reach normal levels of 500 CD4 T cells/µl, even after a decade of otherwise effective ART with good viral suppression (Kelley 2009, Lok 2010).

Immunological treatment success is not necessarily linked to maximal viral suppression; even partial suppression can result in improved CD4 T cell count (Kaufmann 1998, Ledergerber 2004). The initial level of viral load is also not significant. What seems to be important is that the viral load remains lower than before treatment (Deeks 2002, Ledergerber 2004). In view of the numerous factors that occur independent of ART that are able to influence therapy success and individual immuno-regeneration (see below), it is generally not wise to look at the CD4 T cell count alone as the deciding criterion for the success of ART. Virological success is more appropriate for judging the efficacy of specific regimens. Once CD4 T cells have "normalized" and plasma viremia remains undetectable, it is unlikely that they will significantly change (Phillips 2002). In a newer study, patients infected with less than <200 copies/mL and CD4 T cell counts 300 cells/µl had a 99.2% probability of maintaining durable 200 CD4 T-cells/µl for four years (Gale 2013). With good CD4 T cells, immunological treatment success therefore does not require constant monitoring.

Discordant response

Failure to achieve therapeutic goals – in terms of immunologic and virologic success – is referred to as a discordant response. The frequencies of such discordant responses in adults are outlined in Table 4.1.

Table 4.1: Prospective cohort studies, treatment response*

Response to ART	Grabar 2000 n = 2236	Moore 2005 n = 1527	Tan 2008 n = 404
Virological and immunological	48%	56%	71%
Discordant: only immunological	19%	12%	16%
Discordant: only virological	17%	15%	9%
No treatment response	16%	17%	5%

* Immunological response was defined as a rise in CD4 T cells >50/µl after 6 months (Grabar 2000) or at least >100/µl during follow-up (Moore 2005, Tan 2007). Virological response: <1000 copies/ml (Grabar 2000) or <500 copies/ml (Moore 2005) or <50 copies (Tan 2008)

Therapies can be virologically successful without immunological improvement; despite undetectable viral load, CD4 T cell counts remain low (Piketty 1998, Grabar 2000, Moore 2005, Tan 2007). Conversely, ART may be extremely effective immunologically and induce significant increases in the CD4 T cell count, while viral load remains detectable. Although therapies have constantly improved, discordant responses appear in one fourth of all treatment-naïve patients. Especially in patient

groups showing virological success but little immunological improvement, it is often not clear how to continue therapy. Mortality seems to be slightly higher in this patient group, but has not been related to AIDS diseases (Gilson 2010). If there is any increase of AIDS incidence in the setting of discordant response, this is restricted to the first six months (Zoufaly 2011). Different CD4 T cell response kinetics are shown in Figures 1a-1d.

The risk factors for a lack of immunologic response can often not be influenced and are also heterogenic (Review: Aiuti 2006). Low CD4 counts at baseline, as well as a low viral load at treatment initiation are only two factors (Florence 2003, Kaufmann 2005, Moore 2005, Kelley 2009). Age may also play a role. In older patients, immuno-logic response is often only moderate, mainly due to thymic degeneration (Lederman 2000, Grabar 2004). Various studies have demonstrated that the probability of not achieving a rise in CD4 count increases with patient age and with progressive decrease in thymus size as detected by CT (Goetz 2001, Piketty 2001, Teixera 2001). Regulatory T cells (Tregs) may also play a role (Saison 2014).

Other possible causes for a lack of immunological response, despite good viral sup-pression, may be immuno- or myelosuppressive concomitant therapies. We have seen patients with less than 50 CD4 T cells/µl for more than a decade, despite viro-logical suppression. A significant immune reconstitution only set in after removing prophylaxis with ganciclovir or cotrimoxazole. Other causes may be autoimmune diseases (Crohn's disease, lupus erythematosus) or liver cirrhosis.
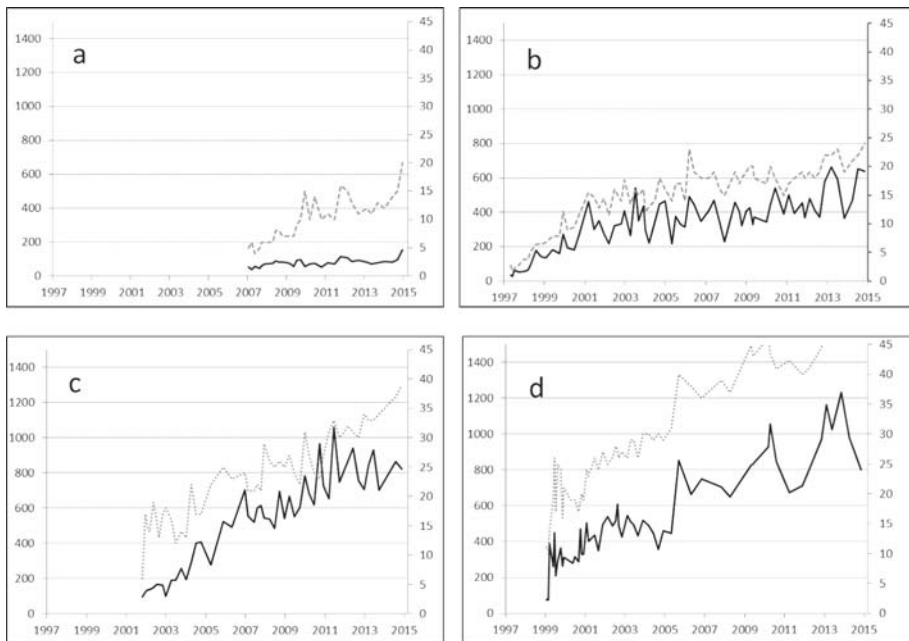
Figures 1a-d: CD4 T cells over years in four selected patients on ART. In all of them, HIV RNA remained fully suppressed for years. The dark line indicates the absolute CD4 T cells/µl (primary axis left), the grey line the relative CD4 T cells % (secondary axis right). a) Poor immune reconstitution (discordant response) in an old patient with very low CD4 T cells at baseline, remaining low despite sustained viral suppression. Of note, the relative CD4 T cells show a slow increase. b) young patient with moderate immune reconstitution, reaching a CD4 T cell plateau. It remains questionable if this plateau truly represents his individual ranges prior to HIV infection. c) and d) very good immune reconstitution in two patients, despite very low CD4 T cells at baseline. There seems to be no plateau. Note the broad intraindividual ranges of both the absolute and relative T cells

However, there is some evidence that certain antiretroviral regimens have unfavorable effects on immune reconstitution. Significant drops in CD4 T cell count were observed in patients with a suppressed viremia who switched to a simplified regimen of TDF+ddI plus nevirapine (Negredo 2004). The reason for this is still not understood, but seems to be related to negative interactions between ddI and tenofovir. Where possible, this combination should be avoided, especially in primary therapy. In two other studies, the CD4 T cell increase with abacavir+3TC or TDF+FTC was significantly better than with AZT+3TC (all combined with efavirenz), despite comparable virological success. This may be related to the myelotoxicity of AZT (DeJesus 2004, Pozniak 2006). In the Swiss cohort, patients on an AZT-containing regimen had 60 CD4 T cells less than patients without AZT over a period of two years (Huttner 2007). Whether it makes sense for patients showing poor immunologic success to switch to AZT-free regimens is an open question. There is no difference between NNRTIs and PIs regarding immune reconstitution and a switch is ineffective (Torti 2011).

What about newer agents? One meta-analysis showed that an increase of CD4 T cells on maraviroc was better than with other agents, and led to several other studies (Wilkin 2008). In these studies patients with poor immune reconstitution received an additional dose of maraviroc. The results were disappointing (Lanzafame 2009, Stepanyuk 2009, Wilkin 2010, Vitiello 2012, Hunt 2013). The same applies to raltegravir (Byakwaga 2011, Hatano 2011, Negredo 2013) and T-20 (Joly 2010), none of them showing any positive effects on immune reconstitution.

Some reports show that the thymic function and corresponding immune reconstitution can be stimulated by growth hormone (Tesselaar 2008, Napolitano 2008). Such approaches are still experimental and not recommended as routine. Whether higher CD4 T cell counts have clinical benefits or not remains unknown. However, the example with interleukin-2 (see section on immune therapy) may call for caution, as in this case higher CD4 T cell counts had no positive effect on the frequency of opportunistic infections.

Practical considerations in dealing with viral load and CD4 count

- Viral load (VL) is the most important parameter in treatment monitoring.
- If possible use only one type of assay (in the same lab) – bear in mind that there is considerable methodological variability (up to half a log).
- Virological success should be monitored one month after initiation or modification of ART.
- VL should be below 50 copies/ml after 3–4 months (in those with high initial viral load, after 6 months at the latest) – if it is not undetectable, investigate.
- The greater the decrease in viral load, the more durable the response to ART.
- Transient, low-level increases in VL (blips) are usually insignificant – but VL should be monitored at short intervals (e.g., 4–6 weeks after such blips).
- The older the patient, the more likely a discordant response (low VL with no significant increase in CD4 count).
- In contrast to VL, increase in CD4 T cells, i.e., immunological success, is difficult to influence. A hectic switch of antiviral agents will not help!
- CD4 T cells are probably more predictive of the individual risk for AIDS.
- Once CD4 T cell count is good, it requires less frequent monitoring. With higher CD4 counts, values may vary considerably from one measurement to the next (which may mislead the patient to either a false sense of euphoria or unnecessary concern).
- To help avoid false euphoria or concern, look at the big picture – measurements over time, not one specific measurement alone, for CD4 cells and viral loads.

Clinical treatment success and failure

Clinical treatment success is dependent on virologic and immunologic therapeutic success. In individual patients, clinical response is not always easy to assess. After all, there is no way to show what might have occurred if treatment had not been started. As an asymptomatic patient cannot feel much better, it may be difficult to find good arguments to continue treatment in the presence of side effects, which, at least temporarily, may affect quality of life.

Clinical success is almost always evaluated via clinical endpoints (AIDS-defining illnesses, death), although the improvement on ART in a patient with considerable constitutional symptoms should also be seen as clinical success. With regard to risk of disease progression, the immunologic response is at least as important as the virologic response. However, the extent of virologic success is of great significance. In the Swiss Cohort, of those with a constantly undetectable viral load, the proportion of patients who went on to develop AIDS or die was 6.6% after 30 months. In contrast, this proportion was 9% in patients with viral rebound and up to 20% if the viral load was never suppressed to undetectable levels (Ledergerber 1999). The importance of a sustained virological treatment success for clinical benefit has also been reported from other cohorts (Thiebaud 2000, Lohse 2006).

Table 4.2: Risk of progression, as defined by immunologic and virologic treatment response (See previous table caption for definitions). 95% confidence intervals in parentheses

	Grabar 2000	Piketty 2001	Moore 2005
Baseline CD4 T cells (median)	150	73	180-250
Response to ART			
Virologic and immunologic	1	1	1
Immunologic response only	1.6 (1.0–2.5)	6.5 (1.2–35.8)	1.9 (1.1–3.0)
Virologic response only	2.0 (1.3–3.1)	9.7 (1.6–58.4)	2.5 (1.5–4.0)
No treatment response	3.4 (2.3–5.0)	51.0 (11.3–229.8)	3.5 (2.3–5.3)

Clinical endpoints: progression/death (Grabar 2000, Piketty 2001), death (Moore 2005)

Clinical failure is usually defined as the development of an AIDS-associated condition or death. However, illness is not always indicative of clinical treatment failure. This is particularly true for the immune reconstitution inflammatory syndrome (IRIS), where a pre-existing, subclinical infection becomes apparent during the first weeks after ART initiation (see chapter on *AIDS*). An OI with increased CD4 T cells does not necessarily mean that the ART has failed, but that the immune system is doing its job, to put it in simple terms. On the other hand, if a patient develops serious side effects or dies, this should clearly be evaluated as a clinical failure. Fortunately, this is rare.

Table 4.3: Causes of death in HIV+ patients in France (Morlat 2014)

	2000 (n=964)	2005 (n=1042)	2010 (n=728)
AIDS-defining events	47%	36%	25%
Non-AIDS-defining cancers	11%	17%	22%
Liver diseases	13%	15%	11%
Cardiovascular diseases	7%	8%	10%
Suicide	4%	5%	3%

Many serious and life-threatening events that affect HIV+ patients on ART today are related to hepatic or cardiovascular complications (Reisler 2003). Table 4.3 shows the causes of death in patients in France in the years 2000–2010. As seen there, other diseases than AIDS such as tumors or (mostly hepatic) liver diseases are becoming more important.

What can be achieved today?

Every HIV clinician sees the remarkable strides made possible by ART reflected in his or her own patients (see example below). In many areas, the incidence of AIDS has been reduced to less than a tenth of what it was at its height (Mocroft 2000). Some illnesses that occur only with severe immunodeficiency are rarely seen today. CMV retinitis or MAC disease have become unusual. AIDS cases in Western countries occur mainly in patients who are not being treated with antiretroviral therapy – usually because they are unaware of their infection or have not acknowledged it. These so-called late presenters now make up a large proportion of the cases of AIDS (see below). In patients who are continuously followed in specialized centers, AIDS has become a rare occurrence.

Table 4.4: Patient (female, 41 yrs old) showing remarkable advances due to ART*

		CD4 T cells	Viral load
Feb 95	AZT+ddC	23 (4%)	NA
Nov 96	AIDS: Toxoplasmosis, MAC, Candida esophagitis	12 (1%)	815,000
Feb 97	d4T+3TC+SQV	35 (8%)	500
Jun 97	Stopped HAART due to polyneuropathy		
Jul 97	AZT+3TC+IDV	17 (4%)	141,000
Mar 98		147 (22%)	<50
Mar 99	AZT+3TC+IDV/r+NVP	558 (24%)	100
Mar 00		942 (31%)	<50
Apr 05	AZT+3TC+LPV/r+NVP	744 (30%)	130
Jan 14		912 (30%)	<50

* Excellent immune reconstitution despite severe immunodeficiency and several AIDS-defining illnesses. All prophylaxes (MAC, toxoplasmosis, PCP) have been discontinued

In ART-CC, a collaboration of several large cohorts, life expectancy of a 20 year-old HIV+ patient increased from 36.1 to 49.4 years between 1996-1999 and 2003–2005 (ART-CC 2008). Life expectancy of HIV+ patients in many industrialized countries is approaching that of the general population (Porter 2008, Lodwick 2010, van Sighem 2010, Obel 2011, Hogg 2012, Nakagawa 2012).

However, all analyses show that a gap still exists between certain patient groups compared to the general population. This applies not only to patients with hepatitis coinfection or active drug or alcohol consumption, but also to black patients or patients with low CD4 T cell count when starting ART (Lohse 2007, ART-CC 2008, Harrison 2010). Even in Western countries, there remain considerable differences in the overall mortality of HIV+ patients. Higher mortality rate in North American, compared with European, may be because of the inclusion of more socially marginalized patients with higher mortality risk (May 2012). One of the most important mortality risk for HIV+ patients in industrialized countries remains still neglected: smoking. HIV+ smokers lose more life-years to smoking than to HIV. The excess mortality of smokers is tripled (Helleberg 2013).

Data from prospective controlled studies on the dramatic improvement of the clinical outcome in HIV+ patients is still limited, as there have not been many randomized

trials with clinical endpoints (Hammer 1997, Cameron 1998, Stellbrink 2000). The results seen in these studies, due to their design, led to the licensing of the PIs. In ABT-247, a multi-center trial, 1090 clinically advanced patients received ritonavir or placebo. The probability of AIDS and death after 29 weeks was 21.9% in the ritonavir arm and nearly double (37.5%) in the placebo arm (Cameron 1998).

Studies of mono- or dual therapy are no longer considered ethically justifiable and the number of clinical endpoints that occur is fortunately now extremely low. As a result, the duration of any contemporary study to prove clinical benefit of one combination over another would have to be extended over a long period of time. Unrealistically large study populations are now required given the extremely low probability of progression – only rarely will such investigations be undertaken in the future (Raffi 2001). Two of the few trials that could confirm the benefits of ART on clinical endpoints were the SMART and the START trial (see sections 6.5 and 6.10). However, all large cohorts such as EuroSIDA, the Swiss Cohort and the US HOPS Cohort have clearly shown the benefit of ART (Table 4.5). The Swiss Cohort showed that the effect of ART increases over time – after more than two years on ART, the risk of disease progression was only 4% of the risk without ART (Sterne 2005). Numerous cohort studies have shown that during recent years there has been no further decline in AIDS and mortality rates. It seems that, in many patients, ART is simply begun too late. Even in 2006, almost half of the patients initiating ART have less than 200 CD4 T cells/µl (May 2006).

Table 4.5: Decline in morbidity and mortality in large cohorts

	Where (n)	Patients (Period)	Mortality (/100 PY)	Morbidity (/100 PY)
Palella 1998	USA (1,255)	<100 CD4 T cells/µl (1994–97)	29.4 → 8.8	21.9 → 3.7*
Ledergerber 1999	Switzerland (2,410)	6 months before versus 3 months after ART (1995-97)	NA	15.1 → 7.7
Mocroft 2000	Europe (7,331)	All (1994–98)	NA	30.7 → 2.5
Mocroft 2002	Europe (8,556)	All (1994–2001)	15.6 → 2.7	NA
D'Arminio 2005	Worldwide (12,574)	The first 3 months after versus 3 years after ART	NA	12.9 → 1.3
D:A:D 2010	Worldwide (33,308)	All (1999–2007)	1.7 → 1.0	NA

* MAC, PCP, CMV. Mortality/Morbidity each per 100 PY = patient years

The effect on AIDS-defining diseases appears to be different. The most obvious is the decline in the incidence of viral OIs, although this is not as pronounced for fungal infections (D'Arminio 2005). With regard to opportunistic infections and malignancies, the effect of ART is equally apparent on their clinical course as it is on their incidence. Illnesses such as cryptosporidiosis or PML can be cured, while Kaposi sarcoma can resolve completely without specific therapy. Prophylaxis of pneumocystis pneumonia, toxoplasmic encephalitis, CMV, or MAC infection can usually be safely withdrawn at the adequate CD4 counts. These effects are discussed in more detail in the corresponding chapters.

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6.4.2. Treatment goal: "HIV cure"

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The cure of HIV-infected patients remains the holy grail of HIV medicine. With the introduction of combination ART it has been calculated that the calculated time to eradication of all reservoirs is 70 years. Thus, it is clear that strategies beyond the current ART regimen will be necessary. Many researchers share the opinion that a cure has to be the major goal for the future.

The cure of the so-called Berlin patient Timothy Brown, published in 2008, shows that a cure is at least theoretically possible. Brown had suffered from acute myeloid leukemia and underwent allogeneic stem cell transplantation. The healthy stem cell donor was homozygous for the Δ32 mutation, a genetic defect leading to the absence of CCR5 co-receptor from his cells – after the transplant the viral load of in the Berlin patient (which was very high before ART initiation) has disappeared for at least four years (Hütter 2009, Allers 2011, Symons 2014). The virus was undetectable in the blood, in the lymph nodes and in the intestinal mucosa (Yukl 2013), suggesting that targeted CCR5 disruption can lead to an HIV cure. There is no doubt that in clinical practice, an allogeneic stem cell transplant is not an appropriate way for a HIV cure (Cillo 2013). It is not only complicated and expensive, but also highly risky (mortality up to 30%), making this approach not very practical (Zhou 2013). However, although not reproducible until today, the case of the Berlin patient stirred hope for future academic purposes.

Sterilizing or functional cure?

Eradication of all viruses from the body would be the definitive cure. But is this really necessary? Much would have been achieved if the immune system is able to control HIV without help of medication – i.e., in some viral infections, like herpes, low viral levels persist for a lifetime. This is why a difference is being made today between a "sterilizing cure" and "functional cure" (Reviews: Richman 2009, Lewin 2011). A functional cure is achieved in the so called "post treatment controllers" (PTC), in whom viremia remained controlled for several years after the interruption of ART. Currently at least four strategies are being pursued and partly combined. These are 1. Eradication of latently infected cells and 2. Eradication of residual replication, as well as 3. Improvement of the HIV-specific immune response and 4. Attempts to make cells more resistant against HIV infection.

A few patients have already reached functional cure. These so-called "elite controllers", some found in most large HIV centers, have normal CD4 T cells for many years and even more impressive, a viral load below the limit of detection without therapy. Only when investigating with ultrasensitive methods or examining the lymph nodes can a relatively tiny amount of virus be found. Co-receptor defects explain only a few of the cases, and efficient antiviral immunity capable of controlling HIV reaction was observed occasionally (Smith 2015) (see Pathogenesis).

However, despite maintaining very low levels of plasma viremia, elite controllers have elevated immune activation and accelerated atherosclerosis. In a prospective trial, controllers had a statistically significant decrease in ultrasensitive plasma and rectal HIV RNA levels with ART (Hatano 2013). Moreover, markers of T cell activation/dysfunction in blood and gut mucosa also decreased substantially with ART. Similar reductions were observed in the subset of "elite" controllers with pre-ART plasma HIV RNA levels below conventional assays (<40 copies/mL). These observations raise the question whether a functional cure is comparable to well-tolerated ART and whether the degree of HIV suppression in elite controllers is equivalent to that achieved by ART when it comes to clinical outcomes.

Can (very) early ART lead to a cure?

In 2013, the case of the perinatal infected "Mississippi Baby" gained worldwide attraction. This infant had been antiretrovirally treated only 31 hours after birth (Persaud 2013). The baseline viral load of 19,812 copies/ml felt down to 265 copies/ml at day 19 and was then undetectable for 18 months. The baby was then lost to the health care system for the next six months. Unexpectedly, the viral suppression remained undetectable when tested for HIV upon return. More than two years this girl had no signs of the virus in her blood despite cessation of treatment. An ultrasensitive assay revealed 4 copies HIV-DNA/million PBMCs but no HIV-specific immune responses. Protective HLA types as seen in elite controllers were not observed. The finding encouraged scientists hoping to find a way to save children from a lifetime of ART. However, the virus resurfaced in the patient 27 months after ART stopped (Ledford 2014). It became obvious that the "post-treatment control" had only been transient. It remains unclear what led to the abrupt rebound. Moreover, similar pediatric cases were published in which virologic rebound occurred within days of discontinuation of ART, despite immediate treatment after delivery (Butler 2015).

Also in 2013, PTC cases had been published from France (Sáez-Cirión 2013). In the so-called VISCONTI cohort (Viro-Immunological Sustained CONtrol after Treatment Interruption), 14 HIV+ patients were reported in which prolonged ART had been initiated within the first 35–70 days post infection. Viremia remained controlled (less than 500 copies/ml) for a median of 7.5 years after treatment interruption. Most of these PTCs lacked the protective HLA B alleles that are overrepresented in elite controllers. Thus, it seems that early and prolonged ART may allow some individuals with a rather unfavorable background to achieve long-term infection control. Further studies from France and Thailand also demonstrated that the viral reservoir remains limited with early ART (Hocquelorx 2013, Ananworanich 2013). But how often does treatment of primary HIV infection lead to post-treatment control? Unfortunately, it remained unclear how many patients in total were included in the VISCONTI cohort. Forteen patients out of 100, 1,000, 10,000? More recent data suggest a low likelihood of PTC even when ART is started within 12 weeks of HIV-1 infection (Maenza 2015).

Moreover, the hitherto largest randomized trial in this field yielded only moderate success of early ART (SPARTAC 2013). A total of 366 patients with primary HIV infection (less than 6 months after seroconversion) were randomized for 12–48 weeks ART or to remain untreated. A 48-week course of ART delayed disease progression which was defined as CD4 T cells of less than 350 cells/µl or long-term ART initiation. However, there were no significant differences in the incidence of AIDS, death, or serious adverse events and the delay in disease progression was lost soon after ART interruption. Although the risk/benefit of initiating ART in primary HIV infection remains a matter of discussion (Lodi 2012, Jain 2013), once a decision for ART has been made, therapy should be continued (see also chapter on acute infection).

A cure in chronically infected patients?

The acute HIV infection is rarely diagnosed. The main question is what can be achieved in patients with chronic infection. Several barriers to a cure in these patients have to be overcome (Katlama 2013), such as the intrinsic stability of the viral genome in latently infected cells such as long-lived memory T cells, and the sustained low-level viral replication in different compartments. Not to mention severe methological problems measuring the latent reservoir. It remains unclear what should be measured in which cells with which tools (Siliciano 2013).

Proviral DNA measured by PCR from PBMC detects much more (300-fold) provirus

than the viral outgrowth assays (VOA) which measures replication competent virus. The lack of a precise correlation between VOA and PCR-based proviral DNA assays raise the possibility that the successful clearance of latently infected cells may be masked by a large pool of cells with defective proviruses (Eriksson 2013). These defective proviruses are detected by PCR but may not require eradication to accomplish an effective cure. Less than 1% of proviruses are induced to release infectious virus after maximum in vitro activation. However, analysis of a large number of proviral clones from treated patients showed 12% with intact genomes and normal long terminal repeat (LTR) function, indicating that they may become activated in vivo (Ho 2013). A better understanding of the discrepancy between infected cell frequencies measured by viral outgrowth versus PCR assays is an urgent priority in HIV cure research (Eriksson 2013).

The latent reservoirs

At this point in time, eradication of HIV, the removal of all HIV from the body, is a theoretical goal. The main reason is that latently HIV-infected cells comprise a life-long reservoir (Saksena 2003). Even after years of suppression, viral transcription can be detected (Finzi 1999, Furtado 1999, Sigal 2011). This is particularly true in blood cells, but also in the lymph nodes and in sperm (Lafeuillade 2001, Nunnari 2002), where HIV may persist hiding from immune recognition (Fukazawa 2015). Replication also takes place in cells of the gastrointestinal tract, even if no virus is detected in the blood. Even after myoablative chemotherapy and autologous stem cell transplantation, latent reservoirs persist (Cillo 2013). After stopping ART in such patients, a rebound is seen rapidly (Henrich 2013+2014), and possibly occurs at multiple sites (Rothenberger 2015). In addition, latently infected reservoirs consist of very heterogenic cell populations, among the T memory and stem cells (Buzon 2014). The stability of these cells is probably independent of residual virus replication. Theoretically, how long does it take until the last latently infected cells are removed? A half-life of 44.2 months for the latently infected cell reservoir was measured in a study with 62 patients, whose viral load had been successfully suppressed on ART for a period of seven years (Siliciano 2003). The calculated time to eradication of these reservoirs was 73.4 years. Even in patients with no measurable blips during at least three years of stable ART and with a tendency for a more rapid decrease of viral load, the time to eradication was 51.2 years. Virus in resting CD4 memory cells with minimal evolution persists, even after close to 9 years on ART (Nottet 2009). Moreover, recent research suggest that the latent reservoir is larger than previously thought (Dolgin 2013).

Intensification strategies

Many studies have investigated whether viral decay rates can be improved or whether any change at all can be effected by intensifying therapy. Different strategies were tried, such as additional administration of integrase or entry inhibitors, but also of other compounds to try to to empty the latent reservoirs. These studies are discussed below.

Mega-HAART, entry and/or integrase inhibitors

In a trial with patients with good viral suppression and additional PIs or NNRTIs in their ART, an ultrasensitive single copy assay showed no further reduction of viral load by intensification (Dinoso 2009). The level of viral load depends not so much on the applied regime, but on on the pre-therapeutical setpoint (Maldarelli 2007). Additional administration of the entry inhibitor T-20 did not show any effects either (Ghandi 2010). Resting T cells are also not affected by T-20 nor by a combination with valproic acid (Archin 2010).

Maraviroc, as a potential immune-modulating CCR5 antagonist, was also investigated as an intensification strategy. One study showed no relevant effects on the latent reservoirs (Puertas 2014) and other studies showed no or even unfavorable effects on immune activation (Sauzullo 2010, Wilkin 2010, Hunt 2013). One study with acutely infected patients showed hardly any effect either on virologic or immunologic parameters (Evering 2010). Another carefully designed study with 40 patients with acute HIV infection compared a triple regime plus raltegravir plus maraviroc with a classic triple regimen. Intensive therapy showed no advantages regarding residual viremia or the degree of immune reconstitution or immune activation (Markowitz 2014). Obviously it is not a question of the number of ARVs.

Hopes for additional effects of the integrase inhibitor raltegravir were raised by a study in which treatment-naïve patients on a raltegravir regimen achieved a viral load below detection significantly more rapidly than those on efavirenz (Murray 2007). Several prospective studies in which raltegravir was added to an existing ART showed no additional antiviral effect by means of ultrasensitive viral load assays (Gandhi 2009, MacMahon 2010, Gandhi 2012). Immune activation was also not influenced by raltegravir (Luna 2009, Massanella 2011). Results are contradictory regarding the question of whether proviral DNA decreases more rapidly. While two small studies showed positive effects (Arponen 2008, Reigadas 2010), several larger studies did not confirm these results (Buzon 2010, Hatano 2011, Chege 2012).

Several studies showed an increase of episomal DNA while on raltegravir. This DNA, also referred to as 2-long terminal repeat (2-LTR) circular, develops when integrase inhibitors block the DNA integration process into the chromatin. Evidence of this episomal DNA (2-LTR circles) in approximately 30% of patients receiving raltegravir plus effective ART, shows that an active viral increase was stopped (Buzon 2010, Reigadas 2010, Llibre 2012, Hatano 2013). A recent study, however, found no increased 2-LTR circles during raltegravir intensification (Besson 2012). Another study demonstrated that resting CD4 T cells were not achieved with raltegravir or with a combination that included valproic acid (Archin 2010) (see below). Sites such as the CNS or gut are not influenced (Yukl 2010, Lee 2011, Yilmaz 2011).

"Kick and Kill" or reservoir eradicators

As shown above, it is very doubtful that eradication is possible with currently available regimens (Shen 2008, Lewin 2011). Intensification or extension to a four- or five-drug therapy has not had meaningful results. Therefore, the old "Kick and Kill" strategy is being revived, in which infected cells are first activated in hope of them being recognized by the immune system and killed more rapidly (Deeks 2012).

Several attempts to empty viral reservoirs using different methods (IL-2, hydroxyurea or OKT) have not been successful (Kulkosky 2002, Pomerantz 2002). A pilot study on valproic acid, an epileptic drug, caused a stir in the summer of 2005. Implemented as an inhibitor of histone deacetylase 1 (HDAC), it suggested a clearance of HIV from resting T cells (Lehrmann 2005). In three out of four patients the number of infected resting CD4 T cells decreased significantly and half-life was reduced to 2-3 months compared to other studies showing a longer half-life of 44 months on ART (Siciliano 2003). Smaller follow-up studies (Steel 2006, Siliciano 2007, Archin 2010) did not confirm these results. More recently, a randomized crossover study finally put an end to the discussion, showing no effect at all of valproic acid in 56 patients (Routy 2010). With the end of valproate, more selective and possibly more potent HDAC inhibitors are being investigated. Results are conflicting (Archin 2012, Blazkova 2012). Vorinostat, an agent that has been approved as a treatment of malignant mesothelioma, was active in one study *in vivo* (Archin 2012) but failed to do so in another (Elliott 2013). Vorinostat was able to increase HIV transcription ("kick"), but without

"kill" – the pool of latently infected cells was not reduced. Romidepsin seems to be more effective (Wei 2013, Søgaard 2014) and is tested as well as panobinostat and other HDACi (Edelstein 2009, Rasmussen 2013). Further chemical classes able to activate latent infected cells are quinolone derivatives (Xing 2012) protein phosphatase-1 targeting compounds (Tyagi 2015) or disulfiram (Spival 2012).

It may be necessary to activate HIV-specific CTLs (Shan 2012, Deng 2014) for the "kill" part. There are attempts with therapeutical vaccines that simultaneously improve HIV-specific immune response (Garcia 2012). Recently it was shown that acutely infected patients retain a broad-spectrum viral-specific CTL response and that appropriate boosting of this response may be required for the elimination of the latent reservoir (Deng 2015).

Attempts with immunoglobins (Lindkvist 2009) or broadly neutralizing antibodies are also being postulated. Even the old substance interferon is being discussed again as an immune modulator (Sandler 2014). In one study 9 out of 20 patients receiving pegylated interferon during a HAART interruption, demonstrated viral load levels below 400 copies/ml after 12 weeks of IFN monotherapy (Azzoni 2013).

Gentherapeutic approaches are also under investigation. In a pilot trial, the infusion of autologous, gene-modified CD4 T cells in which the CCR5 gene was rendered permanently dysfunctional by a zinc-finger nuclease was safe (Tebas 2014). The observed relative survival advantage of the gene-modified cells during treatment interruption suggests that genome editing at the CCR5 locus confers a selective advantage to CD4 T cells in patients infected with HIV. Many more approaches are under investigation, the most promising among them are:

a) zinc-finger nucleases that can efficiently excise integrated HIV-1 from the human genome in infected cells

b) "designer" T cells that can target and kill HIV Env-expressing cells and thus improve the HIV-specific immune response (Sahu 2013, Yang 2014)

c) induction of broadly neutralizing antibodies that can effectively suppress viremia in untreated patients (Horwitz 2013).

Summary:

An HIV cure is not around the corner. Within the next years, we will see more and more patients classified as post-treatment controllers or "functionally cured". However, this will apply only to a small group of patients. Latently infected cells differ minutely from non-infected cells, which cannot be easily discerned via those methods available in most clinics. They are also non-specific. Washing out the reservoirs or eliminating all the infected memory cells has either been unsuccessful or too toxic. Removing the HIV genome from infected cells with special recombinants has been successful in the laboratory and in the animal model (Hauber 2013); but there is still a long way to go before this can be used in the clinic (Sarkar 2007). Given the complexity of the immune system which is far away from being completely understood, a solution for the majority of the patients is a distant prospect.

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6.5. When to start ART

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Since the introduction of ART, practice of treatment initiation is ever-changing. Facing encouraging results with early AZT in 1995, David Ho initiated the slogan "hit hard, hit early", and almost all clinicians were taking him at his word. However, over the following years it became obvious that the tolerability of first generation ART regimens was poor. Patients complained about high pill burden and physicians became more defensive. There was controversial debate about the best time for initiation and the indication for antiretroviral therapy was based on clinical assessment, CD4 T cell count and viral load. The risk of AIDS and other HIV-associated complications had to be weighed against the risks of long-term toxicity and viral resistance. In Europe, the median CD4 T cell count at initiation of ART was only 200/µl in the first years of the last decade, after being 270/µl in 1998 (May 2006).

In more recent years, the pendulum has been swinging back. With regard to new drugs that are more potent and better to tolerate, there is a strong trend towards earlier treatment initiation. In 2007/2008, most guidelines have determined that a CD4 T cell count of <350 cells/µl, instead of 200 cells/µl, is the definitive threshold for initiation of ART in all asymptomatic patients. During the last years, the threshold was raised up to 500 cells/µl in many guidelines (US/WHO). However, patients in resource-limited countries are still starting their ART at CD4 cells lower than 200/µl (Mugglin 2012).

Facing the early results of the landmark START trial published in May 2015 (see below), the discussion whether and when to start ART may come to an end. The question "When to start ART" may change to "Why not yet started ART?". There is no doubt that current guidelines in the USA and (especially in) Europe (see Table 5.1.) will be modified again in the near future.

Guidelines are not set in stone. Decisions must still be made on a case-by-case basis. In some cases, therapy might (or even should) be deferred. Last but not least, the patient should be ready to start. Experience as well as some intuition of the treating physician is mandatory.

Table 5.1: Recommendations from various guidelines on when to initiate therapy

Clinical	CD4 T cells/µl	Initiation of HAART is...
CDC B+C	All values	"Is always recommended" (DHHS, EACS)
CDC A	<350	"Is always recommended" (DHHS, EACS)
CDC A	350-500	"Is recommended" (rating: strong) (DHSS) "Should be considered in asymptomatic patients, recommended in patients with several conditions like hepatitis coinfection, malignant or renal diseases, high risk of cardiovascular/malignant diseases" (EACS)
CDC A	>500	"Is recommended (rating: moderate) in asymptomatic patients, recommended (rating: strong) in patients with hepatitis coinfection, renal and other diseases" (DHSS) "Should be considered in asymptomatic patients, recommended if one of the points listed in 350–500 apply" (EACS)

DHHS: US Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. April 2015. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. EACS: European AIDS Clinical Society (EACS). Guidelines for the clinical management and treatment of HIV-infected adults in Europe. November 2014. http://www.eacs.eu

How high is the individual risk of progression?

The following table lists the (selected) risks of developing AIDS within six months, as identified in 3,326 patients from the pre-HAART era (Phillips 2004). The range of the individual risk of progression, calculated by using CD4 T cells, viral load and age only, varies widely – from 0 to almost 50%. This may also demonstrate how helpful these surrogate markers can be.

Table 5.2: Predicted six-month percentage risk of developing AIDS, according to age, viral load and CD4 T cell count (data from the pre-HAART era)

	100 CD4/µl	200 CD4/µl	350 CD4/µl
35 years			
Viral load 10,000 copies/ml	5.3	2.0	1.1
Viral load 100,000 copies/ml	10.6	4.1	2.3
55 years			
Viral load 10,000 copies/ml	10.7	4.6	1.8
Viral load 100,000 copies/ml	20.5	9.2	3.6

(From: Phillips et al, CASCADE Collaboration. AIDS 2004, 18:51-8.)

But even after initiation of ART individual risk may vary considerably. Table 5.3 shows individual risks after initiation of ART for different age groups. These data were derived from 12 cohorts in Europe and North America, in which more than 20,000 patients started antiretroviral therapy between 1995 and 2003 (May 2007). It is of note that the data apply only to asymptomatic patients without intravenous drug use (IVDU). In patients with AIDS and in IVDUs, progression risks can be much higher. On the other hand, it seems possible that these data overestimate the individual risk as risk may be lower with the newer drug combinations. Moreover, treatment interruptions were not taken into account. Thus the values in Table 5.3 are only rough estimates and should be interpreted with caution. However, they could be helpful in any discussion with the patient, of course without browbeating or scaring them with statistics.

Table 5.3: Probability (%) of experiencing a new AIDS-defining disease or death by the end of 1 year (5 years) after the patient starts ART. Only valid for patients without previous AIDS and non-IVDUs

	<25 CD4/µl	25–49 CD4/µl	50–99 CD4/µl	100–199 CD4/µl	200–350 CD4/µl	>350 CD4/µl
16–29 years						
VL <100.000	10 (19)	8 (17)	7 (16)	5 (11)	2 (7)	2 (6)
VL >100.000	12 (23)	10 (21)	9 (19)	6 (13)	3 (8)	2 (7)
30–39 years						
VL <100.000	12 (22)	10 (19)	8 (18)	5 (12)	3 (8)	2 (6)
VL >100.000	14 (26)	12 (23)	10 (22)	6 (15)	3 (10)	2 (8)
40–49 years						
VL <100.000	13 (25)	11 (22)	10 (20)	6 (14)	3 (9)	2 (7)
VL >100.000	16 (29)	13 (26)	12 (24)	7 (17)	4 (11)	3 (9)
>50 years						
VL <100.000	16 (29)	13 (26)	12 (24)	7 (17)	4 (11)	3 (9)
VL >100.000	19 (35)	16 (31)	14 (29)	9 (21)	5 (13)	3 (11)

From http://www.art-cohort-collaboration.org. VL is copies/mL, CD4 is cells/µl

One important caveat of cohort studies is the fact that the individual treatment success of the patient is not taken into account. This was shown by an analysis of 13 cohort studies from Europe and North America including 9,323 adult treatment-naïve patients who started ART with a combination of at least three drugs. At 6 months after starting ART, the current CD4 T cell count and viral load, but not values at baseline, were strongly associated with subsequent disease progression (Chene 2003).

To evaluate the individual risk for a treatment-naïve patient, one can check www.art-cohort-collaboration.org (May 2007). Only a few parameters are needed. It is also possible to calculate the risk after 6 months on ART.

Practical experiences

Even if the indication for ART seems obvious or even urgent, it should be clarified whether the patient is indeed prepared to start treatment (treatment readiness). The problem is not necessarily the initiation of ART, but the longer-term maintenance. The decision to initiate treatment is often made prematurely. It is usually unwise to prescribe lifelong medication to a patient in the very first consultation. One should first attain an overall picture of the patient, and try to get to know something about lifestyle and motives – why they have come to see a doctor and what they expect.

On the other hand, the patient's wish to start therapy should be respected. If after a detailed discussion a well-informed patient wants to begin treatment, even though the results justify waiting, ART should not be withheld. For many patients, treatment can be a psychological support. Not everybody can sleep peacefully at night knowing that inside them a hundred million new viruses are being produced every day and a huge number of helper cells are being destroyed.

However, if a vacation is planned, it may be better to delay therapy, so that treatment response and side effects can be adequately monitored. On the other hand, patients may sometimes find one reason after another (stress at work, exams, change of job, etc) to delay initiation of treatment. Many patients are afraid of AIDS, but often just as afraid of ART ("the pills are the beginning of the end!"). They may have irrational or simply false expectations of ART and its consequences – starting therapy does not mean that one will be subjected to daily infusions and no longer able to work. Therapy should be explained to every patient from the outset. It is also useful to define individual threshold values for the commencement of therapy with patients early on, so that therapy is started only when these levels are reached. In our experience, patients who are not ready at the moment are more motivated by this approach.

As a rule, as much time as is needed should be taken for the decision to start therapy. We recommend that patients come for several consultations to get prepared for treatment. There are two exceptions: acute HIV infection (see chapter on *Acute Infection*) and severe immunodeficiency. However, even in the presence of most AIDS-defining conditions, the acute disease should often be treated first before initiating ART, as the potential for complications with TB, PCP, toxoplasmosis or CMV therapies unnecessarily jeopardize treatment options. In asymptomatic patients with very low CD4 T cells, it makes sense to start first with a PCP prophylaxis. Over the next (few) days, one can perform an exam (X-ray, ultrasound, fundoscopy, etc) and check the patient's readiness. Does the patient come back? Are they really motivated?

Practical tips for initiation of ART

Below 350 CD4 T cells/µl or an HIV-associated complication
• Start immediately with ART.
• Do not wait until OI occurs or until acute OI therapy is finished
• Get to know the patient (What took him/her so long to start treatment?), give proper counselling and start treatment with prophylaxes in advance
• Address fears and anxieties before starting therapy

Above 350 CD4 T cells/µl without any other problem
• Talk early about ART so the patient knows what to expect (1–3 pills OD)
• Talk about the START study and the risk observed in these patients
• Is the patient ready for therapy? How compliant will he be?
• If the patient is reluctant and anxious, more time must is needed for preparation before beginning therapy. If the patient is not ready, check the reasons. Define thresholds below which ART can be initiated
• Do not only consider the absolute CD4 T cells, but observe other individual factors: Hepatitis coinfection? Older age? Malignancy? Pregnancy? If so, start!
• Is the patient sexually active? Is there a negative partner? It may be also a good reason (and motivation) to start to reduce rates of transmission
• Try not to start therapy before a holiday or other big event, but do not allow the patient put off therapy indefinitely
• Check to see if the patient is suitable for a clinical trial

However, in times of well-tolerated antiretroviral therapies, is it worth exposing patients to the dangers of AIDS for the sake of a little more quality of life? And is quality of life really better without ART? In the SMART study quality of life was worse without. Remember also that in an observational cohort collaboration study on 34,384 ART-naive individuals, the mean CD4 T cell decline was -78 (95% CI, -80 to -76) cells/µl per year. The decline was strongly associated with a higher current viral load: for every 1 log10 copies/ml higher, CD4 T cells declined by an additional 37.6 cells/µl per year (COHERE 2014). This means that even a patient with 700 CD4 T cells/µl will reach the thresholds of 350 or 500/µl within a few years. How much long-term toxicity is really saved by two or maybe even five years without ART exposure when lifelong ART will be given over decades? Will this exposure reduction be relevant in the older ages of this patient? Will it be relevant in the year 2050 that he had initiated ART in 2016 or 2018? Probably not. Talk with him. A well informed patient will adhere better.

Asymptomatic patients, >350 CD4 cells/µl

Below 350 CD4 T cells/µl, there is broad consensus that all asymptomatic patients should initiate ART. But even above this threshold, ART should be considered. Although rather low, in the long run the risk of developing AIDS cannot be excluded. There is no reason to think the patient 100% safe, even at very high CD4 T cells. A look at the calculation presented above (May 2007), gives a rough idea about the individual risk. After ART initiation, a 45 year-old asymptomatic patient with 200–350 CD4 T cells/µl, a viral load below 100,000 copies/ml has an AIDS mortality risk of 3.1% after one year, and 8.7% after five years. Starting ART above 350 CD4 T cells/µl, reduces the risk to 2.0% and 7.3% for the same patient. Such a reduction of just 1 or 2% may seem insignificant at first. The best data for this patient goup come from HTPN-052, a trial with 1,763 HIV-discordant couples in the US, Africa and Asia. The requirements were that HIV+ partners were treatment-naïve with CD4

T cells between 350 and 550/µl. They were then randomized for ART, either imme-diately or in a rather late stage when CD4 T cells reached below 250/µl or even after manifestation of AIDS (Cohen 2011, Grinsztejn 2014). Although the trial's primary endpoint was HIV transmission, preliminary results showed that the numbers of severe diseases and death were significantly lower in the group starting ART imme-diately (57 versus 77, p=0.07). With regard to AIDS cases, the difference was signif-icant (40 versus 61, p=0.03). However, a major reason for this difference was caused by extrapulmonary tuberculosis (3 versus 17), which, at 55%, was most frequently observed in India.

Asymptomatic patients, >500 CD4 cells/µl: START Study

Large but very complex cohort studies have yielded conflicting results with regard to the benefits of starting ART in patients at high CD4 T cell ranges. Whereas in the US early treatment was of clinical benefit (Kitahata 2009), this was not observed in Europe (Sterne 2009). It seems obvious, that the hitherto largest trial, the START study, will end this debate (START 2015). In this study, worldwide 4,685 patients with more than 500 CD4 T cells/µl (median 651/µl, median viral load 12,759 copies/ml) were randomized to initiate ART immediately or to wait until CD4 T cells declined to below 350 CD4 cells/µl or until symptoms appeared. The ART regimen was chosen by the treating physician. The primary composite end point was any serious AIDS-related event, serious non-AIDS-related event, or death from any cause. In May 2015, the data and safety monitoring board recommended that patients in the deferred-initiation group be offered antiretroviral therapy. In the immediate ini-tiation group the composite primary end point was reached in significantly fewer patients than in the deferred initiation group (42 versus 96 events, p <0.0001). The clinical endpoints are shown in the Table 5.4.

Table 5.4: Clinical endpoints in the START study, numbers per 100 person-years (absolute numbers)

	Immediate ART (n=2,326)	Deferred ART (n=2,359)	Hazard Ratio (95% CI)	P Value
Death from any cause	0.17 (12)	0.30 (21)	0.58 (0.28–1.17)	0.13
Serious non-AIDS-related event	0.42 (29)	0.67 (47)	0.61 (0.38–0.97)	0.04
Serious AIDS-related event	0.20 (14)	0.72 (50)	0.28 (0.15–0.50)	<0.001
TB	0.09 (6)	0.28 (20)	0.29 (0.12–0.73)	0.008
Kaposi sarcoma	0.01 (1)	0.16 (11)	0.09 (0.01–0.71)	0.02
Malignant lymphoma	0.04 (3)	0.14 (10)	0.30 (0.08–1.10)	0.07
Cancer not related to AIDS	0.13 (9)	0.26 (18)	0.50 (0.22–1.11)	0.09

As can be seen, the benefit was mainly driven by a reduction of TB, KS and lym-phoma incidence. However, the beneficial effect of immediate ART was evident also for serious non–AIDS-related events, and no increased rate of adverse effects associ-ated with this strategy was observed. There was no evidence that the beneficial effect differed according to age, sex, race, region of the world, CD4 T cell count, viral load, or risk factors for serious non-AIDS diseases. According to the authors, these results indicate that ART should be recommended for all HIV+ patients regardless of the CD4 T cell count.

New guidelines will have to consider these results. It remains to be seen if there will be small patient group (very high CD4 T cells, very low plasma viremia) for whom ART will not be recommended.

Late Presenters: AIDS and/or <350 CD4 T cells/µl

Although treatment possibilities have dramatically improved, many patients still present at a very late stage of infection. Questions about beginning an optimal therapy are superfluous as these patients are more or less classified as urgent. There is no consensus regarding the definition of "late presenter". In most cases, a CD4-cell count below 200/µl and/or a manifest AIDS disease at the time of HIV diagnosis will do. Some authors also classify the groups "late testers", "very late presenters" and even "long-term non-presenters". At the second "HIV in Europe" conference in November 2009, it was agreed that those patients with a CD4 cell count below 350/µl at initial presentation are to be referred to as late presenters (Antinori 2011). In the US and Europe they still constitute more than half of all patients (Althoff 2011, Mocroft 2013).

Incidence and risk factors of a late HIV diagnosis

How frequent are late presenters? In COHERE, a collaboration of observational HIV cohorts in Europe, among 84,524 individuals from 23 cohorts in 35 countries, 53.8% were classified late presenters. The rate decreased only moderately from 57.3% in 2000 to 51.7% in 2010/2011 (Mocroft 2013). Lacking an overall valid definition, rates between 10-44% are currently being reported in different European countries and the US with a recently slightly downward trend (Table 5.5). Several studies have looked at the risk factors of late diagnosis (Table 5.6). The characteristics (advanced age, migrant origin, heterosexual transmission, see above) indicate more complex reasons for a late diagnosis.

They probably involve patients (less access to health system, lack of information, fear of stigmatization), as well as doctors and members of the health care system (among others lack of HIV awareness with certain patient groups). Several studies enforce the notion that, even with high-risk patients, many chances of diagnosing HIV at an earlier stage are missed (Duffus 2009, Jenness 2009). As much as 76% of 263 African patients living in London had visited a general doctor a year before HIV was diagnosed. Of note, 38% were in outpatient care and 15% had received inpatient treatment in the year before HIV diagnosis (Burns 2008).

Table 5.5: Frequency of late diagnosis in Europe

Country	Period (n)	Definition of late diagnosis	% (ADE)	Trend over time
Italy (Borghi 2008)	1992–2006 (884)	CD4 <200 cells/µl or AIDS <3 Mo	39 (24)	Decline from 43 to 35%
France (Delpierre 2008)	1996–2006 (6.805)	CD4 <200 cells/µl or AIDS <1 year	38 (17)	Decline from 43 to 32%
Spain (Carnicer 2009)	1987–2006 (6.186)	AIDS <3 months	(44)	Not stated
Great Britain (HPA 2009)	2008 (7.218)	CD4 <200 cells/µl	32	Not stated
USA (CDC 2009)	1996–05 (281.421)	CD4 <200 cells/µl or AIDS <1 year	38	Decline from 43 to 36%
Great Britain (UK Chic 2010)	1996–2006 (15.775)	CD4 <200 cells/µl	27 (10)	Not stated
Switzerland (Wolbers 2009)	1998–2007 (1.915)	CD4 <200 cells/µl	31	No clear trend

ADE = AIDS-defining event

Table 5.6: Risk factors for late diagnosis in Western industrialized countries

Country (reference)	Risk factors
Italien (Borghi 2008)	Advanced age, male, foreign origin
France (Wilson 2014)	Older age, heterosexual transmission, migration
Spain (Carnicer 2009)	Male, age under 30 or over 40 years, MSM or heterosexual transmission. Protective: IVDU
USA (CDC 2009)	Advanced age, male, ethnic origin non-white
Great Britain (UK Chic 2010)	Heterosexual transmission
Schweiz (Wolbers 2009)	Advanced age, ethnic origin non-white. Protective: MSM, IVDU, living alone
Germany (Zoufaly 2011)	Higher age, heterosexual transmission, migration background

Morbidity, mortality – consequences of a late HIV diagnosis

Up to 90% of AIDS-defining diseases today, appear with viremic – mainly untreated – patients. This applies greatly to classical opportunistic infections such as PCP or CMV retinitis, but also to tuberculosis or Non-Hodgkin lymphoma (ART-CC 2009). In the German Lymphoma Cohort, two-thirds of patients with newly diagnosed NHL had not previously received ART. 40% of patients with AIDS, a group associated with the highest mortality rate even today, are diagnosed with NHL and HIV infection simultaneously (Hoffmann 2009). In a British analysis counting 387 deaths of HIV+ patients in the years 2004/2005, as many as 24% of all deaths and 35% of HIV/AIDS-related deaths were ascribed to a late HIV diagnosis (Lucas 2008). An account analysis showed that, treating expenditures increased by 200% with less than 200 CD4 T cells at the time of HIV diagnosis (Krentz 2004). This may be attributed to the immune reconstitution syndrome (IRIS) frequently observed in late presenters (see chapter on *AIDS*).

There is no doubt that a late HIV diagnosis is associated with higher mortality and morbidity risk. The risk increases with lower CD4 T cells at therapy initiation (Egger 2002, Sterne 2009). An analysis of therapy-naïve patients in three major European cohort trials observed 8.3 new AIDS cases per 100 patient years in patients with less than 200 CD4 cells/µl at the beginning of therapy – and only 1.8/100 patient years in those with at least 350 CD4 T cells/µl. The mortality rate was slightly higher with 2.9 versus 0.7/100 (Phillips 2001). Several other cohort trials also found a clear association between CD4 T cells at therapy initiation and AIDS and mortality rates (Cozzi-Lepri 2001, Kaplan 2003, Palella 2003, Braitstein 2006, Mocroft 2013). The lesser the CD4 T cell count, the higher the risk for the following time period, over many years (Lanoy 2007). Increased mortality remains with very low rates (less than 25 CD4 T cells/µl) even six years after starting ART and maybe longer (ART-CC 2007). A complete reconstitution of the immune system is rarely the case if the patient's initial situation is poor – the worse the immune system, the more unlikely a complete recovery (Garcia 2004, Kaufmann 2005, Gras 2007). Viral suppression over several years cannot change that. In a study with patients on ART showing a constant low viral load below 1000 copies/ml for at least 4 years, 44% of patients with less than 100 CD4 cells/µl at initiation of ART failed to reach 500 CD4 T cells/µl even after 7.5 years. Patients with 100-200 CD4 T cells/µl still showed a risk of immune non-recovery of 25% (Kelley 2009). In our own study, a low CD4 T cell nadir remained associated with a lower CD4 cell recovery even after 15 years (Erdbeer 2014).

Another risk factor, besides low CD4 T cells, is advanced age, which has been observed

frequently with late presenters. The ability to regenerate the immune system decreases with age and is probably caused by degeneration of the thymus (Lederman 2000, Viard 2001, Grabar 2004). A consequence of a late start of ART can also mean that the antigen-specific immune reconstitution against HIV, as well as opportunistic viruses, remain poor. Many studies suggest that the qualitative immune reconstitution cannot keep up with the quantitative (Gorochov 1998, Lange 2002). But why does the risk of AIDS drop so dramatically with rising CD4 T cell count? How can patients with severe immunosuppression safely discontinue a prophylaxis, as soon as their CD4 T cell count is above 200/µl? Clinical observations seem to show differently, at least for the time being.

However, the relevance of a limited immune constitution in the long run is not yet clear. Recent data from the ClinSurv Cohort suggests that a discordant response (low CD4 T cells in spite of good viral suppression) is only associated with higher AIDS risk in the first few months. With virally well-suppressed patients, the CD4 T cells are no longer a good surrogate marker for risk of AIDS (Zoufaly 2009).

In contrast to the immunologic response, virologic response in combination with poor starting conditions is generally not worse than with other patients. Nevertheless, 89% out of 760 patients with AIDS at HIV diagnosis showed a viral load below 500 copies/ml after initiating ART (Mussini 2008).

When to start ART in Late Presenters?

Patients with a poor immunological state should begin ART quickly. This recommendation applies for CDC stage C (AIDS-defining diseases) and for all stage B diseases. However, it has not yet been agreed on how quickly one should start ART within the context of an acute opportunistic infection (OI). Up to now, many therapists preferred to tend to the acute disease first and to wait a few weeks before beginning ART. They hoped to avoid the unnecessary high complication potential of OI therapies. The first randomized trial addressing this idea has made this strategy questionable (Zolopa 2009). In ACTG A5164, 282 patients with acute OI (63% PCP, cases of tuberculosis were omitted) were randomized to start ART either immediately or at earliest time after completing OI therapy. On average, the "immediate" group started ART 12 days after initiation of OI therapy, whereas the "later" treated group after 45 days. Although the intervals were not so wide apart, distinct differences could be observed after 48 weeks: the group treated immediately showed significantly less fatalities and less new cases of AIDS. The risk to have to adjust ART was slightly higher, but not the number of severe undesired incidents, hospitalization or cases of IRIS. The authors concluded that patients with an acute OI (at least of PCP) should immediately start ART.

Regarding tuberculosis, at least five large randomized trials worldwide have discussed the optimal time to start ART (Abdool 2011, Blanc 2011, Havlir 2011, Török 2011, Wondwossen 2012). The general overview is as follows: Neither mortality nor AIDS-related mortality are significantly improved by immediate initiation of therapy. Patients showing below 50 CD4 T cells at diagnosis of tuberculosis seem to pose an exception. It must always be considered that immediate initiation always implies the risk of a paradoxical worsening of tuberculosis associated with IRIS, reaching up to 30% in some trials. Negative effects on survival have been observed in the case of tuberculosis meningitis (Török 2012). The same applies for cryptococcal meningitis (Makadzange 2010).

It is likely that differentiated recommendations depending on the OI must be given (Lawn 2011). There is also some controversial debate, as to whether patients with malignant lymphomas and newly diagnosed HIV infections should receive ART immediately or after chemotherapy (see chapter on *Lymphoma*).

ART for late presenters – What to start with?

An active OI is an obligatory exclusion criteria in almost every clinical trial. Thus, this patient group is always underrepresented in evaluation of clinical efficacy data. The question if late presenters should be treated with a special antiretroviral therapy is therefore not clear and depends more than with other patients on individual decision-making (Manzardo 2007) (see above on "What to Start?"). Regarding immunologic success, no relevant difference was measured between NNRTI- and PI-based regimens with late presenters (Landay 2003, Samri 2007). New ARV classes are also considered for late presenters. In favor of raltegravir are its low interaction potential, its overall tolerance and effectiveness in reducing viral load compared to efavirenz, especially in the first weeks (Murray 2007). However, as described in chapter 6.4, there is no evidence for a better immune reconstitution with drugs such as raltegravir or maraviroc or T-20 (not indicated in Europe for first-line therapy).

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6.6. What to start with

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Once the decision has been made to start, the next question is, what to start with? More than two dozen drugs are now available for first-line therapy, and the number of theoretically possible combinations seems to be almost infinite. In many guidelines, more than ten different combinations are recommended as "preferred", while numerous more are listed as "alternatives" in first-line.

Recommended first-line regimens

Combinations that we currently recommend for first-line therapy (as of January 2015) are shown in Table 6.1. In the list, there is no order of preference. Moreover, many other combinations are possible. These other combinations may be acceptable in individual cases or in investigational studies, but general recommendations for their use are not given. Problematic drugs or combinations that are not advisable for use are listed at the end of this chapter.

Table 6.1: ART combinations suitable for initial therapy (in no order of preference)

2 NRTIs	plus a	third agent	
TDF + FTC		PI/r:	Atazanavir/r , Darunavir/r, Lopinavir/r
ABC[1] + 3TC		NNRTI:	Efavirenz[2], Nevirapine[3], Rilpivirine[4]
Alternative:		INI:	Dolutegravir, Elvitegravir/c, Raltegravir
TDF + 3TC			

[1] Only when HLA typing is possible; caution when risk for cardiovascular events is high.
[2] Caution in women of childbearing age (teratogenicity).
[3] Beware of hepatotoxicity when CD4 T cells are high (women >250, men >400/μl).
[4] Caution if plasma viremia is high (>100,000 copies/ml)

Part 1: First line therapy – practical rules

All current initial regimens consist of two nucleoside analogs (NRTIs) combined with either a boosted PI, an NNRTI or an integrase inhibitor. All classes have their pros and cons – there is no one gold standard. When choosing primary therapy, besides the antiviral potency and tolerability, many other factors are involved. Individual factors, such as compliance, concurrent illnesses and concomitant medications, as well as the needs of the individual patient, should be included in the decision. Primary (first-line) therapy is of great significance and needs to be well prepared. It is at this time that the chance of viral suppression followed by long-term maintenance of suppression is greatest. However, many patients are very nervous at this point. Even today, knowledge about HIV therapy is often limited and expectations are often unrealistic ("do I need injections?", "will I be able to work?").

What should be clarified first

Dosing issues

For many patients the numbers of pills or requirements for food intake are important. The range of licensed and recommended initial regimens varies from 2 to 5 pills per day. Some patients find it unacceptable to have to take pills at certain times during the day with fatty foods as required with rilpivirine. A patient who works in

Practical tips for first-line therapy

- The first regimen offers the best chance of suppression. The viral load should decrease to below detection levels within 3-6 months.
- If possible, do not prescribe medication in the first consult with a new patient who brings their results. Do you know the patient well enough? Is (s)he really motivated? Will (s)he come back?
- Do not rush – the patient must be ready for ART. If in doubt, wait and continue to monitor levels.
- For every patient, prescribe the ART they are able to take. Do not insist on theoretically superior combinations. Once-daily treatment should be considered if it is important for the patient. What about eating habits, shift work?
- The pros and cons (side effects) of different combinations should be discussed – make enough time for this.
- The toxicity profiles should not overlap whenever possible – never use several allergenic drugs simultaneously.
- Ask about other medication (and drug use) – are relevant interactions to be expected?
- Is there a resistance test available?
- Concomitant illnesses should also be checked – what about the liver (hepatitis), kidneys?
- All drugs are started on the same day – no "lead-in" mono- or dual therapy.
- Be sure to check whether the patient would be eligible for a clinical study. All patients, especially if treatment-naïve, should be encouraged to participate in clinical trials.

shifts should not take efavirenz. Patients today are more demanding than before – justifiably so. There are now alternatives. Even the size or consistency of tablets can be a problem. A lifelong therapy must fit into daily life. It's about decades, not months. All these details must be discussed before initiating therapy as ART needs to become one more part of normal daily life.

Adherence

Compliance is defined as a patient's consent and acceptance of therapy. In the mid-90's a new term, "adherence", from the English, was adopted. Since then, the more politically correct term – "adherence" is frequently used. This term refers to both physician and patient working together to set up a treatment concept acceptable to both parties and emphasizes that responsibility for failure of the therapy is not automatically the patient's fault.

Adherence includes all factors that influence staying on a regimen, in terms of acceptability, under these three headlines:

1. The success of a treatment is endangered if medication is taken irregularly
2. Clinicians tend to overestimate a patient's adherence
3. Adherence diminishes with the complexity of the treatment

Is the patient able to take the pills on his own? Did he understand that ART is a lifelong treatment that should not be stopped when he feels better? Did he realize that there is no need to tolerate severe side effects? What is realistic, given his private and social background?

No doubt: adherence is the Achilles' heel of every antiretroviral therapy. Non-adherence is the main, if not the major factor for developing resistance and treatment failure (Turner 2000). Partial viral suppression with insufficient drug levels is an ideal condition under which resistance grows. ART must be taken regularly, correctly or

not at all. Taking either more than 90% or less than 69% of the treatment are both associated with a lower risk of resistance (Sethi 2003).

Not only drug users, those dependent on alcohol or patients with side effects are considered "risky patients" when it comes to adherence. In several studies, depressed patients, patients living alone and younger patients have been identified as problem groups (Murri 2001, Glass 2006). Positive factors are the physician's experience, the patient's confidence in the positive effects of ART, and social support. Race, sex or stage of disease does not seem to be relevant. The individual's general view of illness and health, accepting modern medicine and fear of side effects are further considerations. However, all these factors vary greatly, and in the end, adherence is difficult to predict in individual cases (Lerner 1998). The physician must rely on experience and intuition.

The importance of taking drugs regularly has been demonstrated in numerous studies. In one study with 99 patients, in which compliance was evaluated via an electronic monitoring system, the rate of viral treatment failure was only 22% in patients with a compliance level of at least 95% (95% of doses taken). Failure rates of 61% and as much as 80% were measured with a patient's adherence between 80–94% and <80% (Paterson 2000).

However, it must be taken into consideration that this much-cited study is outdated. Newer drugs, such as darunavir, with longer half-lives, higher resistance barriers and better overall pharmacokinetics may forgive a clearly higher non-compliance (Nelson 2010). In the previously mentioned study, clinicians misjudged their patient's compliance in 41% of the cases. Nurses did better – judging incorrectly in only 30% of the cases (Paterson 2000). Adherence tends to be overestimated in other studies as well (Miller 2002).

The importance of adherence was also demonstrated in patients with directly observed therapy (DOT) or directly administered ART (DAART), applied in some penal institutions in the US. In institutions in Florida, 100% of the patients in a DOT study achieved a viral load below 400 copies/ml after 48 weeks, compared to 81% in an unmonitored control group (Fischl 2001). According to one randomized study, response improved in drug-addicted patients receiving DAART (Maru 2009). However, more recent data indicate that effects of DAART with PI based regimens are marginal and disappear rapidly as soon as the patient is on his own (Gross 2009, Smith-Rohrberg 2009, Berg 2011). Only transient effects were seen in studies evaluating DOT in HIV+ methadone patients or in African patients (Nachega 2010, Berg 2011, Nahvi 2012). The virologic benefit of these strategies wanes following transition to self-administered therapy.
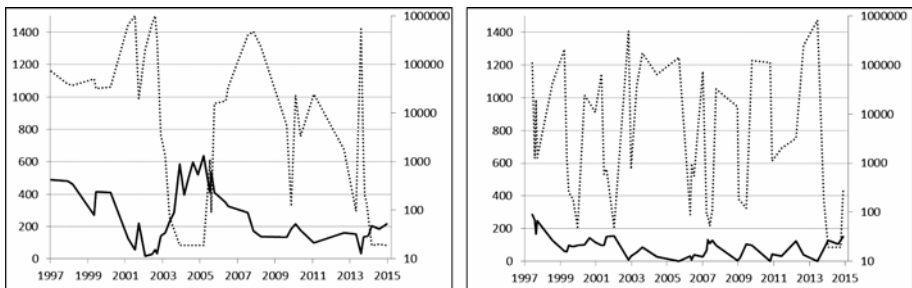
Figure 1: Absolute CD4 T cells (black, primary axis, left) and viral load (grey, dashed lines) in two HIV+ patients with adherence problems. Left: Female caucasian patient with only transient adherence. Right: African patient who takes ART only for weeks, followed by long interruptions and total absences from medical care. Over the years, in both patients, several AIDS defining illnesses and resistance mutations will have occurred

Poor adherence not only leads to virologic failure. It also bears immunological consequences (Mannheimer 2002). Moreover, poor adherence has clinical effects beyond surrogate markers. In a Spanish study, patients who did not take more than 10% of their drugs showed a four-fold increase of mortality risk (Garcia 2002). This data has been confirmed in other studies (Maher 1999, Hogg 2000, Wood 2004). Hospital stays are also less frequent in patients with high adherence to ART (Paterson 2000). In addition, it should be considered that non-adherent patients increase the risk of transmission of primary resistant viruses.

The basic mechanisms for development of resistance should be explained to patients. Intensive early adherence counseling at ART initiation results in a sustained, significant impact on adherence and less virologic treatment failure (Chung 2011). One must emphasize that, in contrast to other chronic illnesses, resistance mutations do not disappear once they have developed. Diabetes and hypertension make effective examples. These diseases may "tolerate" forgetting some tablets occasionally, but HIV is different. Blood glucose and blood pressure levels can easily be lowered again the next day, but with HIV this strategy may not work. Even short-term lapses can have irreversible consequences. And every new occurrence of resistance complicates therapy. Patients have to be made aware of these dangers. Such conversations should be repeated from time to time and become a standard component of routine care. Cooperation with special treatment discussion groups offered by patient-centered support organizations can be useful. The 12-step table below provides additional suggestions. In addition, a number of strategies on improving adherence have been investigated. They range from employment of additional nurses and patient community to telephoning patients regularly (Review: Kenya 2011). The effect of these strategies, however, depends on the individual setting of the patient (Collier 2005, Chung 2011, Pop-Eleches 2011).

If adherence remains poor

Despite all efforts, some patients will not succeed in improving their adherence. Physicians and other healthcare providers should not take this personally or feel offended. Although it may be difficult to accept the patient's views on life, disease and treatment, healthcare providers must keep tolerance and acceptance as key components in their interactions with patients. Some providers, especially those who treat selective patient populations in university settings, tend to forget the reality of routine medical practice. Rigidly upholding the principles of modern medicine usually does not help here and putting patients under pressure achieves even less. It is important to clearly outline and explain, advise, help, question and listen.

The question of whether noncompliant patients should continue to be treated with antiretroviral therapy is not always easy to address. On the one hand, there are patients who benefit even from suboptimal therapy; on the other hand, drugs are expensive and should not be prescribed too readily. Restraint should be applied until the reason for poor compliance is understood. Perhaps referral to counseling (peer support?) is needed.

Duesbergians – a sect

Patients who refuse antiretroviral treatment on principle are a special case. These patients are frequently not on treatment thanks to (shockingly misdirected) doctors, who call themselves "Duesbergians" (after the US virologist and AIDS dissident Peter Duesberg, who denies any association between AIDS and illness). In such cases, it can be very difficult to leave patients to their fate. Informative consultations should be as detailed as possible and preferably documented in writing.

Twelve steps to improve compliance

- Every patient should receive a written (comprehensible) treatment plan, which should be reviewed at the end of the visit. It should include a telephone number to call (or email address) in case of problems or questions.
- Patient and clinician should agree on the treatment plan. The patient's concerns, questions and criticisms should be discussed.
- The patient should have the impression that the treatment regimen is not randomly chosen, but tailored to his/her individual needs.
- The explanation of a new or modified treatment plan takes time, and should not be rushed – all questions should be answered.
- The reasons why adherence is so important should be explained. It makes sense to repeat such conversations – they should not only take place when initiating or modifying treatment, but should be part of routine care.
- Possible side effects should be explained, as well as what can be done to alleviate them.
- Support groups and other types of assistance should be specified and suggested.
- It is important to tell the patient to come back if any problems are encountered with ART – it is better to try to solve them together rather than have the patient try to deal with them alone at home.
- The patient should know that the treatment regimen must be taken in its entirety (avoid, "Last month I left out the orange tablets").
- Prescriptions should be documented, in order to get a rough idea of adherence. Irregularities should be addressed openly. Pills counted, bottles checked?
- During all stages of therapy, the patient should be informed of treatment success as seen by reduction of viral load and rise in CD4 T cell count.
- Detect the early signs of depression and treat appropriately!

An example: An approximately 40-year-old patient with a long history of untreated HIV, 30 CD4 T cells/µl and cerebral toxoplasmosis (TE), which improved significantly after 4 weeks of acute treatment (the last MRI still showed scattered lesions) introduced his case to the HIV outpatient department. Clinically, he was relatively well and fully oriented and due for discharge that day. In a conversation, the patient categorically refused to start the urgently recommended antiretroviral therapy. His Duesbergian physician had advised him against HIV therapy ("You can die from AZT, and the other drugs are not much better, etc"). He refused antibiotics on principle as well. This was why the patient would not continue the TE maintenance therapy, which had made him suffer from diarrhea (NB, probably cryptosporidiosis), skin problems (seborrhoic dermatitis, thrush), and extreme loss of weight (MAC?) since his first day in hospital. It was very important for him to have a break from all medication.

In such cases, we make sure the patients sign the information sheets. Every patient is allowed to and should decide for himself (if fully cognizant and capable) – they must be fully informed about what they are doing. It is important to give the patient control: if they change their mind, they may return! In our experience, arguing with medical Duesbergians leads to nothing at all. This sect has a very restricted view of the world and stick to their repetitive mantra-like arguments. Discussing with them is time-consuming and a waste of energy. Fortunately, these cases have become rarer. The initial widespread skepticism towards ART has decreased significantly, due to its overwhelming success in the last few years. Concerning Peter Duesberg, he is relatively quiet, as far as his HIV activities go. The sect is in decline.

Resistance testing

Prior to initation of first-line therapy, at least one resistance test should be available. In Europe, the prevalence of transmitted drug resistance mutations (TDRM) is around 10–15% (see Resistance Chapter). These mutations should be considered when choosing an ART regimen as a single NNRTI mutation such as K103N could compromise a whole combination. Although TDRM can persist in the absence of drugs for considerable time periods, many of them disappear over time, mainly due to fitness-costs. For example, the reversion rates of key mutations such as M184V (which reduces the replicative capacity of the virus) are very high. Thus, in untreated patients it is recommended to perform resistance testing as soon as possible.

Concurrent illnesses

Before starting treatment, concurrent illnesses should be identified (anamnesis, examination). This is fundamental in helping make the right choice (Table 6.2).

For example, a patient with diarrhea should not be given fosamprenavir or lopinavir. Use tenofovir or indinavir with caution in patients with renal disease. Atazanavir may also be associated with renal diseases (Mocroft 2010). ddI and d4T are contraindicated in patients with a history of pancreatitis or polyneuropathy and are no longer recommended in first-line therapy. Non-insulin-dependent diabetes can become insulin-dependent with PI treatment. Patients with osteoporosis or osteopenia should avoid tenofovir. If caution is needed with abacavir in individuals with an increased risk of myocardial infarction, as recommend by some experts (Behrens 2010), is not clear (see abacavir).

Liver disease and chronic hepatitis must also be taken into account, because the risk of developing severe hepatotoxicity on nevirapine or ritonavir is high (Sulkowski 2000). Caution is also required with boosted PIs. However, one study conducted in over 1000 patients found no difference between lopinavir/r and an unboosted PI such as nelfinavir in patients coinfected with hepatitis C (Sulkowski 2004).

In coinfections with HBV, 3TC, or even better, TDF+FTC should be utilized (Avihingsanon 2010). Long-term monitoring of HBV over a span of five years or longer is useful with tenofovir (de Vries-Sluijs 2010). However, in HBV-coinfected patients starting ART, two HBV drugs should be used in order to reduce the risk of HBV resistance. Avoid Combivir® or Kivexa® in cases of hepatitis B coinfection when no other HBV agent is on board – 3TC alone is not enough for HBV. Last but not least, a wish for parenthood should be considered. Women of child-bearing age should avoid efavirenz.

Table 6.2: Concurrent illnesses requiring caution with specific drugs (not only in first-line therapy). There are no absolute contraindications

Illness	Caution with
Active hepatitis B	Nevirapine, boosted PIs (beneficial: Tenofovir+ FTC)
Active illicit drug use	NNRTIs, ritonavir, cobicistat (possibly beneficial: raltegravir)
Anemia	AZT, possibly also 3TC
Arterial hypertension	Indinavir
Cancer requiring chemotherapy	Boosted PIs, boosted INIs (possibly beneficial: raltegravir)
Chronic diarrhea, intestinal diseases	Lopinavir, fosamprenavir, other PIs
Diabetes mellitus	PIs
Kidney disease	Tenofovir, atazanavir, elvitegravir/c
Myocardial infarction	Abacavir, ddI, PIs (potentially beneficial: nevirapine)
Osteoporosis	Tenofovir
Pancreatitis	ddI
Polyneuropathy	d4T, ddI
Psychoses, other CNS illnesses	Efavirenz, possibly rilpivirine

Interactions with medications and drugs

Interactions are important in when choosing regimens. Whereas interactions between antiretroviral drugs are well known, those with other medications are often less well characterized (see section on interactions). The urgent need for more research was demonstrated in a study investigating the interactions between ART and lipid lowering agents. In healthy volunteers, the measurement of plasma levels showed that levels of simvastatin were elevated by 3059% after concurrent dosing with ritonavir or saquinavir (Fichtenbaum 2002). Several cases of fatal rhabdomyolysis on simvastatin, atorvastatin and PIs such as atazanavir or lopinavir have been described (Review: Chauvin 2013). There are even case reports on pravastatin and rosuvastatin (Mikhail 2009, de Kanter 2011), so boosted PIs or INIs such as elvitegravir/c should be utilized with caution. The same applies to several HCV drugs such as daclatasvir. Many other drugs should be avoided in combination with particular antiretroviral drugs, as incalculable interactions may occur. These include certain contraceptives. Even drugs that seem unproblematic at first glance can have unfavorable effects. For example, the plasma levels of saquinavir can be reduced by half with administration of garlic capsules (Piscitelli 2002). One noteworthy interaction is between PIs and inhaled or intranasal corticosteroids. This interaction can result in adrenal insufficiency and iatrogenic Cushing's syndrome (Saberi 2013). Even a seemingly harmless agent such as vitamin C can influence plasma levels. A small study in healthy volunteers showed that vitamin C can significantly lower (14%) unboosted indinavir levels (Slain 2005). Coumarin derivative anticoagulants, such as warfarin can also be a problem; ritonavir can significantly lower plasma levels (Llibre 2002). Further typical problem drugs include migraine remedies, prokinetic drugs and sedatives/hypnotics. One fatal case was described with ergotamine and ritonavir (Pardo 2003). The simultaneous administration of ART and PDE-5 inhibitors (sildenafil, vardenafil, tadalafil) can also be problematic.

Drugs or alcohol can interact with ART (Neuman 2006, Mass 2006). For those in substitution programs, the methadone requirement may be significantly increased by certain antiretroviral drugs, such as nevirapine and efavirenz (Clarke 2001). To a lesser extent, this is also true for ritonavir and nelfinavir. There is inconsistent data on lopinavir but it may also require dose adjustments. Raltegravir, seems to have no effects (Anderson 2010).

Other interactions have even more dangerous consequences. Several deaths have been reported after simultaneous dosing with ritonavir and amphetamines or MDMA/ecstasy, the popular narcotic gamma hydroxybutyric acid (GHB) or "liquid ecstasy" (Henry 1998, Harrington 1999, Hales 2000). Ritonavir in particular inhibits the metabolism of amphetamines, ketamines or LSD (Antoniou 2002). Clinicians and patients are well advised to have an open conversation about drug use before starting therapy. Marijuana and THC appear to have a low potential for interactions (Kosel 2002). Amphetamines seem to be particularly dangerous and neurotoxic in HIV+ patients (Chana 2006).

Not every agent can be discussed here. Many are described in the respective drug chapters and in the *Interactions* chapter. It is always recommended to check the package insert. Initiation of ART provides a good opportunity to re-evaluate existing prescribed medications.

Additive toxicities

Although toxicity of newer antiviral agents has been markedly reduced, compared to first generation compounds such as AZT, ddI or d4T, several potential additive toxicities should be considered in the choice of therapy. If other myelotoxic drugs

(i.e., valgancyclovir, cotrimoxazole) are necessary, caution is required with AZT. When treating hepatitis C with interferon and ribavirin, ddI must be avoided. Ribavirin should not be combined with AZT or d4T. d4T should generally be avoided due to its potentially high toxicity. Tenofovir, indinavir, possibly also atazanavir should also be avoided with potentially nephrotoxic drugs. Interactions with the transport of creatinine have been identified with rilpivirine, dolutegravir, and cobicistat. Although these interactions can cause mild-to-moderate increases in serum creatinine concentrations that do not translate into real decreases in glomerular filtration, these interactions must be considered.

Lastly, it is not advisable during primary therapy to start with potential allergy-inducing agents if anti-infectious prophylaxis with cotrimoxazole or other sulphonamides is necessary. Included here are all NNRTIs and abacavir, but also fosamprenavir and darunavir. In these cases, it is better to avoid these ARVs. Otherwise, it can be difficult to clearly identify the causative agent for a drug-induced exanthema.

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Part 2: What drug classes should be used?

All combinations currently used as initial regimens consist of two NRTIs plus either a PI, an NNRTI or an integrase inhibitor. Advantages and problems of these three strategies are outlined in Table 6.3. There are considerable differences between these strategies with respect to pill burden, food restrictions, side effects, resistance risk, drug interactions and the amount of available data in special patient populations.

A third NRTI (triple nuke) is only used in exceptional cases and is only briefly mentioned here. All other combinations such as NRTI-free regimens or dual therapies are currently (January 2015) not justified for use outside the framework of clinical studies. Large, sufficiently powered, randomized studies directly comparing these different strategies are listed in Table 6.4. It is obvious that the amount of data differs from agent to agent. Efavirenz-based regimens were the comparator arm in many studies. On the other hand, for nevirapine and especially for rilpivirine, data derived from class-comparing studies is much more limited. With regard to PIs, most studies were performed with atazanavir/r and darunavir/r. Lopinavir/r was mainly used in resource-poor settings. Some of these studies are also shown in the table as they may be relevant in special settings. In contrast, the validity of previous milestone trials such as Atlantic (van Leeuwen 2003) is considered limited today due to outdated combinations and are not mentioned here.

In most of the trials, the antiviral potency of the regimens was comparable, measured by the number of patients with viral load below the limit of detection. Although there were considerable differences with regard to tolerability and the rate of resistance mutations, these studies do not provide enough evidence to compromise one of the three drug classes.

Table 6.3: Combining drug classes: Advantages (↑) and disadvantages (↓)

2 Nukes + PI	2 Nukes + NNRTI	2 Nukes + INI
↑ a lot of data, including clinical endpoints and severely immunocompromised pts.	↑ equivalent, perhaps even better suppression of viral load than with PIs	↑ very good efficacy, excellent tolerability
↑ long-term data available	↑ low pill burden, STRs available	↑ few interactions with some INIs
↑ high genetic resistance barrier	↑ leaves PI options	↑ maintains options
↓ high pill burden (for the older PIs), some once-daily regimens not licensed	↓ clinical effect not proven (only surrogate marker studies)	↓ limited long-term data
↓ No single tablet regimen (STR) available	↓ some restrictions for CD4 (NVP) and VL (RPV)	↓ limited long-term data
↓ frequent drug interactions	↓ less data in severely immuno-compromised patients	↓ once-daily with raltegravir not possible
↓ some PIs with cross-resistance, leaving limited options	↓ rapidly occurring complete cross-resistance, low resistance barrier	↓ No clinical endpoints
↓ lipodystrophy, dyslipidemia with most PIs	↓ strict monitoring required initially (esp. nevirapine), allergies frequent	↓ relatively low resistance barrier

Table 6.4: Randomized studies on approved agents of different classes as initial regimen

Study	Agent (n)	Main results: Virologic Failure (VF) and severe Adverse Events (AEs)
Large well-powered studies		
ACTG 5142 (Riddler 2008)	EFV versus LPV/r (250 + 253)	Less VF with EFV, severe AEs same (but more lipoatrophy on EFV)
ACTG 5202 (Daar 2011)	EFV versus ATV/r (929 + 928)	VF same, more severe AEs on EFV (in c. with ABC+3TC), but better lipid profile
ARTEN (Soriano 2011)	NVP versus ATV/r (376 + 193)	VF same, slightly more severe AEs and re-sistances with NVP, better lipids with NVP
NNRTIs versus INIs		
STARTMRK (Rockstroh 2011)	EFV versus RAL (282 + 281)	VF same, more AEs with EFV
GS 236-102 (Wohl 2014)	EFV versus ELV/c (352 + 348)	VF and tolerability same (more CNS -toxicity, less nausea with EFV)
SINGLE (Walmsley 2013)	EFV versus DTG (419 + 411)	VF better with DTG, more AEs and discontinuations with EFV
INIs versus PIs		
GS 236-103 (Clumeck 2014)	ELV/c versus ATV/r (353 + 355)	VF and tolerability same
FLAMINGO (Clotet 2014)	DTG versus DRVr (217 + 200)	VF risk low (no resistance mutations!), Tolerability of DTG better (less diarrhea)
ACTG 5257 (Lennox 2014)	RAL versus ATV/DRV/r (603 + 1206)	VF same, tolerability of RAL better than both PIs
Smaller trials or trials in resource-limited countries or in subgroups		
ALTAIR (Puls 2010)	EFV versus ATV/r (114 + 105)	VF same, AEs same (slightly less increase of peripheral fats with EFV)
KISS (Maggiolo 2009)	EFV versus ATV/r (124 + 62)	VF same, AEs same
PHIDISA II (2010)	EFV versus LPV/r (888 + 883)	VF same, clinical endpoints same (South Africa <200 CD4 T cells/AIDS)
Sierra-Madero (2010)	EFV versus LPV/r (95 + 94)	Less VF on EFV than on LPV/r, better lipid profile on EFV (Mexico, <200 CD4 T cells)
NEWART (De Jesus 2010)	NVP versus ATV/r (75 + 77)	VF same, lipids better with NVP
OCTANE II (Lockman 2012)	NVP versus LPV/r (249 + 251)	VF same, but more severe AEs with NVP (African women <200 CD4 T cells)
Lubumbashi (Clumeck 2012)	NVP versus LPV/r (425)	Less VF under LPV/r, clinical endpoints comparable (Zaire)

Note: Different (partly randomized) NRTI backbones were utilized, in some cases there were other trial arms. VF=Virologic failure, AE=Adverse events. Note: The MERIT study is not mentioned here, as maraviroc is not licensed for first-line therapy in Europe

NNRTIs versus PI/s

In ACTG 5142, an advantage of efavirenz over lopinavir/r was observed after 96 weeks (12% more patients got to below 50 copies/ml). However, if ART failed, resistance was less frequent and CD4 T cells increased more in the LPV/r arm. The ACTG 5142 trial showed that NNRTIs were possibly more effective than boosted PIs, because they were better tolerated. Resistance, however, occurs faster on NNRTIs than on PIs, which is probably due to the low resistance barrier. This phenomenon was observed in trials such as FIRST, ARTEN and ACTG 5202 (Gardner 2008, Daar 2011, Soriano 2011).

These observations were confirmed in a systematic evaluation of 20 studies that included 7,949 patients (see Table 6.5). All of the patients had been treated with either an NNRTI or a boosted PI, and had additionally received 3TC or FTC. Virologic failure was as frequent on NNRTIs as on PIs (4.9% versus 5.3% of patients, p=0.50). However, major differences were observed in patients with virologic failure whose genotypic resistance testing was successful. Mutations were significantly higher with NNRTIs. This applied for NRTI key mutations like the M184V and K65R, and also for other resistance mutations.

Table 6.5: Rates of resistance mutations at therapy failure on first-line regimens containing NNRTIs or PIs, in percentages (Gupta 2008)

	NNRTIs	PIs	p
M184V	35.3 (29.3–41.6)	21.0 (14.4–28.8)	<0.001
K65R	5.3 (2.4–9.9)	0 (0–3.6)	0.01
Resistance to third agent (NNRTI or PI)	53.0 (46–60)	0.9 (0–6.2)	<0.001

INSTIs versus NNRTIs or PIs

In large, randomized trials such as STARTMRK, GS102 and SINGLE, the three integrase inhibitors raltegravir, elvitegravir/c and dolutegravir were tested against the standard therapy efavirenz (Rockstroh 2011, Wohl 2014, Walmsley 2013). All three integrase inhibitors were better tolerated. More patients discontinued efavirenz, mainly due to CNS toxicity. In FLAMINGO, GS103 and ACTG 5257, the integrase inhibitors were also tested against boosted PIs such as atazanavir/r or darunavir/r (Clotet 2014, Clumeck 2014, Lennox 2014). Dolutegravir und raltegravir were superior, mainly with regard to tolerability.

It is important to consider, however, that tolerability depends on the specific setting and context of each study. During recent years, a switch to another regimen has become easier. The growing repertoire of antiviral agents implicates that the "tolerance threshold" of both patients and their physicians declines. Especially the tolerability of efavirenz has been reduced during recent years – in SINGLE, around 10% of all patients discontinued efavirenz due to CNS toxicity. These high rates have not been observed in older studies.

So what about resistance-associated virological failure in these studies, the most relevant endpoint? Data suggest that resistance-associated mutations (RAMs) are less frequently seen with raltegravir and elvitegravir (1–2%) than with NNRTIs (1–9%). Compared to boosted PIs, the rates are higher. In ACTG 5257 (ARDENT), a three-armed trial, raltegravir was superior to both PIs, mainly due to better tolerability, but there were more RAMs (18/603 = 3%) seen with the integrase inhibitor (Lennox 2014). With dolutegravir, however, no RAMs were seen in all clinical trials, suggesting that the resistance barrier of this compound is as high as that of boosted PIs. In the FLAMINGO trial, not a single RAM was detected in any of the patients failing on dolutegravir or darunavir/r – a first in HIV therapy (Clotet 2014).

Thus, the pros and cons for the different strategies continue, and controversy over the best first-line therapy persists. One should be warned against cross-trial comparisons, which are often used as marketing strategies to influence health providers on the effectiveness of a specific treatment ("we achieved over 90% tolerance rates in our study"). In a systematic evaluation of 10 large-scale randomized trials with 2,341 therapy-naïve patients receiving AZT+3TC+efavirenz, the success rates (viral load in the ITT analysis <50/copies/ml at 48 weeks) ranged between 37% and 77%. This broad range was seen with use of the same combination in ART-naïve patients! The rates of adverse events also differed considerably. Heterogeneous patient populations and study designs (definition of therapy failure), clinician experience and patient adherence may lead to variations (Hoffmann 2007).

Below, various strategies or primary therapies are discussed. These include:
• Two NRTIs plus an NNRTI
• Two NRTIs plus a PI
• Two NRTIs plus an integrase inhibitor (INI)
• Experimental combinations (nuke-sparing, intensive approaches)
• Three or four NRTIs (triple nuke, quadruple nuke)
• Problematic primary therapies to be avoided

1. Two NRTIs plus an NNRTI

NNRTIs have an equal if not superior effect on surrogate markers compared to PI combinations. In numerous randomized studies, efavirenz-based regimens were superior to unboosted PIs such as indinavir or nelfinavir (Staszewski 1999, Robbins 2003) and at least equivalent to lopinavir/r (Riddler 2003) and atazanavir (Daar 2010). When tested against INIs, efavirenz-based regimens were less successful, especially when compared to dolutegravir (Rockstroh 2011, Walmsley 2013, Wohl 2014). Nevirapine-containing regimens were roughly equivalent to atazanavir/r or lopinavir/r (McIntyre 2010, Soriano 2011). However, nevirapine-based (or rilpivirine-based) regimens were never tested against INIs.

Advantages of NNRTI regimens include the low pill burden and good long-term tolerability. In contrast to PIs, however, data with clinical endpoints is not available. Neither is there any long-term data or studies on severely immunocompromised patients. A disadvantage of NNRTI combinations is the rapid development of cross-resistance. This could result in failure, especially for highly viremic patients, although this has not been confirmed. Resistance upon virological failure is generally more frequent on NNRTIs than on PIs (Gupta 2008, see above).

Allergies are frequent on all NNRTIs. The incidence is highest with nevirapine, but allergies are also seen with efavirenz, etravirine or rilpivirine. Hepatic adverse events requiring careful monitoring (nevirapine) but also central nervous system side effects and potential teratogenicity (efavirenz) should be considered. The 2NN trial showed no significant difference in efficacy between efavirenz and nevirapine in combination with d4T+3TC (van Leth 2004). Rilpivirine seems to be less potent in patients with high baseline viremia.

TDF+FTC plus efavirenz was one of the most frequently used combination for many years. It is available as a single-tablet (STR), fixed-dose regimen Atripla®. During recent years, Atripla® has been less frequently used as many patients complain about CNS adverse events such as dizziness, sleep disorders and depression. With the growing repertoire of ART, patients are less willing to tolerate these well-known side effects. Although the bioequivalence with each individual substance has been shown, the EMA restricted the use of Atripla®. It is only approved for patients with virological suppression under 50 copies/ml for at least three months on their current anti-

retroviral regimen. Furthermore, patients must not have experienced virological failure with an earlier treatment combination or be known to have resistance to any of the three components in Atripla®. There are also generics available for efavirenz. It remains to be seen how many patients will switch from Atripla® back to generic triple-tablet regimens to obtain economic savings.

Instead of FTC one can also use 3TC. In the double-blind, randomized Gilead 903 Study, this combination was effective and less toxic than d4T+3TC plus efavirenz (Gallant 2004). However, the combination of TDF+3TC is seldom used today in Europe and the US, as there is no FDC available. There is no reason to use 3TC instead of FTC.

TDF+FTC plus nevirapine is also still a frequently prescribed regimen. However, there is less data available than for efavirenz. Smaller trials observed an increased risk for therapy failure and for development of resistance, especially when viral load was high (Lapadula 2008, Rey 2009). The large ARTEN trial also showed a higher risk for resistance with TDF+FTC plus nevirapine, but an altogether comparable efficacy to TDF-FTC plus atazanavir/r (Soriano 2011). In African woman with low CD4 T cells this regimen demonstrated equivalent antiretroviral efficacy but higher rates of treatment discontinuation and new drug resistance compared with lopinavir/r plus TDF/FTC (Lockman 2012). In favor of nevirapine are its good lipid profile (Podzamczer 2012) and the excellent long-term tolerability, despite some risk for severe allergies and hepatotoxicity in the first weeks. Since 2011, an extended-release tablet of nevirapine is on the market which can be taken once-daily. The old 200 mg tablets (for BD use) are available as generics.

TDF+3TC plus rilpivirine is available as the FDC tablet Eviplera® since November 2011. In a double-blind randomized trial (ECHO), this combination proved as effective as TDF+FTC and efavirenz, showing a slightly better tolerance regarding lipids and CNS side effects (Molina 2011, Cohen 2011). In the STaR Study, Eviplera® demonstrated non-inferior efficacy and improved tolerability compared to Atripla® – at 48 weeks, 2.5% versus 8.7% of patients had discontinued their ART due to adverse events (Cohen 2014). In addition, the lipid profile seems to better (Tebas 2014). However, resistance mutations and virological failure rates were higher, especially in highly viremic patients. This is why approval is limited to therapy-naïve patients with viral loads below 100,000 copies/ml. Eviplera® must be taken with a fatty meal in order to be absorbed properly. This may also be a drawback for some patients.

ABC+3TC plus efavirenz is an alternative first-line therapy, if HLA testing to predict hypersensitivity to abacavir is available. The combination ABC+3TC plus efavirenz has been evaluated with success in numerous large trials such as CNA30024 (DeJesus 2004), ZODIAC (Moyle 2005) and ABCDE (Podzamczer 2006). More recently, studies such as ACTG 5202 and ASSERT showed slightly less efficacy than on comparable regimens (Post 2010, Daat 2011). Tolerability is good – in ASSERT, less renal and bone side effects and were observed than with TDF+FTC plus efavirenz (Post 2010, Stellbrink 2010). Data on ABC+3TC plus nevirapine or rilpivirine are so far limited.

AZT+3TC plus efavirenz or nevirapine were among the most frequently used regimens for many years. They have been evaluated in numerous milestone trials (006, Combine, ACTG 384, 5095, 934). Side effects have limited its use. In the 934 Study, anemia and gastrointestinal problems significantly compromised the efficacy of AZT+3TC compared to TDF+FTC (Arribas 2008). Side effects such as increased lipids and lipoatrophy are significantly reduced by switching to TDF+FTC (Fischer 2010). Another disadvantage of these combinations including AZT is the fact that QD dosing is not possible. These regimens can only be recommended if there are good reasons not to use tenofovir or abacavir. One argument for this combination is economy – all agents are available as generics, making this a cost-saving option.

2. Two NRTIs plus a PI

The combination of two NRTIs plus one protease inhibitor is the only three-drug combination ART that is supported by efficacy data from randomized studies with clinical endpoints (Hammer 1997, Cameron 1998, Stellbrink 2000). Given the high resistance barrier and the robustness of these regimens, many experts still prefer to use boosted PIs today, particularly in advanced patients with high viral load.

Darunavir, atazanavir or lopinavir/r are the main agents. Lopinavir is coformulated with ritonavir, darunavir and atazanavir can also be boosted with cobicistat. Recently, the FDA and EMA have granted marketing approval to two fixed-dose combinations. Evotaz® is a combination of atazanavir and cobicistat, Prezcobix® or Rezolsta® contains cobicistat and darunavir. Saquinavir and fosamprenavir do not play an important role, nelfinavir and amprenavir have been taken from the market. Tipranavir is only used in specific salvage settings. Resistance on boosted PIs is significantly less than with NNRTIs or integrase inhibitors; PI/r resistance hardly exists (Gupta 2008). The slightly higher pill burden and frequent gastrointestinal side effects, which complicate adherence, are disadvantages of a PI-containing therapy. Often small factors are important when choosing the right PI, see Table 6.6.

Table 6.6: Frequently used PIs. Issues which may have an impact on treatment decision

	DRV/r/c	LPV/r	ATV/r/c	SQV/r	FPV/r
Pill number/day	1–2	4	1–2	6	4
Once daily dosing?	yes	yes	yes	no	no (US: yes)
Intake with food?	Irrelevant	Irrelevant	yes	yes	Irrelevant
Important side effects	Diarrhea (mild)	Diarrhea	Hyperbilirubin., icterus	Diarrhea (mild)	Diarrhea
Main study	ARTEMIS	Diverse	CASTLE	GEMINI	KLEAN

The following briefly describes the most common combinations:

TDF+FTC plus darunavir/r/c was licenced for initial therapy in 2009 and is one of the preferred first-line regimens in most guidelines. The combination proved at least as effective as TDF+FTC plus lopinavir/r in the ARTEMIS trial. With regard to tolerance (diarrhea, lipid changes) it was even better (Ortiz 2008). The effects remain stable out to 192 weeks (Orkin 2013). In a small study, the metabolic profile was comparable to atazanavir (Aberg 2013). The resistance barrier is very high and resistance mutations during first-line are rarely seen. Gastrointestinal symptoms may occur in some patients. In FLAMINGO and ACTG 5257, these problems led to slightly inferior results compared to the integrase inhibitors dolutegravir and raltegravir, respectively (Clotet 2014, Lennox 2014). An advantage of this combination is the once-daily dosing. Darunavir can also be boosted with cobicistat (Kakuda 2014). Recently, the FDA and EMA have granted marketing approval to the fixed-dose combination of darunavir and cobicistat (Prezcobix® or Rezolsta®), reducing the pill burden to two tablets with this regimen.

TDF+FTC plus atazanavir/r/c: In the CASTLE trial, atazanavir/r proved virologically equal to lopinavir/r, but with better lipids and similar tolerance (Molina 2010). Although a randomized study showed no difference between unboosted and boosted atazanavir (Malan 2008, Squires 2009), boosting with ritonavir is recommended. The main arguments in favour of this combination are the low number of pills and the good lipid profile (compared to lopinavir/r) which, however, does not differ from darunavir (Aberg 2013). The major disadvantage is hyperbilirubinemia, which often manifests as harmless but disturbing icterus. In ACTG 5257, at least 8% of the patients on atazanavir/r (combined with TDF+FTC or ABC+3TC) discontinued their ART due

to icterus (Lennox 2014). Atazanavir can also be boosted with cobicistat. Recently, the FDA and EMA have granted marketing approval to the fixed-dose combination of atazanavir and cobicistat (Evotaz®), reducing the pill burden to two tablets with this regimen.

TDF+FTC or ABC+3TC plus lopinavir/r have been categorized in many guidelines as a preferred combination. However, after the results of CASTLE, ARTEMIS and ACTG 5142 (see above), lopinavir/r was down-graded in the US (by the DHSS) to an "alternative" regimen. More data is available for TDF+FTC as a backbone for lopinavir/r, although the HEAT study did not find significant differences compared to ABC+3TC (Smith 2008). Since 2009 lopinavir/r has also been licensed for once-daily use, after several studies showed similar efficacy and tolerability (Molina 2007, Gathe 2009). However, there is some evidence that the potency of once-daily dosing is slightly less than with BID (Ortiz 2008, Flexner 2010). Lopinavir/r lost its main disadvantage of requiring cool storage compared to other boosted PIs with the introduction of the Norvir® tablets in 2010. The main problem with this regimen are the sometimes intense diarrhea, leading to high discontinuation rates. Recently, some studies on dual therapy with lopinavir/r plus 3TC have been published (see below Nuke-S sparing). AbbVie is currently working on a coformulation.

ABC+3TC (or TDF+FTC) plus fosamprenavir/r: In the KLEAN study, this combination proved almost equal to ABC+3TC plus lopinavir/r in regard to both efficacy and tolerability. Better rates of diarrhea or cholesterol levels were, however, not achieved (Eron 2006). In the ALERT study, fosamprenavir/r was as effective as atazanavir/r, both combined with a TDF+FTC backbone (Smith 2006). In Europe, once daily use of fosamprenavir/r has not been licensed, although using a low booster of 100 mg ritonavir should be possible (Hicks 2009, Cohen 2010). There is no clear argument for this combination.

TDF+FTC plus saquinavir/r: Saquinavir was the first PI which showed a survival benefit (Stellbrink 2000). In the relatively small GEMINI study saquinavir/r with a TDF+FTC backbone proved to be non-inferior to lopinavir/r (Walmsley 2009). The even smaller BASIC study showed that a once-daily dosing (1000/100) was comparable to atazanavir/r with regard to lipid profiles (Vrouenraetes 2009). The main disadvantage of saquinavir-based regimens is the twice-daily dosing, the high pill burden and a QT prolongation (ECG monitoring required!), which is why the combination is very rarely used today. Recently, however, generics of saquinavir have been introduced to the market.

3. Two NRTIs plus one integrase inhibitor

Raltegravir was licensed as the first integrase strand transfer inhibitor (INI) for first-line treatment in 2009. In the meantime, elvitegravir and dolutegravir were licensed by the FDA and the EMA. Tolerance and efficacy are both excellent. Convincing long-term data covering a period of 3-5 years, especially regarding tolerability, are lacking. However, there is no doubt that INI-based regimens will be of growing importance in first-line therapy during the next years.

TDF+FTC (TDF+3TC) plus raltegravir: in the large STARTMRK trial, raltegravir proved at least as effective as efavirenz (Lennox 2010). Viral load decreased more rapidly in the raltegravir arm and CD4 T cell counts increased. In addition, tolerance was better and effects lasted over 196 weeks (Rockstroh 2011). It should be noted that data is available for raltegravir with TDF-based backbones while data for ABC+3TC or other backbones is still very limited. A pilot study with ABC+3TC plus raltegravir, however, showed no negative effects (Young 2010). Unfortunately, once-daily dosing of raltegravir is not possible (Vispo 2010, Eron 2011). This is why MSD is working hard on a new formulation, allowing QD use (2 600 mg tablets). A main

advantage of raltegravir-based regimens are the excellent tolerability and the low potential for interactions. This may be used in patients with comedications, especially chemotherapies or tuberculostatics.

TDF+FTC plus elvitegravir/c: was approved as a single tablet regimen (STR, Stribild®) in June 2013. Two large Phase III trials yielded excellent results: In 236-0102 and 0103, elvitegravir/c has shown at least comparable efficacy over 144 weeks with efavirenz and atazanavir/r (Clumeck 2014, Wohl 2014). Tolerability was good, except for some more cases of nausea and diarrhea. However, the combination of tenofovir and cobicistat may be problematic as both agents interact primarily with distinct renal transporters. Cobicistat inhibits renal tubular secretion of creatinine and increases serum creatinine levels, resulting in a decrease in estimated glomerular filtration rate (GFR) without a true decline in GFR. Thus, it may be difficult to distinguish between these effects and the "true" renal toxicity of tenofovir. There exist detailed renal monitoring and dosing guidance (see also Drugs).

ABC+3TC (or TDF+FTC) plus dolutegravir: since the approval of dolutegravir in early 2014, these combinations have become an important option in first-line therapy. In SPRING-2, non-inferiority to raltegravir was shown in a double-blinded design (Raffi 2013). Encouraging data were also reported from FLAMINGO, when dolutegravir was superior to once-daily darunavir/r, mainly due to better tolerability (Clotet 2014). This was also the case with Atripla®, as shown in the SINGLE Study (Walmsley 2014). Of note, in all of these trials, there has been no report of treatment-emergent resistance with dolutegravir to this date. ABC+3TC plus dolutegravir are available as a single-tablet regimen, named Triumeq®. As with all regimens containing abacavir, HLA-testing is mandatory prior to initiation. When combined with TDF+FTC, slight increases of creatinine levels are seen, due to an inhibition of tubular secretion. Compared to other regimens, long-term data with this combination is relatively limited.

4. Experimental combinations

Antiretroviral therapies need to be more effective and tolerable. However, the pipeline of new ARVs is limited. New strategies are needed, including new combinations with old (approved) agents. Two new approaches have attracted great interest over the last years: combinations without any NRTIs (nuke-sparing), and so-called induction therapies. Both approaches will be discussed below.

NRTI-sparing and dual therapies

All classical ART regimens have to date included a backbone of two nucleoside or nucleotide analogs. This is mainly historical: nucleoside analogs were the first drugs on the market, and by the time NNRTIs and PIs were under development, treatment with two nucleoside analogs was standard. With growing knowledge of the mitochondrial toxicity of nucleoside analogs, omission of NRTIs ("nuke sparing") is increasingly being investigated, even for first-line therapy. A slightly modified strategy is followed by the so-called dual therapies in which only one NRTI is given instead of two. This is usually 3TC, for two reasons: as it is less toxic than others and it is available as generics.

Studies evaluating NRTI-sparing in ART naïve patients are shown in Table 6.7. – most of them were small pilot studies that were underpowered to show non-inferiority of NRTI-sparing strategies compared to standard therapy. However, in 2014 two large landmark studies, namely MODERN and NEAT001, have been published. These trials tested the combination of maraviroc or raltegravir with the boosted PI darunavir/r. They will be discussed in a more detail as well as other strategies such as the combination of NNRTI plus PI.

Table 6.7: Prospective studies on NRTI-sparing regimens in treatment-naïve patients and patients with little prior treatment experience (intent-to-treat analyses)

	n (naïve)	combination	(study)	%, <50 copies/ml
NNRTI + PI				
Staszewski 1999	148*	EFV+IDV	(006 Study)	47% at 48 Weeks
Boyd 2003	61*	EFV+IDV/r	(HIVNAT 009)	69% at 96 Weeks
Allavena 2005	86*	EFV+LPV/r	(BIKS)	73% at 48 Weeks (<400)
Riddler 2008	253	**EFV+LPV/r**	**(ACTG 5142)**	83% at 96 Weeks
Harris 2009	14	NVP+LPV/r	(CTN 177)	78% at 48 Weeks
Ward 2006	63	EFV+ATV/r	(BMS 121)	63% at 48 Weeks
INI/CCR5 + PI				
Mills 2013	60	MVC+ATV/r	(A4001078)	75% at 48 Weeks
Calcagno 2013	19	MVC+LPV/r	(VEMAN)	83% at 12 Weeks
Taiwo 2013	24	MVC+DRV/r	(MIDAS)	88% at 24 Weeks
Stellbrink 2014	396	**MVC+DRV/r**	**(MODERN)**	77% at 48 Weeks
Kozal 2012	63	RAL+ATV	(SPARTAN)	75% at 24 Weeks
Reynes 2012	103	**RAL+LPV/r**	**(PROGRESS)**	66% at 96 Weeks
Taiwo 2011	112	RAL+DRV/r	(ACTG 5262)	26% VF at 48 Weeks
Raffi 2014	401	**RAL+DRV/r**	**(NEAT 001)**	89% at 96 Weeks

*Patients all PI--naïve. VF=Virologic Failure. **Bold** = most relevant studies

NNRTI plus PI: these combinations have been evaluated over the years in small (underpowered) studies. ACTG 5142 was the first large study providing convincing evidence for the NRTI-sparing strategy (Riddler 2008, Haubrich 2011, see above). This study showed that a combination of lopinavir/r and efavirenz was not inferior to two NRTIs with either lopinavir/r or efavirenz. This was also shown by a smaller study (Harris 2009). In contrast, a randomized African trial found that different NRTI-sparing regimens (different NNRTIs plus PIs) were inferior to standard ART regimens (Duvivier 2008). It is still unclear whether side effects really improve with these regimens. A substudy of HIVNAT 009 reported that lipoatrophy resolved, and that visceral fat and subcutaneous limb fat increased (Boyd 2005). In CTN 177 NRTI-sparing regimens had a favorable effect on lactate levels (Harris 2005). In ACTG 5142 rates of lipoatrophy were lower in the NRTI-sparing arm (Haubrich 2009). However, adverse events in total were not reduced and dyslipidemia was observed even more frequently (Riddler 2008). Poor response rates were observed with double PI therapies, which is why this NRTI-sparing approach will probably not be further investigated (Landman 2009, Ulbricht 2011). In total, data on NNRTIs plus PI remains limited. These combinations should not be used outside clinical trials.

INSTI plus PI: Many studies are ongoing, especially with raltegravir, combined with the main PIs darunavir, lopinavir or atazanavir. Several trials with dolutegravir are ongoing. What does the current data say?

The randomized PROGRESS study evaluating 206 ART-naïve patients showed a more rapid and impressive reduction of viral load after 8 weeks with raltegravir and lopinavir/r than with the classic combination of TDF+FTC plus lopinavir/r. After 48 weeks, the antiviral effect was comparable. The results remained stable out until 96 weeks (Reynes 2013). There was a statistically significantly lower mean reduction in estimated GFR and less changes in bone mineral density with NRTI sparing.

In NEAT 001, a randomized, open-label, non-inferiority trial in treatment-naive adults in 15 European countries, 805 patients were treated with raltegravir BD plus darunavir/r or with TDF+FTC plus darunavir/r. Kaplan-Meier estimated proportions of treatment failure by week 96 were 17.8% and 13.8%. A few more patients on NRTI-

sparing showed virological failure and a total of 5/401 developed INI resistance mutations. Most of them had a high baseline viral load and low CD4 T cells. The frequency of serious or treatment-modifying adverse events were similar. Lipids tended to be worse with NRTI-sparing while GFR was better. The authors concluded that NRTI-sparing with raltegravir and darunavir/r was non-inferior to standard treatment and represents a treatment option for patients with CD4 T cell counts higher than 200 cells/µl (Raffi 2014).

PIs should always be boosted when NRTI-sparing regimens are used. In the SPARTAN trial, 4/63 (6.3%) patients developed raltegravir resistance mutations on a combination of raltegravir and unboosted atazanavir (Kozal 2012). This trial was stopped prematurely. A striking observation in SPARTAN was the high rate of severe hyperbilirubinemia (grade 4), with 21% on atazanavir plus raltegravir, compared to no cases in the TDF+FTC plus atazanavir/r. This combination can therefore not be recommended now.

CCR5 antagonists plus PI/r: Promising but preliminary results were shown with the combinations of atazanavir/r, lopinavir/r or darunavir, together with low-dose maraviroc. Resistance mutations were not observed in this early pilot studies (Calcagno 2013, Mills 2013, Taiwo 2013). However, MODERN, the first large study evaluating maraviroc plus darunavir/r in 797 patients, led to disappointing results (Stellbrink 2014). At 48 weeks, only 77% of the patients had achieved a viral load below 50 copies/ml with NRTI-sparing, compared to 87% in the standard arm with TDF+FTC plus darunavir/r. The vast majority of patients had low level viremia at the time of virological failure. Although non of them developed any resistance mutations, the data safety monitoring board decided to stop the trial in October 2013. Since thena, NRTI-sparing with maraviroc and boosted PIs cannot be recommended in first-line (pretreated patients see below).

Dual Therapy (NRTI sparing with only one NRTI): 3TC is generic and thus very attractive for diverse combinations. The best data are available for lopinavir. In GARDEL, the dual combination lopinavir/r+3TC (400/100+150 BID) was tested in 426 treatment naïve patients against lopinavir plus 2 NRTIs (Cahn 2014). At 48 weeks, 88% versus 84% of the patients had a viral load of less 50 copies/ml (patients with high baseline viral load: 87 versus 79%). More patients in the standard arm had discontinued their therapy due to adverse events. Lopinavir/r+3TC seems to be an interesting, less expensive option, especially in areas with limited ressources. It remains to be seen how guidelines in industrialized countries will deal with the GARDEL results.

Monotherapy, alternating therapy

Can it get any easier? Several studies introduced a very avant-garde concept in the summer of 2003: monotherapy with boosted PIs. With respect to the high resistance barrier of boosted PIs, success was considerable (Gathe 2009). Lipoatrophy can be avoided (Kolta 2011). However, in many studies, low-level viremia was found to be more frequent on monotherapies. In the MONARK study, only 64% (compared to 75% on AZT+3TC+lopinavir/r) of patients on lopinavir/r achieved a viral load of less than 50 copies/ml at 48 weeks (Delfraissy 2008). At 96 weeks it was only 47% (Ghosn 2009). Darunavir/r also started to show weaker effects in a small pilot study (Patterson 2009). According to one overview, the overall efficacy of monotherapy is slightly less effective to standard ART (Bierman 2009). This strategy is not recommended for treatment-naïve patients. In view of the constantly growing choice of well-tolerated combinations, it is difficult to find good arguments for monotherapy other than cost aspects.

Another approach is alternating therapy, which involves changing treatment every few weeks. In the SWATCH Study (Martinez-Picado 2003) a total of 161 patients were randomized to a regimen of d4T+ddI+efavirenz or AZT+3TC+nelfinavir. A third arm changed between the two regimens every three months when the viral load was below the level of detection. After 48 weeks, virologic failure in the alternating arm was significantly reduced. There was no difference for any other parameters (CD4 T cells, side effects, adherence, and quality of life). Considering the fact that several therapies are well-tolerated, alternating strategies, which can be very confusing for the patient, have never gained much attraction.

Induction with 4 or 5 drugs

Some experts speculate on whether more intensive approaches than conventional triple combinations are necessary for patients with high viral load. Because of fear of rapid development of resistance, some physicians give initial treatment with an induction of four or even five drugs, and then simplify to a triple combination once the viral load has dropped below the level of detection.

This theoretical concept has not yet been validated, and is based on hypotheses or smaller proof-of-concept studies (Ramratnam 2004) in which it has been shown that the viral load falls faster under intensive combinations than under standard therapies with three active drugs. Approaches in which multiple individual drugs (usually nucleoside analogs) are given have to be distinguished from approaches in which three instead of two classes of drugs are used.

Multiple individual drugs: Current data indicates that there is no benefit to using this strategy. Giving two PIs or two NNRTIs instead of one sometimes produces even negative results (Katzenstein 2000, van Leth 2004). There is also no evidence in favor of giving three instead of two NRTIs (Staszewski 2003, Orkin 2004, Mallolas 2008, Hammer 2010). In ACTG 5095 with 765 patients, there was clearly no difference between Combivir® plus efavirenz and Trizivir® plus efavirenz, not even when the starting viral load was higher, or with regard to resistance (Gulick 2005).

More drug classes: The data on whether to use three or two drug classes is less clear. Large studies on this subject, such as ACTG 388 (Fischl 2003), ACTG 384 (Robbins 2003, Schafer 2003), INITIO (Yeni 2006) or FIRST (May Arthur 2006) were conducted with old combinations with nelfinavir as the main PI and ddI+d4T as the backbone. Therefore, validity of these studies is limited. A more recent randomized study with additional doses of T-20 in late presenters showed some effect on the viral load after 24 weeks, but these results were not sustained through week 48 (Joly 2010). In two recent trials trial, a PI based regimen, intensified with raltegravir and maraviroc, initiated during early infection fails to significantly further impact virologic or immunologic responses beyond those achieved with standard PI-based ART (Markovitz 2013, Chéret 2015).

In summary, it is questionable whether intensification of therapy leads to any improvement at all and produces anything more than toxicity and cost. The studies above indicate that supposed improved efficacy (not shown in many trials) is counterbalanced by more side effects. Indeed, there is the risk of scaring patients away with the higher number of pills and side effects. It is unclear whether and in which patients such intensification of therapy is useful, and which drugs would be optimal.

5. Three or four NRTIs – triple nuke or quadruple nuke

Triple or quadruple nuke therapies have some theoretical advantages: fewer interactions, no side effects typical of PIs or NNRTIs, and the fact that all other drug classes can be spared for later. The major disadvantage of triple nuke therapies is that

they are virologically less potent than other combinations. While this may not be the case with quadruple nukes, the increasing knowledge of the mitochondrial toxicity of NRTIs makes pure nuke therapies less attractive.

AZT+3TC+ABC in a single tablet Trizivir® (BID) is the classic triple nuke therapy. Since ACTG 5095, Trizivir® is no longer equivalent (Gulick 2004) and clearly less effective than AZT+3TC plus efavirenz. This also applies for developing countries, where Trizivir® is still occasionally propagated (Munderi 2011).

AZT+3TC+TDF: We have some experience with this approach. Given the different resistance pathways of AZT and TDF, the thymidine analog seems to be protective against tenofovir-associated mutations (Mauss 2005, see chapter on resistance). However, larger studies have not been conducted.

AZT+3TC+ABC+TDF: Some studies have reported good responses and low rates of virologic failure on this quadruple nuke therapy (Moyle 2006, Gulick 2007). However, these studies were not powered to demonstrate equivalence to other combination regimens. In two randomized studies, discontinuation rates were high, due to adverse events (Mallolas 2008, Puls 2010). In the ALTAIR study, it proved less effective than the standard ART regimen (Puls 2010). The long-term toxicity and efficacy of these combinations is still unknown.

TDF+3TC+ABC+ddI should be avoided (Jemsek 2004, Gallant 2005, Khanlou 2005). In up to 49% of patients, early virologic treatment failure was seen, probably due to a low genetic resistance barrier (Landman 2005). This is also true for treatment-experienced patients who want to simplify their therapy (Hoogewerf 2003, Perez-Elias 2005). Conclusion: Pure NRTI combinations are not recommended for first-line therapy. Triple nuke is poorer in comparison to regimens of at least two classes and the results of some of the single-class combinations are truly not good. Data on quadruple nukes is too limited. However, triple and quadruple nuke therapy remains under consideration for maintenance therapy (see Chapter 7).

6. Suboptimal first-line therapies

Combinations generally considered to be suboptimal include all forms of mono- and dual therapy, especially two nucleoside analogs, but also one nucleoside analog plus one NNRTI (Montaner 1998). When using NRTIs, it is important to make sure that they are not competing for the same pocket. The thymidine analogs AZT and d4T are even antagonistic (Pollard 2002). The same is true for FTC and 3TC. According to a warning letter by the company BMS in March 2011, d4T should generally be avoided (not only in first-line).

Full dose ritonavir can be rejected as an active agent, as tolerability is very poor. There is no longer a reason to use ddI, indinavir or nelfinavir in a first-line regimen. Some drugs, such as T-20, etravirine, and tipranavir are not licensed for use in primary therapy. Drugs such as ddC (HIVID®), saquinavir-SGC (Fortovase®) and amprenavir (Agenerase®) have been taken off the market.

NNRTI combinations act non-competitively at the same site, and furthermore all can cause a rash, making differential diagnosis difficult. Efavirenz levels seem to be lowered considerably in combination with nevirapine (Veldkamp 2001). In the wake of the 2NN study, it seems clear that the combination of efavirenz and nevirapine should be avoided. The study arm with this combination fared worse than the other arms, mainly due to toxicity (Van Leth 2004).

TDF in a triple-nuke combination should not be administered. Many studies have reported poor response rates, particularly in combination with ABC+3TC (Hoogewerf 2003, Jemsek 2004, Khanlou 2005, Gallant 2005) (see Triple Nukes).

TDF+ddI: at least five trials looking at TDF+ddI plus an NNRTI resulted in a high failure rate, and some were stopped prematurely (Leon 2005, Podzamczer 2005, Maitland 2005, van Lunzen 2005, Torti 2005). BMS even issued a warning letter concerning TDF+ddI. There are unfavorable interactions between both agents. Toxicity is high (Blanchard 2003, Martinez 2004) – the combination of TDF+ddI no longer has a place in antiretroviral therapy. The same is true for FTC+ddI (Campbell 2013).

Starting gradually: All drugs should be started simultaneously. Highly significant differences were shown between patients who had received three drugs immediately compared to those patients who were started on only two drugs (Gulick 1998, Ait-Khaled 2002). This is significant in the long-term. A large cohort study showed that the risk of virologic failure was doubled even years later if dual therapy had been the starting regimen, even for as little as a few weeks (Phillips 2002). Initiating triple therapy gradually, as is sometimes practiced due to concerns of side effects, is wrong and dangerous.

Avoidable mistakes in first-line therapy

- Mono- or dual therapy (except in controlled trials) as well as a gradual introduction of therapy – always start with a complete ART regimen
- Starting at a lowered dose (except for nevirapine)
- T-20, delavirdine, tipranavir, etravirine, maraviroc (not licensed for primary therapy in Europe)
- ddC (HIVID®), SQV-SGC (Fortovase®), amprenavir (Agenerase®) – distribution has been stopped
- Ritonavir (not tolerated – only for use as low-dose booster)
- AZT+d4T and 3TC+FTC (antagonistic effects)
- D4T in general
- TDF+ddI (diverse reasons), d4T+ddI (toxicities)
- TDF in triple-nuke therapy (especially without thymidine analogs)
- Simultaneous introduction of ABC and NNRTIs without prior HLA testing (allergy potential)
- Efavirenz+nevirapine (too toxic)
- Efavirenz or nevirapine+raltegravir (low resistance barrier)

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6.7. When to switch

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Antiretroviral therapy has to be modified frequently, even though the rates of modification and interruptions have declined in recent years. In EuroSIDA, among almost 1200 patients who began ART after 1999, at one year after initiation, only 70% of patients remained on their original regimen. 24% had changed, and 6% were off all treatment (Mocroft 2005). In an evaluation of the Swiss Cohort, 42% of 1318 patients beginning ART between 2005 and 2008 had modified therapy after one year, 22% of them due to side effects (Elzi 2010). In general, ART is switched for three main reasons (interruptions will be discussed separately):
• Acute side effects
• Long-term toxicity (or concerns regarding them)
• Virologic treatment failure

6.7.1. Switching due to acute side effects

Not every acute side effect requires immediate modification. Mild nausea or diarrhea at the beginning can and should be tolerated. Gastrointestinal side effects that occur during the first weeks often improve spontaneously or can be treated symptomatically. The same is true for some allergic reactions and for mild CNS disorders. Talking with the patient, suggestions on how to tolerate or palliate certain problems with the idea that these will not continue indefinitely will help. However, certain adverse drug events almost always require discontinuation (see box).

Side effects that almost always require discontinuation/change of ART
• Severe diarrhea, which persists despite loperamide even after several weeks (usually with nelfinavir, lopinavir/r, fosamprenavir/r)
• Severe nausea, which persists despite metoclopramide, which requires continuous treatment or leads to significant weight loss (usually AZT, ddI)
• Persistent sleeping disorder (efavirenz)
• Polyneuropathy (d4T, ddI, possibly also 3TC), often resolves very slowly
• Severe anaemia (AZT)
• Severe, progressive muscular weakness (d4T, ddI)
• Pancreatitis (ddI, ddI+TDF, d4T+ddI, in rare cases lopinavir/r)
• Lactic acidosis (most often d4T+ddI, but also all other NRTIs)
• Severe allergies with involvement of mucous membranes, fever (typically abacavir, all NNRTIs, more rarely fosamprenavir or darunavir)
• QT prolongation (saquinavir, but also other ARVs)
• Renal failure (tenofovir/STRs, indinavir), nephrolithiasis (indinavir)
• Hepatotoxicity with transaminases >5 x normal values (nevirapine, tipranavir)
• Jaundice (nevirapine, atazanavir, indinavir, tipranavir)
• Rhabdomyolysis (raltegravir)
• Severe repetitive onychitis (indinavir, possibly also 3TC)
• Depression, psychosis (efavirenz, possibly also AZT)

6.7.2. Switching due to concerns over long-term toxicity

In the last few years, many clinicians have started to change virologically successful combinations out of concern for cumulative long-term toxicities, especially in cases of lipodystrophy and dyslipidemia. The switch strategy is based on the assumption that not all antiretroviral agents have similar toxicities. The most important switch studies are discussed below.

PI replacement with other agents

PIs may cause side effects in the long-term. Among these are lipodystrophy with abdominal fat accumulation and at the back of the neck, but also gastrointestinal side effects and dyslipidemia. Many randomized studies replacing a successful PI-based regimen by other drugs have been performed during recent years (Table 7.1). Taken together, these studies show that lipid levels are most likely to improve after switching to other agents, in particular rilpivirine, nevirapine and integrase inhibitors, and to a lesser extent, if ever, efavirenz. Quality of life and treatment satisfaction improved significantly in the switch arms of most studies, probably due to the reduced pill burden. In cases of lipodystrophy the effects are clearly poorer and less-well characterized.

Table 7.1: Randomized studies on switching from PIs to other drugs

Source	witch	n	Effect of switch on	
			VL control	Lipids (L), Lipodystropy (LD)
PI → NNRTIs				
Barreiro 2000	NVP	138	Advantage	L unchanged, LD better
Ruiz 2001	NVP	106	n.s.	L better, LD unchanged
Arranz-Caso 2005	NVP	160	n.s.	L better, LD better
Becker 2001	EFV	346	Advantage	L unchanged
Molina 2005	EFV	355	Advantage	L/LD n.a., AEs similar
Negredo 2002	EFV/NVP	77	n.s.	L only better with NVP better, LD unchanged
Calza 2005	EFV/NVP	130	n.s.	L even worse (if PI-patients received statins)
Palella 2014	RPV	476	n.s.	L better
PI → Triple Nuke				
Clumeck 2001		211	Advantage	L better, LD subjectively better
Opravil 2002		163	Disadvantage (Trend)	L better, LD unchanged
Katlama 2003		209*	n.s.	L better, LD better
PI → NNRTIs or Triple Nuke				
Martinez 2003	EFV/NVP/ ABC	460	Trend against ABC	L only better with ABC, LD unchanged
PI → INSTIs				
Eron 2010	RAL	350	Disadvantage	L better
Martinez 2010+2012	RAL	139	n.s.	L (and some biomarkers) better
Arribas 2014	EVG/c	433	Advantage	AEs similar

In all studies (except Martinez 2003), randomization was against continuing PIs. If available, 48 weeks results are shown. All patients were on PIs for several months at the time of the switch, with undetectable viral load. VL=viral load in the switch arm versus the continuing arm. LD=lipodystrophy, L=lipids, n.a.=not available, n.s.=not significant. *Here only 62% of patients were taking a PI, the remaining patients were on NNRTIs or a triple nuke regimen

Switching from a PI to other drugs poses an increased risk of virologic failure, particularly with prior NRTI treatment and the associated resistance mutations. One example of what could happen when the drug is changed for strategic reasons is shown in Table 7.2. This case demonstrates how careful one must be when switching drugs, if there is a past history of inadequate treatment (i.e., dual therapy).

Table 7.2: Example of what could happen on switching drugs (n.k.=not known)

Date	ART	CD4 cells	Viral load
1996-98	AZT+ddC	n.d.	n.d.
Since 1998	AZT+3TC+NFV (always under the limit of detection)	n.d.	n.d.
Nov. 2002	Findings: significant lipoatrophy. Decision to switch	688	<50
Feb. 2003	ABC+3TC+NFV	788	<50
Apr. 2003	ABC+TDF**+NVP** (=targeted regimen, notes below)	871	<50
May 2003	Severe rash, ALT/AST >500 U/l	n.d.	<50
Jun. 2003	ABC+TDF+3TC		
Aug. 2003	Resistance: M41L+D67N+M184V+L210W+T215Y	679	37,400
Sep. 2003	AZT+3TC+NFV	n.d.	59,100
Oct. 2003		n.d.	<50
Oct. 2004		743	<50

Notes: On account of possible allergies to both ABC and NVP, ART was changed in February 2003 in two steps. Rash with hepatic involvement occurred on NVP, so in July 2003 NVP was replaced by 3TC – a triple nuke. The resistance mutations then detected were acquired almost certainly from the earlier treatment with AZT+ddC, but sufficiently suppressed while on PI therapy

There is a risk of a higher failure rate when switching from PI based regimens to triple nuke, especially in patients with prior NRTI pretreatment (Bommenell 2011). Caution is need even with INSTIs. A higher failure rate was also seen in the SWITCHMRK trials in patients switching to the integrase inhibitor raltegravir (Eron 2010). In these two large-scale Phase II studies, a total of 702 patients on a stable and functioning lopinavir-containing regimen were randomized to change to raltegravir or to continue with lopinavir. Lipids improved with the switch, but after 24 weeks a non-inferiority of raltegravir compared to lopinavir/r in efficacy was not seen. In the ITT analysis, only 82% of patients on raltegravir compared to 88% on the continued PI maintained viral load below the limit of detection after 24 weeks. The viral load breakthrough applied especially for pre-treated patients with previous therapy failure. A smaller open-label randomized study in Spain did not make the same observations, however. Patients had been below detection for a longer period (Martinez 2010). In STRATEGY-PI, a trial in which patients were randomized to the INSTI elvitegravir/c or to remain on their PI regimen, no rebounds were seen. However, in this study patients with complex pre-treatment were excluded, in order to avoid sobering results like SWITCHMRK, (Arribas 2014). With elvitegravir/c, less diarrhea but more nausea was observed.

It is thus important to consider potential side effects of new agents when a switch from a PI is planned. With all NNRTIs, allergic reactions are possible. Efavirenz may be associated with adverse CNS events. There is the risk of a hypersensitivity reaction with abacavir if HLA typing is not available. Of note, there is still no data on a change or a PI substitution with maraviroc or dolutegravir yet.

Possibly the PI does not always have to be replaced with another drug class. In cases of dyslipidemia with lopinavir or fosamprenavir, switching to atazanavir could make sense as it is associated with a comparably good lipid profile (Gatell 2007, Soriano 2008, Mallolas 2009). Darunavir has a metabolic profile similar to atazanavir (Aberg 2012), however, there are no switch studies. There may be additional favorable effects

on lipids if atazanavir is not boosted, which seems to work well with pretreated patients with a viral load below detection (Sension 2009, Ghosn 2010, Wohl 2014). A new alternative could also be boosting of atazanavir (or darunavir) with cobicistat. However, patients must be informed about the risk of jaundice, which is typical for atazanavir.

Replacement of thymidine analogs with other NRTIs

The thymidine analogs AZT and d4T, which play a leading role in mitochondrial toxicity, is frequently replaced with other nucleoside analogs. An overview is given in Table 7.3.

Table 7.3: Randomized clinical studies on switching from d4T or AZT to other drugs

Source	N	Switch	Wk	Effect of switch
Carr 2002 Martin 2004	106	ABC instead of d4T or AZT	104	LA better, lipids unchanged
John 2003	37	AZT instead of d4T and ABC instead of PI	48	LA of limbs slightly better, lipids and abdominal fat unchanged
Moyle 2003	30	ABC instead of d4T or PI/NNRTI, or AZT+ABC instead of d4T+PI	48	LA better (when replacing d4T) Lipids better (when replacing PI)
McComsey 2004*	118	AZT or ABC instead of d4T	48	LA better, lactate better
Moyle 2006	105	TDF or ABC instead of d4T or AZT	48	LA better, lipids better on TDF
Valantin 2010	91	TDF+FTC instead of 2 NRTIs	16	Lipids better
Fisher 2009	234	TDF+FTC instead of AZT+3TC	48	LA better, lipids better
Ribera 2013	80	TDF+FTC instead of AZT+3TC	72	LA better
Ribera 2008	62	TDF instead of D4T	48	Lipids better, lactate better, LA slowly better
McComsey 2011	50	TDF or uridine instead of AZT or d4T	48	LA better, but reduction in bone density
Tebas 2009	101	ABC or nuke sparing instead of d4T or AZT	48	LA better
Milinkovic 2007	58	TDF or d4T reduction (30 mg) instead of d4T	24	LA, lipids better (TDF effects better than d4T reduction)

No study showed any difference with respect to virologic failure. Wk=weeks, LA=lipoatrophy. In McComsey 2004 and Moyle 2005, only patients with LA were investigated. *not randomized

Despite their heterogeneity, most studies show that lipoatrophy improves if d4T, and probably also AZT, is replaced (review: Curran 2011). In particular, the subcutaneous fat of the limbs increases, although at first the improvement is often unrecognizable clinically and can only be detected in DEXA scans (Martin 2004). Histological investigations have shown that the elevated rate of apoptosis in adipocytes normalizes when d4T is replaced (Cherry 2005, McComsey 2005).

Based on the available data, it seems advisable to replace d4T with another nucleoside analog. According to a warning letter by the company BMS of March 2011, d4T should only be used if other antiretroviral substances can not be used and duration of treatment should be as short as possible, and patients should change to a more suitable therapy alternative whenever possible. Unfortunately, it still plays a role in

resource-limited regions for the time being. A dose reduction may be able to reduce adverse events (McComsey 2008). With regard to AZT, a replacement should be considered when lipoatrophy or anemia becomes manifest. To avoid a hypersensitivity reaction the patient's HLA status should be known before switching to abacavir (Carr 2002).

Switching to tenofovir

Studies on ART-naïve patients have shown that the short-term mitochondrial toxicity of tenofovir is lower than that of d4T or AZT (Gallant 2004+2006). In the 903 Study, lipids improved in patients that were switched from d4T to tenofovir. There was also an increase of the mean limb fat after three years (Madruga 2007). Several studies, some of them randomized trials, point in the same direction. Lipids, lipoatrophy, mitochondrial toxicity and patient satisfaction improve on tenofovir (Milinkovic 2007, DeJesus 2008, Ribera 2008, Fischer 2010, McComsey 2012, Martinez 2012). Recently, a double-blind, placebo-controlled, randomized study showed unexpected results. In ACTG A5206, the addition of tenofovir alone to existing virologically-suppressed ART regimens improved lipid parameters compared to placebo (Tungsiripat 2010). However, the mechanism of the lipid-lowering effect warrants further study. In a retrospective study, replacing d4T with tenofovir improved both lipids and liver enzymes (Schewe 2006). There is also one trial showing that switching from ABC+3TC to FTC+TDF in persons with hypercholesterolemia on efavirenz maintains virological control and significantly improves key lipid parameters (Moyle 2015).

It must be noted that also negative effects may arise when switching to tenofovir. Randomized studies in treatment-naïve patients have observed a stronger reduction of bone density on tenofovir, compared to other NRTIs (Martin 2009, Stellbrink 2010). This negative effect is also seen in pretreated patients and there are also studies showing significant decreases in bone density after a switch to tenofovir (McComsey 2011, Haskelberg 2012, Rasmussen 2012). Bone turnover markers do improve when tenofovir is replaced by raltegravir or abacavir (Bloch 2012, Negredo 2015). The potential nephrotoxicity of tenofovir is another point.

Switching to tenofovir-containing triple-nuke combinations must be avoided in general, as several studies have shown a high risk for an increase in viral load when switching to this combination (Hoogewerf 2003, Perez-Elias 2005), even after many years on successful ART. The resistance barrier is too low, as the patients example above shows.

In practice, changes are often made that go further than PI and d4T/AZT, such as replacement of ddI, simply due to concerns over long-term toxicity. Such switching is based on laboratory studies showing a certain hierarchy with respect to mitochondrial toxicity.

A lot of attention is being drawn to simplification of therapy, in which mono- or nuke-sparing strategies are being used (see below). So far, there is no clear clinical evidence to show that this procedure has any benefit for the patient. If the patient has no complaints, a switch to monotherapy or NRTI-sparing can not be justified and subjects the patient to unnecessary risks. Below, current data on this topic is discussed.

Switching to NRTI-sparing

NRTI (Nuke)-sparing or dual therapy is the attempt to completely avoid or to reduce NRTIs in antiretroviral therapy. During recent years, many trials have been evaluated NRTI-sparing in pretreated patients with sustained virological suppression who switched on such regimens. There are also large trials which have evaluated this strategy in patients with failing regimens.

The NRTI-sparing combination of PI+NNRTI will not be further discussed here. Results with lopinavir/r and efavirenz or nevirapine were at best mixed. In some studies, this combination led to higher discontinuation rates due to increased virologic failure and other side effects (Fischl 2007, Tebas 2007+2009) and cannot be recommended.

PI plus INSTI: In ART naive patients, the NEAT001 study has shown promising results with raltegravir and darunavir/r, at least in patients with low baseline viral load and limited immunodeficiency (Raffi 2014). This argues for this approach also in pre-treated patients. However, data is limited.

Small, uncontrolled studies have evaluated atazanavir or darunavir/r plus raltegravir with encouraging results (Ruane 2009, Allavena 2009, Ripamonti 2009). Larger trials are ongoing. In the randomized KITE study on 60 virologically suppressed patients on ART, switching therapy to lopinavir/r plus raltegravir produced similar sustained virologic suppression and immunologic profile as continuous ART. Adverse events were comparable between arms. However, the NRTI-sparing arm experienced higher triglyceridemia but favourable effects on lipodystrophy and bone mineral density (Martin 2013). In the SPARE Study no favourable effect on renal function was seen with darunavir/r plus raltegravir (Nishijima 2014). Taken together, it remains unclear whether the combination of PI plus INSTI may improve long-term tolerability of ART. Positive effects on lipids are unlikely.

NNRTI plus INSTI (plus maraviroc): In a pilot study on 39 patients from France, the combination of nevirapine and raltegravir showed (somewhat surprisingly, given the low resistance barrier of both drugs) as sustained virological potency. The patients were treated for many years and had been below 50 copies/ml for at least six months on a standard nevirapine-based regimen. Some patients replaced the PI/r, some also TDF+FTC. After the switch on this combination, only one case of virological failure has occurred during 27 months (Reliquet 2014). Similar results were found in another pilot trial and in a retrospective study evaluating 25 and 91 patients on etravirine and raltegravir (Monteiro 2014, Calin 2013). The combination of NNRTI plus ralte-gravir plus maraviroc has been mainly tested in salvage settings. In an Italian study on etravirine plus raltegravir plus maraviroc, 25/26 remained below 50 copies/ml after four years (Nozza 2014).

NRTI-sparing without PI and NNRTIs: these combinations are experimental. They may go wrong, as shown recently by the ROCnRAL study. In this one-arm pilot trial, 44 patients with lipodystrophy were enrolled. The median time on ART was 15 years and all patients had R5 virus and an undetectable viral load for more than 5 years (Katlama 2014). After the switch to "Nuke+PI Sparing" with raltegravir plus maraviroc, 7 patients showed treatment failure, among them 3 with resistance muta-tions against raltegravir. These observations led to the premature discontinuation of this study. ROCnRAL but also experiences from an Italian study (Nozza 2014) clearly demonstrate, that caution is needed even in patients with long-lasting viral suppression. Die NNNB Study ("NoNucNoBoost") is currently evaluating whether the combination of raltegravir and maraviroc is feasible in patients without prior treatment and possible resistance mutations. In this pilot trial, ART naive patients are treated with TDF+FTC and maraviroc plus raltegravir for 24 weeks. In the case of viral suppression, patients will switch to maraviroc plus raltegravir only. In a preliminary analysis, 10/10 patients remained below 50 copies/ml at week 48 and the study was opened for further 30 patients (Cotte 2013).

Dual Therapy with 3TC: What worked well in the GARDEL study in ART naive patients (see above), did so in treatment-experienced patients. In two Spanish trials, OLE and SALT, 239 and 286 patients were enrolled. Patients had a viral

load below 50 copies/ml for at least 6 months on a standard ART and were randomized to remain on their PI regimen or to switch to lopinavir/r+3TC/FTC or atazanavir/r+3TC. At weeks, similar virological efficacy was shown for both regimens, compared to continued ART. Blips were observed at the same rates. However, tolerability was not improved with dual therapy (Gatell 2014, Perez-Molina 2014). AbbVie is currently working on a co-formulation lopinavir/r/3TC.

Taken together: In treatment-experienced patients with sustained virological suppression and without resistance problems, many of these new NRTI sparing strategies do work. But not in all patients! Without good reasons and outside clinical trials it seems too early to allow definite recommendation. During the first weeks, careful monitoring is needed in order to minimize the risk for new resistance mutations. It remains unclear whether lipodystrophy improves with these strategies. Data is too limited to draw definite conclusions.

6.7.3. Switching due to virologic failure

Any change in treatment due to virologic failure requires experience, a certain degree of finesse and decisiveness. There are many possibilities for mistakes here. On the one hand, there is a threat of acquiring more resistance (if they have not already developed), but on the other hand, young physicians often want to quickly change treatment, which is not always necessarily the right solution. In many cases a frequent change of therapy confuses the patient and causes anxiety. If the problem is adherence, switching the regimen without talking about adherence may not be the solution. A switch only brings up more misunderstandings and, consequently, may generate later resistance. It is always important to explain to patients, who often tend to be skeptical ("should I save the other drugs for later?") when and why treatment changes must be made.

As a rule of thumb, ART should be changed quickly with insufficient viral suppression and/or a rise in plasma viremia, as otherwise future options could be limited. One speaks of insufficient viral suppression or virologic failure if the viral load is repeatedly above the level of detection. A switch is not recommended with temporary viremia (blips – more on this topic in the chapter *Principles of Therapy*).

Even single point mutations can be a problem. Tenofovir, abacavir, 3TC, FTC and ddI lose their efficacy in the presence of the K65R mutation, which is often selected by tenofovir-containing triple-nuke therapies. Viral replication with insufficient plasma levels is the best breeding ground for resistance. Therefore, it is recommended to act fast if a clear virologic failure occurs. The longer one waits, the more complicated it becomes. An insufficient viral suppression means, as stated before, a repeated viral load above 50 copies/ml. Some clinicians tolerate levels of up to 500 or even 1000 copies/ml for months. We believe such hesitation is not justified in most cases when patients have good options and good adherence. A patient's frequent assertions of not having symptoms should not count too much, either. Obviously, such thoughts do not always play a role in clinical reality. In an analysis in Great Britain 34% out of 694 patients remained on a virologically unsuccessful combination for over 6 months. Factors associated with an early switch were low CD4 T cells, a high viral load and older age (Lee 2008).

To date, only a few randomized trials have investigated strategies in patients in whom several ART combinations have failed: either the patients change immediately or when the viral load reaches a certain level (early versus deferred switch). The preliminary results of some small randomized studies indicate that even in such cases one can wait a short time (Nasta 2006, Tenorio 2009). However, these trials were small. It seems difficult to recruit physicians and patients to participate in such strategy trials.

Arguments for a rapid switch in the case of virologic failure	Arguments for a later switch in the case of virologic failure
The virus becomes incapable of generating more resistance	New therapies bear the risk of new toxicities/intolerance, which can lead to a termination of therapy
Options are maintained	Most patients are immunologically stable for a long time with low viremia (clinically)
The switch is more successful with less resistance	Replication fitness is reduced on failing treatment
The lower the viral load at time of switch, the better the response to the new therapy	Resistance testing is often not possible with low viral load, even though they are there, so you may switch "blindly"
The following regimens do not have to be as complex as the present one – some things can be simplified (QD, no more d4T/ddI, etc.)	It is sometimes difficult to explain to the patient why change of a well-tolerated and simple regimen is necessary

With failing PI therapies there is more time. In the prospective Johns Hopkins Cohort there was no association between a deferral of ART modification and mortality in the course of treatment in patients on a PI showing virologic failure (Petersen 2008). This is why in the TITAN Study, the number of acquired PI mutations had no effect on the success of darunavir/r, although it did play a role for lopinavir/r (De Meyer 2008).

In cases of clinical treatment failure (disease progression) or immunological failure (stagnation or decrease in the level of CD4 T cells) where the viral load remains below 50 copies/ml, the value of a change in therapy is unclear. Some combinations such as TDF+ddI are clearly unfavorable for immunological reconstitution (Negredo 2004). This may also be the case for AZT-containing regimens; such combinations should be changed.

It is important that when virologic failure occurs, the individual situation of the patient is carefully analyzed. In particular, several questions need to be addressed:

What are the reasons for the measurable viral load? A viral load above 50 copies/ml does not necessarily mean that resistance mutations have developed. A frequent cause may be a blip (see section on Goals and Principles of ART). These transient and, almost always, small increases in viral load usually have no relevance. However, a measurable viral load may be due to treatment failure. It may indicate insufficient plasma drug levels (measure these if possible). This may be due to drug malabsorption, drug interactions or simply insufficient dosing (e.g., in larger, heavy patients).

How is the patient's adherence? Adherence is critical. Any difficulties related to the regimen should be openly addressed. Is it the number of pills? Do restrictions in food intake cause problems? Would once-daily treatment be better? Are there other reasons, such as depression? Any misunderstanding on how to take the drugs? The risks of resistance development as a result of non-compliance should be reiterated. If plasma levels are sufficient and viral load remains detectable (monitor blips at short intervals – within a few weeks), treatment should be changed as soon as possible.

How vulnerable is the present combination? NNRTI regimens are extremely sensitive, and cross-resistance can develop particularly rapidly for the whole class. A prompt change in therapy is more vital than with the other drug classes. Delaying

this by even a few days or weeks may be too long. Rapid development of resistance can also be expected with 3TC/FTC and probably with the integrase inhibitor raltegravir. A PI-containing regimen without an NNRTI may allow a little more time, but the credo still applies. The higher the viral load at the time of modification, the lower the chance of success. One should not wait too long.

What options does the patient have, and what are the consequences of the change in therapy? The more options that remain available the sooner they should be utilized. Therapy can sometimes be intensified quite easily (e.g., adding abacavir plus an NNRTI). In such cases, the decision to change or intensify a regimen is less difficult. On the other hand, it may be advisable in certain circumstances to continue therapy in a patient, even if the plasma viremia is not completely suppressed. Often, the viral load does not rise above the baseline value, and the CD4 T cells remain stable or even increase. Some experts advocate waiting in these cases. Resistance to nucleoside analogs are to be expected, so NNRTIs and PIs can be saved by waiting.

Even when multiple resistance mutations are already present, one is probably able to wait (see above). Especially in patients with adherence problems, it does not make sense to run through new drug classes. Adherence will not automatically be better with newer regimens. One should talk with the patient, find out what needs to be made better, and clarify if they are really ready for intensification or modification of therapy.

Virologic failure: to be considered before changing therapy

- How resistance-sensitive is the present therapy? NNRTIs, 3TC/FTC, raltegravir, elvitegravir: rapid development of resistance, change quickly
- The lower the viral load, the greater the prospect of success with a change
- Are you sure it is virologic failure and not a temporary blip? Check viral load within 2–4 weeks!
- Are there other reasons for a detectable virus load? What about malabsorption/drug uptake?
- Do you know all the other therapies the patient is taking? Ask. Whether a gastric stimulant prescribed by the family doctor (i.e., PPIs) or herbal agents prescribed by an alternative doctor, it should all be laid out
- Has the patient been adherent to current ART or have there been misunderstandings? Was the therapy discontinued *ad hoc*?
- What do the plasma levels say and what does the patient say?
- What options are there and what does a change mean for the patient? Is the patient able to start a new therapy?
- Does a reasonably up-to-date resistance test exist? (if not, do one)
- If relevant mutations to the current agents have already developed, calmly wait and prepare the patient for a new regimen, possibly with more adherence counseling

6.8. How to switch ART

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Changing a regimen that is successful but intolerable due to side effects is usually easy. The suspected drug is replaced with another drug of the same class. It becomes more difficult if alternate drugs are contraindicated because of potential toxicity or if resistance mutations to these drugs are suspected. In such cases, changes need to be individualized. This is particularly true in subjects with a treatment history of 15 years or longer, who probably harbor multi-resistant viruses. Even physicians with lots of experience in treatment should discuss these complex individual cases with their colleagues. In many large centers, so-called weekly ART meetings have been established. At these meetings, both virologists (to translate resistance testing) and clinicians (with personal experience of the individual patient's situation) can discuss these complex cases. This chapter discusses two important reasons for switching where certain principles should apply: changing due to virologic failure, and changing to simplify the regimen. Switching due to possible side effects has been discussed in previous chapters.

6.8.1. Switching due to virologic failure

The same principles apply as when initiating therapy: compliance, dosing issues, concurrent diseases, co-medications and drug interactions. It is also essential to consider treatment history and possible existing resistance mutations. Although desirable before any change in treatment, resistance tests in cases of virologic failure are not always practical. It is therefore useful to become familiar with the most important resistance mutations, particularly for nucleoside analogs:

Table 8.1: Expected resistance mutations with different nuke backbones

Failing nuke backbone	Mutations
AZT/d4T+3TC AZT+3TC+ABC	M184V and then successive TAMs, the more one waits
TDF+3TC/FTC	K65R and/or M184V
ABC+3TC	L74V, less frequently K65R and/or M184V
AZT/d4T+ddI	TAMs, Q151M, T69ins
TDF+ABC/ddI	K65R

Table 8.2: Changing first-line therapy without knowledge of resistance mutations*

Failing initial therapy	Potentially successful change
2 NRTIs + NNRTI	Replace NNRTI by a boosted PI (rapid switch) or 1–2 new/active NRTIs plus boosted PI or boosted PI plus RAL
2 NRTIs + 1 PI/r	1–2 new/active NRTIs plus INSTI (preferably DTG)
2 NRTIs + 1 INSTI	1–2 new/active NRTIs plus boosted PI (rapid switch)

* In individual cases, other modifications or simply waiting may be advisable. For complex cases, see chapter on Salvage Therapy

The basic principles for changing therapy in cases of virologic failure apply: the faster the change, the better; the virus should be given as little time as possible to generate more resistance mutations. Resistance patterns become more complex the longer one waits (Wallis 2010). In addition, the more drugs that are changed, the higher the likelihood of success for the new regimen.

Table 8.2 provides a rough guide on how to proceed without knowing the specific resistance mutations. Of note, in individual cases, other modifications or simply waiting may be advisable. For complex cases, see also the following chapter on Salvage Therapy.

Virological failure with NNRT-based regimens

There is usually complete cross-resistance with efavirenz and nevirapine. Resistance develops quickly. This applies also for rilpivirine which is vulnerable especially in highly viremic patients. Continuation in the presence of these resistance mutations is of no use as they have no impact on the replicative fitness of the virus. Moreover, accumulation of further resistance mutations may compromise the efficacy of second generation NNRTIs such as etravirine. Therefore, NNRTIs should be discontinued if resistance occurs or quickly be replaced by etravirine if the situation allows (etravirine is only approved for use in combination with boosted PIs). Reduction of etravirine activity seems to take longer in patients experiencing therapy failure on nevirapine *vs.* efavirenz (Cozzi-Lepri 2011). In patients with an isolated K103N mutation, rilpivirine remains effective as shown by a small case series (Rokx 2014). In patients with long pretreatment with NRTIs and NNRTIs, however, a boosted PI should be used in the case of virological failure. This boosted PI can also be combined with an INSTI such as raltegravir, without continuing NRTIs. In SECOND-LINE (2013), an open-label non-inferiority trial at 37 sites worldwide, 558 patients with a failing NNRT-regimen, were randomized to receive lopinavir/r plus either two or three NRTIs (control group) or raltegravir. The NRTI sparing raltegravir regimen was no less efficacious than the standard of care and was safe and well tolerated. Both strategies maintained efficacy greater than 75% and results were sustained until 96 weeks (Amin 2015).

The largest study on patients with NNRTI failure was performed in Sub-Saharan Africa (Paton 2014). In this open-label, three-arm trial, 1,277 patients were randomized to receive a) lopinavir/r plus clinician-selected NRTIs b) a PI plus raltegravir or c) PI monotherapy after 12 weeks of raltegravir induction. The primary end point (chosen due to the resource-limited setting) was "good HIV disease control", defined as survival with no new AIDS events, a CD4 T cell count of more than 250 cells/µl and a viral load of less than 10,000 copies/ml. Good HIV disease control was achieved in 60%, 64% and 55% of the patients. NRTIs retained substantial virologic activity without evidence of increased toxicity, and there was no advantage to replacing them with raltegravir. Virologic control was inferior with PI monotherapy. Although these results may not fully transferable to industrial countries, they show that NRTI-sparing can be an alternative and that monotherapy in patients with virological failure is not a good idea. PI-monotherapies which can be an option as a maintenance strategy in patients with virological suppression (see below) have shown sobering results in another study (Bunupuradah 2013).

Virological failure with PI-based regimens

There are also relevant cross-resistance mutations for PIs. In the case of virological failure with first-generation PIs such as saquinavir or indinavir, these agents can be replaced by lopinavir/r or darunavir/r. If these PIs fail, an INSTI regimen is necessary. For switching and sequencing PIs refer also to the salvage section of the next chapter.

On account of the high resistance barrier of lopinavir/r and darunavir/r, the regimen need not be rapidly changed in cases of low level viremia (LLV). LLV during PI therapy does not always indicate virological failure. Even in the presence of the NRTI mutation M184V, ART can be continued. One study showed that if M184V is detected alone, cytidine analogs 3TC or FTC can be continued, provided a boosted PI is initiated. The effect of the boosted PI is enough to achieve virological success – 3TC seems to be able to conserve M184V that in turn lowers viral fitness (Hull 2009). If enough new agents are active, it may be reasonable to omit NRTIs in treatment-experienced patients failing a PI regimen, as shown by the ACTG OPTIONS Study (Tashima 2013).

In patients with a truly failing PI-regimen (repeated viremias above 200 copies/ml, detection of PI resistance mutations), a new INSTI regimen is recommended. A new NNRTI alone is often not sufficient (Abgrall 2007, Khaykin 2008). The two INSTIs raltegravir and elvitegravir/c were of similar potency in patients with virological failure (most patients were on PI-based regimens). In 145, a double-blinded randomized study, patients were randomized to elvitegravir QD or raltegravir BID with a fully active boosted PI plus a third agent. The proportion of subjects maintained HIV-1 RNA <50 copies/mL through week 96 were 48% and 45% (Elion 2013). Dolutegravir seems to be even more potent, as shown by the large SAILING trial, a double-blinded non-inferiority study in 715 patients with virological failure and resistance to two or more classes of antiretroviral drugs. Patients received dolutegravir 50 mg QD or raltegravir 400 mg BID, with investigator-selected background therapy. At week 48, 71% patients on dolutegravir had HIV-RNA less than 50 copies/ml, versus 64% patients on raltegravir. Superiority of dolutegravir versus raltegravir was concluded. Of note, significantly fewer patients had virological failure with INSTI RAMs on dolutegravir (treatment-emergent integrase-inhibitor resistance on dolutegravir (four vs 17 patients). Dolutegravir seems to have the highest potential in pre-treated patients with PI-failing regimens (see next chapter).

Virological failure with INSTI-based regimens

Failure of an INSTI-based regimen in first-line is a rare event. With elvitegravir or raltegravir, the risk seems to be around 1–2%. If these regimens fail, a rapid switch is recommended, in order to preserve the efficacy of dolutegravir. Dolutegravir remains effective in patients with limited INSTI RAMs (see Salvage Chapter). In the case of concomitant NRTI resistance mutations, a boosted PI should be considered.

6.8.2. Simplification – do maintenance therapies work?

Can HIV infection be treated in a similar fashion to mycobacterias, with a sequence of intense induction therapy followed by less toxic (and less expensive) maintenance therapy? The idea is appealing, and has circulated almost since the existence of combination ART. Between 1998 and 2003, the answer was clearly that maintenance therapies do not work. Three randomized studies (Trilège, ADAM, ACTG 343) destroyed any hope that ART might be reduced to two or even one drug. By today's standards, one could object that outdated agents such as saquinavir, indinavir or nelfinavir were used (Havlir 1998, Reijers 1998, Flander 2002).

In the last few years better drugs have been licensed. In particular, lopinavir and darunavir with high resistance barriers cast a different light on the negative image of maintenance therapies. Randomized studies already exist for lopinavir/r and darunavir/r, but other boosted PIs such as atazanavir/r have also been investigated as PI/r monotherapy (see Table 8.3).

Tabelle 8.3: Newer studies on maintenance therapies (PI/r monotherapy)

Source	N	"Maintenance"	Wk	Less than 50 HIV RNA copies/ml?
Nunes 2009 (KalMo)	60	LPV/r versus 2 NRTIs+LPV/r	96	80 vs 87% (ITT, VL <80)
Campo 2009 (M03-613)	155	LPV/r versus CBV+EFV	96	60 vs 63% (ITT), but low-level viremia more frequently
Pulido 2008 (OK04 Study)	205	LPV/r versus 2 NRTIs+LPV/r	48	85 vs 90% (ITT), Non-inferiority shown, but more frequent low viremia
Meynard 2010 (KALESOLO)	186	LPV/r versus ART-continuitation	48	84 vs 88% (ITT), Non-inferiority not shown, more frequent low viremia
Gutmann 2010	60	LPV/r versus ART-continuitation	24	21% VF on Mono. Especially those with low CD4 nadir, study discontinued.
Cahn 2011	80	LPV/r versus ART-continuitation	48	98 vs 95% (LOCV, VL <200)
Clumeck 2011 (MONET)	256	DRV/r versus 2 NRTIs+DRV/r	96	78 vs 82% (ITT), Non-inferiority not clearly shown completely
Valentin 2012 (MONOI)	225	DRV/r versus 2 NRTIs+DRV/r	96	84 vs. 88%, Non-inferiority not clearly shown (low viremia more frequently)
Castagna 2014 (MODAt)	103	ATV/r versus 2 NRTIs + ATV/r	48	73 vs 85% (ITT), when re-intensification considered no VF: 92 vs 85%

ITT = Intention to treat, VF = Virological Failure

Studies show that in most cases virologic suppression can be maintained when simplifying to a PI/r monotherapy. In the OK04 study with lopinavir/r, even a reduction of lipoatrophy rates was achieved. The observation period was extended to four years (Cameron 2007, Pulido 2008). However, other studies failed to show any effect on lipoatrophy (Bernadino 2013). In MONARK, bone density improved after a switch to darunavir/r monotherapy (Guarladi 2014).

However, some patients on lopinavir/r show low levels of viremia, especially in combination with low CD4 T cells. They tend to show poor compliance (Campo 2007, Pulido 2008, Gutmann 2010). The same was observed with therapy-naïve patients (see above).

For darunavir, the results of two large randomized studies MONET and MONOI with identical design are published (Clumeck 2011, Valentin 2012). In MONET, non-inferiority of the monotherapy could not completely be shown after 96 weeks, at least regarding the primary endpoints (Clumeck 2011). In total, 82% of patients were below 50 copies/ml in the standard arm at week 96, compared to 78% on darunavir monotherapy. At week 144, the difference was 6% (Arribas 2012). When virologically successful therapies were not evaluated as failure, a difference was not observed. The results can be explained by a possibly low adherence in the mono-arm (with significantly more HCV-coinfected patients).

In MONOI, transient viremia was more frequent on monotherapy and a permanent control under 50 copies/ml without blips was observed in 59% versus 70% of patients at week 96 (Valantin 2012). Virologic failure was associated with levels of proviral DNA at baseline (Marcelin 2011), but also with low adherence (Lambert-Niclot 2011). Of note, darunavir RAMs were not observed either in MONET or in MONOI (Lambert-Niclot 2012, Pulido 2012). Possibly, darunavir levels are lower without NRTIs (Garvey 2010). In MONOI, lipoatrophy improved in some patients (Valantin 2012). Less data

is available for other PIs. For indinavir/r, saquinavir/r and fosamprenavir/r there are one-arm pilot studies with weak results (Kahlert 2004, Patricia 2010, Saumoy 2011). The Ataritmo study observed an elevated viral load in cerebrospinal fluid within some patients on atazanavir with otherwise well suppressed viral loads. In the OREY study, 9/63 patients developed virological failure (Pulido 2009).

In a systematic review of 10 randomized trials of 1,964 patients with HIV RNA suppression at baseline, PI monotherapy showed a higher risk of HIV RNA elevations, and small numbers with HIV RNA detectable in CSF and concomitantly in the plasma. However, there was no increased risk of treatment-emergent drug resistance (Arribas 2014). The risk of treatment emergent NRTI or PI resistance was 11/973 (1.1%) for patients on PI monotherapy, versus 7/991 (0.7%) for patients on triple therapy. HIV-1 RNA suppression rates after intensification were similar between PI monotherapy and triple therapy.

More recently, new approaches such as dual therapies (ususally with 3TC as a single NRTI) have been evaluated. In studies like SALT and OLE, atazanavir/r or lopinavir/r have been combined with 3TC (see above), the results are encouraging. In contrast, dual therapy with an NNRTI is not recommended. In the COOL study, many patients developed virological failure on TDF plus efavirenz (Girard 2006).

Conclusion: Monotherapies with boosted PIs such as lopinavir/r and darunavir/r are slightly less effective than classic therapies (review: Mathis 2011). In most cases, low-level viremia without resistance appears that does disappear upon intensification (Arribas 2014). Risk factors for monotherapy failure are poor adherence, a prior virological failure and a low CD4 T cell nadir. Monotherapies as a strategy can not be justified at this time. In individual cases, however, they may be able to reduce adverse events. Dual therapy of a boosted PI and 3TC are promising, coformulations are in development. These combinations may have the potential to reduce some of the long-term toxic effects associated with NRTIs, preserve future treatment options, and reduce the cost of antiretroviral therapy.

Switching to simplify – triple-nukes revisited

Triple nuke therapy, fairly obsolete for first-line therapy, may be justifiable in maintenance therapy. Several randomized studies have not detected any virologic disadvantage (Katlama 2003, Markowitz 2005, Sprenger 2010).

In the ESS40013 study, a total of 448 patients were treated with AZT+3TC+ABC plus efavirenz. After 36 or 44 weeks, 282 patients with undetectable viral load were randomized to continue with the same therapy or to stop efavirenz. After 96 weeks, 79% versus 77% of patients were still below 50 copies/ml, proving that triple nuke was not inferior (Markowitz 2005). Similar results were also seen in the TRIZAL and FREE study, in which 209 patients were randomized (Katlama 2003, Sprenger 2010). In the Swiss Cohort, the failure rate was low in 495 patients with suppressed viral load and switch to Trizivir®. Patients with earlier exposure to mono- or dual-NRTI therapy, low CD4 T cell count at time of switch, or AIDS were at increased risk of treatment failure, limiting the use of Trizivir® in these patient groups (Wolbers 2007). Some long-term data for the quadruple-nuke strategy with Trizivir® plus tenofovir (d'Ettore 2007, Llibre 2008) also exist.

Taken together, maintenance therapy using Trizivir® seems feasible. However, the benefit remains questionable. Three or four NRTIs are possibly more toxic than other strategies. Strategies such as monotherapy with boosted PIs are not yet justifiable outside clinical trials.

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6.9. Salvage therapy

C H R I S T I A N   H O F F M A N N

Background

The term "salvage therapy" is not clearly defined in HIV medicine. As in oncology the term is currently used to refer to varying situations. Some speak of salvage only if all drug classes have failed, whereas others employ the term from second-line therapy onward. Today, many clinicians talk about salvage when there is resistance to at least two or three antiretroviral drug classes.

Triple class resistance (TCR) is present when viral resistance-associated mutations (RAMs) against the three conventional drug classes NRTIs, NNRTIs and PIs have developed. Triple class failure (TCF) means that the viral load remains detectable although these three drug classes have been used. Analogous to MDR tuberculosis, triple class resistant viruses with additional resistance mutations are also referred to as multi-drug resistant (MDR) viruses. However, these terms are not uniformly defined.

Significant progress has been made for patients with TCR and/or MDR viruses over the last few years. Several agents are now available, showing remarkable effects in patients with multiple RAMs. Even in intensely pretreated patients all efforts should be made to get viral loads to below the limit of detection (Youle 2006).

The number of patients with TCF is in decline and not, as often presumed, increasing (Lohse 2005+2006). Today, TCF or TCR are mainly observed in patients who were treated with mono- or dual therapy in the 90s (Napravnik 2007). In an analysis of almost 92,000 patients in Europe, the TCF rate was only 3.0% during the years 2000–2009. Since 2005, the rate of patients who do not achieve a viral load of less than 50 copies/ml due to TCF has plateaued at low prevalence (Plato 2012). New cases of TCF are rare. The rate of patients without any options is very low and usually less than 1% (De Luca 2013).

Given that this patient group is small, it is difficult to do studies with sufficient power. Homogenous populations do not really exist and every patient has his own individual therapy history and resistance pattern. In larger centers as many as 50 different combinations are used. This makes it difficult to test new salvage agents in Phase II/III studies. The design of these studies is another problem: as the single use of an experimental drug within a failing regimen is ethically questionable, ART must always be optimized (OBT, optimized background therapy). If the OBT is too good, the effect of the new drug may be hidden, as many patients achieve a good viral suppression just on OBT. If the OBT is poor, the effect of the new drug may only be temporary or too weak – the window through which the efficacy of a new salvage drug can be seen is small. The failure of the CCR5 antagonist vicriviroc (Gathe 2010) is only one of many examples. This shows how difficult it has become to bring a new drug to market.

Preface

First a few words about daily practice: it should not be forgotten that patients with TCF, who often have a long history of being on treatment and who now find themselves once again on the precipice, need encouragement. It is important not to leave these patients without hope. It usually takes years to progress from virologic treatment failure to immunologic and finally clinical failure (see Principles of Therapy). Fortunately these patients – most having been treated for fifteen years or longer,

having experienced a lot – are often not nearly as nervous as the often young HIV doctor. They have learned that there is almost always more to come.

Much is possible now in individual cases. Table 9.1 shows an example illustrating the history of antiretroviral therapy – although treatment always remained up to date, viral load of less than 100,000 copies/ml was not always achieved. Finally, with the application of a new agent the patient experienced their first success after more than a decade of having a high level plasma viremia. Viral load has now been below the limit of detection for more than four years.

Table 9.1: Case report showing what is possible today

Date	ART	CD4 cells	Viral load
Jun 95	AZT (later, ddC, ddI)	0	N.A.
Jun 96	AZT+ddC+RTV	25	62,000
Oct 96	D4T+3TC+IDV	10	167,000
Jul 97	D4T+ddI+3TC+NVP+IDV	173	69,000
Jan 99	D4T+ddI+ABC+3TC+SQV/r	212	106,000
Sep 99	D4T+ABC+3TC+DLV+LPV/r	231	74,000
Dec 01	TDF+ddI+DLV+HU	174	84,000
Jun 03	TDF+3TC+FPV/r	143	145,000
Oct 03	TDF+3TC+ddI+TPV/r	77	733,000
May 04	AZT+3TC+TDF+LPV/r+T-20+DLV	43	123,000
Dec 04	AZT+3TC+TDF	32	204,000
Dec 07	AZT+3TC+TDF+DRV/r+RAL+T-20	7	>1,000,000
Jan 08		54	<50
Apr 09	AZT+3TC+TDF+DRV/r+RAL+ETV	83	<50
Mar 12		134	<50
Apr 14	TDF+FTC+RPV+DRV/r+DTG	183	<50
Mar 15		254	<50

Comment: Not all treatment modifications are shown. The switch in 2007 was deferred until DRV and RAL were available in order to use both agents simultaneously. T-20 was recycled when resistance testing did not clearly show if darunavir was still active. Although not foreseeable how long this therapy success will last, the complete and durable suppression of the patient's viral load is remarkable after so many years. A pill reduction was done in 2014 (now 4 pills/day). Further deescalation of the current treatment seems risky at present

Patients with TCF probably have a worse prognosis than patients without TCF (Lohse 2007). In a population-based study from the Danish HIV Cohort on all patients who experienced TCF between 1995 and 2004 (n=179), the total number of genotypic resistance mutations and specific single mutations predicted mortality. In a regression model adjusted for CD4 T cell count, HIV RNA, year of TCF, age, gender and previous ART regimen, harboring at least 9 (versus less) mutations was associated with increased mortality (Lohse 2007). However, things have changed. There are new options for patients with TCF.

Moreover, other studies did not find an association between number of resistance mutations and mortality (Lucas 2004). With good CD4 T cell counts, even despite TCR viruses, the risk of developing AIDS is relatively small (Ledergerber 2004). TCR viruses have less ability to replicate and are probably less aggressive (Prado 2005). Moreover, further progress has to be expected. New drug classes such as attachment or maturation inhibitors but also neutralizing antibodies will represent new options. So, in cases of TCR or MDR, be patient.

It is, however, important that patients with MDR viruses are very carefully monitored and undergo regular (monthly) full-body exams – something that is often neg-

lected these days in the discussions on blood values and resistance testing, etc. Loss of weight, Stage B symptoms, oral candidiasis, OHL and cognitive worsening are early signs of disease progression that need to be watched for. If possible, these patients should be treated in large centers that have access to clinical studies.

Salvage with the newer drugs

A wide range of agents for the treatment of patients with limited options has been licenced in the last few years. These agents include the PIs tipranavir/r and darunavir/r, the NNRTI etravirine, the CCR5 antagonist maraviroc and the integrase inhibitors raltegravir and, more importantly, dolutegravir. They have revolutionized salvage therapy and have become indispensable in the struggle against resistant viruses. Other strategies have proved less effective. The most important results on salvage therapy from large-scale studies within the last few years are shown in Tables 9.2 and 9.3.

Table 9.2: Large randomized studies in salvage therapy

References	Study (Agent)	Main inclusion criteria
Lalezari 2003, Lazzarin 2003, Nelson 2005	TORO 1+2 (T-20)	TCF or TCR or both, VL >5000
Hicks 2006	RESIST 1+2 (tipranavir/r)	TCF and 1-2 primary PI-resistance, VL >1000
Clotet 2007	POWER 1+2 (darunavir/r)	TCF and ≥ 1 primary PI-resistance, VL >1000
Lazzarin 2007, Madruga 2007, Katlama 2009	DUET 1+2 (etravirine)	≥1 NNRTI-resistance and ≥3 primary PI-resistance, VL >5000
Gulick 2008, Fätkenheuer 2008	MOTIVATE 1+2 (maraviroc)	TCR or TCF or both, VL >5000 (prior treatment interruption at baseline allowed), only R5-tropic viruses
Cooper 2008, Steigbigl 2008	BENCHMRK 1+2 (raltegravir)	TCR, VL >1000

TCR=Triple Class Resistance, TCF=Triple Class Failure, VL=Viral load

Of note, inclusion criteria for these studies varied widely. In some studies inclusion was coupled to certain resistance mutations, others included triple-class failure. There were great differences in patient populations and the definition of TCF was not consistent. The proportion of patients additionally receiving T-20 ranged from 20–44%. Different resistance scores were also used in order to determine the number of active agents in background therapy.

Accordingly, response rates vary considerably even in the placebo arms. The rates of all patients with a plasma viremia less than 50 copies/ml at 48 weeks ranged from 10% to 40%, with addition of T-20 from 11% to 62%. The response rates of patients who had received only one active agent and placebo varied from 1–24%.

Cross-trial comparisons regarding the efficacy of the new agents need to be avoided, although this is attempted for marketing reasons. According to these trials, darunavir/r is not better than tipranavir/r. Raltegravir does not have a higher efficacy than maraviroc. The individual study matters greatly.

Table 9.3: Large randomized studies in salvage therapy, main results

	POWER	RESIST	MOTIVATE	BENCHMRK	DUET
Agent tested	DRV	TPV	MVC	RAL	ETV
Total n	245	1509	1049	701	612
Baseline characteristics					
Median VL, log RNA/ml	4.5–4.6	4.7	4.9	4.5–4.7	4.8
Median CD4 T cells/µl	153–163	195–196	187–195	102–140	99–109
0-1 active drug, %*	49–55	43–45	38–44	48–51	54
Background-Therapy					
With de novo T-20, %	29–33	18–23	40–44	20	25
With darunavir, %	100	0	0	25–50	100
With tipranavir, %	0	100	14–16	19–23	0
Response at 48 Wks*					
In total, %	45 vs. 10	23 vs. 10	44 vs. 17	64 vs. 34	61 vs. 40
With de novo T-20, %	58 vs. 11	28 vs. 14	61 vs. 27	84 vs. 62	71 vs. 59
0-1 active drug, %	37 vs. 1	n.a.	37 vs. 6***	48 vs. 12	57 vs. 24

*Definition of an active drug varied considerably (different resistance scores were used); **Response at 48 weeks defined as viral load <50 copies/ml; ***Data at week 24. n.a.=not applicable

During recent years, the INSTIs were evaluated in randomized studies in pretreated patients with virological failure. However, in many of these trials, patients were not as heavily pretreated as in the above mentioned trials such as TORO, MOTIVATE or BENCHMRK (Table 9.2). In the 145-Study, in which raltegravir and elvitegravir were tested in a double-blinded design (with similar results), the main inclusion criteria were a viral load of more than 1.000 copies/ml on ART for at least 30 days, documented resistance *or* at least 6 months of ART (Elion 2014). In the SAILING Study, in which superiority of dolutegravir over raltegravir was shown, patients were enrolled when they had at least 400 copies/ml and RAMs against only two classes. At least one fully active agent was required (Cahn 2014).

However, studies like SAILING, although not conducted in "true" salvage patients, provide practical information for the concrete treatment of these patients (see below).

What to do in patients with TCR

First of all, a resistance test should be available that was not done during a treatment interruption. Older resistance tests should also be reviewed. Resistance mutations detected earlier presumably still exist even if they are no longer detected. It is also important to check incompatibilities of the last years to spare the patient unnecessary side effects and dangerous re-exposure.

Some pilot studies reported success when only new drugs are used. In the French TRIO study, 103 extensively pretreated patients with TCF were treated with RAL+ETV+MVC, out of which 86% achieved plasma viremia below 50 copies/ml at 48 weeks (Yazdanpanah 2009). In a smaller Italian study with 28 patients on the same combination RAL+ETV+MVC, this got to 96% after 4 years (Nozza 2014).

Does it necessarily have to be new drugs? Before switching, physicians should go over the classes, one by one, depending on the individual resistance profile, even the old ones. Table 9.4 shows an overview of the major salvage strategies with regard to each class.

Table 9.4: Salvage strategies in patients with TCR to NRTIs, NNRTIs and PIs

Drug	Strategies to consider, remarks
NRTIs	Try to conserve mutations that reduce replication fitness, such as M184V with 3TC or FTC. Consider AZT and TDF simultaneously, due to diverging resistance pathways
NNRTIs	At <3 NNRTI mutations, consider etravirine (approved only with a boosted PI/r), otherwise discontinue NNRTIs
PIs	Darunavir/r (good data with etravirine) or tipranavir/r
Maraviroc	Tropism test? Due to non-detected dual-tropic viruses, use 2 definitively active agents such as raltegravir and T-20 (if nothing else works), remember dose adaptions when boosting with PIs
INSTIs	At least 1–2 active agents additionally needed, be aware of rapid resistance development, dolutegravir most potent
T-20	Consider when uncertain that at least one other agent beyond dolutegravir or maraviroc is active

NRTIs: Even if 3TC or FTC are no longer effective according to the resistance test, it might make sense in many cases to continue treatment with them. In this way, HIV is forced to conserve the M184V mutation, which reduces the replication fitness (Eron 2004, Campbell 2005, Castagna 2006). Due to diverging resistance pathways, another consideration may be to use AZT and TDF. This also applies when patients have already been treated with these agents. By adding AZT, viral load can be decreased to below detection in the presence of resensitizing K65R (Stephan 2010). However, recent studies evaluating if partially active or inactive NRTIs contribute to treatment response have yielded conflicting results (Imaz 2011, Scherrer 2011).

NNRTIs: In the case of NNRTIs, with less than three NNRTI resistance mutations, etravirine seems to be a good option in combination with a boosted PI (most effective with darunavir/r). In other cases it is recommended to discontinue NNRTIs. There is little doubt that once generated, resistance remains. However, with pregnant women who have received nevirapine once for transmission prophylaxis there was no elevated rate of treatment failure on nevirapine-containing regimens if ART was initiated more than 6 months after delivery – at least theoretically, it seems possible for NNRTI resistances to disappear provided one waits a long enough time (Lockman 2007). However, there is no other data on recycling NNRTIs besides those for transmission prophylaxis.

PIs: In the case of PIs, the boosted PIs darunavir and tipranavir are strongly recommended. These PIs probably have distinct resistance profiles. When resistance findings are unclear, they should be discussed with the virologist. If darunavir/r and tipranavir/r are not available or if they are not tolerated, one can try lopinavir/r; other PIs are probably not suitable.

INSTIs: in patients naïve to INSTIs, all three agents, namely raltegravir, elvitegravir and dolutegravir can be considered. If INSTI RAMs are already present, sequencing of raltegravir and elvitegravir makes no sense (DeJesus 2007, Garrido 2012). This seems to be different with dolutegravir. The VIKING trials have shown that higher doses of dolutegravir (50 mg BID instead of 50 mg QD) may help to overcome raltegravir resistance. In VIKING III, a single-arm, open-label phase III study in which therapy-experienced patients with INSTI-resistant virus received DTG 50 mg BID while continuing their failing regimen (without raltegravir or elvitegravir), viral load

declined by 1.43 log at day 7. In total, 69% of the patients achieved <50 copies/ml at week 24 (Castagna 2014), an exceptional results in this patient population. There is no doubt that dolutegravir should be considered in any patient with multiple RAMs.

Maraviroc, T-20: If at least one other agent is still active, it seems sufficient to treat with only one of the new agents, either maraviroc or an INSTI, to reduce the viral load to below the limit of detection. That way, one could keep the option with the other drug that could be then combined with T-20 in the future. In the case of maraviroc, a recent tropism test should be available. If maraviroc and INSTIs are the only active agents according to the resistance test, they could and should be administered together. Fortunately, there is no relevant interaction (Baroncelli 2010). If maraviroc can not be used due to tropism and dolutegravir efficacy seems to be uncertain, one should consider T-20.

It is also important to strategize. What comes after the current regimen, and what can you do if that fails? To what extent is the patient standing with his back against the wall, immunologically? How high is the risk of progression to AIDS? The lower the CD4 T cells and the higher the viral load the more active agents are required to control the virus. If CD4 T cells are very low, it may be better to put all stakes into one option with as many active agents as possible (at least two), instead of saving up for future options.

Such complex decisions should be discussed in a team of experienced HIV physicians with a virologist who can shed some light onto the resistance situation. The treating physician should be present as well, as they are familiar with the individual situation, know the patient's adherence history and understand what can be expected from the patient.

Practical tips for salvage therapy

- First question: what is the treatment history, what level of success was there and for how long? Perform resistance testing (not during treatment interruption).
- Choose as many new active drugs as possible when changing therapy. The less option you have, the more you should combine them.
- Do not add one new drug to a failing regimen. If the clinical and immunological situation allows, wait for a second active drug.
- Do not wait too long to switch, thus giving the virus the opportunity to develop further mutations – the higher the viral load at the time of switch, the more difficult the chances for success.
- Do not be too demanding toward the patient! Not everyone is suitable for Mega-ART.
- Patients should be treated in larger centers where new drugs and experience are available.
- Encourage the patient. New treatments may become available soon. A "watch and wait" approach may be possible.
- Do not allow reversion to wild-type virus – even a failing regimen should be continued in the absence of further options.

The following strategies were used with some success. Today, after the introduction of new drugs, however, they play a minor role.

Double PI salvage regimens

Since the introduction of darunavir/r and tipranavir/r, double PI regimens have lost their standing in salvage therapy. In many double PI regimens, old agents such as indinavir or saquinavir were used. Others were found to be of poor potency, among

them lopinavir/r plus atazanavir (Ulbricht 2011), lopinavir/r plus fosamprenavir (Kashuba 2005) or atazanavir/r plus fosamprenavir (Landman 2009). The best data are available for lopinavir/r plus saquinavir (Staszewski 2006) and atazanavir/r plus saquinavir (von Hentig 2007, Manosuthi 2008).

However, there is no longer any reason to put a patient on a double PI. Simplifying therapy should be considered for patients on such regimens. One study showed that patients with stable viral suppression on a double PI can change to darunavir/r as single PI without risk (Cohen 2009). This would also save costs as darunavir, albeit a more expensive PIs, is still less expensive than two older PIs together.

Mega-ART with T-20, treatment interruptions

Intensified treatment combinations with more than three drugs – often described as mega- or giga-ART – may indeed be effective. Only well-informed and highly motivated patients can be considered for mega-ART regimens, and such approaches are often unrealistic in clinical practice. There is some evidence from the small INTENSE study that, in some cases, induction with T-20 is of benefit (Clotet 2008).

So, do structured treatment interruptions (STI) before initiation of such intensified regimens provide additional benefit? The answer is clearly no. After some encouraging results from the early GIGHAART Study (Katlama 2004) there is an overwhelming amount of data showing that STIs do not have a positive effect in heavily pretreated patients. In the CPRC064 Study in which patients interrupted treatment for four months prior to going on a salvage regimen, no differences were found between patients who took an STI and those who did not (Lawrence 2003). However, it was disconcerting to see that patients who interrupted treatment not only had worse CD4 counts but also had a significantly higher frequency of severe clinical events during the follow-up period. Other randomized studies did not find any virologic benefit by interrupting treatment prior to starting an intensified salvage regimen (Ruiz 2003, Beatty 2006, Benson 2006, Walmsley 2007, Holodiny 2011). This approach is no longer an option.

Utilizing NNRTI hypersusceptibility

Viral strains are considered "hypersusceptible" to certain drugs if the IC50 (50% inhibitory concentration) for the drug is lower than that of the wild-type in phenotypic resistance tests. NNRTI hypersusceptibility was first described in 2000 (Whitcomb 2000). It generally occurs very rarely with NRTIs but quite frequently with NNRTIs, and mostly in viruses that have developed resistance mutations against NRTIs (Albrecht 2001, Haubrich 2002). In an analysis of more than 17,000 blood samples the prevalence of hypersusceptibility in NRTI-naïve patients to efavirenz and nevirapine was 9% and 11%, respectively. In NRTI-experienced patients, it was 26% and 21% (Whitcomb 2002). Studies show that NRTI mutations, predominantly at codons 215, 208 and 118, are independently associated with NNRTI hypersusceptibility (Shulman 2004, Clark 2006).

There seems to be some evidence that patients with NNRTI hypersusceptibility have better virologic response (Haubrich 2002, Clark 2006). Even if the real significance and molecular correlates for NNRTI hypersusceptibility remain uncertain, the consequence is clear: patients with NRTI mutations (preferably TAMs) and without NNRTI resistance should receive an NNRTI if possible.

Watch-and-wait or even simplifying ART

Sometimes even the most intensified salvage regimen is not effective. Viral load cannot be suppressed to undetectable levels. What should be done in these cases? The answer is to keep going as long as the patient can tolerate the therapy. Multi-

drug resistant viruses are typically slightly less aggressive than wild-type, at least for a certain period of time. A drug such as 3TC also has a positive effect on the viral load even in the presence of a confirmed M184V resistance (Campbell 2005). An Italian study enrolled 50 patients with a viral load of at least 1000 copies/ml on a 3TC-containing regimen, with evidence of the M184V mutation and at least 500 CD4 T cells/µl (Castagna 2006, Gianotti 2008). Patients were randomized to completely interrupt treatment or to continue with 300 mg 3TC alone because the M184V mutation reduces the replicative fitness of HIV. Patients on 3TC indeed had a significantly lower increase in viral load (0.6 versus 1.2 logs) and lost significantly less CD4 T cells. The M184V mutation was maintained in all patients on 3TC, and no other mutations accumulated. In contrast, a shift to wild-type was observed in all patients without 3TC. The benefit was sustained until week 144 (Castagna 2007) when 3TC was continued on a daily basis. Regarding FTC, daily doses also seems to be effective, but not when given weekly (Soria 2010).

Given these results, ART should never be stopped completely in very immunocompromised patients who are then at risk of developing opportunistic infections. In fact, all efforts should be made in such cases to at least partially control the virus. Waiting, even on a suboptimal regimen, is a strategy that can be used to gain valuable time until new drugs are available. In such cases, ART is not being taken in vain: suboptimal ART is better than none at all, and some suppression of viral load better than none. Patients benefit even with only a slight reduction in viral load (Deeks 2000, PLATO II).

A trial of patients with at least 2500 copies/ml on ART who were randomized to interrupt or continue ART for at least 12 weeks showed an immunological benefit for those who remained on their regimen. CD4 T cells dropped only by 15, compared to 128 cells/µl in patients on an STI (Deeks 2001). In a large cohort study, CD4 T cells did not drop as long as the viral load remained below 10,000 copies/ml or at least 1.5 logs below the individual set point (Lederberger 2004).

How intensively should treatment be continued? Which drugs can be discontinued in this watch-and-wait setting? The quadruple nuke strategys seems to be safe, as indicated by a retrospective study (Llibre 2008). NNRTIs such as nevirapine or efavirenz can be stopped if resistance mutations are found, because replicative fitness is not influenced by NNRTI mutations (Piketty 2004). Moreover, accumulation of further RAMs should be avoided as these may compromise newer NNRTIs such as etravirine. The same is probably true for integrase inhibitors (Wirden 2009). In particular, efficacy of dolutegravir should not be compromised by continuing a failing regimen containing INSTIs such as raltegravir or elvitegravir.

What about PIs? There is data from a small pilot study showing that PI discontinuation may be safe (Deeks 2005). 18 patients, in whom the viral load remained high despite more than 6 months on ART (good compliance, appropriate efficacy), had the PIs removed from their respective ART regimens while the NRTIs were continued. Within the first two weeks, none of the patients had an increase of more than 0.5 logs, and even after 16 weeks, no increase was observed in most patients (in only 5/18 patients was there an increase of between 0.5 and 1.0 logs; in the others there was no increase, maybe even a fall). A negative immunological effect was also seen in a few patients, but this was only moderate. Repeated resistance tests showed that all PI mutations persisted in all patients in the first 12 weeks, although PIs were not being taken. One retrospective study on HIV-infected children, in which the PIs had been discontinued, was based on the same idea as the Deeks study. Here, it was also seen that on continuous NRTI therapy, there was no increase in viral load (LeGrand 2005). Another study, however, showed that PIs maintained activity (Opravil 2009).

Table 9.5: Example of a successful watch-and-wait strategy over almost three years

Date	(HA)ART	CD4 T cells	Viral load
until 1997	AZT, AZT+ddC, AZT+ddI	40 (nadir)	107,000
Mar 97	AZT+3TC+SQV-HGC	84	259,000
Oct 97	d4T+3TC+SQV+NFV	211	67,000
Jun 98	d4T+3TC+NVP+IDV/r	406	1200
Jan 00	AZT+3TC+ABC+NVP+IDV/r	370	1030
Mar 02	AZT+3TC+ABC+TDF+NVP+IDV/r	429	3350
Sep 02	d4T+ddI+3TC+NVP+LPV/r	283	5000
Nov 02*		348	7600
Jan 03		315	16,400
Feb 03	AZT+3TC+ABC	379	6640
May 03		241	2400
Dec 04	AZT+3TC+ABC+TDF**	298	4200
Jan 06		323	5800

* Resistance testing showed a total of 20 mutations, with genotypic resistance against all drugs tested. Compliance was very good and plasma levels were always adequate. ** TDF was added because of chronic HBV infection. Note: the patient's viral load has been below the limit of detection since April 2006, when he started AZT+3TC+TDF+TPV/r+RAL

Results from one of our own patients where this approach has been successful for almost three years are shown in Table 9.5. Resistance testing after two years showed that there were no changes in the MDR virus. Watch-and-wait on a simple NRTI regimen seems feasible in some patients for a limited period of time. The reasons for this phenomenon, however, are still not understood but it is possible that multiresistant viruses cannot easily mutate back. With PI therapy alone, this does not appear to be effective – in 5/5 patients, in whom only the nucleoside analog was stopped, viral load increased significantly (Deeks 2005).

As total patient numbers are still very small in the data presented to date, many observers remain skeptical. The main question is how long and in which patients these strategies might be successful. It is thus advisable to monitor CD4 T cells at short intervals.

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6.10. When to stop ART
A review of treatment interruption

CHRISTIAN HOFFMANN

Treatment interruptions are common. They are an important part of antiretroviral therapies whether as a clinician one approves of them or not. In the ART Collaboration Cohort (21,801 patients from 18 cohorts from Europe and North America 2002-2009), the probability of treatment interruptions was 11% after three years of ART (Abgrall 2012). Rates of interruption were markedly higher for intravenous drug users (than men who have sex with men) and in patients younger than 30 years of age. Many patients have adherence problems. The following chapter provides an overview of the current knowledge in patients with chronic HIV infection. For treatment interruptions in patients with acute HIV infection, refer to the chapter *Acute HIV infection*.

Viral load and CD4 T cells during treatment interruptions

Almost all patients who stop treatment experience a rebound in viral load within a few weeks, even patients in whom this has been undetectable for several years. Viral load is usually detectable again within 10–20 days (Chun 1999, Davey 1999, Harrigan 1999). The viral load in compartments such as the CNS, as well as in semen and vaginal fluids, parallels that in the plasma (Garcia 1999) and is detectable in semen within only a few weeks (Ananworanich 2011). Patients should therefore be informed about the higher risk of transmitting HIV (Burman 2008). Some cases report infections during interruption (Bernasconi 2001). There may be an increased risk of maternofetal transmission, even if ART is interrupted in the first trimester (Galli 2009).

Frequently, an initial overshooting rebound is observed (De Jong 1997), and only after a few weeks does the viral load settle to its original, pre-treatment level (Hatano 2000). The rebounding virus evidently does not originate from latent reservoirs; other cell populations must exist from which this new virus is produced so quickly (Chun 2000, Ho 2000, Imamichi 2001).

Treatment interruptions can have serious immunological consequences. Often, CD4 T cell counts drop within a short time to pre-treatment levels. The ground that has been gained on ART is rapidly lost. The drop is bi-phasic, and the drop more pronounced in the first few months (Fagard 2005, Wit 2005, Skiest 2006). CD4 T cell losses vary greatly between patients but may reach -200 or -300/µl within a few weeks. The higher and faster the CD4 T cells increase on ART, the more rapid their decline (Tebas 2002). The CD4 nadir is also important. The lower it was and the older the patient, the more rapidly the count drops again (Maggiolo 2004, Molina 2006, Skiest 2006, Touloumi 2006). Probably there is also an association with high proviral DNA level at treatment interruption (Piketty 2010).

The loss of CD4 T cells during an interruption may not be regained as quickly. In a prospective study, we saw a significant disadvantage for patients undergoing treatment interruptions. After a follow up of 18 months, CD4 T cells were more than 120/µl less in these patients than in matched patients who had not interrupted treatment (Wolf 2005). This has also been seen in the SMART trial (see below) and in other studies (Kaufmann 2011). The following examples illustrate that this disadvantage may persist for a long time.
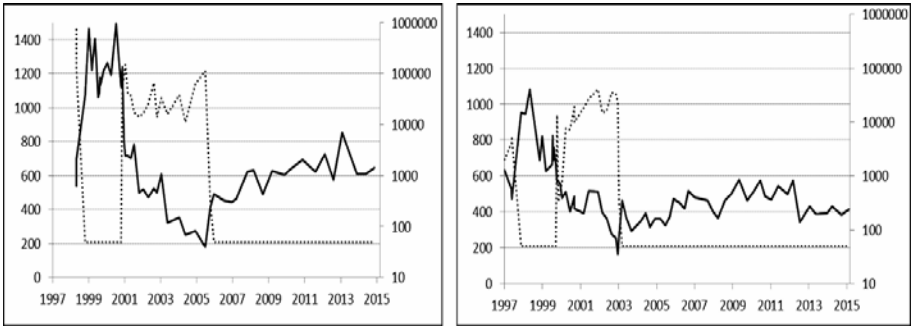
Figure 1: CD4 T-cells (black line, left axis) and HIV RNA (grey dashed line, right axis) in two asympto-matic HIV+ patients. Left: This female patient was treated during acute HIV infection. Right: This male patient initiated ART in chronic infection. Both patients interrupted their ART regimens for some years. During treatment interruptions a marked decline of the CD4 T cells was seen which was not fully reco-vered after re-initiation. In both patients there seems to be a plateau which is lower than the initial values.

The risks: resistance, clinical problems, AIDS

Viral resistance always has to be anticipated whenever there is viral replication in the presence of suboptimal drug levels, and thereby resistant mutants gain a selec-tive advantage over the wild-type virus. As a result, there are concerns that resist-ance could develop both during the washout phase of medication (increasing viral replication with insufficient plasma levels) and on re-initiation of treatment (con-tinued replication despite sufficient plasma levels).

However, in the case of single treatment interruptions, the probability of this does not appear to be particularly high, as shown in 1999 by the small French COMET Study, one of the first studies on treatment interruption (Neumann 1999). But there is no certainty as to whether interruptions might not eventually lead to develop-ment of resistant isolates, which merely require more time until they are able to dominate. Mathematical models show that this risk – at least theoretically – is not low, especially if viral load rises to high levels (Dorman 2000, Bonhoeffer 2000).

The risk of resistance is probably higher for repeated treatment interruptions. In several studies, these have led particularly to NNRTI- or 3TC-resistance (Martinez-Picado 2002, Schweighardt 2002, Ruiz 2007). The risk seems particularly high for strategies involving stopping and starting at fixed intervals (see below). Table 10.1 describes the example of a patient who was clinically well and who interrupted treat-ment. It was probably the repeated stopping and starting of ART that ultimately led to resistance in this case.

The sharp increase in viral load that may often occur can present as a retroviral syn-drome. The symptoms are similar to acute HIV infection, with lymphadenopathy, fever, asthenia and malaise (Colven 2000, Zeller 2001). Thrombocytopenia occurs in 25% of cases, especially when low thrombocytes existed previously (Ananworanich 2003, Bouldouyre 2009). The blood count needs to be monitored, especially in patients with a history of thrombocytopenia.

Finally, attention should be paid to patients who are coinfected with hepatitis B. If the HBV treatment with 3TC, FTC or tenofovir is interrupted, HBV rebound can result in fulminant and life-threatening hepatitis (Sellier 2004, Dore 2010). It is advis-able to monitor these patients very carefully and read the liver enzymes at least every two weeks.

Table 10.1: Example of the development of resistance due to repeated ART interruptions*

Date	ART/comments	CD4 T cells	Viral load
Jun 97	AZT+3TC+SQV	288	67,000
Oct 99	ART stopped, patient feeling well	540	<50
Dec 99	Diagnosis of autoimmune hyperthyroidism	400	63,000
Jan 00	AZT+3TC+NVP (+ carbimazole)	260	74,000
Feb 00	Diagnosis of anemia (Hb 7.3 g/dl)	347	1500
	ART stopped again		
Mar 00	d4T+3TC+NVP (+ carbimazole)		
Apr 00	Resistance mutations K103N, M184V	360	2400

* During the first treatment interruption the patient developed autoimmune hyperthyroidism, the treatment of which led to anemia after re-initiation of ART, so ART was interrupted again. As a result, resistance developed against NNRTIs and 3TC. Autoimmune phenomena in the context of treatment interruption as seen in this patient have not previously been described

The risk of AIDS seems to be low for single interruptions provided the immune defect is only moderate. In the Swiss Cohort, the risk of progression was not increased (Taffe 2002). In 133 patients who interrupted treatment we observed no increased risk of AIDS after 24 months compared to 262 matched controls (Wolf 2005). However, almost all patients in this study were immunologically stable throughout. The risk is probably higher in patients with severe immunodeficiency (Deeks 2001, Lawrence 2003). The CPRC064 Study in which 270 patients with MDR virus and clear immunodeficiency (median 144 CD4 T cells/μl) were randomized before a salvage regimen either to a four-month treatment interruption or not was stopped because of high risk of progression. In comparison with the control group, a significantly higher progression to AIDS (17 versus 5) occurred in the group interrupting therapy. In a multivariate analysis, two factors were predictive for death or progression: treatment interruption and the CD4 T cell count at the time of interruption. The risk increased by 1.4 with every drop of 50 CD4 T cells. This study demonstrates that severely immunocompromised patients are particularly at risk of developing AIDS during treatment interruptions of several months. Treatment interruptions should be avoided in such patients. Data from the SMART Study show that even with higher CD4 T cells treatment interruptions can lead to the development of AIDS (see below).

STI for immunologic reasons: no effects

Hardly any patient became as famous as the acutely-infected man treated in a Berlin practice a few years ago who, with a viral load of approximately 80,000 copies/ml, began an ART regimen consisting of ddI, indinavir and hydroxyurea. The virus rapidly became undetectable. After several problems – and two short treatment interruptions – ART was completely stopped after 176 days. Surprisingly, even without drugs plasma viremia remained below the level of detection for more than five years. Although virus was still detectable in lymph nodes, thus excluding eradication, the immune system in this case – referred to as the Berlin Patient (not to be confused with Timothy Brown, another patient from Berlin who had been cures by an allogeneic stem cell transplantation) – was obviously capable of durable control of infection (Lisziewicz 1999). But why? Was it the early initiation of therapy, the hydroxyurea, or the treatment interruptions? No one knows the answer, even today. There may be a completely different explanation: it is possible that certain host factors in this patients that have not yet been elucidated could have influenced the course of disease – completely independently of ART, STI or hydroxyurea (Bloch 2006).

STIs have been extensively investigated in acutely-infected patients (see chapter on *Acute HIV Infection*). The theory of "endogenous vaccination" seems plausible. Transient increases in viral load could strengthen HIV-specific immune responses, which decline with increasing viral suppression on ART.

In several pilot studies from 2000/2001 successive interruptions seemed to indeed prolong the time to viral rebound or decrease the rate of rebound and, in parallel, there were measurable improvements in HIV-specific CD4 or CD8 T cell immune responses (Haslett 2000, Garcia 2001, Lori 2000, Ortiz 1999, Papasavvas 2000, Ruiz 2000). However, almost none of these studies included more than 2–6 patients, and a control group was usually missing. STIs were finally put to the test in the Spanish-Swiss SSITT Study (Oxenius 2002): 133 patients were monitored throughout four ten-week treatment cycles, each consisting of eight weeks ART and two weeks of treatment interruption. After this, ART was permanently interrupted. Treatment success – defined as a viral load below 5000 copies/ml without ART after 52 weeks – occurred in 21/99 patients. However, 5/21 patients had a low viral load even before the initiation of ART. Most importantly, none of the 32 patients with a pre-ART viral load above 60,000 copies/ml achieved a viral load of less than 5000 copies/ml. The viral load set point was lowered in only a few patients, usually those with low initial viral load, despite repeated STIs. In contrast to acute infection, improvement of HIV-specific immune response seems unlikely in the setting of chronic HIV infection. SSITT clearly showed that treatment interruptions on immunological grounds alone are not justified and are dangerous.

Approaches with immunomodulatory drugs such as hydroxyurea (Foli 2004), mycophenolate (Garcia 2004), steroids (Ulmer 2005) or IL-2 (Henry 2006, Kilby 2006, Angus 2008) took place to lengthen the period of STIs. These approaches have not delivered positive results or are still in the experimental phases and are not justified outside studies. The same holds true for vaccination strategies (Harrer 2005, Jacobson 2006, Goujard 2007, Harrer 2008).

STI as a salvage strategy for MDR virus: obsolete

In most patients with MDR virus, treatment interruption leads to a gradual shift back to wild-type and a loss of resistance. Resistance testing during treatment interruption is often of little use since mutations disappear from the blood as early as two weeks after treatment interruption (Devereux 1999). In modestly immunosuppressed patients, this shift is observed more frequently and faster. In more advanced stages of disease and with a longer duration of treatment, it lasts longer (Miller 2000, Izopet 2000), and sometimes after a longer interruption of therapy, no shift can be seen (Halfon 2005). When the shift is visible, PI mutations are the first to disappear, while NNRTI mutations are more protracted because they hardly affect viral fitness (Deeks 2001, Birk 2001). It is assumed that the wild-type merely dominates the resistant mutants. Special PCR methods can detect low quantities of resistant virus during STI (Izopet 2000) and when treatment is restarted resistance mutations rapidly re-dominate (Delaugerre 2001). Only a few cases have been described in which resistance mutations were apparently flushed out completely. There is one patient (Walter 2002) who was not able to attain sufficient viral suppression despite intensified ART, who then interrupted treatment. During the following seven months of treatment interruption there was a gradual reversion to wild-type, and after re-starting ART (which, according to previous resistance testing, should have had no effect) the viral load was successfully suppressed for several years.

Can patients with MDR improve the effect of the salvage regimen if they have had a previous interruption of treatment? At least two studies have shown that the shift

resulting from treatment interruptions can be beneficial for salvage strategies (Miller 2000, Katlama 2004). However, this data is in contrast to that of numerous other studies in which an increased risk of AIDS was occasionally seen during treatment interruptions (Lawrence 2003+2006, Ruiz 2003, Ghosn 2005, Beatty 2006, Benson 2006, Walmsley 2007, Holodny 2011). In view of the risk of AIDS and the lack of evidence regarding the benefits, treatment interruptions are no longer justified.

STI for reduction of toxicity

Every antiretroviral therapy can cause side effects. Is it possible to reduce toxicity by treatment interruptions? Increased transaminases or lipid levels can drop quite rapidly after stopping treatment (Hatano 2000, Wolf 2005). However, it is not clear whether this is relevant in reducing the risk of cardiovascular disease. In SMART (see below), the risk of cardiovascular and metabolic complications during STIs was actually higher. In contrast to other studies, no relevant improvement of lipids was observed (Lampe 2010). In SMART but also in other trials, biomarkers for cardiovascular events were even elevated during treatment interruptions (Baker 2011, Olmo 2012). Thus, it seems questionable that, through solitary or repeated interruptions, the cardiovascular risk profile can be improved.

What about lipodystrophy and mitochondrial toxicity? At least two studies have shown that, after a few months, mitochondrial DNA can regenerate itself following a treatment break (Cote 2002, Mussini 2005, Kim 2007). In contrast, another study showed no effect (Negredo 2006). Whether or not a clinically manifest lipodystrophy improves, remains to be seen. At least short treatment interruptions have not had any effect on morphological changes (Hatano 2000). A six-month ART interruption markedly improved adipose tissue function, although fat distribution did not visibly change (Kim 2007). Substudies from the SMART trial (see below), so far the largest, showed a moderate positive effect on peripheral fat, lipids and bone mineral density during CD4-guided treatment interruptions (Martinez 2010). Another subtrial showed more reduction of bone density on continued therapy than during interruption – however, numbers of a slightly reduced fracture risk during interruptions are still small (Grund 2009).

Conclusion: Although a treatment interruption is theoretically the solution to long-term toxicity on ART, a convincing argument has not been provided by the data so far. Nevertheless, we will try to outline some relevant data. It is essential to distinguish between structured intermittent treatment with fixed intervals and interruptions that are individualized based on CD4 T cell count, in which case the interruption period depends on the patient's immunological situation.

Structured Intermittent Treatment (SIT, Fixed Intervals): In the initial phase immediately following ART interruption the viral load usually remains low. Plasma viremia only reaches pre-treatment levels after about four, sometimes six weeks. The risk of developing resistance is presumably small at lower levels of viral replication (Bonhoeffer 2000). Does this indicate that ultra-short treatment interruptions could be utilized to reduce drug use, costs and long-term toxicity? In two NIH pilot studies on SIT in chronically infected patients ART was administered as seven days of treatment and seven days interruption (7-on-7-off). At 44-84 weeks, neither the viral load nor the proviral DNA increased (Dybul 2001+2004). CD4 T cells and HIV-specific immune responses remained unchanged suggesting that the immune system is probably unaffected by such ultra-short breaks in treatment. A significant reduction in lipid levels did, however, occur. Some patients experienced several blips (temporary increases in viral load) to above 100 copies/ml. It is impossible to predict whether

this treatment strategy might result in a higher risk of resistance in the long term. There are still no larger studies, and it has become suspiciously quiet in this area. In addition, patients in the NIH studies were carefully selected, with good immune status and many years of viral suppression. This strategy is probably only applicable to a selected group of patients. A three-arm study from Thailand showed a negative experience with the 7-on-7-off approach (Cardiello 2005). In this study, 19/36 patients experienced virologic treatment failure within a short period of time, and this treatment arm was consequently stopped prematurely. The main reason for this appears to lie in the fact that the majority of patients were NRTI-experienced. This means that if NRTIs are unstable, such on-off strategies are problematic.

ART only on weekdays? This approach was taken by the randomized FOTO Study (Five On, Two Off) in which TDF+FTC plus efavirenz was either given daily or from Monday to Friday and stopped at the weekends (i.e., sparing 28%). 60 patients were enrolled who showed an undetectable viral load for at least three months prior to the study. After 48 weeks, viral load increased in one patient despite low trough levels (Cohen 2007+2009).

In contrast, longer interruptions, over several weeks, with fixed intermittent treatment seem to be unfavorable. Results from a randomized NIH study with fixed intervals (each with one month of STI, two months of treatment) were disconcerting (Dybul 2003). The SIT arm contained significantly more patients with virologic treatment failure. Resistance mutations developed particularly against NNRTIs and 3TC, so that the study was stopped early. In the SSITT Study (2 weeks STI, 2 months ART) some resistance was seen (Yerli 2003), likewise in an Italian study (Palmisano 2007), but not in the French WINDOW Study (two months each of STI and therapy) (Marchou 2006). In the DART trial, the risk of AIDS was increased during the three months of treatment interruption (DART 2008).

CD4 T cell driven interruptions: Beside fixed intervals, whether short or long, there is another approach whereby interruptions are individualized based on CD4 T cell count. In other words, in patients with a good CD4 count, ART is interrupted until the CD4 count drops below some immunological cut-off and only then is it resumed. Over the last few years, many non-randomized studies with differing cut-off points and very heterogeneous patient populations came to the conclusion that this approach is safe and allows for a considerable reduction in drug exposure (Maggiolo 2004, Skiest 2004, Fernandez 2005, Mussini 2005). In the meantime, a few randomized studies compare such CD4-driven intervals with continuous administration of ART. The relevant data and results of these studies are given in Table 10.2.

It is clear that the results of these randomized studies differ considerably. While TIBET, Staccato or ACTG 5170 produced the verdict that CD4 T cell-driven interruptions are safe, two other studies, Trivacan and SMART came to other conclusions. In particular, the results of the SMART Study, which started in 2002, caused a sensation. In this, the largest randomized HIV study of all time, the cut-off levels for stopping ART were 350 cells/µl, and 250 cells/µl for re-initiating it. In the end, 318 centers in 53 countries recruited a total of 5472 patients. In 2006 an independent data safety monitoring board concluded that therapeutic interruptions result in an increased risk of AIDS – in the interruption arm, approximately twice as many AIDS illnesses were observed at follow-up, over an average of 18 months. This included severe opportunistic infections as well as malignant tumors. In fact, the overall risk was low, but so significantly elevated that the unusual and far-reaching decision was made to end the study.

In addition it was observed that cardiovascular incidents in the interruption arm did not become less frequent, but actually increased. The clinical incidents in SMART (http:/www.smart-trial.org/news.htm) are shown in the following table.

Table 10.2: Randomized studies of CD4 T cell-guided structured treatment interruptions

Source	n	CD4 BL	CD4 Restart	Clinical findings in the STI arms
TIBET Ruiz 2007	201	>500 >6 Mo	<350 or VL >100,000	Some retroviral syndromes, some de novo NNRTI resistance, otherwise clinically safe (not a single AIDS case)
SMART El Sadr 2006	5472	>350	<250	Morbidity and mortality risk low, but significantly raised. See Table 10.3
Trivacan Danel 2006	326	>350	<250	Morbidity significantly raised (double) due to invasive bacterial infections.
Staccato Ananworanich 2006	430	>350	<350	Clinically safe (slightly more side effects in ART arm; more candidiasis in STI arm). No evidence of resistance
ACTG 5170 Skiest 2007	167	>350	<250	In general safe, with risks only elevated when CD4 nadir was low
LOTTI Maggiolo 2009	329	>700	<350	Clinically safe. More pneumonias but less cardiovascular events, no evidence of resistance

FU=follow up; Mo=months; BL=baseline

Table 10.3: Different events occurring in SMART, per 100 patient years (El Sadr 2006)

	STI (n)	Control (n)	Odds ratio
Progression of disease or death	3.7 (120)	1.3 (47)	2.6 (1.9–3.7)*
Death	1.5 (55)	0.8 (30)	1.8 (1.2–2.9) *
Cardiovascular/renal/hepatic events	1.8 (65)	1.1 (39)	1.7 (1.1–2.5)*
Grade IV toxicity	5.0 (173)	4.2 (148)	1.2 (1.0–1.5)*

* Significant difference. ** 95% Confidence interval

Quality of life did not improve with therapy interruptions – it even declined (Burman 2008). More recent studies showed that clinical and immunological disadvantages remained, even when ART was resumed (El Sadr 2008).

However, even after SMART, not all questions were answered. A striking fact was the high incidence of clinical occurrences compared to Staccato, a study involving 430 patients. As measured by the AIDS/mortality rates of ART, there should have been at least 17 cases in Staccato – instead there was not one. Moreover the significantly higher risk of an AIDS-defining malignancy during therapy interruption (Silverberg 2007) was questionable as the majority of the patients who developed KS or lymphoma in SMART had already suffered from AIDS illnesses before. Why were these patients enrolled in the SMART study?

One can only speculate about the increased cardiovascular, renal and hepatic incidents in the interruption group. How many patients interrupted therapy that should not have? How many patients with chronic hepatitis B experienced a HBV rebound during interruption, how many patients with previous HIVAN developed renal problems, how many patients decided to stop concomitant medications (statins) that led to a cardiovascular event? However, there are some newer studies that show an increase of inflammatory or coagulation parameters during therapy interruption (Kuller 2008, Calmy 2009, Baker 2011, Olmo 2012). Cystatin C, a parameter for renal dysfunction, also increases (Mocroft 2009).

Despite all these questions, the conclusion remains that **it is difficult to find a reasonable argument for treatment interruption.** Especially the argument that therapy interruptions improve quality of life is no longer the case. One can discuss higher values for initiation and interruptions, but there will certainly not be any second SMART with new starting/stopping values for some time.

Patients should always be encouraged to continue ART. Thanks to the new classes, the options have widened, enabling us to respond to side effects. If the patient, after discussion, still wishes to interrupt therapy the wish should be respected. The interruption will happen anyway with or without the doctor's agreement. A monitored interruption is better than one done secretly behind the physician's back. Under strict surveillance the risk for complications is rather low, but again, the patient should consider the possibilities of changing treatment vs leaving it.

Practical tips for treatment interruptions

- If there are no problems with ART, there is no reason to stop it.
- If there are problems with ART, better switch than stop it.
- To reverse resistance or for immunologic reasons, i.e., from a strategic point of view, STIs are not useful.
- A positive effect on cardiovascular incidents or lipodystrophy has not been confirmed. From the SMART Study, this seems highly unlikely.
- The patient's wish for a break should be respected. A supervised treatment interruption is better than one undertaken without the awareness of the clinician.
- Beforehand, information should be provided on possible clinical (retroviral syndrome, AIDS), immunologic (loss of CD4 T cells) and virologic (resistance) consequences.
- Patients must be aware that the risk of infection increases – even after a longer suppression, viral load returns to initial levels after 4-6 weeks without ART.
- Beware of HBV coinfection (danger of hepatitis flare-ups).
- CD4 T cells (including percentage), viral load, and blood count (i.e., thrombocytes) should be monitored monthly during interruptions.
- Risk of resistance is possibly higher with NNRTIs (choose robust regimens and stop NNRTIs several days earlier if possible – consider the half-life of the drugs).
- Patients who started ART "too early" according to current standards can probably interrupt safely.
- Resistance testing during treatment interruptions is not useful – it usually only measures the wild-type.
- Start with ART again, but not too late.

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6.11. Monitoring

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Which parameters should be included in routine laboratory monitoring of HIV-positive patients? What results can be expected? This section deals with viral load, CD4 T cells, routine checks, and plasma levels. Resistance and tropism tests are the subject of a separate chapter (see *HIV Resistance Testing*). For the tests to be performed on initial presentation see *The New Patient*.

Viral Load

Viral load is the amount of HIV RNA in the blood. Alongside the CD4 T cell count, viral load has become the most important surrogate marker for HIV infection (Hughes 1997, Mellors 1997, Lyles 2000, Ghani 2001, Phillips 2004). It provides information on how high the risk is for disease progression. Above all, however, it is the critical value in determining the success of therapy. Viral load assays measure the amount of HIV RNA (viral genetic material), which correlates directly with the number of virions. The units are viral copies/ml (or genome equivalents). This is reported either as a direct whole number or as a logarithmic number. A change of one or more logs refers to the change in viral load by one or more decimal powers. Many labs provide both values, the number and the log. Reporting in international units/ml is also possible but in contrast to hepatitis B and C less common.

Number of copies	Log_{10}
20	1.3
50	1.7
100	2.0
400	2.6
1,000	3.0
10,000	4.0
50,000	4.7
100,000	5.0
1,000,000	6.0

Assessment

The higher the viral load, the higher the risk of decrease in CD4 T cells, with subsequent disease progression or occurrence of AIDS-related illnesses (Mellors 1997, Lyles 2000, Phillips 2004). A viral load above 100,000 copies/ml (sometimes even above 50,000 copies/ml) is considered to be high; a value below 10,000 copies/ml (sometimes below 5000 copies/ml), low. However, these thresholds are not absolute and only provide points of reference.

The effects of plasma viremia on immune status can vary greatly between individuals. There are some patients whose CD4 T cells remain stable for relatively long periods despite having a high viral load, while others experience a rapid drop, although the viral load is relatively low. Even in the so-called elite controllers in which the viral load is undetectable without ART a slow but constant drop in the CD4 cells can be observed (Stellbrink 2008).

Viral load is probably lower in women than in men. In a meta-analysis, the difference was 41% or 0.23 logs (95% CI 0.16-0.31 logs) (Napravnik 2002). The reason for this phenomenon remains unclear and whether it should have an impact on the indication for treatment is still the subject of debate.

Methods

For viral load measuring usually nucleic acid amplification tests (NAT) such as Reverse Transcription-Polymerase Chain Reaction (PCR) and related techniques are used. Briefly, after extraction the viral RNA is transformed into several enzymatic steps and then amplified to measurable amounts. Detection and quantification occurs after binding of marked DNA fragments. Characteristics of commercially available assays widely used in laboratories are listed in Table 11.1. The testing systems differ both in levels of detection and in the linear range within which measurement is reliable or reproducible. The branched DNA (bDNA) method frequently used in the early years is no longer available.

The market for assay systems is very dynamic. New assay and devices have become available, existing ones were further developed during recent years. Furthermore, besides already established manufacturers with expertise in the field, additional diagnostic companies such as Qiagen, Hologic and Cepheid are trying to gain market share. Experience will show whether their testing systems are reliable or not. An important improvement with regard to a higher degree of security is the "dual target" strategy initially introduced by Roche Diagnostics. This means that not one section of the viral RNA, like before, but two sections can be amplified at the same time. If amplification fails in one section on account of the high variability of the HIV genome (the result in this case would be incorrect negative), it will be amplified in the second section.

Recent further developments also concern a reduction below detection level which is at 20 copies/ml in the most sensitive tests. Clinical relevance of a viral load below 50 copies/ml is questionable due to lack of data. It should be noted that a higher sensitivity can lead to insecurity in patients and clinicians and to more frequent control tests. Although intra-assay variability is fairly good for all three methods, methodological variations should be carefully considered. Differences of less than 0.5 logs are not considered significant. A decrease from 4.3 to 3.9 logs, for example (corresponding to a decrease from approximately 20,000 to 8,000 viral copies/ml) does not necessarily signify a drop in viral load. The same holds for increases in viral load. Changes of up to threefold can therefore be irrelevant. Patients should be made aware of this.

Considerable differences exist between the methods (Coste 1996) and to change from one method to another is generally not advisable. Different subtypes are also detected with varying success according to the method employed (Parekh 1999, Alvarez 2015, Ndiaye, 2015). One should be particularly cautious in patients from Africa and Asia with non-B subtypes in whom the viral load at first presentation can be unexpectedly low. In such cases, use of a different assay may actually be indicated. However, newer versions with improved primers and probes are probably superior in measuring even unusual HIV subtypes with adequate sensitivity.

All assays have a linear dynamic range, outside of which precise numbers are not so reliable. The following rule applies: use one method, one laboratory. The laboratory should be experienced and routinely perform a sufficiently large number of tests. Pre-analytical aspects concerning specimen collection, transport and storage should be taken into account to ensure correct viral load measurement. In particular, it should be noted that for obtaining plasma whole blood should be centrifuged within an adequate time interval (optimally within 24 hours). It is recommended to contact the laboratory ahead of time on these issues. Apparent low-level HIV RNA viraemia can be related to long sample processing time (Portman 2012).

Viral load measurement is also vulnerable to contamination. If other examinations such as CD4 T cell count is done in the same lab, it is recommended to send a separated EDTA tube.

Table 11.1: Methods of measurement

Company	Test	Technology	Detection limit (copies/ml)	Linear Range (copies/ml)
Roche Diagnostics	COBAS TaqMan HIV-1 Test; v2.0	RT-PCR	20	20–10,000,000
Siemens Healthcare Diagnostics	Versant HIV-1 RNA 1.0 Assay (kPCR)	RT-PCR	37	37–11,000,000
Abbott Molecular	Abbott RealTime HIV-1	RT-PCR	40	40–10,000,000
Biomérieux	NucliSENS EasyQ HIV v. 2.0	NASBA	250	25–7,900,000

Influencing factors

Apart from methodological variability a host of other factors may influence levels of viral load including vaccinations and concurrent infections. During active OIs viral load is often high. One study showed a 5- to 160-fold elevated viral load during active tuberculosis (Goletti 1996). Viral load can also increase significantly during syphilis and declines after successful treatment (Buchacz 2004, Kofoed 2006, Palacios 2007). In a large retrospective study, 26% of transient viremia in patients on ART were caused by intercurrent infections (Easterbrook 2002). In these situations, determining the viral load does not make much sense.

Following immunizations, i.e., for influenza (O'Brien 1995) or pneumococcus (Farber 1996), the viral load may be transiently elevated (Kolber 2002). As the peak occurs one to three weeks after immunization, routine measurements of viral load should be avoided within four weeks of immunization. It should be noted that not every increase is indicative of virologic treatment failure and resistance. Slight transient increases in viral load, or blips, are usually of no consequence, as numerous studies in the last few years have shown (see chapter on *Goals and Principles of Therapy*). The possibility of mixing up samples always has to be considered. Unusually implausible results should be double-checked with the laboratory, and if no cause is found there, they need to be monitored – people make mistakes. Should there be any doubt on an individual result; the lab should be asked to repeat the measurement from the same blood sample.

Viral kinetics on ART

The introduction of viral load measurement in 1996-1997 fundamentally changed HIV therapy. The breakthrough studies by David Ho and his group showed that HIV infection has significant *in vivo* dynamics (Ho 1995, Perelson 1996). The changes in viral load on antiretroviral therapy clearly reflect the dynamics of the process of viral production and elimination. The concentration of HIV-1 in plasma is usually reduced by 99% as early as two weeks after the initiation of ART (Perelson 1997). In one large cohort, the viral load in 84% of patients was already below 1000 copies/ml after four weeks. The decrease in viral load follows biphasic kinetics. In the first phase, i.e., within the first three to six weeks, an extremely rapid drop occurs, followed by a longer phase during which the viral load gradually decreases further (Wu 1999).

The higher the viral load at initiation of therapy, the longer it takes to drop below the level of detection. In one study, the range was between 15 days with a baseline viral load of 1000 and 113 days with a baseline of 1 million viral copies/ml (Rizzardi 2000). The following figure shows a typical biphasic decrease in viral load after initial high levels.
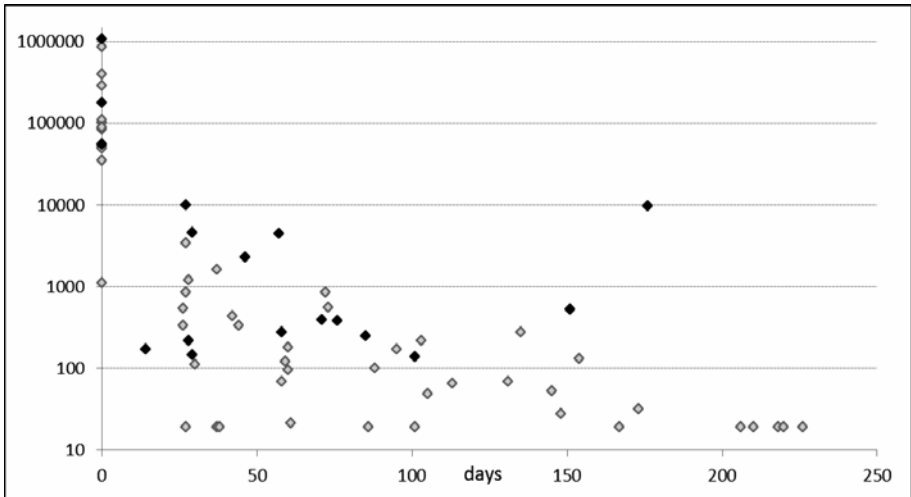
Figure 1: Viral load kinetics during the first months on first-line ART. The grey values derive from 10 patients who achieved a sustained virological suppression, the black values from 3 patients in which resistance mutations occurred during primary therapy (all 3 had NNRTI-based regimens)

Numerous studies have focused on whether durable treatment success can be predicted early (Thiebaut 2000, Demeter 2001, Kitchen 2001, Lepri 2001). In a study on 124 patients, a decrease of less than 0.72 logs after one week was predictive of virologic treatment failure in more than 99% of patients (Polis 2001). According to another prospective study, it is possible to predict virologic response at 48 weeks even after 7 days (Haubrich 2011). However, this has little clinical relevance, and in our opinion it is pointless to start measurement of viral load only one or two weeks after initiation of therapy.

Many studies have evaluated the question whether long-term virological success can be predicted at early phases (Thibaut 2000, Demeter, Kitchen 2011, Lepri 2001). Many of them suggest that changes during the first days after treatment initiation are major correlates of longer-term virological responses. In a study on 124 HIV+ patients initiating a PI-based ART, a decline of less than 0.72 logs after 6 days was highly predictive for consecutive virological failure (Polis 2001). In another prospective trial, week 1 HIV-RNA change was associated with virologic failure above 50 copies/ml at weeks 24 and 48 (Haubrich 2011).

However, such an early measurement is not clinical routine. We recommend measuring viral load every four weeks until it has dropped to below detection of 20–50 copies/ml. Once that is achieved, measurement every three to four months is enough. Eventually, longer intervals are possible (Chaiwarith 2010). In case of rebound, closer monitoring becomes necessary. Within the first 4 weeks of therapy initiation the viral load should be reduced by a factor of 100, after 3-4 months (6 months if viral load was high) it should be below the level of detection. Viral load can also be measured fairly reliably in body fluids other than blood or plasma (for example cerebrospinal, vaginal or seminal fluid). However, such tests are usually performed for scientific purposes and are not officially licensed for other reasons.

> **Practical tips for dealing with viral load**
> **(see also chapter *Goals and Principles of Therapy*)**
> - Use only one assay, if possible.
> - Use only one experienced laboratory, if possible, no home-brewed assays.
> - Watch for assay variability (up to half a log) and explain this to the patient.
> - Monitor viral load every four weeks with new ART until the viral load is below the level of detection (50 copies/ml).
> - Measure viral load sparingly – on successful ART, every three months are sufficient.
> - Not on ART, measurement every three months is usually sufficient.
> - Do not measure shortly after vaccinations or with concurrent infections.
> - Implausible results should be rechecked after 2–4 weeks.
> - Consider differences between subtypes (in some cases it may be useful to use another method).

CD4 T cells

CD4 T cells are T lymphocytes that express the CD4 receptor on their surface. This lymphocyte subpopulation is also referred to as T helper cells. Alongside viral load, measurement of the CD4 T cell level is the most important parameter or surrogate marker in HIV medicine. It allows for a reliable estimate of the individual risk of developing AIDS. Two reference values are generally accepted: above 400–500 CD4 T cells/µl, severe AIDS-related diseases are very rare; below 200 CD4 T cells/µl, the risk of AIDS-related morbidity increases significantly with increased duration of immunosuppression. Most AIDS-related illnesses occur below 100 CD4 T cells/µl.

Several points should be considered when measuring CD4 T cells (usually by flow cytometry). Blood samples should be processed within 18 hours. The lower normal values are between 400 and 500 cells/µl, depending on the laboratory. Samples should always be sent to the same (experienced) laboratory. The same applies for viral load as for CD4 T cells: the higher the level, the greater the variability. Differences of 50–100 cells/µl are not unusual. In one study, the 95% confidence intervals with a real value of 500 cells/µl were between 297 and 841 cells/µl. At 200 CD4 T cells/µl, the 95% confidence interval was between 118 and 337 cells/µl (Hoover 1993).

Measurement of CD4 T cells should only be repeated in the case of highly implausible values. As long as the viral load remains below the level of detection, there is no need to be concerned even with decreases in CD4 T cells. In such cases, the relative values (CD4 percentages) and the CD4/CD8 ratio (ratio of CD4 to CD8 T cells) should be referred to; these are usually more robust and less prone to fluctuation. As a general point of reference, with values above 500 CD4 T cells/µl, fluctuations of more than 29% are to be expected, with less than 200 CD4 T cells/µl fluctuations of up to than 14%. Individual laboratories may define the normal ranges for the relative values and the ratio differently. If there are considerable discrepancies between absolute and relative CD4 T cells, any decisions involving treatment should be carefully considered – if in doubt, it is better to check the values again. The remaining differential blood count should also be scrutinized carefully – is leucopenia or leukocytosis present? Figure 2 shows CD4 T cells in two untreated patients.

Clinicians sometimes forget that the result of the CD4 T cell count is of existential importance for the patient. To go to the doctor and discuss the test results can involve a great deal of stress for many patients. Insensitively informing the patient of a supposedly bad result can lead to further negative results. From the start, patients must be informed about the possible physiological and method-related variability of laboratory tests. In the case of unexpectedly good results, every effort should be
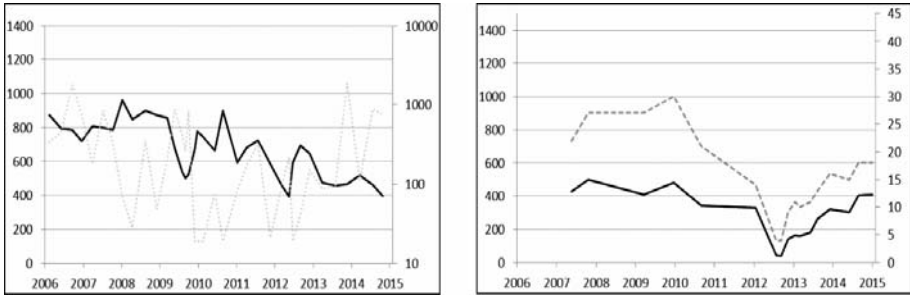
Figure 2: Untreated patients: Decline of the absolute CD4 T cells (black). Left: A female patient with a relatively slow decay over almost a decade. Note the considerable variation and the low viral load which was below 50 copies/ml without any ART at several time points. Right: A male patient with a rapid decline within only a few months in 2012/2013, developing AIDS (toxoplasma gondii encephalitis). With an earlier initiation of ART (which was started in 2013), this complication could have been avoided. This case argues for carefully monitoring in untreated patients (grey: relative CD4 T-cells)

made to contain euphoria. In the long run, this saves time and discussions, and the patient is spared unnecessary ups and downs. We do not consider it advisable for non-physician personnel (without extensive HIV experience) to inform patients of results.

Once CD4 T cell counts within the normal range are reached in addition to adequate viral suppression, measurements every six months should suffice, in our opinion. The probability of CD4 T cells dropping to values below 350/µl is extremely low in such cases (Phillips 2003). Among HIV+ patients with HIV-1 RNA <200 copies/ml and CD4 T cell counts above 300 cells/µl, the probability of maintaining durable CD4 above 200 cells/µl for 4 years was 99.2%. These data support less frequent CD4 monitoring during viral suppression. In the USA, CD4 T cell measurement is considered to be optional in these patients (Whitlock 2013). Patients who might sometimes insist on more frequent monitoring of immune status can be assured that there are usually no detrimental changes in the CD4 T cell count as long as HIV remains suppressed.

Influencing factors

Several other factors can influence CD4 T cell counts apart from laboratory-related variables. These include concurrent infections, leucopenia of varying etiology and steroids or other immunosuppressive therapies. Extreme exertion, surgical procedures or pregnancy can also lead to lower values. Even diurnal variation occurs; CD4 T cells are lower at noon, and highest in the evening around 8 p.m. (Malone 1990). Psychological stress seems to play a negligible role, even though patients often assume the contrary.

Kinetics of CD4 T cells on ART

If untreated, a continuous CD4 T cell decline is seen in the majority of the patients. However, there are discontinuous cases in which the decline may be very rapid after a long stable period (see Figure 2). In an observational cohort collaboration study on 34,384 ART-naive individuals, the mean CD4 T cell decline was -78 (95% CI, -80 to -76) cells/µl per year. The decline was strongly associated with a higher current viral load: for every 1 log10 copies/ml higher, CD4 T cells declined by an additional 37.6 cells/µl per year (COHERE 2014). Of note, neither sex, race nor transmission by injecting drug use was associated with change in either the viral load or CD4 T cell count. Different kinetics are shown in Figure 3.

Similarly to viral load, a biphasic increase in CD4 T cells occurs following the initiation of ART (Renaud 1999, Le Moing 2002), with a rapid increase within the first three to four months and a much slower rise thereafter. In a study of almost 1000 patients, the CD4 T cell count increased by 21/µl per month during the first three months. In the following 21 months, this rate was only 5.5 CD4 T cells/µl per month (Le Moing 2002). The initial rapid increase in CD4 T cells is probably due to redistribution, which is followed by the new production of naïve T cells (Pakker 1998). Diminished apoptosis may also play a role (Roger 2002). It is still being debated whether the immune system steadily continues its recovery even after a long period of viral suppression, or whether a plateau is reached after three to four years beyond which there is less improvement (Smith 2004, Viard 2004).

Several factors can influence the extent of immune reconstitution during ART. The degree of viral suppression is crucial – the lower the viral load, the more pronounced the effect (Le Moin 2002). The absolute increase is higher if CD4 T cell counts were high at the start of ART (Kaufmann 2000). Naïve T cells still present at initiation of therapy are a particularly important factor for long-term immune reconstitution (Notermans 1999).

Age is also important (Grabar 2004). The larger the thymus and the more active the process of thymopoiesis, the more significant the rise in CD4 T cells is likely to be (Kolte 2002); due to age-related degeneration of the thymus, CD4 T cells in older patients do not increase as much as those in younger ones (Viard 2001). However, we have seen both 20-year-old patients with very poor CD4 T cell count recovery and 60-year-old patients with very good, above average increases in CD4 T cells. The regenerative capacity of the human immune system seems to vary considerably, and no method to date has been capable of reliably predicting this capacity.
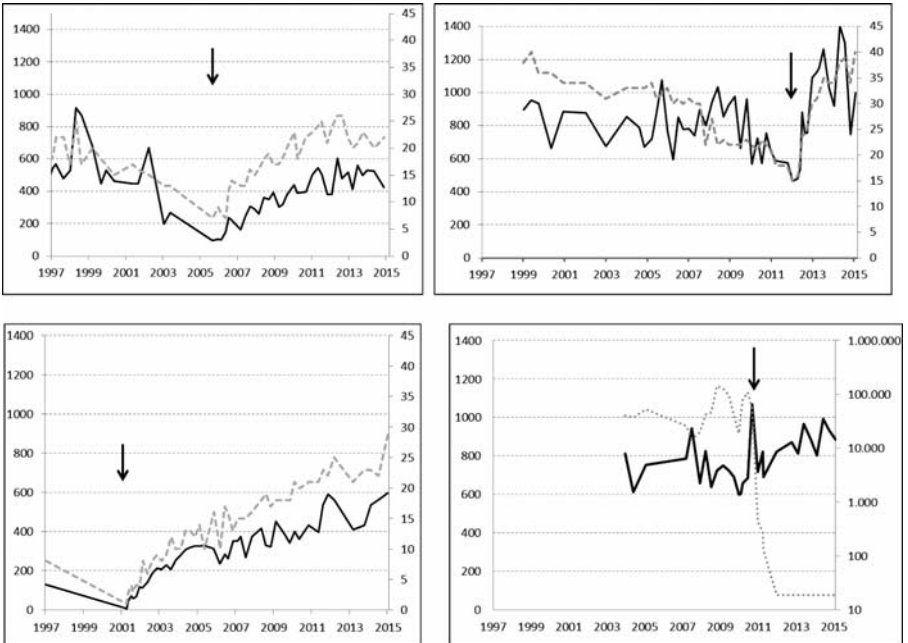
Figure 3a-d: Increase of the absolute (black) and relative (grey) CD4 T cell counts in patients on suppressive ART. Arrows show the time point of ART initiation. Considerable variations, especially in the high ranges. It may be helpful to inform the patients about these physiological changes. Lower right: This patient developed Kaposi sarcoma at high CD4 T cells and consequently initiated ART (in grey: viral load)
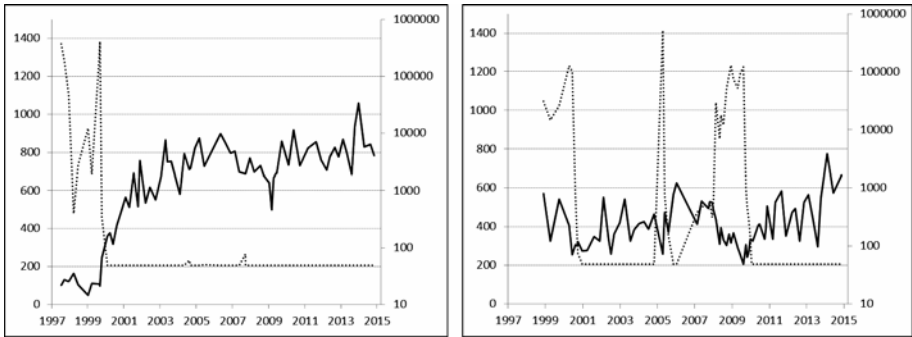
Figure 4: Viral load (dashed line, right axis, logarithmic scale) and absolute (black, left axis) CD4 T -cells in patients on long-term ART. On the left a patient with adherence problems during the early years which disappeared after two AIDS- defining events in 1999, thereafter good adherence and a rapid and sustained immune reconstitution. A plateau was reached during the last decade. It remains questionable whether CD4 T cell monitoring is necessary in this patients. On the right an old patient (>60 years) with two treatment interruptions and only moderate immune reconstitution

It is possible that some antiretroviral therapies such as ddI+tenofovir are associated with less immune reconstitution than others. In addition, current studies are evaluating if immune reconstitution is better during treatment with CCR5 antagonists. Immunosuppressive concurrent medications should also be considered (see chapter on *Goals and Principles of Therapy*).

More CD4 T cell courses are shown in the chapter *Goals and Principles of Therapy*. Beyond the measurement of the CD4 T cell count and lymphocyte subpopulations, a number of other assays allow detailed testing of the qualitative or functional capacity of the immune system, for example in response to specific antigens (Telenti 2002). These often cumbersome methods are not currently necessary for routine diagnostics and their use remains questionable. However, they could one day help to better describe individual immune status and, for example, identify those patients who are at risk of developing opportunistic infections despite good CD4 cell counts.

Practical tips for dealing with CD4 T cells

- As with viral load, use only one (experienced) laboratory.
- The higher the values, the greater the variability (consider numerous factors) – compare the relative (percentage) values and CD4/CD8 ratio with previous results.
- Do not disconcert the patient when there are apparent decreases – if viral suppression is sufficient, the drop is usually not HIV-related. Only highly implausible results should be repeated.
- If the viral load is below the level of detection, three-monthly measurements of CD4 T cells are sufficient.
- In the presence of good viral suppression and normal CD4 T cells, CD4 T cells (not viral load) may also be checked less frequently. The value as a surrogate marker is limited in these patients.
- In untreated patients they remain an important surrogate!
- The patient should have time to discuss the CD4 count and viral load with the physician.

Routine checks – What else should be monitored?

Besides the CD4 T cell count and viral load several other parameters should be monitored in the HIV-positive patient. The following recommendations apply to clinically asymptomatic patients with normal results on routine laboratory evaluation, who have been on stable treatment for several months or who are not taking antiretroviral therapy. Of course, if treatment is started or changed or if the patient develops complaints more frequent monitoring is required. Depending on the problem additional tests may be necessary. On the other hand, the rate of new lab abnormalities decreases as more time elapses post-ART initiation (Taiwo 2012). This suggests that as time on initial ART increases, monitoring frequency may be reduced in subgroups without early abnormalities.

A complete physical examination should be performed regularly, and this often leads to the discovery of important findings such as Kaposi's lesions or mycoses (thrush). The lower the CD4 T cells, the more frequently patients should be examined.

In patients with less than 200 CD4 T cells/µl, we usually perform fundoscopies every three to six months to exclude CMV retinitis. Close cooperation with an HIV-experienced ophthalmologist is essential. The better the CD4 T cells, the less often fundoscopies are necessary – in our opinion when CD4 counts have normalized these can be stopped completely. In contrast, regular gynecological examinations with PAP smears are recommended regardless of CD4 count. Many experts now also recommend rectal examination (including proctoscopy) for the early detection of precancerous lesions and anal cancer.

However, such guidelines or recommendations can be interpreted very differently. In our opinion, in cases of good immune status unless there is a specific suspicion, routine X-rays, ultrasound examinations (exception: patients with chronic hepatitis, as hepatocellular carcinoma is not rare in such cases), multiple serologies or lactate measurements are not necessary. An annual ECG is only indicated in our view in patients with a specific risk profile (see chapter on *HIV and Cardiac Disease*). The tuberculin test (the Mendel-Mantoux skin test with 5 IE once a year) should only be repeated if it is negative initially.

Table 11.2: Minimal evaluations per year in stable asymptomatic patients

	Patient on ART per year	Untreated per year
Blood count, LDH, ALT, AST, creatinine, bilirubin, AP, lipase, GGT, glucose	4 x	2–4 x
Viral load	4 x	2–4 x
CD4 T cells	2–4 x	2–4 x
Lipids	1–2 x	1 x
Physical examination, urine status	2–4 x	1–2 x
Gynecological examination	1 x	1 x
Fundoscopy if CD4 T cells <200/µl	1–2 x	4 x

With regard to the growing age of the HIV+ population, it is essential not to forget cancer screening. In many countries, for example, colonoscopy is recommended for early detection of colorectal cancer for every individual older than 50–55 years (colonoscopy should be performed every 10 years). For further information see WHO website, http://www.who.int/cancer/detection/en/

Therapeutic Drug Monitoring (TDM)

Plasma levels of many antiretroviral drugs may vary considerably for diverse reasons (e.g., adherence, metabolism, absorption). Measurement of drug concentrations in serum or plasma is also referred to as therapeutic drug monitoring (TDM).

Sufficient plasma levels are essential for success of virologic treatment (Acosta 2000). In the VIRADAPT Study adequate PI concentrations were even more crucial than knowledge of resistance mutations (Durant 2000). The importance of sufficient plasma levels has also been shown for NNRTIs (Marzolini 2001, Veldkamp 2001). This information however dates to the early years of ART.

Whether TDM improves virologic response today is not clear (Kredo 2009). Only a few large randomized studies exist that have provided data regarding this question. One randomized trial showed no benefit in 183 patients experiencing therapy failure, who had switched to a new PI and had either adjusted or not adjusted the dose of PIs when their levels were low. After 48 weeks the number of patients with viral loads below the limit of detection did not increase with TDM. A positive effect on viral load was merely restricted to a small subgroup of patients with only partial PI effects (Albrecht 2011). Another randomized trial also showed no positive effects on viral suppression (Best 2007). Favorable effects of TDM continue to remain questionable and the method is still regarded as experimental (Review: Liu 2010).

On the other hand, very high plasma levels correlate with a higher rate of side effects. Reported renal problems with indinavir (Dielemann 1999), gastrointestinal disturbances with ritonavir (Gatti 1999), hepatotoxicity with nevirapine (Gonzalez 2002) or CNS problems with efavirenz (Marzolini 2001) were all associated with high plasma levels. For this reason, TDM will remain a tool for therapy observation: not every interaction between antiretroviral drugs or with concomitant drugs has been investigated. Measurement of plasma levels may currently be reasonable in the following situations (German-Austrian ART guidelines):

• Complex drug combinations including boosted PIs
• Patients with very high or low body weight
• Side effects
• Treatment failure (resistance?)
• Suspected absorption or adherence problems
• Severe liver or renal diseases
• ART in children, pregnancy
• Use of new drugs (unknown interactions)

Several problems associated with TDM limit its broader use. The measurement of NRTIs, for example, is not possible since they are converted to the active metabolites only intracellularly. Intracellular measurements are difficult and are not available in routine clinical practice. There is no valid data available for newer antiretroviral agents such as integrase inhibitors, maraviroc or T-20.

Measuring NNRTIs or PIs may therefore currently determine levels of only one component of a failing combination. Further problems include not only viral strains with different levels of resistance, different inhibitory concentrations, variable protein binding, and time-dependent variability of plasma levels, but also methodological problems with the assays, as well as lack of clearly defined limits. Many uncertainties thus remain in the assessment of therapeutic drug plasma levels. Until data from randomized studies is available, proving the clinical value of TDM, both the measurement and interpretation of results should be left to specialized centers.

Before performing TDM it is important to consider what the question is to answer. If efficacy of ART is under evaluation, trough levels are important – trough level should be measured just before the administration of the next dose. If toxicity is the issue, peak levels 1–3 hours after intake are of interest.

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6.12. Prevention of HIV infection

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Approximately 35 years after AIDS was first described, a prophylactic vaccination remains a distant prospect. In 2007 two highly promising vaccine studies were prematurely halted. Only a few other trials are ongoing. It seems doubtful now that a vaccine to effectively prevent HIV infection will be discovered anytime soon – the moderate but surprisingly successful RV144 vaccine study will not change that (Rerks-Ngarm 2009, see chapter on *Preventive Vaccination*). Several experts believe that a promising vaccine candidate does not exist currently.

The finding that HIV superinfection occurs at approximately the same rate as primary HIV incidence (Redd 2013) has significant implications for HIV vaccine research. When HIV itself does not provide any protection from re-infection – how can a protective vaccine for uninfected persons be found? Some experts believe that a vaccine may never come. Neither blind hope nor overambitious time schedules have proved very helpful. Some vaccine studies up until now can in fact be regarded as counterproductive, fatiguing both sponsors and the community.

Considering all this, prevention will continue to be the central means of controlling the HIV epidemic. However, common prevention strategies focused on the ABC guidelines (abstinence, be faithful, condom use) have reached their limits. Despite significant efforts – according to UNAIDS, the incidence has dropped from 3.4 million in the year 2001 to 2.1 million new infections in 2013 – the rate of new HIV infections worldwide remains unacceptably high, highlighting the need for new HIV prevention strategies.

In every major city in the US or in Europe syphilis outbreaks in HIV-infected patients have been reported. Acute hepatitis C among MSM is common. It seems clear that advertisements and brochures alone are not the solution, especially when these simple publicity mechanisms are not maintained. High-risk groups are not being reached effectively. Prevention remains an arduous business and success is not immediately visible nor is it profitable in economic terms, although the savings in people needing treatment, i.e., people not becoming infected, could be enormous. In any case, sexual behavior is not easily modified by a few advertisements or brochures.

For some time, preventive medicine in HIV has been taking completely new and sometimes unusual paths to reach focus groups. Terms such as serosorting, seropositioning, dipping or strategic positioning show that the medical world is learning to face sexual reality. People have sex and not everyone cares about, follows, or can follow the ABC guidelines. Recent studies with serosorting, choosing sexual partners according to perceived HIV serostatus, show that new prevention strategies can be developed (Morin 2008).

The following focuses mainly on medical prevention strategies. In 2010 there were groundbreaking new findings in this area regarding PrEP and microbicides. In 2011, the protective effect of ART, which had been expected for a while, finally became evident and now may have a substantial impact on HIV prevention.

Treatment as prevention (TasP)

Little in HIV medicine of the last few years has met with such wide response as the results of the HTPN 052 trial. Spontaneous, standing ovations as seen during the International AIDS Conference in Rome in July 2011 are seldom seen in the world of science. The reputable journal "Science" described the results as "the breakthrough in 2011" and "The Economist" even wrote about the "end of AIDS". What caused

this commotion? Results of a trial were published in the summer of 2011, which had investigated the protective effect of ART (Cohen 2011). HTPN-052 was a trial with 1763 HIV-discordant couples in the US, India, Brazil, Thailand and five African countries. The HIV-infected partners were treatment-naïve and had CD4 T cell counts between 350 and 550/µl. Approximately 97% of the couples were heterosexual and most of the volunteers were between 26 and 40 years. The couples received thorough instructions and the use of condoms was counselled at monthly sessions. Then the HIV-infected partners were randomized to start ART, either immediately or when CD4 T cells fell to below 250/µl or when AIDS had manifested. The primary endpoint was defined as new infections of the HIV-negative partners that clearly originated from the infected partners ("linked infections"). In a first evaluation in February 2011 after a follow-up of 1.7 years, 39 infections were observed, among them 28 identified as "linked". Dramatic differences were observed. There was only one infection in the arm in which infected partners had received ART immediately. Later investigations showed that the infection was probably caused before or just after the infected partner had started ART. Even when counting this case, the results gave evidence for a 96% protection with ART, a result unachieved up to then by any of the other prevention strategies, including PrEP or vaccination (Karim 2011).

Thus, antiretroviral therapy is an important contribution to prevention, possibly the most important. However, this was already suggested by a large number of uncontrolled studies prior to the HTPN trial. These studies are discussed here briefly:

- In a group of 415 HIV-discordant couples in Uganda 90 new infections were diagnosed over a period of 30 months. Not a single infection was caused by an infected partner with a viral load below 1500 copies/ml. With every additional log of HIV RNA, infection risk increased by a factor of 2.45 (Quinn 2000).
- In a study in Thailand with 493 HIV-discordant couples, the factor was 1.81. No infection from a partner with less than 1094 copies/ml was recorded (Tovanabutra 2002).
- In a study in Spain with 393 heterosexual HIV-discordant couples, a transmission rate of 8.6% was observed between 1991 and 2003. No infection was recorded when infected partners were receiving combination ART (Castilla 2005).
- Among 534 MSM in San Francisco, infectiousness based on the probability of transmission per couple decreased by 60% between 1994 and 1998 (Porco 2004). The HIV incidence decreased in spite of the reported higher number of partners and risk contacts, even though not all of the HIV+ partners were on ART.
- In a Spanish study with 62 HIV-discordant couples (22 HIV+ women, 40 HIV+ men, all of them on ART), 76 "natural" pregnancies were diagnosed. Not a single HIV infection of a non-infected partner was recorded (Barreiro 2006).

The above-mentioned clinical studies show clearly that the lower the viral load in the plasma, the less infectious the patient. In a meta-analysis of 11 cohorts with 5,021 heterosexual couples (and 461 HIV transmissions) the transmission rate of patients on ART was 0.46 per 100 person years (5 cases). No transmission was detected from anyone who was below 400 copies/ml (Attia 2009).

Test and treat?

At the end of 2008 a statistical paper caused great discussion. A research group led by the WHO director Kevin De Cock calculated how to, at least theoretically, curtail and even eliminate the worldwide HIV epidemic (Granich 2008, De Cock 2009). For this ambitious goal they concentrated totally on the preventive effect of antiretroviral therapies. They compared the common treatment strategy used today, beginning ART only on symptomatic patients or on those who have less than a certain number of CD4 T cells, to a theoretical strategy that seems simple enough. Every

person is tested for HIV once a year and if found positive, starts ART immediately, irrespective of CD4 T cells or viral load. The study was based on population data in South Africa, where 17% of the adult population is HIV-infected and on data from a successful intervention in Malawi. Other preconditions of the calculation model are that infectiousness of treated versus not-treated patients was estimated at 1%. The case-reproduction number, the so-called R0 number of new infections caused by one infection, was crucial for this calculation. A simple assumption that an R0 of <1 is required in order to reduce the incidence and to eventually eliminate HIV means that an incidence rate of less than one new case per 1000 person years was determined in order to eliminate HIV.

At present, every untreated HIV-infected individual causes another 7 HIV infections (R0=7) in the course of their lifetime. R0 could be reduced to 4 if every person received regular treatment with therapy starting at 200 CD4 T cells/µl, or even to 3 if therapy starts at 350 CD4 T cells/µl. However, an R0 reduction to less than 1 is impossible by this method and curtailing the epidemic with ART alone remains unrealistic. This could change however, with regular testing and immediate treatment of positively-diagnosed individuals – elimination of the epidemic could be possible by 2020, even in a country as severely affected as South Africa. Compared with common practice in 2008 where ART is begun only at a certain level of CD4 T cells, immediate treatment could reduce AIDS mortality to half of today's number by 2050. Calculations showed that this initially more expensive strategy could start to be cost-saving by around 2032.

The comments to the WHO publication ranged from "provocative" (Cohen 2008) to "extremely radical" (Garnett 2008). Critics raised concerns over the risks and the absence of ethics (would all actors agree? Could a restricted individual autonomy be complied with? Can changes in sexual habits be maintained?), medical (compliance problems, the dangers of possible resistance, the side effects and "overtreatment" – starting too early) as well as financial (South Africa would have to triple their financial commitments) considerations.

Such calculations are not new. Other groups had arrived at similar results in the past (Velasco-Hernandez 2002, Montaner 2006). What is new is that antiretroviral therapies today are potentially more user-friendly and such programs are probably easier to put into practice than just a few years ago.

In addition, people are realizing that the current preventive measures can only improve slowly and that neither vaccines nor microbicides can be expected in the near future. At present, approximately 80% of the population in sub-Saharan Africa is not aware of their infection. More than 90% do not know if their sexual partners are infected – an invitation for further spread of the epidemic.

Juggling figures like this may seem unhelpful at first. Despite all objections regarding methodological, ethical, financial or logistic considerations, etc., facing 2.1 million new infections per year, a number that is not likely to decline much in the near future, and the failure of several vaccine and prevention studies, one thing has become clear. Antiretroviral therapy has turned into one of the most important components of prevention.

Initiatives like this one of WHO must continue, and new and unusual strategies must be continually developed. It cannot do harm to bring more therapy to the millions of people worldwide, who still desperately need ART and are not receiving it (see chapter on *Global Access*).

ART & viral load in other body fluids

Do viral load in plasma (PVL) and viral load in other body fluids correlate?
Here are some data on male HIV+ patients::

- In a study from Italy the viral load on PI-containing ART regimens decreased by several logs in plasma as well as in semen (Liuzzi 1999).
- In a study with 114 male patients with PVL under 400 copies/ml on ART, only 2 (2%) isolated viral loads were detected in semen, compared to 67% in the untreated control groups.
- Among 255 MSM receiving ART with a PVL below 40 copies/ml, 7 patients (3%) showed an isolated viral load in semen (Marcelin 2009). These 7 patients had been on ART for some time.
- In two longitudinal studies on 157 and 88 HIV+ MSM patients on successful ART for >6 months from France, prevalence of intermittent seminal viral load was 7.6% and 7.5%, respectively (Goshn 2014, Ferraretto 2014).

Some more data on female HIV+ patients:

- In 205 HIV+ women with PVL under 400, 400–9999 and over 10,000 copies/ml, the rate of detectable HIV-1 RNA in the genital tract was 3, 17 and 48%, respectively (Cu-Uvin 2000). In 7 ART-naïve women, the viral load decreased by 0.7–2.1 logs within the first 14 days of ART. Similar results were achieved with 11 Brazilian female patients (Vettore 2006).
- In a group of 290 women with PVL under 500 copies/ml, 44 (15%) had detectable HIV-1 RNA in cervical smears (Neely 2007). In comparison to PI-containing ART the risk with NNRTIs was double.
- Out of 122 samples of cervical vaginal lavage, the viral load in the lavage correlated highly with PVL (Fiore 2003). However, in 25% of cases, virus was found in the lavage even when plasma viremia findings proved negative.

In conclusion, in most cases, viral load in plasma parallels viral load in other bodily fluids. If the viral load in plasma decreases, so does the RNA in semen or the vaginal fluid within a short time. However, intermittent shedding can occur in patients on ART (1–14%). Although there are implications that the detected virus in semen is not completely infectious (Nunnari 2002), one cannot rule out the patient being potentially infectious even on successful ART. Genital infections or inflammation may enhance shedding. In reproductive-aged women, shedding frequency and magnitude are greatest immediately following menses and lowest during ovulation (Curlin 2013).

Putting together these facts with clinical data, transmission with a low viral load seems unlikely. To date, only a few cases have been recorded in which transmission has taken place despite effective ART (Stürmer 2008). These cases show that there is in fact a residual risk. The question is how to manage that risk.

The EKAF paper

In January 2008 a paper was released by the "Eidgenössische Kommission für Aids-Fragen" (EKAF), the Swiss AIDS commission. Just the title of this paper caused a great stir: "HIV-infected individuals without other STDs on effective antiretroviral therapy are not sexually infectious." The original manuscript can be found at http://www.saez.ch/pdf_d/2008/2008-05/2008-05-089.PDF.

EKAF concluded that HIV-infected individuals do not transmit the disease when three conditions are met:

1. ART is adhered to and monitored by a clinician
2. The viral load has been below detection for at least six months
3. There is no other STD

This first official statement from public authorities on this subject had a major impact. Despite its caveats, critics feared that this publication could be misunderstood as an all-clear signal resulting in people being less careful and unnecessarily exposing themselves to risks of HIV infection.

Critics say that the data is not sufficient, especially for the risk of anal sexual contacts. The probability of infection is certainly under 1:100,000, but nevertheless not zero (Wilson 2009). The preventive effect of ART may be endangered by higher risk taking. According to mathematical models, a 10% rise in risk behavior could counter the effects of ART (Blower 2001, Law 2001). However, a meta-analysis came to the conclusion that ART does not increase risk behavior of the patient, even if the viral load is below detection (Crepaz 2004).

HIV clinicians must be prepared for this discussion. Patients are asking more questions: do I have to use a condom for the rest of my life? Here, it is better to give individualized advice. It depends greatly on the non-infected partner as well, as he or she should not be pressured. On the other hand, information of this type can be a relief for many patients and their partners. The EKAF paper may also motivate high-risk patients to finally start antiretroviral treatment (preventing more infections rather than causing new ones initially feared by the release of the paper).

However, it must be repeated that the EKAF statement refers only to stable relationships. Safer sex is still recommended, especially with occasional sexual contacts to avoid other sexually transmittable diseases.

The PARTNER Studies

How high is the transmission risk in reality? The European PARTNER Study is addressing this issue. PARTNER is a large observational multi-centre study of 1,110 HIV serodiscordant couples in which the positive partner is on ART and who do not routinely use condoms. Results presented at CROI 2014 from a planned interim analysis, reported that no linked transmissions have so far occurred after almost 900 couple years of follow-up. Follow-up results included almost 44,500 times with sex without condoms and over 21,000 times when this was with anal sex (Rodger 2014). However, uncertainty over the upper limit of risk remains, particularly over receptive anal sex with ejaculation. Moreover, PARTNER provides only evidence to date on the level of risk for people who have already been having sex without condoms (sometimes for many years). Thus, the findings in this study may not apply 1:1 to others. Additional follow-up in MSM is needed through PARTNER2 (2014–2017) to provide more precise estimates for transmission risk to inform policy and also individual choice on condom use.

Medical prevention strategies besides ART

In general, the risk for sexual transmission of HIV is relatively low and lower than commonly thought. According to a recent meta-analysis, the current per-act risk of HIV transmission via sexual exposures ranges from 4 per 10,000 exposures for insertive penile–vaginal intercourse to 138 for receptive anal intercourse (Patel 2014). The estimated risk of HIV acquisition from sexual exposure was attenuated by 99.2% with the dual use of condoms and antiretroviral treatment of the HIV+ partner. Thus, transmission is a relatively infrequent event. This necessitates studies on large patient collectives and/or extended observation periods, in order to access the effectivity of medical prevention strategies. Some of these studies will be discussed here.

Circumcision

Circumcision of the male foreskin reduces the risk of infection for several diseases in unprotected sexual intercourse (Weiss 2006). At least three randomized trials with heterosexual males in Uganda, Kenya and South Africa demonstrated this in recent years for HIV as well. Remarkably similar results were achieved (Table 12.1).

A meta-analysis of these studies shows a relative risk of 0.44 for circumcision (Mills 2008). The NNT (number needed to treat) required to prevent an event reached a relatively low 72.

Table 12.1: Large randomized studies on circumcision

Place (Reference)	n	Main Results	Reduction of Transmission risk
Kenya (Bailey 2007)	2784	Two-Year HIV Incidence 2.1% (95% CI 1.2–3.0) vs 4.2% (95% CI 3.0–5.4)	53–60%
Uganda (Gray 2007)	4996	Over 24 months HIV incidence 0.66 vs 1.33/100 person years	51–60%
South Africa (Auvert 2005)	3274	Over 18 months HIV incidence 0.85 vs 2.10/100 person years	60–61%

TR = Transmission Risk, partly different definition/calculation

The effect of circumcision is explained by the presence of CD4+ Langerhans cells and primary HIV target cells in the male foreskin. Circumcision reduces the frequency of genital HSV-2 infection (Tobian 2008), which however does not explain the protective effect (Gray 2009). An estimated 2 million HIV infections in Africa alone could be prevented in the next few years (Williams 2006). The WHO recommends circumcision as a preventive means for heterosexual men. A favourable side effect is that circumcision also has a protective effect against HPV-infections (Serwadda 2010, Davis 2013).

Circumcision, however, is not without risk. Complications (infections, postoperative bleeding) occur in 3–4% of cases (Gray 2007). Sexual behavior after circumcision, ethics and logistical problems are only a few aspects (Lie 2006). It must be noted that circumcision reduces the risk for male but not for female partners. The randomized study in Uganda showed a slight increase in infections of the female partners of circumcised males (Waver 2008). This can be mainly explained by couples probably having sexual intercourse earlier than recommended. Several weeks of abstinence are stipulated after the operation.

Is there a protective effect for MSM after circumcision? If there is, the data is less clear compared to the results for heterosexual men. A meta-analysis of 15 greatly varying studies with 53,567 MSM (52% with circumcision) showed no significant difference between circumcised and uncircumcised males (Millet 2008). Another newer study confirms these results (Sanchez 2011). Taken together, it remains unclear whether the protective effects of circumcision apply to the MSM population.

Preventive treatment of HSV and other diseases

Genital infections clearly increase the risk of acquiring HIV. For example, in a large prospective trial, bacterial vaginosis was associated with a greater than 3-fold increased risk of female-to-male HIV-1 transmission (Cohen 2012). Even more relevant is human herpes virus 2 (HSV-2) as this common virus can be easily detected and quantified in genital fluids.. Genital HSV-2 infection is associated with increased cervicovaginal and plasma RNA among coinfected women with genital ulcers, independently of the level of immunodeficiency (LeGoff 2007). According to a meta-analysis, the risk of HIV increases with HSV-2-seropositivity: when HSV-2 antibodies are detected in the blood, the risk increases in male patients 2.7-fold and in female patients 3.1-fold (Freeman 2006). A considerable amount of new HIV infections are due to HSV coinfection, with an estimated 38–69% in female patients and 8–49% in male patients. Considering these data, several studies have been conducted in which the protective effect of HSV therapy has been evaluated both in HIV-negative and HIV-positive populations.

HSV treatment of HIV-negative persons: Is a reduction of the HIV transmission rate in HIV-negative persons possible by suppression of HSV-2? HPTN 039, a double-blind, randomized, Phase III trial investigated this question (Celum 2008). In total, 1871 MSM from the USA and Peru and 1,380 women from Zimbabwe, Zambia and South Africa received 400 mg acyclovir or placebo twice daily. Enrolled subjects were all HIV-negative and HSV-2-positive at the beginning of the trial. Although less HSV ulcers were observed in the active group, the trial failed to show a decline in HIV incidence in the acyclovir -group, with 3.9/100 person-years compared to 3.3/100 in the placebo group. These disappointing results were confirmed by the Mwanza trial with 821 women in Tanzania, in which again no decline was observed (Watson-Jones 2008). It is still not clear why, however, resistance to acyclovir is unlikely (Watson-Jones 2010). Another study showed that short bursts of subclinical genital HSV reactivation are frequent, even during high-dose anti-herpes therapy, and probably account for continued transmission of HSV during suppressive antiviral therapy (Johnston 2012). Taken together, preventing HIV infection with HSV therapy using acyclovir in HIV-negative individuals has proven unsuccessful. The prophylactic use of azithromycin, to prevent bacterial STDs also showed no protective effect against HIV (Kaul 2004).

HSV treatment of HIV+ patients: Can the transmission rate be reduced if the HIV+ partner is treated with acyclovir? A huge study enrolling 3408 discordant African couples showed no effect on the transmission rate, although there was a clearly reduced rate of genital HSV ulcers (Celcum 2010). However, this study did show an interesting side effect, that there is a slight but measurable effect with acyclovir and its derivatives regarding HIV viral load. Compared to placebo, a decline of 0.25 logs was observed. This effect slightly decreased the risk of HIV progression in treatment-naïve patients (Lingappa 2010). The transmission rate was obviously not influenced by the reduction in viral load. Resistances were not induced by acyclovir (Baeten 2011). Antiviral effects were also observed in several other randomized studies. The viral load in blood and cervicovaginal fluids was reduced by 0.26 to 0.53 logs by using acyclovir or valacyclovir (Nagot 2007, Zuckermann 2007, Baeten 2008, Dunne 2008, Delany 2009, Paz-Bailey 2009, Roxby 2012). Valacyclovir also significantly decreased early breast milk HIV-1 RNA among women receiving PMTCT (Drake 2012). In a meta-analysis of seven randomized trials conducted between 2000 and 2009 in which acyclovir or valacyclovir were used as prophylaxis among individuals coinfected with HIV-1 and HSV-2, the summary treatment effect estimate was -0.33 logs, an approximate halving of plasma viral load (Ludema 2011). This effect may be enhanced with high doses of valacyclovir. Of note, the incremental reduction in plasma HIV-1 RNA achieved with valacyclovir was not mediated by greater genital HSV-2 suppression (Perti 2013).

Thus, these studies may possibly lead to the development of new acyclovir derivatives with improved antiviral potency, provided they respond well to HIV (Vanpouille 2010).

Microbicides, lubricants, diaphragms

Microbicides are chemical agents, mostly of topical application, in the form of gels that kill or immobilize HIV and other diseases. Heterogenic mechanisms are being examined, among them are agents that inhibit docking to the target cell or antiviral agents. Microbicides will need to be inexpensive, easy to apply non-toxic, and effective against other STDs, as these increase the risk of HIV transmission. The CAPRISA trial (see below) led to a revival in this field of research.

Classical microbicides: Up to now, there is no product that has delivered convincing protective effects in clinical studies. HIV transmission risk in fact increased with

nonoxynol-9 (Van Damme 2002) or cellulose sulfate (van Damme 2008). PRO 2000, which initially seemed promising (Abdool Karim 2011), had no effect (McCormack 2010). Application of diaphragms and/or lubricants in addition to condoms had no protective effect, as one randomized study showed (Padian 2007).

Antiretroviral microbicides: A breakthrough in research of microbicides was achieved in 2010 in the CAPRISA trial, a double-blind study in which 889 HIV-negative women in South Africa used 1% tenofovir gel (Abdol Karim 2010). Compared to placebo, HIV incidence was reduced from 9.1 to 5.6/100 years. Transmission risk for women applying the gel regularly was reduced by 54% and safety and tolerability were pretty good (Sokal 2013). According to newer estimations (Williams 2011), over 20 years, the use of tenofovir gel in South Africa could avert up to 2 million new infections and 1 million AIDS deaths. Even with low rates of gel use, it is highly cost-effective and compares favorably with other control methods.

This first success ("proof of concept") has led to a focus on antiretroviral agents in the research of microbicides, such as tenofovir and even the more experimental NNRTIs dapivirine and MIV-150, as well as maraviroc and raltegravir (Review: Mertenskötter 2011).

PrEP (Pre-exposure Prophylaxis)

PrEP is an oral prophylactic antiretroviral treatment. It is a novel strategy to stem the spread of HIV with ARVs (mainly used: TDF or TDF+FTC) in at-risk HIV-negative populations such as MSM, female sex workers or injecting drug users. In contrast to PEP (post-exposure prophylaxis, see chapter on PEP), PrEP attempts to prevent transmission before the exposure occurs. The impetus for the development of PrEP was the successful use of prophylaxis for mother-to-infant transmission, as well as animal studies. However, early studies were regarded with skepticism. Pressured by activists and others, a study with Cambodian sex workers was interrupted in 2004 and others in Cameroon and Nigeria in 2005 (Cohen 2004, Sing 2005). Researchers were accused of not providing sufficient information to the participants and of discontinuing treatment once the study was over.

A breakthrough was seen with PrEP at the end of 2010. In the iPrEx Study, 2499 MSM from six countries received either TDF+FTC or placebo. After a median of 1.2 years, 36 versus 64 infections were observed and the risk for infection was reduced by 44% (Grant 2011). Apart from slightly more cases of nausea and weight loss in the active arm, there were no differences. Of note, only in 3/34 patients of those infected in the active group was tenofovir or FTC detected in plasma. Protective effects were also proven in the Partners PrEP trial, a large trial involving 5000 heterosexual couples in Kenyia and Uganda, and the TDF2 trial (Thigpen 2011, Baeten 2012+2014). In the Partners PrEP trial, the placebo arm was stopped in July 2011 and the subjects re-randomized to tenofovir or TDF+FTC. In the Bangkok Tenofovir Study, daily oral TDF reduced the risk of HIV infection in people who inject drugs. Among 2413 participants, 1204 on TDF and 1209 on placebo, 17 and 33 participants became infected (incidence of 0.35 and 0.68 per 100 person-years), indicating a 49% reduction in HIV incidence (Choopanya 2013).

These results, however, have not been without their setbacks. In the FEM-PrEP Trial on African woman, 35 infections in the placebo arm were observed compared to 33 with TDF+FTC. Due to lack of efficacy, this large trial was discontinued in April 2011. The three-armed VOICE Trial investigating women from three African countries also showed no benefit with different interventions, neither with TDF gel, TDF tablets nor with TDF+FTC (Marrazzo 2013). The following table shows an overview of the ongoing large trials:

Table 12.2: Large randomised trials on continuous PrEP, March 2015

Reference	N	Risk groups, region, PrEP regimen	Protective effect
Bangkok Tenofovir Study	2400	IDU in Thailand: TDF	49% with TDF
PARTNERS PrEP (Baeten 2012)	4758	Heterosexual couples in Africa: TDF, TVD	67% with TDF, 75% with TVD
iPREX (Grant 2011)	2499	MSM, world-wide: TVD	44% with TVD
CAPRISA 004 (Abdool 2010)	889	Women in South Africa: Vaginal TDF gel	39% with TDF gel
TDF 2 (Thigpen 2011)	1200	Young women and men in Botswana: TVD	62% with TVD
Africa, FEM-PrEP (van Damme 2012)	2064	Women, Kenia, South Africa, Tanzania: TVD	None (study stopped)
Africa, VOICE/ MTN 003	5029	Women in South Africa, Uganda and Zimbabwe: TVD, TDF, vaginal TDF gel	None

To date it remains unclear why some trials were successful and others not. However, adherence certainly has a strong influence. Simple truth: One can not expect a protective effect if the patient doesn't take the agent. Trials such as iPREX or PARTNERS PrEP showed a clear correlation between blood levels and infection risk. Protection was highest in volunteers with detectable tenofovir levels (Anderson 2012, Donnel 2012, Baeten 2014). In the Bangkok Tenofovir Study, the risk of HIV infection decreased as adherence improved, from 48.9% overall to 83.5% for those with at least 97.5% adherence (Martin 2015). Adherence was poor in the FEM-PrEP trial, as the young women considered their risk of acquiring HIV infection as minimal. The VOICE trial also showed poor adherence as probably the application of gel was considered inconvenient by participants.

What has become clear however is that continuous PrEP is not always beneficial and that success depends on several factors: first, adherence, but also viral load of the infected sexual partner, other STDs, different biological factors, sexual behavior and sexual practices, to name just a few. Hormonal contraceptives seem to have no influence on the transmission rates (McCoy 2012). There are also some concerns about safety. Several studies suggested that tenofovir-based PrEP moderately but significantly reduces bone density and renal function (Mugwanya 2015, Mulligan 2015). And what about development and transmission of resistance in an unidentified HIV infection? Fortunately, drug resistance was rare in iPrEx on-study seroconverters, and only as low frequency minor variants (Liegler 2014). In PARTNERS PrEP, however, 5/26 seroconverters with detectable plasma drug levels had virus with resistance mutations associated with their PrEP regimen, mainly M184V (Lehman 2015).

In early 2015, two randomized trials (PROUD and Ipergay) provided further evidence for the efficacy of PrEP in high-risk populations. In a pragmatic open-label trial, the PROUD Study, PrEP was evaluated in MSM in a real-world setting, and the use of public clinics, with minimal extra research funds, was key to its design (McCormack 2015). In total, 545 MSM reporting anal intercourse without condoms in the previous 90 days were randomized to receive open-label daily TDF+FTC either immediately (IMM) or after a deferral (DEF) period of 12 months. In October 2014 the DSMB recommended that all MSM in the deferral group be offered PrEP. At this point, 3

and 19 HIV infections had been observed in the IMM and DEF arm (1.3 versus 8.9/ 100 PY), yielding a relative reduction of 86%.

In the French Ipergay trial, another strategy was evaluated for the first time, a so-called "on demand", event-driven PrEP strategy. In total, 400 high risk adult MSM who reported anal sex without condoms were randomized to take two pills of TDF+FTC or placebo 2 to 24 hours before each sexual intercourse, then another pill 24 hours later and a fourth pill 48 hours after the first drug intake (Molina 2015). In November 2014, after a median follow-up of only 8.8 months, the placebo arm was also discontinued as the incidence of HIV-infection was 6.75 versus 0.94 per 100 patient-years in the TDF+FTC arm, indicating a relative reduction of 86% in the incidence of HIV with on-demand PrEP. Sixteen patients had acquired HIV infection after enrollment, 14 in the placebo arm and 2 in the TDF/FTC arm.

Taken together, PrEP represents a very effective and safe prevention strategy if the person is adherent. In July 2012, FDA approved TDF+FTC to reduce the risk of HIV infection in uninfected individuals who are at high risk of HIV infection and who may engage in sexual activity with HIV+ partners. In July 2014, the WHO released guidelines recommending the offer of oral PrEP to high-risk populations http://www.who.int/hiv/pub/guidelines/keypopulations/en/. In Europe, where TDF+FTC are not yet available for prevention, community organisations are currently calling stakeholders to make PrEP available and accessible.

Evaluation of long-acting medications and alternative formulations for PrEP is underway and may lead to the wider implementation and impact of PrEP. Physicians must be prepared to talk about PrEP. Patients and their partners will be asking for it. However, many questions remain that have not been answered by the above-mentioned studies.

How should PrEP be administered? And who will cover the expense? Will PrEP be sold on the black market? Who should distribute it (walk-in clinics, doctors, pharmacists?) and how to make it more accessible to high risk groups? What compose these high risk groups? Will the success of PrEP lower the use of condoms? Is the dose studied and the form (every day or before and after sex as "on-demand") the best way? Is TDF+FTC the only option? Other questions regarding long term tolerance, safety during pregnancy, administration in young people or patients with hepatitis B remain unanswered.

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6.13. Global access to HIV treatment

ROB CAMP

We all know this data, which we see every time we go to an international meeting:

- Some 5,600 people become infected with HIV every day, 600 of whom are under 15 years of age, half of them are women, 30% are under 25.
- Approximately 15 million people are currently receiving ART, and while that is more than laudable (just three years ago it was half that!), the rest (approx. 21 million) are waiting, or do not know they have HIV.
- There are some 36.9 million people infected, 2.0 million newly infected in 2014. 1.2 million people died of AIDS-related causes in 2014.
- An investment of $35 billion/year is envisioned for the global "fast track" response by 2020.

Access to drugs depends not only on financial and human resources. It depends also on people being aware of their HIV status, knowledgeable about treatment, and empowered to seek it. Thus public information and education are important elements in widening access, alongside efforts to build or strengthen health services. Stigma has been and remains a major stumbling block in wanting, seeking and taking the treatment regimen correctly. The campaign for universal access to life-saving drugs for HIV and AIDS, started originally by grassroots AIDS activists, is today a major focus of attention of UN agencies and most all influential organizations at national and global levels.

The Declaration of Commitment on HIV/AIDS, unanimously endorsed by the UN General Assembly in 2001, embraced equitable access to care and treatment as a fundamental component of a comprehensive and effective global HIV response. Since then many countries, through the support of intergovernmental organizations and donors, have definitively demonstrated the ability to deliver HIV treatment in very resource-limited settings. Access to treatment has helped mobilize communities in response to HIV, preserved the health and viability of people and households vulnerable to HIV, and strengthened HIV prevention efforts in many parts of the world. The UN underscored this goal in 2011, upholding their belief in TRIPS flexibility regarding public health drugs and global trade agreements.

In the goal to reach universal access to HIV prevention, treatment, care and support, leadership at a national level is required to establish policies that support treatment scale-up, and is now a very central part of being able to achieve the new funding levels needed to eradicate HIV:

- increasing the number of people who choose to know their HIV status;
- reducing HIV stigma;
- building human capacity to sustain treatment through education and training and better use of human resources;
- improving supply management and integrating HIV care with other health services.

In 2012, the international community committed to a new goal of 15x15, 15 million people on ART by 2015, which was reached some 9 months ahead of target. The goal of universal access is also part of Millennium Development Goal (MDG) 6 which includes halting and beginning to reverse the spread of HIV/AIDS by 2015.

The updated 2011–2015 global health strategy was released in June 2011. This strategy outlines four key targets that countries need to achieve if universal access and MDG 6 are to be realised: reduce new infections by 50 percent among young people (15–24 years), reduce TB-related mortality by 50 percent, eliminate new infections in children, and reduce HIV-related mortality. And of course, the 90/90/90 program, of having 90% of those with HIV tested, 90% of those tested starting treatment, and 90% of those undetectable.

Major Players

PEPFAR Update

The President's Emergency Plan for AIDS Relief (PEPFAR) was launched in 2003 to combat global HIV/AIDS, and is the largest commitment by any nation to combat a single disease in history. During PEPFAR's initial phase in 2004–2008, the United States invested nearly $19 billion in PEPFAR (which includes bilateral HIV/AIDS and tuberculosis programs, as well as contributions to the Global Fund to Fight AIDS, Tuberculosis and Malaria). For 2015, $6.83 billion was enacted for bilateral HIV/AIDS programs (see chart; $ here are US$).

PEPFAR is the cornerstone of the US Global Health Initiative, which has committed almost $66 billion to support countries in improving and expanding access to health services. PEPFAR focuses now on sustainability, and serves as a platform for expanded responses to a broad range of global health needs. PEPFAR partnerships in more than 70 countries have directly supported care for millions of people affected by HIV/AIDS. Until July 2015, PEPFAR supported prevention of mother-to-child transmission programs that allowed more than 1,000,000 infants of HIV+ mothers to be born without HIV. PEPFAR has also directly supported HIV counseling and testing for nearly 56.7 million people, including community-based services and rapid tests, providing what may be an important entry point to prevention, treatment, and care.

Table 13.1: FY 2009 – FY 2015 PEPFAR Funding ($ in millions)

Programs	2009	2010	2011[2]	2012	2013	2014	2015	Total
Bilateral HIV/AIDS Programs[1]	5,503	5,574	5,440	5,333	4,726	4,940	5,238	51,791
Global Fund	1,000	1,050	1,046	1,050	1,569	1,650	1,350	11,968
Bilateral TB Programs	177	243	239	256	233	243	242	2,163
TOTAL PEPFAR (w/o Malaria)	6,680	6,867	6,725	6,639	6,527	6,833	6,830	65,921

[1] Bilateral HIV/AIDS Programs includes funding for bilateral country/regional programs, UNAIDS, IAVI, Microbicides and NIH HIV/AIDS research.
[2] Includes enacted funding for FY 2009-2015.
Note: All funding amounts have been rounded to the nearest million, so the numbers shown in the table may not sum to the totals. *Accessed September 2015

PEPFAR will have dispersed more than 1 billion condoms in the years 2012 and 2013. The commitment to voluntary male circumcision has grown to reach 6.5 million men. A new component is the $210 million partnership with Gates and Nike for engaging adolescent girls and young women called DREAMS.

They speak on prevention, *vis-a-vis* most at-risk populations, risk reduction, STI screening and treatment and comprehensive services for drug users, criminalization and stigma. They report on Operational Plans, including Training for Health Care Workers, by country and by region.

The Global Fund

The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) is an international financing institution that invests the world's money to save lives. It supports large-scale prevention, treatment and care programs against the three diseases.

Fiscal strategies were reviewed and redesigned in 2013 for accounting and forecasting. Risk management was updated in 2014 in finance, procurement and supply chain management.

In 2014, The Global Fund received $3.5 billion in contributions, including a newer model of engaging emerging economies like Indonesia and Vietnam to contribute

(vs receive, see MSF below). Total new grants for 2014 fell by close to 25%, to $2.5 billion due to the transition to this new funding model and better cash management procedures of moneys already disbursed. There is almost ¾ of another billion USD signed but not committed to.

From their 2014 Press Statement on Results, "Through the (new) funding model, the Global Fund is pursuing a differentiated approach to investing. It is weighing economic scenarios against epidemiological intelligence that points to diseases, especially HIV and tuberculosis, becoming less generalized and more concentrated in certain locations and in key populations within a country. While certain middle-income countries and regions are making remarkable progress, others are falling back. Achieving control over these diseases calls for a diversified and differentiated approach, aligned with the Post-2015 development agenda."

Finland, Greece, Hungary, Slovenia and Spain are some of the European countries with zero GFATM contributions from 2014–2016. Ireland and Italy are in the "promised but not yet paid" column.

UNAIDS

UNAIDS provides technical support to countries, assisting them with expertise and planning for national AIDS programs, to help 'make the money work' for the people on the ground. UNAIDS tracks, evaluates and projects the financial resource requirements at global, regional and country levels to generate reliable and timely information on the epidemic and the response. Based on these evaluations, UNAIDS produces guidelines and progress reports. Much of the international data we juggle is set and approved by UNAIDS. They set out plans for "Getting to Zero" and other platitude-ridden slogans and programs. They are making a good effort on tackling major social issues like homophobia, financial sustainability and gender equality. "Together we will end AIDS", whose title is annoyingly cheerleader-ish, actually offers some really important information like the fact that low- and middle-income countries now invest significantly in their HIV/AIDS response, while donor countries have not increased much. The UN adopted a Political Declaration on HIV/AIDS in which member states agreed to increase investments for HIV to between $22–24 billion by 2015. A concerted effort by all countries is needed to meet the targets (slightly under $22B has been reached up until June 2015). Another promising approach would be to expand innovative mechanisms like indirect taxation (airline tickets, mobile phone usage, exchange rate transactions) to support global health initiatives, including HIV. The larger international community must continue to support and strengthen existing financial mechanisms, including the Global Fund and relevant UN organizations. The 15x15 program is a UNAIDS-sponsored program, as is the 90/90/90 idea, mentioned above and further on.

The Bill and Melinda Gates Foundation

The largest private philanthropic organization is located in Seattle, US, "focusing on improving people's health and giving them the chance" to emerge from "hunger and extreme poverty." They have approximately 1376 employees with an endowment of $42.9 billion. They have committed $33.5 billion since inception and in 2014 committed grants to the tune of $3.9 billion in over 100 countries (the 2013 annual report at http://www.gatesfoundation.org/Who-We-Are/Resources-and-Media/Annual-Reports). Much of these moneys are for non-AIDS-specific works, including development (reducing poverty and hunger). In health (58% of the total spending), they fight and prevent enteric and diarrheal diseases, malaria, pneumonia, TB, neglected and infectious diseases, working on integrated heath solutions, improving delivery of existing tools and supporting research and development in

Health products and health equipment	21%
Medicines and pharmceutical products	19%
Human Resources	15%
Other	12%
Program Management	11%
Training	9%
Infrastructure and other equipment	8%
Monitoring and Evaluation	4%

Figure 13.1: Like many of the mechanisms described in this chapter, The Global Fund dedicates significant resources to non-drugs support, like training and human resources (GFATM Funding and Spending 2013)

new interventions like vaccines, drugs and diagnostics (http://www.gatesfoundation.org).

Drugs available from whom and where

FDA's qualification of generics

Generic drugs are important options that allow greater access to health care. They have the same high quality, strength, purity and stability as brand-name drugs. Generic manufacturing, packaging, and testing sites must pass the same quality standards as those of brand name drugs.

For PEPFAR use, all drugs need FDA approval. As of 2 July 2015, FDA had approved 187 generic drugs for use in the PEPFAR program that are approved in as short a time as six weeks. While quality, strength, purity and stability are guaranteed, administration, delivery and correct use is another issue. For example, the latest generic approved was another version of nevirapine. And there the rub. Generics companies copy what is easiest and cheap, not necessarily the most innovative, or the most optimal treatments (there are no integrase inhibitors yet). We must continue to try to remind the generics companies that what is best for the patient will continue selling for years, while less-than-optimal combinations will have limited life-times, and might do harm via side effects along the way. Although the WHO pulled d4T from its list of recommended products in 2009, the switch to more effective treatments like TDF took time (the latest was an EFV/FTC/TDF FDC in Feb 2015).

Lopinavir/r and atazanavir/r are the only PIs approved. Sadly, Janssen under J&J never bothered to outlicense any of its products (darunavir, etravirine, rilpivirine). There are a handful of generics companies with an abacavir approval. As HLA testing for abacavir HSR is not easily available in the Global South, it is very important to train both the medical professional as well as users on diagnosis of HSR and what to do if it occurs, and the importance of never re-starting it once HSR is suspected, things that from an international regulatory agency would be hard to monitor. And although REMS programs from FDA or EMA would accept information on side effects from the Global South (which has up to 6 times the amount of people on drug), they probably contribute little to the overall numbers and therefore safety of these drugs. Mentioned below is VigiAccess from WHO.

In 2015 (until 1 September), FDA has issued no warning letters to any generics company regarding its HIV products.

In 2015, FDA inspectors along with EMA will continue to perform manufacturer-related inspections. Enforcement actions from suspension to closures can be considered.

WHO

3 fronts – HIV, viral hepatitis and STIs

WHO works on diagnostics (testing) and prevention as well as treatments for these disease areas. The strategies for WHO 2016–2021 will be drafted, after a lengthy input survey period, by Fall 2015 and voted on by the World Health Assembly by mid-2016.

Prequalification and quality assurance of antiretroviral products – a fundamental human right

WHO's Prequalification Programme conducts evaluation and inspection activities and builds national capacity for manufacturing and monitoring high-quality medicines. WHO began reviewing HIV antiretroviral drugs for prequalification in 2001. In 2005–2006, WHO conducted a quality assurance survey of antiretroviral medicines in Cameroon, the Democratic Republic of the Congo, Kenya, Nigeria, Uganda, United Republic of Tanzania and Zambia. Of the 395 samples tested, none had quality deficiencies that would pose a risk to the people taking them. They have now opened as well a pharmacovigilance website called VigiAccess where adverse events due to medicinal products collected by 110 national drug authorities are housed.

Invitations to manufacturers to submit an expression of interest (EOI) for product evaluation are issued not only for HIV/AIDS-related care and treatment products, but also for anti-malarial medicines, anti-tuberculosis medicines, influenza-specific antiviral medicines and reproductive health products.

On the WHO List of Prequalified Medicinal Products is an extended list of 354 products (http://apps.who.int/prequal/query/ProductRegistry.aspx, accessed 1 Sep 2015) for HIV/AIDS, made by both originator companies and generics companies. Prequalification may be better described as pre-, on-going, and post-qualification, as they do inspections at all these time points.

On the list are many drugs for OIs (acyclovir, ceftriaxone, ciprofloxacin, amongst others). WHO also approves medicines quality control laboratories (QCLs): 38 QCLs are currently prequalified all around the world.

Table 13.2: Total antiretroviral therapy in low- and middle-income countries by region, December 2014

Region	Estimated number of people receiving ART	Estimated number of people needing ART (people living with HIV)	ART coverage
Sub-Saharan Africa	10 680 000	25 800 000	41.4%
Latin America and the Caribbean	908 000	1 980 000	45.85%
East, South and South-East Asia	1 774 000	5 000 000	35.5%
E. Europe and Central Asia	284 000	1 500 000	18.9%
North Africa and the Middle East	32 000	240 000	13.3%
Total	13 678 000	34 500 000	39.7%

Source: UNAIDS, How AIDS changed everything

A chasm

Improved treatment in line with scientific evidence and recognized international standards of care

Médecins Sans Frontières (MSF, Doctors without Borders) are on the front lines in clinics and health centers in more than 70 countries, their advocacy is not of the ivory tower type. In the filed on the ground, they work on innovative approaches to tackling the major health challenges posed by HIV, TB, malaria, flu, neglected tropical diseases and emerging pathogens. They believe that not continuing to invest today in improved treatment and protocols will cost lives down the road, increase a double standard in HIV care and lead to increased costs. They were the first to talk about the risk that donors may not continue to support or try to delay the implementation of proven and recommended medical strategies for the sake of short-term savings. They recommend:

- Supporting initiation of ART for all people with HIV. No CD4 count level.
- Implementing a tenofovir-based first-line regimen to allow people to stay on their first regimen as long as possible with fewer side effects and delay the need for more costly second-line regimens. They also now recommend dolutegravir over efavirenz for tolerance as well as prevention.
- Providing access to viral load testing to support adherence and detect treatment failure earlier, thereby preventing resistance and needless switching to expensive sub-optimal second-line treatment.
- Preserve India's role as the pharmacy of the developing world (TTIP).
- TPP must not impose restrictive IP protections.
- There must be greater transparency and accountability in Global Fund management and procurement.
- The Global Fund must support developing countries to combat HIV, TB and malaria, not reclassify countries to a higher (middle-income) level to offset funding shortages.
- The World Trade Organization must extend its TRIPS waiver for least developed countries.
- TB and HCV testing and treatment programs must be better integrated with HIV.

Their July 2015 report on the seven next steps ("or fail") is a must read at http://www.msfaccess.org/sites/default/files/HIV_Brief_HIV_Fail_Derail_or_Prevail_ENG_2015.pdf.

How to ensure that prices of drugs and diagnostics remain reasonable?

The international community needs to support policies that will enable funds to stretch as far as possible to meet needs and contain costs in the short- and long-term by, amongst other measures, ensuring a competitive supply for drugs.

In accordance with the Doha Declaration on TRIPS and Public Health, governments can authorize governmental use or compulsory licenses to ensure generic production of patented products (as in Brazil and Thailand).

Companies and governments can support the Medicines Patent Pool for antiretroviral medicines at www.medicinespatentpool.org. This mechanism brings together patents held by different owners and makes them available to others for generic production and further development. Gilead was the first company to sign on, in July 2011, following the US NIH, although their most recent agreements for TAF etc, is presently very limited in scope. They signed with ViiV Healthcare in early 2014. Roche, Abbvie and BMS have signed. This Pool could save lower income countries more than $1 billion a year in drug costs. Shamefully, J&J (Janssen) and BI have not joined the savings party.

Prices of first-line regimens in low-income countries

The decline in drug prices since 2008 can be attributed to the scaling up of treatment programs, increased competition between a growing number of products prequalified by WHO, new pricing policies by pharmaceutical companies and successful negotiations between the William J. Clinton Foundation (CHAI) and major generic manufacturers. The median price paid for TDF+3TC+efavirenz (prequalified by WHO) in low-income countries in July 2014 was $100 per person per year for the fixed-dose combination. Combinations with d4T (stavudine) and ddI (didanosine) have fallen off the pricing scales finally.

Second-line regimens

Second-line regimens are still significantly more expensive than first-line regimens in low- and middle-income countries. In 2014, the cost of a regimen of AZT+3TC+atazanavir/r, is $243 in low-income countries and significantly higher in middle-income countries. Prices paid for second-line regimens can vary significantly between countries.

In the UK, a study showed that first-line treatments can last 8 years or longer (UK Chic 2010). If ARV access in the developing world started in earnest in 2002 (without all the management and strategic tools of retention and adherence used in the North, like viral load measurements), we are beyond the 8-year mark. What to do with the people who need to move to a new regimen (whose number is growing now that everyone should be on treatment)? Of the regimens that fail, NNRTIs fail at a rate almost three times higher than the rate of PIs. Most people in resource-limited settings are on an NNRTI-containing regimen (nevirapine or efavirenz). MSF estimates that regimen failure is "largely under-diagnosed" due to limited lab facilities for viral load testing, which can only lead to resistance and harder-to-construct post-first-line regimens.

How to expand treatment to more people plus switch those currently failing to a more expensive second-line regimen, all within a framework of cutting back on donor spending?

As the absolute numbers of people who need access to second-line regimens continue to grow, addressing the high cost of second-line regimens will become increasingly important to ensure the cost-effective use of available resources. A third-line treatment can be up to 8 times as expensive as a second-line treatment. Obviously, management, retention and adherence issues need to be fully incorporated into care in order to keep everyone on treatments and help them not need to advance to more complicated and expensive regimens.

Future Funding

As funding stalls, major funders – US, UK, Netherlands, France, Germany, Norway and Sweden – are fatigued. The good news is that countries are now starting to help support their own programs, ranging from a little more than 40% in Sub-Saharan Africa to more than 95% in Latin America and the Caribbean (UNAIDS 2015).

New strategies have to be developed – small taxes on currency transactions (Oxfam's Robin Hood tax), an airline ticket tax, a Global Health charge on alcohol and tobacco consumption (Hill 2012) etc. Product(Red) is a fund-raising mechanism tied to the Global Fund that coordinates profits from sales from businesses, and has recently reached the $320 million mark.

It is perhaps more important than ever that we all contribute, whether economically or advocacy-based, to be able to optimally treat everyone.

Europe gets involved

The European Union can impact access to medicines for developing countries through its policies, legislation and bilateral and regional trade agreements. The EU can adopt appropriate measures to improve access to existing medical tools (medicines, diagnostics, vaccines) as well as stimulate the research and development of better tools for people in resource-starved countries. The Working Group on Innovation, Access to Medicines and Poverty-Related Diseases will create a meaningful dialogue between Members of the European Parliament, the European Commission, and civil society. The EP Working Group is working hard on not allowing TTIP to harm medicines and health access.

How do we get there? From rhetoric to reality

Successes in controlling the epidemic can be attributed to a comprehensive response and commitment from all sectors of society, according to on-the-ground experts in sub-Saharan Africa. Buy-in from the highest political offices is important in creating polices that place HIV on the national agenda. For instance, in Rwanda, all government departments were mandated to carry HIV messages over a long period, which helped stabilize spread of the disease. The Rwandan Minister of Health reminds us to include youth in the messaging.

Although extensively reviewing the latest International AIDS Conference is impossible here, there was an effort to demonstrate the importance of including patients and their broader communities in the delivery of services (www.aids2015.org), *aka* locally-mobilized resources, which has to include building civil society capacity to monitor government spending as well as simply for any significant long-term community-driven transformation. From the Vancouver 2015 consensus statement, "We call on leaders the world over to implement HIV science and commit to providing access to immediate HIV treatment to all people living with HIV. We call on donors and governments to use existing resources for maximum impact and to mobilize sufficient resources globally to support ARV access for all, UN 90/90/90 goals for testing, treatment and adherence, and a comprehensive HIV response. We call on clinicians to build models of care that move beyond the clinic to reach all who want and need ARVs. We call on civil society to mobilize in support of immediate rights-based access to treatment for all."

Sadly, what I wrote in 2012 is still true today in Eastern Europe & Central Asia: While the disease is becoming better controlled in many parts of the world, the opposite is true in Eastern Europe, where there is a higher incidence driven by a take-no-prisoners attitude in intravenous drug use policy. Ukraine gives a positive spin on how to do it with combination prevention and substitution therapies. Educating political leadership in these countries (including Russia) is important since so few resources are allocated to fighting HIV. This is not one epidemic with one simple answer. There may be some basic ingredients for "getting there":

• Cascading implementation structures from national to grassroots level
• Ensuring increasing national government budget allocation to HIV responses while donors support ongoing gaps (ie, country ownership)
• Mobilizing all sectors of society to play their part in HIV
• Integrating principles of good governance from the outset to ensure accountability at all levels.

The unconscionable health gap: a global plan for justice

If the health gap is unfair and unacceptable, how can the international community be galvanized to make a genuine difference? A "global plan for justice" would be a voluntary compact between states and their partners. It would simply encourage the

WHO to exercise its constitutional powers and leadership. This global plan for justice would guarantee a universal package of essential services, comprising core components like essential vaccines and medicines, basic survival needs, and adaption to climate change (Gostin 2010).

The amount of people who need access to ART in the next few years is clear – if 15 million people are treated, 22 million are not. We need to keep up the pressure on all actors – donor organizations as well as individual nations, manufacturers, health care workers and affected communities of all sizes – to do their part in order to provide the most current and useful prevention and treatment strategies to the adequate and most at-risk populations. In order to achieve this, we can not sit idly by and hope for the best – we must continue to push that boulder up the hill for as long as it takes so everyone who needs it has access to prevention including PrEP, treatment and care as early and for as long as necessary.

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7. Management of Side Effects

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Antiretroviral Therapy (ART) has become more and more tolerable. Side effects has been become less frequent and less severe. While in the past up to 25% of ART disruption due to side effects were observed within the first year of ART (d'Arminio Monforte 2000, Yuan 2006), treatment cessation due to side effects has become less frequent (Carr 2009, Cooke 2014).

Nevertheless side effects and tolerability play an important role within clinical care of HIV+ patients. Regular visits help to address this factor for improving treatment success. Biannual visits are recommended by most current guidelines. Standard evaluation should evaluate premedical history, physical examination, vital signs and allergies. Routine investigations include a full blood count, liver, pancreas and renal function tests, electrolytes (plus phosphate in patients on tenofovir-containing regimens) as well as fasting cholesterol, triglycerides and glucose levels. Remarkably, a urine dipstick can detect proteinuria in patients on TDF-containing treatment regimens. While the routine clinical visit is recommended at least quarterly, more frequent visits maybe necessary when beginning or after switching an ART regimen. In contrast, patients on a stable and tolerable ART may be seen less frequently. However, with the increased life expectancy of HIV+ patients, comorbidities are coming to the fore.

The following chapter addresses some ART-specific side effects. Our aim is to give advice regarding the clinical routine. Therefore we structured this chapter according to organ (dys)functions and symptoms.

Gastrointestinal side effects

Gastrointestinal (GI) problems are the most common side effects even if they have become less frequent, as older NRTIs like AZT, ddI or d4T are no longer recommended (Robinson 2008, Chubineh 2008). GI side may effects appear more frequently during the early stages of therapy. Typical symptoms include abdominal discomfort or pain, appetite loss, diarrhea, nausea and vomiting or constipation. Diarrhea occurs frequently with older PIs and rarely occurs with integrase inhibitors like raltegravir, elvitegravir and dolutegravir (Lee 2012) but can be seen with 3TC too.

In most cases, symptoms occur early on therapy. Patients should be informed that these side effects usually resolve after 1-6 weeks of treatment. If gastrointestinal side effects appear for the first time after longer periods on ART, other causes such as gastritis and infectious diarrhea need to be considered. Fructose or other intolerances should be addressed. However, if no other causes of diarrhea could be found, a switch of ART may be considered.

Nausea and vomiting

If administration on an empty stomach leads to nausea and vomiting, most drugs can be taken with meals. Only the NNRTI efavirenz has to be administered on empty stomach; small quantities of low-fat salty crackers may improve nausea. Ginger, peppermint or chamomile teas or sweets may also be helpful, as well as more frequent but smaller meals. Fatty foods and dairy products should be avoided, as well as coffee, smoking, alcohol, NSAIDs like acetylsalicylic acid, ibuprofen, diclofenac and very spicy foods.

Whenever possible, other causes of nausea should be excluded (e.g., by gastroscopy) and switching ART may be discussed. Metoclopramide has been proven to be useful.

Dimenhydrinate, cimetidine, ranitidine or ondansetron can also be taken. Antiemetic drugs can not only be administered if the patient is feeling ill, but taken regularly prophylactically, ideally 30 to 45 minutes before ART. If taken on a regular basis, attention should be paid to side effects such as dyskinesia. After a few weeks, doses of antiemetics should be reduced. If nausea persists for long time, a gastroscopy needs to be performed.

Diarrhea

While diarrhea was a frequent problem for adherence in former times, modern ART regimens offer more tolerable options. Whenever diarrhea persists beyond the first 4-6 weeks and other causes of diarrhea are excluded (GI infection, fructose, lactose or other problems), another more tolerable ART option should be investigated. If switching regimen is not possible, different options maybe helpful:

- Difficult digestive food (particularly those high in fat or glucose) should be avoided. "Home" remedies maybe discussed (see Table 1).
- In the case of significant dehydration and electrolyte loss, cola and salty crackers, sports drinks, herbal teas or electrolyte solutions may be helpful. Oral rehydration solution can easily be made from the juice of 5 oranges, 800 ml of boiled water or tea (cooled to room temperature), a teaspoon of iodized salt and two tablespoons of sugar.
- Oat bran tablets have been proven to be useful and cheap for PI-associated diarrhea. They can be taken together with ART (daily dose 1500 mg). Pancrelipase, a synthetic pancreatic enzyme, or 500 mg calcium BD (Turner 2004) have also been shown to be effective for PI-associated diarrhea. However, as calcium binds with many other agents, it should be taken 2 hours apart from HIV medication.
- Oral supplements of glutamine (10–30 g/day) or L-alanyl-L-glutamine (up to 44 g/day) alleviate diarrhea and can also boost levels of antiretroviral drugs in the blood (Bushen 2004, Heiser 2004). Glutamine can be purchased in drugstores or ordered online.
- Loperamide inhibits bowel movement (initially 2–4 mg, followed by 2 mg, up to a maximum of 16 mg daily). Opium tincture is an alternative (initially 5 drops, maximum 15 to 20 drops), but care must be taken in regard of obstipation.

Table 1: "Approved" homespun remedies

Pectin
In apples (raw and pared), bananas (purée), carrots (purée, cooked, soup), St. John's bread (oatmeal gruel or rice gruel with St. John's flour). Pectin is a dietary fiber, which is not digested, it binds water and toxic agents and lessens the diarrhea.
Gruel
Soupy oatmeal or rice
Tanning agents
Black or green tea, dried blueberries (tea, powder), dark chocolate

Hepatotoxicity

Hepatotoxicity is a common side effect of ART. Severe hepatotoxicity occurs in up to 10% of patients (Price 2010, Josh 2011), mostly in patients with preexisting liver dysfunction (Soriano 2008). While liver failure is rare overall (Nunez 2005), fatal liver damage has been associated with nevirapine, ritonavir and tipranavir (Bjornsson

2006, Rachlis 2007, Chan-Tack 2008). Mild liver enzyme increase is a common side effect and has been reported for other drugs, such as maraviroc, raltegravir, dolute-gravir, elvitegravir, rilpivirine and others. Hypersensitivity reactions are correlated with NNRTI use, are not dose-dependant, occur mostly within the first 4–12 weeks and symptoms resolve usually after stopping the drug (Joshi 2011). There are black box warnings for hypersensitivity for nevirapine and rilpivirine (Cohen 2011, Molina 2011), the NRTI abacavir as well as the CCR5 inhibitor maraviroc. Boosted PIs such as atazanavir/r can lead to hepatotoxicity at any stage during the course of treatment. Common risk factors for hepatotoxicity are elevated liver enzymes before initiating treatment, chronic hepatitis B or C, concomitant hepatotoxic medication, PI therapy, older age, higher BMI, female gender, thrombocytopenia, high alcohol intake, high viral load or renal dysfunction (Sulkowski 2002, Servoss 2006, Nunez 2010).

NNRTIs

Liver toxicity occurs more commonly on nevirapine than on other antiretroviral drugs. Clinically asymptomatic and symptomatic liver toxicity, including rapidly occurring fatal liver failure have been observed (Bjornsson 2006). Serious and fatal liver toxicity has been reported even during post-exposure prophylaxis (PEP), but not after single-dose nevirapine (McKoy 2009). Symptomatic hepatotoxicity seems to depend on different risk factors: Female gender, body mass index <18.5 (Sanne 2005) or chronic hepatitis C (Torti 2007) and higher CD4 T cells at treatment initi-ation. A retrospective analysis of the Boehringer Ingelheim database showed a higher risk for females with CD4 T cell counts >250 cells/µl and for males >400/µl. Although these findings have not been confirmed by other studies (Manfredi 2006, Peters 2010), the *Indications and Usage* section advises against starting nevirapine treatment above these CD4 T cell counts in treatment-naïve patients unless the benefits clearly outweigh the risks (Mallolas 2006, De Lazarri 2008).

In general, nevirapine should be avoided in patients with liver cirrhosis Child–Pugh class B or C (Nunez 2010). Liver toxicity occurs usually early during ART (within 18 weeks of starting) and may progress to liver failure despite laboratory monitoring. If liver enzymes increase to >3.5 times the upper limit of normal (ULN) during treatment, nevirapine should be stopped immediately. Readministration must be discussed carefully and should, whenever possible, be avoided.

In patients treated with efavirenz or rilpivirine, minor enzyme elevations are generally safe and usually resolve so that a treatment change may not be necessary (Gutierrez 2008, Kontorinis 2003, Cohen 2011, Molina 2011).

Protease inhibitors and INSTIs

Atazanavir (as well as indinavir) inhibit the hepatic enzyme UDP glucuronosyl-trans-ferase, inducing non-dangerous hyperbilirubinemia in up to 50% of patients (Torti 2009). UGT1A1*28 variant allele seems to be a predictor of severe hyperbilirubine-mia (Turatti 2012). Atazanavir was safe in end-stage liver disease patients, hepatitis coinfected patients and those with liver fibrosis (Guaraldi 2009, Pineda 2008). While darunavir and atazanavir were not associated with increased liver morbiditiy, other PIs such as tipranavir/r are associated with a higher risk of transaminase elevations (Hicks 2006).

In all cases of unknown liver enzyme increase, hepatitis diagnostics (including HAV, HBV, HCV, HEV), syphilis testing (EBV and CMV) and abdominal ultrasound is recommended. In case of a more chronic enzyme elevation other metabolic diseases, such as Wilson disease, hemochromatosis, alpha-1-antitrypsin deficiency,

autoimmune hepatitis or (non-alcoholic) fatty liver disease must be excluded. In case of acute liver failure or increase in transaminases, more frequent testing is necessary. ART discontinuation may not be necessary, unless acute >5-fold increase of transaminases. Ultimately a liver biopsy can reveal macro- and microvesicular steatosis and mitochondrial alterations in NRTI-induced steatosis and is therefore helpful to distinguish NRTI-induced hepatopathy from other causes.

Moderate liver enzyme elevation has also been reported in several INSTI studies. Dolutegravir, elvitegravir and raltegravir can lead to mild to moderate increase in liver enzymes. Rates of elevated liver enzymes in patients with elvitegravir were comparable to those treated with efavirenz or boosted atazanavir (DeJesus 2012, Sax 2012). Dolutegravir-associated increase in liver enzymes was mostly seen during immune reconstitution and coinfection with viral hepatitis (Curtis, 2014). In any case, transaminase elevation due to INSTI leads only in a very rare number of cases to discontinuation.

Renal problems

Renal complications are mostly seen with tenofovir disoproxil fumarate (TDF) or less likely with atazanavir (see *HIV and Renal Function*). Rilpivirine, cobicistat, and dolutegravir reduce the tubular secretion of creatinine by different mechanisms, inducing a decrease of estimated glomerular filtration rate.

Calculated eGFR results might decrease after beginning a new treatment and will establish a plateau quickly (Sax 2012, Curtis 2014). Clinicians should carefully monitor renal function in order to identify possible alterations suggestive of a true renal functional impairment. Additional renal monitoring (urine dipstick, alpha-1-microglobulin, cystatin C-GFR or the albumin/creatinine ratio) should be used for renal safety monitoring or screening of tubular injury.

Besides renal problems, rhabdomyolysis is a rare but dangerous event. Rhabdomyolysis has been reported during abacavir HSR (Fontaine 2005), statin and boosted PI use as a consequence of CYP450 interactions and in rare cases after raltegravir exposure (Dori 2010). Immediate action should be taken if patients complain about muscle pain and/or otherwise unexplained elevated creatine kinase levels, to avoid more severe kidney injury.

Tenofovir disoproxil fumarate (TDF)

In ART-naïve patients tenofovir is associated with a greater decline in renal function and a higher risk of proximal tubular dysfunction: 4.8% of patients on tenofovir had a more than 50% decline of GFR compared to 2.9% without tenofovir (Horberg 2010). A meta-analysis of 17 studies confirmed an association with a statistically significant loss of renal function with TDF, although the clinical magnitude of this effect was modest (Cooper 2010). Severe cases have been reported with acute renal failure, proximal tubulopathy with Fanconi's syndrome and nephrogenic diabetes insipidus and rarely hypophosphatemic osteomalacia (Rollot 2003, Saumoy 2004). Renal toxicity occurs after some months, rarely at the beginning of therapy. Risk factors include high TDF exposure due to pre-existing renal impairment, low body weight (Nishijima 2012) or coadministration of nephrotoxic drugs (Nelson 2007).

Boosted PIs can interact with the renal transport of organic anions, leading to proximal tubular intracellular accumulation of tenofovir (Izzedine 2004+2007, Rollot 2003). The combination of atazanavir/r plus TDF caused greater GFR decreases compared with EFV (Albini 2012). This was confirmed by another study showing that TDF with a boosted PI leads to a greater initial decline in eGFR than TDF plus

efavirenz; this decline may be worse with atazanavir/r compared to lopinavir/r (Young 2012). Extensive pretreatment with NRTIs might be a risk factor (Saumoy 2004). However, even in patients without any predisposing factors, nephrotoxicity may occur (Barrios 2004).

In cases of renal dysfunction, especially in patients with low body weight, TDF should be avoided, or the dosing interval should be adjusted (see *Drugs*). In case of severe renal dysfunction (creatinine clearance <30 ml/min) TDF should not be administered. As normal creatinine levels may be misleading especially in subjects with low body weight, creatinine clearance needs to be measured before initiating TDF. Renal function tests including urine protein/creatinine ratio (UPC), urine albumin/creatinine ratio (UPA), creatinine clearance, proteinuria, glycosuria, urine dipstick and urine phosphate should be monitored closely. Another tool to analyze the renal function is the measurement of cystatin C and cystatin C-eGFR, to measure the decreased renal function more accurately (Lucas 2014 Driver 2013).

The majority of renal dysfunction in TDF patients is related to pre-existing renal disorders (Brennan 2011). Therefore it is not recommended for use in patients with preexisting renal insufficiency. It should also be avoided with concomitant or recent use of nephrotoxic agents such as aminoglycosides, amphotericin B, foscarnet, ganciclovir, pentamidine, vancomycin, cidofovir or interleukin-2. Usually, abnormalities resolve upon discontinuation (Izzedine 2004, Roling 2006).

Neurological side effects

The most important neurological side effects are peripheral polyneuropathy caused by NRTIs and CNS side effects caused by efavirenz (for other problems see neurological chapters).

Peripheral polyneuropathy

Peripheral polyneuropathy (PNP) is mainly caused by d-NRTIs (ddI, d4T) or AZT and are much less frequent today. Because of their continued use in resource-limited areas, we will review the symptoms and possibilities for palliation. PNP usually presents with a distal symmetrical distribution and sensorimotor paralysis. Patients complain of paresthesia and pain ("tingling") in hands and feet and perioral dysesthesia. The symptoms often begin gradually after several months of therapy. HIV infection itself can lead to PNP, but the drug-induced form becomes apparent much earlier and may develop within a shorter period of time. Patients must be informed that they should consult their treating physician as soon as possible if these complaints develop. Additional risk factors for polyneuropathy, such as vitamin B12 deficiency, alcohol abuse, diabetes mellitus, malnutrition or treatment with other neurotoxic drugs, e.g., INH, should be addressed as well.

Symptoms frequently improve within the first two months following discontinuation of the drugs responsible, but may initially increase in intensity and are not always fully reversible. Because treatment is difficult and because there is no specific therapy, it is important that PNP is recognized early by the doctor, resulting in a rapid change of treatment. The causative agent needs to be stopped.

An easy test in practice is to test vibration with a tuning fork. A 64 Hz tuning fork (Rydel-Seiffer) is applied to the appropriate bony surface (e.g., distal hallux, medial malleolus or lateral malleolus) bilaterally. The patient is asked to report the perception of both, the start of the vibration sensation and the cessation of vibration on dampening. As the intensity of the vibration starts to diminish the two triangles move closer together again. The intensity at which the patient no longer detects the

vibration is read as the number adjacent to the intersection. It can thus be quanti-fied and compared to the results of other tests. Through this simple method first signs of polyneuropathy can be recognized easily.

Apart from symptomatic treatment with metamizole, acetaminophen (paracetamol), carbamazepine, amitriptyline, gabapentine and opioids, methods such as acupuncture or transcutaneous nerve stimulation have been tried with varying success. Vitamin B supplementation can help to improve peripheral polyneuropathy faster. Tight shoes or long periods of standing or walking should be avoided; cold showers may relieve pain before going to bed.

CNS side effects

In up to 40% of patients treatment with efavirenz may lead to CNS side effects such as dizziness, insomnia, nightmares, mood fluctuations, depression, depersonalization, paranoid delusions, confusion and suicidal ideation. It has been shown that efavirenz changes the sleeping pattern (Moyle 2006). These side effects are observed mainly during the first days and weeks of treatment. Discontinuation of therapy becomes necessary in approximately 3% of patients. There is an association between high plasma levels of efavirenz and the occurrence of CNS symptoms (Marzolini 2001). If the CNS side effects persist for more than two to four weeks, an ART switch should be discussed. Otherwise, the dose can be divided into a 400 mg night dose and a 200 mg morning dose. With this schedule, we have observed a relevant reduction in unpleasant CNS side effects in our center.

CNS side effects are possible with etravirine, rilpivirine or dolutegravir as well (Madruga 2007, Cohen 2011, Molina 2011, Mackenzie 2013), but they are less intensive and less frequent. However, a review of current studies did not show increased CNS side effects with dolutegravir compared to other INSTIs or darunavir/r-containing regimens (Curtis 2014).

Allergic and skin reactions

Many ARVs such as NNRTIs, abacavir and boosted PIs (mainly darunavir) but also drugs used for opportunistic infections can cause allergic reactions, which vary in severity, clinical manifestations and frequency.

NNRTIs

Nevirapine may cause a rash in 15 to 30% of patients, leading to discontinuation in about 5%. The rash is seen less frequently with efavirenz, etravirine and rilpivirine therapy, and only rarely must be discontinued (Carr 2001, Cohen 2011, Molina 2011). With etravirine, fatal cases of toxic epidermal necrolysis have been reported as well as hypersensitivity reactions sometimes accompanied by hepatic failure (Borrás-Blasco 2008). It should be immediately discontinued when signs and symptoms of severe skin or hypersensitivity reactions develop.

NNRTI allergy is a reversible, systemic reaction and typically presents as erythematous, maculopapular, pruritic and confluent rash, distributed mainly over the trunk and arms. Fever may precede the rash. Further symptoms include myalgia (sometimes severe), fatigue and mucosal ulceration. The allergy usually begins in the second or third week of treatment. Women are more often and more severely affected (Bersoff-Matcha 2001). If symptoms occur later than 8 weeks after initiation of therapy, other causes should be suspected. Severe reactions such as Stevens Johnson Syndrome, toxic epidermal necrolysis (Lyell's syndrome) or hepatitis are rare.

Treatment should be discontinued in any case, but immediately in cases with mucous membrane involvement, blisters, exfoliation, hepatic dysfunction (transaminases >5 times the upper limit of normal) or fever >39°C.

Approximately 50% of NNRTI allergies resolve without discontinuation of therapy. Antihistamines and glucocorticosteroids may be helpful, prophylactic treatment has no protective effect; rashes were even more common in some studies (Montaner 2003, The Grupo Estudio 2004). Following a severe allergic reaction, the drug responsible for the reaction should never be given again.

Abacavir hypersensitivity

Abacavir might cause a hypersensitivity reaction (HSR). HSR can be life-threatening and occurs in approximately 4–8% of Caucasian patients (Hughes 2008). A higher rate is seen in patients on a once-daily regime, on first-line regimens, with a nevirapine allergy and with acute HIV infection. In over 90% of cases, the HSR occurs after a median of 8 days and within the first 6 weeks. HSR is strongly associated with the presence of the HLA-B*57:01 allele, which has a prevalence of approximately 6% in Caucasians, and a very low prevalence in black populations (Orkin 2010). The prospective PREDICT study involving 1956 patients from 19 countries showed that HLA-B*57:01 screening reduces the risk of hypersensitivity reaction to abacavir (Mallal 2008). Since then, HLA-B*5701 screening has been incorporated into routine care (Phillips 2009). It can prevent significant HSR-related costs and is likely to lead to overall net savings (Wolf 2010).

Abacavir-associated rash is often discrete, in contrast to the skin reactions caused by nevirapine or efavirenz; in 30% of patients it may not occur at all. The majority (80%) of patients have fever. In addition to general malaise (which grows worse day to day), other frequent symptoms include gastrointestinal side effects such as nausea, vomiting, diarrhea and abdominal pain. Respiratory symptoms, such as dyspnea, cough and sore throat are rare. Changes in the blood count, elevation of liver transaminases, alkaline phosphatase, creatinine and LDH may accompany the HSR. There is usually no eosinophilia. One case of Stevens Johnson Syndrome has been described (Bossi 2002). The simultaneous start of abacavir with NNRTIs is unfavorable because of the difficulties of differentiating between allergic reactions to NNRTIs and HSR. If the suspicion of HSR is only vague and abacavir is not stopped, the patient should be seen or spoken to daily. Once the diagnosis of HSR has been established and abacavir stopped, re-exposition can be fatal and is strictly contraindicated.

PIs, INSTIs

Darunavir is a sulfonamide derivative with the risk of antibody cross reactivity (Nishijima 2014). Rash has been reported in 4–11% of patients after being switched to darunavir/r-containing regimens, especially in those who have a history of rashes to NNRTI-containing regimens (Lin 2014). In case of limited alternative treatment options, desensitization may permit continued use of fosamprenavir or darunavir in patients (Marcos Bravo 2009).

Rash is a very rare problem when using INSTI-based regimens. However, a few cases of severe rash have been reported for dolutegravir and raltegravir, were even less frequent than when using efavirenz or darunavir/r containing regimens and need not be considered common (Curtis 2014, Liedtke, 2014).

Enfuvirtide (T-20)

Although T-20 is only rarely used, the most common side effect of T-20 is an injection site reaction (ISR) with erythema, induration, nodules, pruritus, ecchymosis, pain and discomfort. Almost every patient is affected, most of them however only mildly. ISR rarely limits treatment and only 3-7% of patients discontinue therapy (Lazzarin 2003). The practitioner and the patient need to get used to the injection technique and the management of ISRs. Good injection technique (see Table 2) may be most effective in minimizing the incidence and severity, as well as the incidence of associated events, including infections. The appropriate management of ISR can lessen the reaction (Clotet 2004). Desensitization therapy is available for the skin rash that occurs rarely with T-20 (Shahar 2005). Patients traveling to foreign countries should be prepared for questions about their injection material. Taking along a medical certificate stating that the patient is on injection therapy can help to avoid unpleasant situations.

Table 2: Suggestions for prevention and management of injection site reactions (ISR) and other injection-related adverse events (Clotet 2004)

Good injection technique
- Ensure solution is at room temperature
- Avoid muscle by angling needle at 45–90 degrees, depending on body habitus
- Inject slowly
- Maintain sterile technique (wash hands, use gloves, clean injection area and vial caps with alcohol swabs, never touch needle)
- Feel for hard, subcutaneous bumps, avoid injecting into sites of previous ISR
- Avoid indurated or erythematous areas
- Avoid injections on the belt line
- Rotate sites (abdomen, thighs, arms) and never inject two consecutive doses into the same place
- Gentle manual massage after every injection

Interventions for ISR
1. Injection pain
- Topical anesthetic (e.g., lidocaine gel)
- Oral analgesics pre-injection (e.g., ibuprofen or metamizole)
- Numb area with ice or a cool pack before injecting
2. Management of pruritus
- Oral antihistamines
- Emollient creams or lotions (non-alcohol based and fragrance-free)

Osseous side effects

Avascular necrosis

Avascular bone necrosis is most common on the femoral head and less frequent in the humerus. The mechanism is not fully understood. Initial symptoms may be pain and reduced mobility. The postulated association with PIs has not been confirmed (Loiseau-Peres 2002). However, a recent meta-analysis found a two-fold increased risk in patients exposed to PIs (Permpalung 2014). Other risk factors for avascular necrosis are alcohol overuse, hyperlipidemia, steroid treatment, hypercoagulability, hemoglobinopathy, trauma, nicotine abuse and chronic pancreatitis. Virological (viral load) or immunological parameters are not associated with a risk of developing avascular necrosis (Mondy 2003). The incidence of asymptomatic avascular necrosis is approximately 4.4% in HIV+ patients, significantly more frequent than in the general population (Lawson-Ayayin 2005, Cazanave 2008). Once diagnosis is

confirmed, patients should be referred to an orthopedic surgeon as soon as possible. Different treatment strategies are available for reducing bone and joint damage as well as pain, depending on the stage of disease, localization and grade of severity. In the early stages, reduced weight bearing with crutches is often sufficient. Surgical core decompression is an option: several holes are drilled in the femoral neck or head, causing new blood vessels to develop and thereby reducing the pressure within the bone. In the more advanced stages, the chances of success decrease with the size of the necrosis. The alternative option of osteotomy has the disadvantage of reducing the mobility of patients over long periods of time. In advanced states, a total endoprosthesis (TEP) is usually necessary.

Further risk factors need to be identified and eliminated. If possible, steroids should be discontinued. Sufficient data are lacking as to whether treatment modification on non–PI therapy is successful (Mondy 2003). Physiotherapy is recommended. Nonsteroidal anti inflammatory drugs (e.g., ibuprofen) are the treatment of choice for analgesia.

Osteopenia and osteomalacia

The loss of bone mineral density (BMD) and rarefaction of the trabecular architecture with consecutive loss of bone stability is highly prevalent and of multifactorial origin. In addition to the classical risk factors (low BMI, nicotine and alcohol consumption, steroids, hypogonadism, vitamin D deficiency, immobilisation, opiate abuse and HCV infection), HIV infection itself and ART affect bone metabolism (Bolland 2007, Grund 2009, Herzman 2009).

Bone density is determined by the measurement of X-ray absorption (e.g., DEXA scan). Results are given as the number of standard deviations (T score) from the mean value in young, healthy individuals. Values between -1 and -2.5 standard deviations (SD) are referred to as osteopenia, values above -2.5 SD as osteoporosis. Osteomalacia is the softening of the bones. Osteopenia and osteomalacia may occur in combination. HIV+ individuals have lower bone density than uninfected individuals (Loiseau-Peres 2002, Fessel 2011). In particular, the use of TDF is associated with bone demineralization (Stellbrink 2010, Haskelberg 2012, Negredo 2015). These (usually mild) effects are also seen in patients taking TDF-containing PrEP (Mulligan 2015). Tenofovir can induce a Fanconi's syndrome with tubular phosphate loss and consequently osteomalacia, despite no sign of vitamin D deficiency (Wanner 2009). There is also evidence that long-term use of PIs is associated with BMD loss (Duvivier 2009, Kinai 2014). Loss of BMD is associated with increased rates of bone fractures (Triant 2008), mainly affecting spine, hip and wrists.

In 2015, detailed recommendations for guidance on the screening, diagnosis, monitoring and management of bone disease in HIV+ patients were published (Brown 2015). Risk of fragility fracture should be assessed primarily using the Fracture Risk Assessment Tool (FRAX, http://www.shef.ac.uk/FRAX/), without dual-energy X-ray absorptiometry (DEXA scan), in all HIV+ men aged 40-49 years and HIV+ premenopausal women aged ≥40 years. DEXA scans should be performed in men aged ≥50 years, postmenopausal women, patients with a history of fragility fracture, patients receiving glucocorticoid treatment, and patients at high risk of falls.

Vitamin D replacement (800–2000 IU daily or 20,000 IU weekly combined with calcium) is recommended in persons with insufficient dietary intake. Caution is needed when prescribing calcium tablets at a dose of 1200 mg/day, as there is an increased risk of major cardiovascular events. Patients should be advised to exercise and offered methods on how to give up alcohol and nicotine. In patients receiving TDF, vitamin D replacement antagonized the TDF-induced loss of BMD and decreased

serum parathyroid hormone levels (Havens 2012). However, according to experts, TDF or boosted PIs should both be avoided in at-risk patients (Brown 2015).

In cases of osteoporosis, bisphosphonates (e.g., alendronate at 70 mg QW) should be administered (McComsey 2007, Huang 2009). Alendronate tablets should be taken on an empty stomach 30 minutes before breakfast, and an upright position should be maintained for at least 30 minutes. No calcium should be taken on that day. Antiretroviral therapy should not be taken together with calcium. Because testosterone suppresses osteoclasts, hypogonadism should be treated.

Hematological changes

Anemia, cytopenia

HIV infection itself may cause pancytopenia. Low CD4 T cell count may therefore be rarely due to a severe leucopenia. In this case, the percentage of the CD4 T cells and the CD4/CD8 ratio is normal. The myelosuppressive potential of AZT is known (de Jesus 2004). Most commonly affected are patients with advanced HIV infection and preexisting myelosuppression on chemotherapy or comedication with other myelotoxic drugs such as cotrimoxazole, pyrimethamine, amphotericin B, ribavirin, and interferon or with other antiretrovirals. 5-10% of patients on AZT develop anemia – usually during the first 3 months of therapy, but sometimes even after years on treatment (Carr 2001). MCV is always elevated, even in patients on AZT without anemia, and can be therefore an indicator of adherence. For thrombocytopenia see chapter on *HIV-associated Thrombocytopenia*.

Increased bleeding episodes

HIV+ patients with hemophilia A or B, after some weeks of treatment with PIs, may have increased episodes of spontaneous bleeding into joints and soft tissues. Rarely, intracranial and gastrointestinal bleeding has occurred. The etiology is unclear (Review: Wilde 2000).

During clinical trials with tipranavir/r, the manufacturer received 14 reports of intracranial hemorrhage, among them 8 fatal cases, in 13 out of 6840 HIV+ individuals. Most of them occurred more than one year after initiating therapy. Tipranavir was observed *in vitro* to inhibit human platelet aggregation (Graff 2008). Tipranavir/r should be avoided in patients with CNS lesions, head trauma, recent neurosurgery, coagulaopathy, hypertension or alcohol abuse, or those who were receiving anticoagulant or antiplatelet agents.

Lactic acidosis

Lactic acidosis is a rare but life-threatening complication due to mitochondrial toxicity. It occurs most frequently on treatment with d4T and ddI, and less so in patients on AZT, abacavir and 3TC (Garrabou 2009). Risk factors are obesity, female sex, pregnancy and therapy with ribavirin or hydroxyurea, a diminished creatinine clearance and a low CD4 T cell nadir (Bonnet 2003, Butt 2003, Wohl 2006).

Cases of severe lactic acidosis can occur without prior symptomatic hyperlactatemia. Lactate levels do not need to be monitored routinely, as increases are not predictive and may lead to unnecessary changes in treatment (Brinkman 2001, Vrouenraets 2002). In contrast, lactate levels should be tested immediately in symptomatic patients complaining of fatigue, sudden weight loss, abdominal disturbances, nausea, vomiting or sudden dyspnea, in pregnant women on NRTI treatment and in patients on NRTIs post-lactic acidosis (Carr 2003).

Lipodystrophy syndrome

The HIV lipodystrophy syndrome include metabolic complications and altered fat distribution. It is a possible side effect of ART. Fortunately, modern regimens are much less likely to lead to fat tissue abnormalities. The metabolic abnormalities may harbor a significant risk of developing cardiovascular disease. In addition, several studies report a reduced quality of life in patients with body habitus changes leading to a reduced treatment adherence. Despite the impact of lipodystrophy syndrome on HIV management, little is known about the pathogenesis, its prevention, diagnosis and treatment. Current data indicate a rather multifactorial pathogenesis where HIV infection, ART, and patient-related factors are all major contributors. The lack of a clear and easy definition reflects the clinical heterogeneity, limits a clear diagnosis and impairs the comparison of results among clinical studies. Therapeutic and prevention strategies have so far been of only limited clinical success, where avoiding the use of thymidine analogues appears to be most effective in avoiding peripheral fat loss. General recommendations include dietary changes and lifestyle modifications, ART modification (replacing PIs with NNRTIs or replacing d4T and AZT with abacavir or tenofovir or switching to an NRTI-free regimen, e.g., INSTI/NNRTI, see chapter on *ART*), and finally, the use of metabolically active drugs.

Clinical manifestation

Lipodystrophy was originally described as a condition characterized by regional or generalized loss of subcutaneous fat. Non-HIV-associated forms, such as congenital or familial partial lipodystrophy, have a very low prevalence. Generally, these forms are associated with complex metabolic abnormalities and are difficult to treat. The term "lipodystrophy syndrome" was introduced to describe a complex medical condition including an apparent abnormal fat redistribution and metabolic disturbances in HIV+ patients receiving protease inhibitor therapy (Carr 1998). Now, years after its first description, there is still no consensus on a case definition for lipodystrophy syndrome in HIV. Thus, the diagnosis of lipodystrophy in clinical practice often relies on a more individual interpretation than on an evaluated classification. Finally, changes in fat distribution have to be considered as being part of a rather dynamic process. In most cases, peripheral lipoatrophy is clinically diagnosed when significant fat loss of about 30% has already occurred.

HIV-associated lipodystrophy includes both clinical and metabolic alterations. The most prominent clinical sign is a loss of subcutaneous fat (lipoatrophy) in the face (periorbital, temporal), limbs, and buttocks. Prospective studies in patients on thymidine analogues have demonstrated an initial increase in limb fat during the first months of therapy, followed by a progressive decline over the ensuing years (Mallon 2003), which is mostly persistent (Grunfeld 2010). Peripheral fat loss can be accompanied by an accumulation of visceral fat, which can cause mild gastrointestinal symptoms. Initially truncal fat increases on therapy and then remains stable (Mallon 2003). Visceral obesity, as a singular feature of abnormal fat redistribution, appears to occur in only a minority of patients. Fat accumulation may also be found as dorsocervical fat pads (buffalo hump) or within the muscle and the liver. Female HIV+ patients sometimes complain about painful breast enlargement, attributed to the lipodystrophy syndrome. Whether gynecomastia in male patients is a component of the syndrome remains unclear. There is now accumulating evidence that the major clinical components – lipoatrophy, central adiposity and the combination of both – result from different pathogenetic developmental processes.

The risk of the syndrome increases with the duration of treatment, the age of the

patient and the level of immunodeficiency. Children can be affected, like adults, with clinical fat redistribution shortly after initiation or change of ART. The evolution of the individual clinical components of the lipodystrophy syndrome is variable. The nucleoside analogue linked most strongly to lipoatrophy is d4T, particularly when used in combination with ddI. Tenofovir combined with 3TC and efavirenz is associated with less loss of limb fat than d4T in a similar combination in therapy-naïve HIV+ patients (Gallant 2004).

Hyperlipidemia is a frequent side effect of antiretroviral therapies, especially those that include PIs (Nduka 2015). Newer drugs such as dolutegravir, elvitegravir, raltegravir, maraviroc, second-generation NNRTIs such as rilpivirine do not disadvantages in lipid metabolism and may therefore offer a favorable safety profile in patients at risk (Curtis 2014, Quercia 2015).

As many HIV+ patients present with already decreased HDL levels, these are not further reduced by antiretroviral drugs, but usually improve, particularly when nevirapine or rilpivirine is used (Pinnetti 2014). Hypertriglyceridemia, especially in patients with evidence of body fat abnormalities, is the leading lipid abnormality alone or in combination with hypercholesterolemia. Several weeks after initiation or change of HIV therapy, lipid levels usually reach a plateau and remain stable. Part of this increase can be considered as reconstitution of health, as some patients return to the lipid levels they had before seroconversion. All PIs can potentially lead to hyperlipidemia, although to different extents. For example, atazanavir and darunavir appear to be less frequently associated with dyslipidemia and insulin resistance (Aberg 2012). In contrast, ritonavir often leads to hypertriglyceridemia correlating to the drug levels. Boosted lopinavir leads to an approximate 18% mean increase in total cholesterol and 40% mean increase in triglycerides in patients on first-line therapy (Randell 2010).

Diagnosis

Both the lack of a formal definition and uncertainty about the pathogenesis and possible long-term consequences leads to a continuing discussion about appropriate guidelines for the assessment and management of HIV lipodystrophy syndrome and its metabolic abnormalities. Outside clinical studies, the diagnosis relies principally on the occurrence of apparent clinical signs and the patient reporting them. A standardized data collection form may assist in diagnosis (Grinspoon 2005). This appears sufficient for routine clinical assessment, especially when the body habitus changes develop rapidly and severely. For clinical investigations however, especially in epidemiological and intervention studies, more reliable measurements are required. A multicenter study to develop an objective and broadly applicable case definition proposes a model including age, sex, duration of HIV infection, HIV disease stage, waist-to-hip ratio, anion gap, serum HDL cholesterol, trunk-to-peripheral-fat ratio, percentage leg fat, and intra-abdominal to extra-abdominal fat ratio. Using these parameters, the diagnosis of lipodystrophy had a 79% sensitivity and 80% specificity (Carr 2003).

Despite individual limitations, several techniques are suitable for measuring regional fat distribution. These include dual energy x-ray absorptiometry (DEXA), computer tomography (CT), magnetic resonance imaging (MRI) and sonography. Anthropometric measurements are safe, portable, cheap and much easier to perform than imaging techniques. Waist circumference or sagittal diameter is more sensitive and specific than waist-to-hip ratio. Repeated measurements of skinfold thickness can be useful for individual long-term monitoring but needs to be performed by an experienced person.

The main imaging techniques (MRI, CT, DEXA) differentiate tissues on the basis of density. Single-slice measurements of the abdomen and extremities (subcutaneous adipose tissue = SAT, visceral adipose tissue = VAT) and more complex three-dimensional reconstructions have been used to calculate regional or total body fat. Limitations of these methods include most notably their expense, availability and radiation exposure (CT). Consequently, CT and MRI should only be considered in routine clinical practice for selected patients (e.g., extended dorsocervical fat pads, differential diagnosis of non-benign processes and infections).

DEXA is appropriate for examining appendicular fat, comprised almost entirely of SAT, and has been successfully employed in epidemiological studies. However, SAT and VAT cannot be distinguished by DEXA, limiting the evaluation of changes in truncal fat. Application of sonography to measure specific adipose compartments, including those in the face, requires experienced investigators and has been minimally applied in HIV infection so far. Bioelectrical impedance analysis estimates the whole body composition and cannot be recommended for measurement of abnormal fat distribution.

Patients should routinely be questioned and examined for cardiovascular risk factors such as smoking, hypertension, adiposity, type 2 diabetes, and family history. For an accurate assessment of blood lipid levels, it is recommended to obtain blood after fasting for at least 8 hours. Total cholesterol and triglycerides together with LDL and HDL cholesterol should be obtained prior to the initiation of, or switch to, any new antiretroviral therapy and repeated 3 to 6 months later. Fasting glucose should be assessed with at least a similar frequency. The oral glucose tolerance test (OGTT) is a reliable and accurate instrument for evaluating insulin resistance and glucose intolerance. An OGTT may be indicated in patients with suspected insulin resistance such as those with obesity (BMI >27 kg/m^2), a history of gestational diabetes and a fasting glucose level of 110 to 126 mg/dl (impaired fasting glucose). The diagnosis of diabetes is based on fasting glucose levels >126 mg/dl, glucose levels of >200 mg/dl independent of fasting status, or a 2-hour OGTT glucose level above 200 mg/dl. Screening of HbA1c appears to be less reliable, as in seronegative patients (Kim 2009, Eckhardt 2011). Additional factors that could lead to or assist in the development of hyperlipidemia and/or insulin resistance always need to be considered (e.g., alcohol consumption, thyroid dysfunction, liver and kidney disease, hypogonadism, concurrent medication such as steroids, -receptor blockers, thiazides, etc).

Therapy

So far, most attempts to improve or reverse abnormal fat distribution by modification of ART have shown limited clinical success (see ART subchapter *When to switch ART*). In particular, peripheral fat loss appears to be resistant to most therapeutic interventions. The metabolic components of the syndrome may be easier to improve (Table 1). Thus, preventing lipoatrophy by avoiding thymidine analogs (AZT, d4T) is the main goal (Behrens 2008). For more detailed recommendations for improving fat redistribution and treating dyslipidemia, please see the guidelines of the European AIDS Clinical Society (www.europeanaidsclinicalsociety.org). These guidelines emphasize that all traditional cardiovascular risk factors, such as arterial hypertension, hyperlipidemia and type 2 diabetes should be assessed and considered for intervention.

The most advantageous changes of metabolic parameters have been observed by replacement of the PI with nevirapine or abacavir. This option is, however, not always suitable, and the clinical benefit of effective viral suppression and improved immune function needs to be considered in view of drug history, current viral load, and resist-

ance mutations. When options are limited, antiretroviral drugs that may lead to elevation of lipid levels should not be withheld for fear of further exacerbating lipid disorders.

Lipid-lowering agents should be considered for the treatment of severe hypertriglyceridemia, elevated LDL or a combination of both. The clinical benefit, however, of lipid lowering or insulin-sensitizing therapy in HIV+ patients with lipodystrophy remains to be demonstrated. In light of the potentially increased cardiovascular risk to recipients of antiretroviral therapy, the AIDS Clinical Trials Group (ACTG) published recommendations based on the National Cholesterol Education Program (NCEP) for primary and secondary prevention of coronary artery disease in seronegative patients. In addition, more detailed recommendations by the European AIDS Clinical Society have been published to provide guidelines for physicians actively involved in HIV care that will be regularly updated (www.europeanaidsclinicalsociety.org). However, these recommendations should be considered as being rather preliminary, given the limited numbers, size and duration so far of the clinical studies they are based on. It appears reasonable to measure fasting lipid levels annually before and 3-6 months after ART is initiated or changed. Whenever possible, the ART least likely to worsen lipid levels should be selected for patients with dyslipidemia.

Metformin has been evaluated for the treatment of lipodystrophy syndrome. Some studies have revealed a positive effect on the parameters of insulin resistance and the potential reduction of intra-abdominal (and subcutaneous) fat, although not clinically significant. Together with exercise training, metformin has been described to reverse the muscular adiposity in HIV+ patients (Driscoll 2004). Metformin, like all biguanides, can theoretically precipitate lactic acidosis and should thus be used with caution. Use of metformin should be avoided in patients with creatinine levels above 1.5 mg/dl, increased aminotransferase levels, or hyperlactatemia.

Surgical intervention (liposuction) for the treatment of local fat hypertrophy has been successfully performed, but appears to be associated with an increased risk of secondary infection (Guaraldi 2011), and recurrence of fat accumulation is possible. For the treatment of facial lipoatrophy, repeated subcutaneous injection of agents such as poly-L-lactic acid (Sculptra®, New-Fill®), a resorbable molecule that promotes collagen formation, has been effectively used in HIV+ patients (Casavantes 2004, Mest 2004, Behrens 2008). In 2004, Sculptra® was approved by the FDA as an injectable filler to correct facial fat loss in people with HIV. We recommend consultation with experienced specialists for surgical treatments and injection therapy. Further evaluation in long-term follow-up studies is necessary to fully assess the value of these methods.

We do not recommend the following drugs for HIV-related lipodystrophy:

• The therapeutic intervention of recombinant human growth hormone (rHGH) (Serostim); the role of rHGH for HIV-associated fat accumulation has not been clearly defined. This therapy is very expensive and its only at best moderate effects disappear after stopping the treatment; there was rapid rebound of visceral fat to levels above baseline after treatment discontinuation (Grunfeld 2007, Lo 2008, Lo 2010).

• Thiazolidinediones (like pioglitazone, rosiglitazone) — although they reduce visceral adipose tissue volume in diabetics, controlled studies have generally not demonstrated favorable effects in HIV+ patients, including those with insulin resistance (Mulligan 2007, Slama 2008).

• Fibrates alone or in combination with statins, because there is not enough efficacy proven, and disagreeable side effects are known.

Lifestyle changes

Dietary interventions are commonly accepted as the first therapeutic option for hyperlipidemia, especially hypertriglyceridemia. Use of NCEP guidelines may reduce total cholesterol and triglycerides by 11 and 21%, respectively. Whenever possible, dietary restriction of total fat to 25–35% of the total caloric intake should be a part of any treatment in conjunction with lipid-lowering drugs. Consultation with professional and experienced dieticians should be considered for HIV+ patients and their partners. Patients with excessive hypertriglyceridemia (>1,000 mg/dl) may benefit from a very low fat diet and alcohol abstinence to reduce the risk of pancreatitis, especially if there is a positive family history or concurrent medications that may harbor a risk of developing pancreatitis. Regular exercise may have beneficial effects, not only on triglycerides and insulin resistance, but probably also on fat redistribution (reduction in truncal fat and intramyocellular fat) and should be considered in all HIV+ patients (Driscoll 2004). All patients should be advised and supported to give up smoking in order to reduce cardiovascular risk. Cessation of smoking is more likely to reduce cardiovascular risk than any choice or change of ART or use of any lipid-lowering drug (Petoumenos 2010).

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8. HIV Resistance and Viral Tropism Testing

PATRICK BRAUN AND EVA WOLF

The goal of antiretroviral therapy is to achieve maximal suppression of viral replication. Viral blips while on suppressive ART are relatively common and mostly due to random biological and statistical fluctuations (see 6.11, *Monitoring of ART*). However, patients with repeated episodes of detectable viremia – suggesting ongoing viral replication rather than viral release from latent reservoirs secondary to immune activation – are at increased risk for the development of drug resistance. The level of viral load on ART is the best predictor of subsequent virological failure; the risk for failure starts to increase at levels between 100 and 300 copies/ml (Nettles 2005, Delaguerre 2009, Garcia-Gasco 2008) and is highest for any drug class at 1000 to 10,000 copies/ml (Prosperi 2011).

The rapid development of resistant variants is due to the high turnover of HIV – in an untreated patient approximately 10 million new viral particles are produced every day (Perelson 1996) – and the exceptionally high error rate of HIV reverse transcriptase. The latter leads to a high mutation rate and constant production of new viral strains, even in the absence of treatment. In the presence of antiretroviral drugs, the development of HIV-1 resistance depends on the selection of resistance-associated mutations (RAMs). If a virus has acquired one or more RAMs leading to reduced drug sensitivity, the mutant virus attains a replication advantage in comparison to wild-type virus when exposed to drugs (Drake 1993). The development of resistant viral strains is one of the main reasons for virological failure of antiretroviral therapy. However, with the strategic use of the newer drug classes, effective regimens are available even in salvage situations.

The discussion about genotypic resistance and viral tropism in this chapter focuses on the methods of resistance and tropism testing, on mutation patterns emerging with ART use, their interpretations and clinical relevance. Most data are derived from patients with subtype B viruses, representing the main subtype in North America, Australia and Europe, but only approximately 10% of the global HIV-1 population. Non-B subtypes have increasingly been investigated, some exhibiting different resistance pathways and patterns (Snoeck 2006).

Assays for resistance testing

There are two established assays for measuring resistance or sensitivity of HIV to specific antiretroviral drugs – the genotypic and the phenotypic resistance tests (Wilson 2003). Genotypic resistance measurements should be distinguished between conventional (population-based) and ultrasensitive sequencing methods.

The conventional genotypic assay accredited by the FDA is:

• ViroSeq® (Abbott Molecular/Applera Corp. of Applied Biosystems and Celera).

Conventional (population-based) genotypic tests can only detect viral mutants when these comprise at least 15-20% of the total virus population. Patients with a low or non-detectable viral load may benefit from sequencing proviral DNA. Concordance of resistance data derived from viral RNA or proviral DNA sequencing is >80% (Boukli 2015, Ferrè 2015). The gold standard for resistance testing in viremic patients, however, continues to use HIV RNA of free virions in EDTA plasma, since resistant viruses may not necessarily be detectable in proviral DNA (Boukli 2015, De La Cruz 2015). Additionally, a large proportion of viruses from proviral DNA is unable to replicate (Ferre 2015).

Minority variants can be analyzed by elaborate ultrasensitive molecular biological methods (allele-specific real-time PCR, single genome sequencing) with limits of detection between 0.1 and 5%. Ultrasensitive sequencing systems like GS FLX (Roche/454 Life Sciences), HiScanSQ (Illumina) and SOLiD (Life Technologies) are currently used for research purposes. With the availability of devices that are able to analyze smaller series at less cost, such as Ion Torrent PGM (Life Technologies) or MiSeq (Illumina), this next-generation technology can become used for routine diagnostics. Prior to general use, analysis and interpretation of test results and the significance in particular of minor variants must be clearly defined and routinely applicable. The clinical relevance of minority populations remains controversial although there is evidence for minor variants with NNRTI mutations (Li 2011). Recent data show that the next-generation sequencing (NGS) results for NNRTIs continue to require cautious interpretation. In a follow-up investigation of the STaR Study (TDF/FTC plus efavirenz or rilpivirine), in which retained baseline samples were reanalyzed by NGS for minor resistant viral populations, these were not in concordance with treatment success (Porter 2015).

Phenotypic resistance tests are expensive and time consuming. While the cost of genotyping ranges anywhere from 260 to 400 €, depending on the assay and laboratory used, phenotyping costs at least double that.

Examples for commercially available **phenotypic resistance tests** are:
• PhenoSense® HIV (Monogram Biosciences)
• PhenoTecT™ (InPheno)
• Phenoscript™ (VIRalliance)
• Antivirogram® (developed by Virco Lab, is no longer available for routine clinical use, only for research and drug discovery)

Depending on the method and on the laboratory, 100–1,000 copies/ml are required for detection of resistance. Tables 1 and 2 show the advantages and disadvantages of phenotypic and genotypic resistance analyses.

Table 1: Advantages and disadvantages of phenotypic resistance analysis

Phenotypic resistance analysis	
Advantages	**Disadvantages**
• Direct measure of drug susceptibility	• Detection of viral mutants only possible when comprising ≥20% of the total virus population
• Measure of drug susceptibility feasible irrespective of the presence of unknown resistance mutations	• Clinical cut-offs not available for all drugs
	• Costly (reimbursement by health insurance often not guaranteed)
• Measure of drug susceptibility feasible irrespective of the complexity of resistance patterns and the presence of resensitizing mutations	• Time-consuming (several weeks)
	• HIV-1 subtyping not possible
	• Interactions between antiviral drugs are not reflected in the test results
	• Test results are not affected by amino acid exchanges, which are only an intermediate step to resistance

Basic principles of phenotyping

Phenotypic resistance tests involve direct quantification of drug sensitivity. Viral replication is measured in cell culture under the selective pressure of increasing concentrations of antiretroviral drugs and is compared to viral replication of wild-type virus. Drug concentrations are expressed as IC_{50} values (50% inhibitory con-

centration), the concentration of drug required to inhibit viral replication in cell cultures by 50%. The sensitivity of the virus is expressed as the IC_{50} divided by the IC_{50} of a wild-type reference virus (fold-change, FC, also known as resistance factor) and compared to the so-called cut-off value. The cut-off value indicates by how much the IC_{50} of an HIV isolate can be increased in comparison to that of the wild-type and still be classified as sensitive. The determination of the cut-off is crucial for the interpretation of the results.

Cut-offs: technical, biological and clinical

Three different cut-offs are currently being used.

The *technical cut-off* is a measure of the methodological variability of the assay.

The *biological cut-off* involves the inter-individual variability of wild-type virus isolates from ART-naïve HIV-positive patients. If the IC_{50} is below the biological cut-off, virological success is very likely. However, an IC_{50} above the biological cut-off does not allow prediction of the virologic response to a drug.

In contrast, the *clinical cut-off* indicates up to what levels of IC_{50} virologic effectiveness can still be expected. Complete resistance to a drug (i.e., to protease inhibitors) generally evolves gradually with the acquisition of several amino acid changes. In general, lower and upper clinical cut-offs are defined. The lower clinical cut-off is the fold-change in IC_{50} (FC) which indicates slightly reduced virological response. An FC above the upper clinical cut-off indicates resistance, and an FC between the two cut-offs indicates partial resistance. Due to limited clinical experience, cut-off data is often lacking for recently approved drugs. In these cases, interpretations are based on biological cut-offs.

In the phenotypic analysis, mutations that do not confer resistance by themselves but provide evidence for transmitted, emerging or reverting resistance have no influence on the measure of resistance.

Basic principles of genotyping

The HIV genome consists of two RNA (ribonucleic acid) strands containing the genetic information of the virus. Within the nucleotide sequence of the HIV genome, a group of three nucleotides, called a codon, code for a particular amino acid in the protein sequence. Resistance mutations are described using a number for each gene, showing the position of the relevant codon, and two letters, the letter preceding the number corresponding to the amino acid specified by the codon at this position in the wild-type virus, while the letter after the number describes the amino acid that is produced from the mutated codon.

A change in the nucleotide sequence of a codon is called a mutation. 'Silent' mutations code for the same amino acid. 'Lethal' mutations cause a defective protein structure leading to a stop of the viral replication cycle. Only those mutations that code for a different amino acid that leads to a change in the protein structure are clinically relevant. This affects protein function and can contribute to the development of resistance to antiretroviral agents. M184V indicates a mutation in codon 184 of the reverse transcriptase gene leading to a valine for methionine substitution in the reverse transcriptase enzyme and rendering the virus resistant to 3TC and FTC. Genotypic assays are based on the analysis of mutations associated with resistance. These are determined by the direct sequencing of the amplified HIV genome or by specific hybridization techniques with wild-type or mutant oligonucleotides. For therapeutic decision making, sequencing of the *pol* region, which encodes for the viral enzymes protease, reverse transcriptase and integrase, and sequencing of the *env* region, which encodes for the glycoproteins of the viral envelope, gp41 and

gp120, are of relevance. Other gene regions, in particular RNase H and *gag*, are reported to be associated with phenotypic drug resistance. However, sequencing of these regions has only been performed in the context of research and is not part of routine diagnostics.

The interpretation of genotypic resistance patterns is based on the correlation between genotype, phenotype and clinical response. There is data available from *in vitro* studies, clinical studies, clinical observations and duplicate testing, in which genotypically localized mutations have been investigated for phenotypic resistance.

Table 2: Pros and cons of genotypic resistance analysis (population-based sequencing)

Genotypic resistance analysis	
Advantages	**Disadvantages**
• Quick analysis (results within days)	• Indirect measurement of resistance
• Widely used (no specific safety requirements for laboratory)	• Detection of viral mutants only possible when comprising ≥20 of the total virus population
• Listing of all changes in the nucleotide sequence	• Complex resistance patterns are often difficult to interpret
• Detection of any mutation – with either evidence of resistance, emerging resistance or reverting resistance	• Unknown mutations are not considered for interpretation
• HIV-1 subtyping possible	• Interpretation systems must be updated regularly
• In general, reimbursement by health insurance (i.e., sequencing of the protease and the RT genes)	

Rules-based interpretation systems

For the phenotypic interpretation of genotypic mutation patterns rules-based interpretation systems are commonly available. Expert panels have developed algorithms based on the literature and clinical outcomes that are updated on an annual or bi-annual basis (Table 3). Interpretation of RAMs in Germany primarily uses the algorithm developed by HIV-GRADE e.V. (Obermeier 2012). The Stanford HIV Drug Resistance Database also provides a database with explanations and statistical analysis of RAMs aside from the algorithm. Most commercial providers of resistance assays have integrated interpretation guidelines into their systems.

Table 3: Genotypic resistance interpretation systems: an overview

Interpretation system	Interpretation	Available free of charge	Internet address: http://
HIV-GRADE (12/2013), Germany	Rules-based	Yes	www.hiv-grade.de
Rega V9 1.0 (HIV-1 V8 0.2 (HIV-2) (10/2013), Belgium	Rules-based	Yes	regaweb.med.kuleuven.be/ software/rega_algorithm
HIVdb Version 7.0 (02/2014), US	Rules-based	Yes	hivdb.stanford.edu/
ANRS (HIV1&2) V24 (02/2014), France	Rules-based	Yes	hivfrenchresistance.org/
EuResist EuResist Network GEIE	Data-based	Yes	euresist.org
GenoSure® MG (Monogram Bioscience)	Rules- and data-based	No	http://www.monogrambio.com/ hiv-tests/genotypic-assays/ genosure-mg
geno2pheno Germany	Data-based (virtual phenotype)	Yes	www.geno2pheno.org/

Data-based interpretation systems and virtual phenotype

Unlike the knowledge-based interpretation algorithms developed by experts, data-based interpretation systems like geno2pheno (Beerenwinkel 2003) or vircoType™ HIV-1 (no longer available post-2013) use technical intelligence, eg. support vector machines or regression models to predict ("virtual") phenotype from genotypic information and hence virologic sensitivity. The virtual phenotype is characterized by phenotypic information derived from genotype without performing a phenotypic resistance test in the laboratory. Phenotypic estimates derive from large databases of paired genotypic and phenotypic information.

Methods of tropism testing

To enter the target cell, HIV binds to the CD4 receptor and so-called chemokine co-receptors, of which CCR5 and CXCR4 are most important. Dependent on the use of coreceptors ("tropism") the virus is classified as CCR5-("R5"-) tropic or CXCR4-("X4"-) tropic. Viral strains using both coreceptors are called dual-tropic. Since tropism tests cannot distinguish between dual-tropic viral isolates and a mixture of R5- and X4-tropic viral isolates, the term dual/mixed (D/M) tropic is used.

Analogous to resistance testing, tropism testing can be performed genotypically or phenotypically (Braun 2007). European guidelines recommend both the enhanced sensitivity Trofile assay and V3 loop population sequencing (Vandekerckhove 2011). Table 4 outlines the advantages and disadvantages of both methods.

Phenotypic tropism testing

Due to its use in clinical trials, Trofile™ is the best-known phenotypic tropism test. Trofile™ ES (Trofile™ with enhanced sensitivity) detects minor viral populations down to a 1% sensitivity. This test has further become available for the use of proviral DNA when viral load is <1000 HIV RNA copies/ml. Another commercially available phenotypic test is Phenoscript® ENV (EuroFins/VIRalliance). An 85% agreement between both assays has been reported (Skrabal 2007). Other non-commercial phenotypic assays have been developed (Mulinge 2013).

Genotypic tropism testing

For genotypic tropism analysis, the V3 domain of the gp120 gene – which is crucial for coreceptor binding and encodes for the viral tropism – is sequenced. This gene sequence primarily defines the viral tropism, though other gp120 regions such as V1/V2 and C4 as well as substitutions at gp41 also play a role. With viral loads between 50 and 200 HIV RNA copies/ml, the preferred method is population-based sequencing of the V3 loop from proviral DNA. Web-based bioinformatic tools are used to predict viral tropism from the respective nucleotide sequence. These tools use methods like the charge rule, support vector machines or decision trees (Garrido 2008). The most popular tropism prediction tools geno2pheno [coreceptor] and WebPSSM are available free of charge:

• geno2pheno [coreceptor]   http://coreceptor.geno2pheno.org/index.php
• WebPSSM   http://indra.mullins.microbiol.washington.edu/webpssm/

Geno2pheno [coreceptor] is widely used and shows good concordance with Trofile™ ES (Prosperi 2010, Sierra 2011, Kagan 2014). In contrast to phenotypic analysis, genotypic analysis cannot distinguish between X4-tropic and dual-tropic or mixed populations. The result of the geno2pheno [coreceptor] tool is the so-called false positive rate (FPR), which is the probability of classifying an R5 virus falsely as X4. A false positive rate of 0.1% means that X4 tropism is very likely, whereas an FPR of 90% means that X4 tropism is very unlikely because an X4 prediction would be false with a 90% probability.

The current FPR cut-offs recommended in national and international guidelines range between 5-10% for X4 prediction and 10–20% for R5 prediction. For tropism testing from proviral DNA, which is used in case of undetectable viral load or low level viremia, the same FPR cut-offs can be used. The European guidelines recommend triplicate PCR amplification and sequencing (which is expensive and labor-intensive). The corresponding FPR when using the geno2pheno [coreceptor] interpretation tool should be 10% to discriminate between R5- and X4-tropic virus. In case of single testing the FPR should be increased to 20% (Vandekerckhove 2011). The German guidelines do not recommend multiple testing. For R5 and X4 prediction an FPR of ≥15% and ≤5% are recommended, respectively. For indeterminate results between 5 and 15% the use of CCR5 antagonists should be carefully weighed against other therapeutic options (Walter 2012).

Ultrasensitive sequencing

As for genotypic resistance testing, there are standard population sequencing (detecting X4-tropic virus variants if they comprise at least 20% of the total virus population) and ultrasensitive methods (such as ultra-deep sequencing with detection limits of a few percent or less).

In a study of ART-naïve patients treated with maraviroc plus atazanavir/r, Trofile™ ES was used for tropism testing. All samples were analyzed using population sequencing (PS) and ultra-deep sequencing (UDS) with FPRs of 5.75% (using geno2pheno [coreceptor] interpretation) and 3.5%, respectively (with 2% as the cut-off for non-R5). In 199 paired results, a concordance with Trofile® ES of 91.7% was found for PS and 89.6% for UDS. Samples, which were classified as non-R5 using Trofile® ES and as R5 using PS had a mean proportion of 2.1% non-R5-viruses (median 0.1%; Portsmouth 2013).

Comparison of genotypic and phenotypic tropism testing

The advantages of genotypic tropism testing are its wide availability and the rapid results. Analyses that have correlated genotypic and phenotypic tropism results with virologic response showed that the two methods can be considered equivalent (Braun 2009, McGovern 2012, Poveda 2012). Both methods were validated on subtype-B infected patients. Larger discrepancies were found in non-B-subtype populations, especially in CRF01_AE, CRF02_AG, A and F. Geno2pheno [coreceptor] and WebPSSM appear to overestimate the use of CXCR4 (Delgado 2011, Mulinge 2013).

Table 4: Advantages (+) and disadvantages (–) of genotypic and phenotypic tropism testing, (examples using geno2pheno and Trofile® ES)

Phenotypic tropism test Trofile® ES	Genotypic tropism test geno2pheno
• Phenotypic analysis using the complete gp160	• Genotypic analysis based on V3 sequence
• Result derives from cell culture	• Prediction of tropism using bioinformatics tools
+ Validated by clinical data	+ Validated by clinical data
+ Differentiation of R5-, X4- and D/M (dual/mixed)-tropic HIV	+ Result based on the exclusion of X4-tropic virus
– Commercial test / expensive	+ Feasible in molecular biology laboratories
– Results within about 3-4 weeks	+ Widely available / less expensive
– Required viral load of 500 – 1,000 copies/ml when using RNA	+ Result within about 5 days
	– Required viral load of 500 – 1000 copies/ml when using RNA
+ Feasible in case of low/undetectable plasma viral load when using proviral DNA	+ Genotyping of proviral DNA in case of low or undetectable viral load

Another advantage of genotypic tropism testing is its feasibility in samples with undetectable plasma viral load. Genotyping of proviral DNA is of clinical importance in successfully treated patients requiring a treatment change due to side effects. According to the results of parallel measurements, X4 tropism tends to be detected slightly more often in cell-associated proviral DNA than in plasma RNA (Verhofstede 2009). By sequencing proviral DNA, good concordance has been shown between Trofile® results and the genotypic tropism predictions from proviral DNA (Obermeier 2008). In the meantime, Trofile® ES has also become available for the testing of proviral DNA.

Mechanisms of resistance

NRTIs are prodrugs that only become effective after being intracellularly converted to triphosphates. Nucleotide analogs require only two instead of three phosphorylation steps. Phosphorylated NRTIs compete with naturally occurring dNTPs (deoxynucleotide triphosphates). The incorporation of a phosphorylated NRTI into the proviral DNA blocks elongation of the DNA, resulting in interruption of the chain. There are two main biochemical mechanisms that lead to NRTI resistance (De Mendoza 2002):

Sterical inhibition is caused by mutations enabling reverse transcriptase to recognize structural differences between NRTIs and dNTPs. Incorporation of NRTIs is then prevented in favor of dNTPs, e.g., in the presence of mutations M184V, Q151M, L74V, or K65R (Naeger 2001, Clavel 2004).

Phosphorolysis via ATP (adenosine triphosphate) or pyrophosphate leads to the excision of the NRTIs already incorporated into the growing DNA chain. This is the case with M41L, D67N, K70R, L210W, T215Y and K219Q (Meyer 2000). Phosphorolysis leads to cross-resistance between NRTIs, the degree of which may differ between agents (AZT, d4T > ABC > ddI > 3TC). Contrary to the excision mutations, K65R leads to a decreased excision of all NRTIs when compared to wild-type, resulting in a greater stability once incorporated. For K65R, the combined effect of its opposing mechanisms (decreased incorporation and decreased excision) results in a decreased susceptibility to NRTIs but an increased susceptibility to AZT (White 2005).

NNRTIs also inhibit the reverse transcriptase. NNRTIs are small molecules that bind to the hydrophobic pocket close to the catalytic domain of this enzyme. Mutations at the NNRTI binding site reduce the affinity of the NNRTIs to the reverse transcriptase, thus leading to a loss of antiviral activity. Whereas a single mutation can confer resistance to first generation NNRTIs, resistance patterns are more complex for second generation NNRTIs (Vingerhoets 2008, Molina 2008).

PIs hinder the cleavage of viral precursor gag-pol-polyprotein by the HIV protease, thereby producing immature, non-infectious viral particles. PI resistance usually develops slowly, as several mutations must accumulate. This is also referred to as the genetic barrier. For PIs, a distinction is made between *major* (or *primary)* and *minor* (or *secondary) mutations*.

Table 5: PI-specific resistance mutations

Major mutations
D30N, V32I, M46IL, I47VA, G48VMALSTQ, I50VL, I54VAMLTS, L76V, V82ATFSMLC, I84VAC, N88S, L90M

Minor mutations (a selection)
L10F, V11I, L23I, L24I, L33FI, K43T, M46V, F53L, Q58E, G73CATS, T74P, N83D, N88DGT, L89V

(HIV Drug Resistance Database, Sequence Analyses Program, version 6.2.0; Table updated on 2014-04-24; http://hivdb.stanford.edu/pages/documentPage/PI_mutationClassification.html)

Major mutations are responsible for phenotypic resistance. They are selected early in the process of resistance and/or are located within the active site of the HIV protease. They reduce the ability of the protease inhibitor to bind to the enzyme. Major or primary mutations may also lead to reduced activity of the protease.

Minor mutations (often referred to as secondary mutations*)* are located outside the active site and usually occur after major mutations. Minor mutations are commonly found at polymorphic sites of non-B subtypes. Minor mutations compensate for the reduction in viral fitness caused by major mutations (Nijhuis 1999, Johnson 2007b). Mutations at positions 20, 36, 63, and 77 are polymorphisms occurring without selective drug pressure particularly in non-B subtypes. Their contribution to resistance is minor and depends on the presence of other mutations.

Entry inhibitors prevent HIV from entering target cells. The first step in cell entry occurs when the HIV envelope glycoprotein gp120 binds to the CD4 receptor leading to conformational changes in gp120 and enabling the binding of the V3 loop of gp120 to the chemokine coreceptors, CCR5 or CXCR4, of the target cell. Interactions between the two heptad repeat regions HR1 and HR2 within the trans-membrane glycoprotein subunit gp41 lead to a conformational change in gp41 and enable fusion of the viral and cellular membranes. **CCR5 antagonists** bind to the CCR5 coreceptor and thereby impede interaction with the viral surface protein gp120 necessary for entry into the target cell. The fusion inhibitor T-20, a synthetic peptide consisting of 36 amino acids, mimics the C-terminal HR2 domain of gp41 and competitively binds to HR1. Thus, interactions between HR1 and HR2 are blocked and the conformational change of gp41 that is necessary for fusion of virions to host cells is inhibited. A single amino acid substitution in HR1 can reduce the efficacy of T-20.

Integrase strand transfer inhibitors (INSTIs) prevent insertion of HIV DNA into the human DNA genome which is catalyzed by viral integrase. INSTIs such as raltegravir, elvitegravir and dolutegravir block the strand transfer step. They bind to the catalytic pocket of the integrase and are transported as a component of the DNA/integrase pre-integration complex into the cell nucleus where strand transfer activity of integrase is inhibited. The selection of key mutations in the integrase gene confers resistance to integrase inhibitors. Strand transfer as well as the preceding step of 3' processing (cleavage of the terminal dinucleotides from both 3' ends of viral cDNA to which integrase binds) can be affected by these mutations. Different resistance pathways have been observed. The accumulation of additional mutations leads to a further decrease in susceptibility (Fransen 2008, Miller 2008).

Transmission of resistant HIV strains

The prevalence of resistance mutations in treatment-naïve patients differs by demographic region. A prevalence of more than 20% has been observed in large US cities with significant populations of MSM and a long history of access to antiretroviral treatment. Data on the incidence and prevalence of primary drug resistance published before 2007 should be interpreted with caution, since a consensus definition of transmitted genotypic drug resistance had not been established. In 2007 (update 2009), an international research group agreed upon criteria defining mutations indicative for transmitted drug resistance (Bennett 2009). This standardization allows for comparisons of epidemiological data across geographic regions and periods of time.

In a systemic review of 215 studies with a total of 43,170 patients until 2009 transmission of resistance was most prevalent in North America (12.9%), followed by Europe (10.9%), Latin America (6.3%), Africa (4.7%) and Asia (4.2%). The most

significant increase was observed in Asia and Africa. Changes with respect to specific drug classes were generally seen over time. Resistance to NRTIs decreased significantly over time in North America, Europe and Latin America, while NNRTI-resistance increased (Frentz 2012).

In the German Seroconverter Study, the prevalence of any resistance mutation was 12.1% between 1996 and 2012 (Bartmeyer 2010, Meixenberger 2011). Whereas the total prevalence remained stable during the observation period, NRTI-resistant virus populations (mainly TAMs) decreased to 6.1% while there was a trend toward more NNRTI resistance (2.4%, most frequently K103N/S). Dual- or triple-class resistance was rare with 1.2% and 0.3%, respectively. The most frequently reported PI mutations were M46L and L90M (Kuecherer 2013). In chronically infected patients of the German RESINA study, the proportion with primary resistance was 10.4% between 2001 and 2012 (Jensen 2013).

European-wide data from the years 2006–2007 derive from SPREAD (Strategy to Control Spread of HIV Drug Resistance), a program established to monitor primary resistance in newly infected patients and ART-naïve patients. In total, at least one resistance-associated mutation was found in 9.7% of newly diagnosed HIV+ patients. NRTI, NNRTI and PI resistance was found in 5.1%, 3.7% and 2.3%, respectively. Two-class resistance was present in less than one percent (Hofstra 2013). Resistance mutations were found in 16.7% of all newly diagnosed patients between 2008 and 2014 in the US (Saduvala 2014).

Ultrasensitive methods such as allele-specific real-time PCR (AS PCR) or ultra-deep sequencing detect resistance mutations more often than conventional sequencing methods. A study from Atlanta found minor resistance mutations in 34 of 205 patients (17%), in which only wild-type virus was identified using conventional sequencing (Johnson 2008). In a British study investigating 165 samples from the years 2003–2006, drug resistance was detected in 13% of samples when using the standard assay compared to 19% when using an assay more sensitive for K103N, Y181C or M184V. In particular, the proportion of M184V isolates increased from 0.6% to 8%. The prevalence of drug resistance was almost similar for treatment naïve patients with either primary or chronic HIV infection (19% and 20%) confirming data showing that primary resistance can persist for a long time (Buckton 2011, Pao 2004). In 2010, the transmission of a virus resistant to INSTIs was reported for the first time. The virus also harbored NRTI, NNRTI and PI resistance mutations. The authors recommended sequencing the integrase gene in cases of transmitted multidrug resistance (Young 2010).

Table 6: Prevalence of resistance prior to initiation of therapy (a selection)

Author	Country or region (study)	Time period	Patient population	N	Prevalence
Kücherer 2013	Germany	1996–2012	Seroconverter	2,060	12.1%
Hofstra 2013	Europe (SPREAD)	2008–2010	Newly diagnosed	2,398	9.2%
Monge 2014	Spain (CoRIS)	2007–2011	ART-naïve	2,781	7.9%
Jensen 2013	Germany (RESINA)	2001–2012	Chronically infected	2,855	10.4%
Margot 2014	US/Western Europe	2000/2003 versus 2013	ART-naïve	2,516	NRTI/INI: each <3%/0.1% NNRTI: 1.9 versus 7.8%
Saduvala 2014	US	2008-2011	Newly diagnosed	16,894	16.7%

The clinical relevance of resistance testing before ART changes has been demonstrated in several prospective, controlled studies using genotypic tests such as VIRADAPT, CPCRA 046 or Havana (Durant 1999, Baxter 2000, Tural 2002) as well as in studies using phenotypic tests like VIRA 3001 (Cohen 2002). For ethical reasons, studies that prospectively examine the benefits of resistance analysis, especially in pre-treated patients for regimens including drug classes such as integrase inhibitors or CCR5 antagonists, are no longer justifiable.

A resistance test before ART initiation is part of routine diagnosis in regions where the transmission of resistant HIV viruses is seen. The impact of transmitted HIV resistance on the initial success of ART was investigated in a retrospective analysis of the Eurocoord-CHAIN project. Of 10,458 patients initiated on ART in 1998, blood samples before therapy were retrospectively examined for resistance. The initial regimens' activities were essential for durable therapeutic success. Patients who were treated with only partially active regimens had a 2.6-fold higher risk of treatment failure (Wittkop 2011).

Relevance of minor variants

A meta-analysis of 10 studies and 985 patients found inferior efficacy of an NNRTI-based first-line therapy in the presence of minor resistant viral variants, especially if these conferred NNRTI resistance (Li 2011). Data from the Swiss cohort in therapy-experienced patients show a continual decline in the relative proportion of patients with resistance mutations over time. In 2003 one of three treatment-experienced patients had resistant viruses; by 2013 this had decreased to one of four. The largest proportion is seen in patients who had started mono- or dual therapy a long time before (Scherrer 2015).

Resistance testing before treatment initiation and at time of virological failure is an integral part of management and treatment of HIV infection.

Interpretation of genotypic resistance profiles

The algorithms cited below are only indicative. Treatment decisions should not be made based on these data alone. We recommend the use of a resistance interpretation system listed in Table 3.

NRTIs

FTC has nearly the same resistance pattern as 3TC: resistance is associated with the mutation M184V (Borroto-Esoda 2007). **M184V** also reduces viral replication capacity (often referred to as reduced viral fitness) by 40-60% (Deval 2004). After 52 weeks with 3TC monotherapy, the viral load remained 0.5 log below the initial levels despite early development of the M184V mutation (Eron 1995). When compared to treatment interruptions, monotherapy with 3TC delays virological and immunological deterioration (Castagna 2006). M184I is often detected before and then replaced by M184V (Schuurmann 1995). M184V occurs more commonly on 3TC than on FTC, especially in combination with TDF (Svicher 2010).

T69I is a rare mutation which causes high-level resistance to 3TC, FTC and possibly also to TDF (Svicher 2010).

Thymidine analog mutations, known as **TAMs**, were first observed with AZT and d4T (Larder 1989, Loveday 1999). They include the mutations M41L, D67N, K70R, L210W, T215Y and K219Q. The combination of certain TAMs also impact the efficacy of ABC, ddI and TDF (Table 8). TAMs do not arise on ABC, ddI or TDF, but can be reselected.

On failing therapy with ABC or ddI the mutation **L74V/I** usually occurs; K65R is less common (Miller 2000, Larder 2001). **Y115F** is a specific ABC-associated resistance mutation, which also affects the susceptibility to TDF. **V75T**, which is associated with an approximately 5-fold increase in resistance to d4T and ddI, is only rarely observed (Lacey 1994).

TDF primarily selects for the **K65R** mutation and leads to an (intermediate) resistance to TDF, ABC, ddI, 3TC, FTC, and possibly d4T (Miller 2004, Garcia-Lerma 2003). Although K65R may emerge on ABC, K65R was rarely seen before the introduction of TDF. This can be explained by the observation that combination therapies containing AZT lead to a lower incidence of the K65R mutation. Prior to TDF, ABC was mainly used as part of the fixed-dose combination Trizivir®. K65R seldom emerges in the presence of TAMs. Since K65R and TAMs represent two antagonistic resistance pathways (see *Mechanisms of resistance*), K65R is rarely observed on the same genome with TAMs and almost never with L74V (Wirden 2005). However, virological failure of other triple-nuke combinations such as TDF+3TC plus ABC or ddI was often associated with the development of the K65R (Landman 2003). The main reason for the high failure rate seems to be the low genetic barrier of these regimens: the emergence of the K65R induces a loss of sensitivity to all three drugs. K65R increases sensitivity to AZT and induces a resensitization in the presence of (few) TAMs (White 2005). On the other hand, TAMs reduce the K65R-associated resistance to TDF, ABC and ddI (Parikh 2007).

As with M184V, K65R reduces the viral replication capacity (RC), which is not the case with TAMs or the L74V/I. The presence of both mutations K65R and M184V led to an RC of only 29% (White 2002, Deval 2004).

Less frequently than K65R, the mutations **K70E or K70G** were observed on failing therapy with TDF, particularly in NRTI-based regimens with ABC and 3TC (Delaugerre 2008). M70E and K65R may be observed simultaneously, but it unusual that these mutations emerge on the same genome (Lloyd 2005). There is one case report of the development of K70E and M184V during therapy with TDF and FTC, which were then replaced by K70G and M184V. Both mutations were located on the same genome and conferred phenotypic resistance to all NRTIs except for AZT or d4T (Bradshaw 2007). **M184V** as well as the **L74V** mutation and the NNRTI-specific mutations, **L100I** and **Y181C**, may have an antagonistic effect on the further development of resistance (Vandamme 1999, Underwood 2005).

M184V induces resensitization to AZT and d4T, resulting in a reduction of IC_{50} by 50 and 30%, respectively. L74V/I with or without M184V leads to a reduction in IC_{50} of about 70%. However, resensitization is of clinical relevance only if there are no more than three other AZT- or d4T-associated mutations (Underwood 2005). The M184V mutation also increases sensitivity to TDF (Miller 2001, Miller 2004). In contrast, the presence of M184V plus multiple NAMs or mutations at positions 65, 74 or 115 increase resistance to ABC (Harrigan 2000).

So-called **multidrug resistance (MDR)** to all NRTIs – except 3TC and probably FTC, is established with **T69SSX**, i.e., the T69S mutation plus an insertion of 2 amino acids (SS, SG or SA) between positions 69 and 70 (Masquelier 2001). The T69SSX insertion induces an approximately 20-fold increase in resistance to TDF (Miller 2001+2004). The MDR mutation **Q151M** is relatively uncommon. Q151M alone leads to intermediate resistance to AZT, d4T, ddI and ABC and involves only a minor loss of TDF activity. Q151M combined with mutations at positions 75, 77 and 116 confers high-grade resistance to AZT, ddI, d4T and ABC and intermediate resistance to TDF. The insertion **T69SSX** together with the mutation M184V, as well as the mutation Q151M together with M184V, leads to a 70% reduction in viral replication capacity (Miller 2003, White 2004). Complete resistance to TDF is caused by the simultane-

ous presence of the Q151M complex in combination with K70Q (Hachiya 2011). TAF (tenofovir alafenamide), like TDF (tenofovir disoproxil fumarate), is a tenofovir prodrug. *In vitro* data suggest that by reaching 5-fold higher intracellular levels of the active substance, the use of TAF may overcome NRTI-resistant viruses (Margot 2013). We are still waiting on clinical data.

In large patient cohorts, quantitative measurements of sensitivity have shown that up to 29% of NRTI-experienced patients have a hypersusceptibility to NNRTIs (i.e., a reduction in the inhibitory concentration by a factor of 0.3–0.6). A reduction in AZT or 3TC sensitivity correlates inversely with an increased NNRTI susceptibility (Shulman 2000). The reverse transcriptase mutations **T215Y, H208Y and V118I** seem predictive for efavirenz hypersusceptibility. This is also true for non-thymidine analog-associated NAMs like K65R, T69X, M184V and in particular for the combination K65R+M184V (Whitcomb 2000, Shulman 2004, Coakley 2005a). However, these results have not influenced treatment strategies.

NNRTIs

First generation NNRTIs

Several mutations have been described with first-generation NNRTIs such as efavirenz and nevirapine. They are listed in Table 9. A single mutation can confer high-level resistance, in particular K101P, K103N/S, V106A/M, Y181C/I/V, Y188C/L and G190A/E/Q/S for nevirapine and L100I, K101P, K103N, V106M, Y188C/L and G190A/E/Q/S for efavirenz (Melikian 2014). Contrary to V106A, V106M is seen more frequently with subtype C as with subtype B viruses (Grossmann 2004). Nevirapine or efavirenz should be stopped in the presence of mutations as the selection of further RAMs may compromise the efficacy of second generation NNRTIs.

Second generation NNRTIs

Etravirine is effective against variants with single NNRTI mutations like K103N, Y188L and/or G190A (Andries 2004, Vingerhoets 2010). Compared to earlier NNRTIs, etravirine has a higher genetic barrier, probably due to flexible binding to the reverse transcriptase site. In a selection experiment, the dominant viral population harbored, after several *in vitro* passages, the mutations V179F (a new variant at this position) and Y181C. Other mutations that have been selected *in vitro* are L100I, E138K, Y188H, G190E, M230L, and V179I (Brilliant 2004, Vingerhoets 2005). Similarly, V179F, V179I and Y181C were seen with virologic failure in the DUET studies. Further RAMs were noted at positions 101 and 138 (Tambuyzer 2010). Using a regression model and a data set of 519 geno-/phenotype pairs, 5 key mutations at 4 positions could be identified: K101P, Y181I/V, G190E and F227C. In addition, K101H, E138G, V179F and M230L proved to be relevant (Melikian 2014).

In the DUET studies, 17 RAMs for etravirine were identified: V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S and M230L. Based on these, an etravirine resistance score was developed. A weighting factor of 3 was attributed to Y181I/V, followed by a weighting factor of 2.5 for L100I, K101P, Y181C, and M230L. The mutations E138A, V106I, G190S, and V179F received a weighting factor of 1.5 and the other mutations were weighted with 1. Total scores of 0-2, 2.5-3.5 and ≥4 corresponded to 74%, 52% and 38% virological response rates in the DUET studies (Vingerhoets 2008).

In a panel of 4,248 NNRTI-resistant clinical HIV-1 isolates, the mutations with the highest weight, Y181I and Y181V, had a low prevalence of 1.5% and 0.9%, respectively. The mutation Y181C, which is selected more frequently in patients taking nevirapine than efavirenz, had a prevalence of 32% (Vingerhoets 2008).

Monogram has developed a weighted sum score including 37 mutations with the following weighting factors. Mutations with the highest level of resistance, i.e., L100I, K101P and Y181C/I/V, received 4 points. E138A/G, V179E, G190Q, M230L and K238N received 3 points; 101E, V106A / I, E138K, V179L, Y188L and G190S received 2 points. V90I, A98G, K101H, K103R, V106M, E138Q, V179D/F/I/M/T, Y181F, V189I, G190A/E/T, H221Y, P225H, and K238T contributed with 1 point. A loss of efficacy is likely with a total score of 4 or higher (Haddad 2010).

Rilpivirine is also effective against single NNRTI RAMs such as K103N, V106A, G190S/A; *in vitro* the following mutations were selected: V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C and M230I/L (Azijn 2009). In a clinical study involving treatment-naïve patients without any (known) NNRTI mutations most of the *in vitro* mutations were confirmed (K101E, K103N, E108I, E138K/R, Y181C und M230L) (Molina 2008). The cross-resistance between rilpivirine and etravirine is greater than 90% (Porter 2013). Six key mutations at 5 positions could be identified for rilpivirine using a data set of 187 geno-/phenotype pairs: L100I, K101P, Y181I/V, G190E and F227C. Similar to etravirine, K101H, E138G, V179F and M230L were further relevant mutations (Melikian 2014). In the Phase III studies ECHO und THRIVE, virological failure was more frequent on rilpivirine than on efavirenz (10.5% versus 5.7%), i.e., in patients with viral load levels >100,000 copies/ml at baseline (17% vs. 7%). RAMs were more common in patients failing on rilpivirine than on efavirenz (63% versus 54%). The most common mutations were E138K (45%), K101E (13%), H221Y (10%), V189I (8%), Y181C (8%) and V90I (8%). In 46%, 31% and 23% of resistant isolates respectively, 1, 2 or 3 NNRTI mutations were detected.

Overall, 15 RAMs were identified as being associated with a decreased susceptibility to rilpivirine: K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C, M230I/L. These RAMs were identified either (a) *in vitro* as HIV-1 SDMs conferring phenotypic resistance to rilpivirine (K101P, Y181I, and Y181V with fold-changes in IC_{50} of 51.7, 15.3, and 12.2, respectively); (b) at the time of virological failure in ECHO and THRIVE (K101E, E138K/G, Y181C, H221Y, V179L, F227C, and M230I/L); (c) in *in vitro* selection experiments in strains with decreased susceptibility to rilpivirine (E138R and E138Q); and (d) in clinical isolates with an increased fold-change to rilpivirine (E138A). Cross-resistance to etravirine was commonly observed among patients failing rilpivirine (>90%) (Rimsky 2012). Besides NNRTI mutations, NRTI mutations were also more frequent among treatment failures on rilpivirine (68% versus 32%) – with primarily M184I on rilpivirine and M184V on efavirenz.

Protease Inhibitors (PIs)

Boosted PIs are a drug class with a high genetic barrier. Generally, several RAMs are required for complete loss in efficacy. The spectrum of PI RAMs is broad. As seen with numerous other antiviral agents, continuation of a failing PI regimen leads to the development of further mutations, which ultimately results in moderate to high cross-resistance between PIs. If treatment is changed early on to another PI combination, i.e., before the accumulation of multiple mutations, the subsequent regimen may be successful. In first-line therapy with ritonavir-boosted PIs the emergence of major PI mutations is rare and has been observed infrequently (Conradie 2004, Friend 2004, Coakley 2005b, Lataillade 2008).

First generation PIs

Lopinavir/r: A few cases report an emerging lopinavir resistance associated with the occurrence of the V82A followed by the mutations V32I, M46M/I and I47A, leading

to virological failure (Friend 2004). On failing monotherapy three viral isolates harbored L76V (Delaugerre 2007).

Lopinavir failure is primarily associated with mutations at the positions 46, 54 and 82, but also with the mutations I50V or V32I in combination with I47A/V/I. (Kempf 2001, Parkin 2003, Kagan 2005, Mo 2005).The mutation I47A, which has rarely been observed since the availability of lopinavir, reduces the binding affinity to lopinavir and results in an 86- to >110-fold loss of sensitivity. In contrast, I47A leads to saquinavir hypersusceptibility due to an enhanced binding affinity to saquinavir (Kagan 2005).

The mutation L76V, selected for by lopinavir (Delaugerre 2009), (fos-)amprenavir or darunavir, can lead to resensitization to atazanavir, saquinavir and tipranavir (Mueller 2004, De Meyer 2008). It has been demonstrated that viruses with the mutation L76V could be successfully controlled by the simultaneous use of two PIs, for example lopinavir (to maintain L76V) and saquinavir (resensitized) despite additional protease mutations (Wiesmann 2011).

Atazanavir is an azapeptidomimetic PI. The resistance profile differs in part from that of other PIs. In patients in whom first-line treatment with unboosted atazanavir failed, the mutation I50L – often combined with A71V, K45R, and/or G73S – was primarily observed (Colonno 2004a+b). I50L leads to an increased susceptibility to other first-generation PIs. Mutants harboring I50L plus A71V showed a 2- to 9-fold increased binding affinity to the HIV protease (Weinheimer 2005). In PI-experienced patients, the I50L mutation was selected for in only one third of patients failing atazanavir (Colonno 2004a/2004b). The accumulation of PI mutations such as L10I/V/F, K20R/M/I, L24I, L33I/F/V, M36I/L/V, M46I/L, M48V, I54V/L, L63P, A71V/T/I, G73C/S/T/A, V82A/F/S/T, L90M, and in particular, I84V, leads to a loss of sensitivity to atazanavir. As with other PIs, the resistance barrier is higher when atazanavir is boosted (Colonno 2004).

In the CASTLE study using atazanavir/r in ART-naïve patients, only two patients developed resistance to atazanavir (M46M/I+N88N/S and V32I+M46I+L90M) (Lataillade 2008). The Reyaphar score can predict response to ritonavir-boosted atazanavir in pre-treated patients, including mutations at 12 positions (L10I/F/R/V, K20I/M/R, L24I, M46I/L, I54L/M/T/V, Q58E, L63P, A71I/L/V/T, G73A/C/F/T, V77I, V82A/F/S/T, I84V and L90M). With less than 5 Reyaphar mutations, the average viral load reduction at 12 weeks was 1.4 logs, compared to only 0.5 log with more than 5 mutations (Pellegrin 2006).

Saquinavir: several RAMs including I84V/A are required to impact efficacy (Valer 2002). In a retrospective study, the presence of 3-4 mutations out of L10F/I/M/R/V, I15A/V, K20I/M/R/T, L24I, I62V, G73ST, 82A/F/S/T, I84V, and L90M was identified as being most strongly associated with reduced response (Marcelin 2007a). In contrast, L76V (observed on failing lopinavir or fosamprenavir) can lead to a clinically relevant resensitization for saquinavir (Wiesmann 2011).

Fosamprenavir: In patients with virologic failure on **amprenavir**, the following mutations have been selected: I54L/M, I50V or V32I plus I47V, often together with the mutation M46I (Maguire 2002). The Zephir study evaluated virological response to treatment with fosamprenavir/r in 121 treatment-experienced patients. With less than three mutations of L10I/F/R/V, L33F, M36I, M46I/L, I54L/M/T/V, I62V, L63P, A71I/L/V/T, G73A/C/F/T, V82A/F/S/T, I84V and L90M, viral load was reduced by 2.4 log 12 weeks after treatment initiation compared to only -0.1 log with 4 or more mutations. In a retrospective study in 73 patients, N88S/D was associated with an increased response (Masquelier 2008). L76V rarely selected by fosamprenavir confers resistance also to lopinavir and darunavir (Wiesmann 2011, Delaugerre 2009).

Second generation PIs

Darunavir has a high genetic barrier and shows good activity against a wide spectrum of PI-resistant viruses. *In vitro*, resistance to darunavir develops more slowly than seen with nelfinavir, amprenavir or lopinavir (De Meyer 2005).

Eleven mutations at 10 positions were associated with a diminished response to darunavir/r, as long as at least three of these developed: V11I, V32I, L33F, I47V, I50V/L, I54L/M, T74P, L76V, I84V and L89V (Johnson 2013). Individual mutations appear to influence susceptibility to darunavir in varying degrees. I50V has the highest impact, followed by I54M, L76V and I84V; V32I, and L33F, and I47V have less influence. The weakest impact was associated with V11I, I54L, G73S and L89V. This weighting, especially of I54L, needs to be validated.

New mutations that have occurred on treatment failure with darunavir are V32I, L33F, I47V, I54L, and L89V. Approximately 50% of these isolates were sensitive to tipranavir. Conversely, over 50% of isolates with reduced tipranavir susceptibility were still sensitive to darunavir (De Meyer 2006). Based on an analysis of the POWER and DUET data, the mutation V82A and E35D are positively associated with response to darunavir (De Meyer 2009, Descamps 2009).

A database analysis of 50,000 paired genotypes and phenotypes showed that between 2006 and 2009 darunavir-associated mutations increased: I50V (from 11 to 15%), I54L (from 17 to 33%) and L76V (from 5 to 9%) (Stawiski 2010).

Tipranavir shows good efficacy against viruses with multiple PI mutations. *In vitro*, L33F and I84V are the first mutations selected by tipranavir, but the loss in sensitivity is only two-fold (Doyon 2005). From data of Phase III trials, an "unweighted" tipranavir mutation score was initially developed, comprising of 21 protease mutations at 16 positions (Baxter 2006). This score was followed by a "weighted" tipranavir score based on clinical data of the RESIST trials (Scherer 2007). The respective model includes mutations of the unweighted score plus five mutations which were related to an increased tipranavir susceptibility (24I, 30N, 50L/V, 54L, 76V). Weight factors were assigned to the mutations according to their contribution to resistance. The weights of the mutations add up to the weighted tipranavir score. The major mutations I47V, I54A/M/V, Q58E, T74P, V82L/T, and N83D contribute significantly to tipranavir resistance.

Integrase strand transfer inhibitors (INSTIs)

Sequence analysis of viruses from treatment-naïve patients showed that the integrase gene is very polymorphic, but most of the relevant positions for resistance, such as 143, 148 and 155, are conserved (Hackett 2008).

Raltegravir: At week 156 of the STARTMRK study in previously therapy-naïve patients, only 4/49 patients had virological failure accompanied by the emergence of raltegravir mutations (Markowitz 2007, Rockstroh 2011). In treatment-experienced patients failing on a raltegravir-based regimen, three key mutations or resistance pathways were observed: N155H, Q148K/R/H and less frequently Y143R/C. Mutations observed along with N155H were L74M, E92Q, T97A, V151I, G163K/R, S230R (Steigbigel 2010). In the presence of Q148K/R/H the following mutations may occur: L74M, T97A, E138A/K, G140A, G140S and G163R, whereas mutations at positions 140 prevail. The mutations N155H and Q148K/R/H do not occur on the same viral genome. The accumulation of additional mutations after the emergence of the key mutations N155H or Q148K/R/H causes an increase in resistance and, depending on the pattern of mutations, also an increase in viral fitness. This is particularly true for the mutation Q148H (Goethals 2008, Hatano 2008). Virus variants harbor-

ing N155H plus secondary mutations are often replaced by variants with higher replicative fitness harboring Q148H+G140S. In order to preserve the efficacy of second generation INSTIs (i.e., dolutegravir), raltegravir should be discontinued after a first key mutation has occurred.

Raltegravir resistance secondary to the mutation Y143H/R/C, for example in combination with E92Q, T97A, V151I, G163R or S230R, is rare (Steigbigel 2010).

Viral populations harboring the mutation N155H can also be replaced by viral populations harboring Y143C/H/R (da Silva 2010). In viruses of the relatively common HIV-1 subtype CRF02_AG, the mutation G118R will lead to raltegravir resistance (RF=25.2) (Malet 2011).

Caution is needed with raltegravir in patients with existing resistance mutations. The genetic barrier is not as high as that of boosted PIs (Gatell 2009). In the SWITCHMRK study patients on a virologically successful lopinavir/r-based ART were randomized to continue PI or to switch to raltegravir. In the switch arm, virological failure was more frequent, likely due to archived RAMs, reducing the efficacy of the NRTI backbone.

Elvitegravir: With failing first-line therapy of elvitegravir/c plus TDF+FTC, the mutations M184V or I, followed by INSTI RAMs are primarily detected. Two Phase III trials documented the following mutations within a 3-year follow-up: E92Q (n=5), E92Q+T66I (n=1), E92Q+Q148R+N155H (n=1), E92Q+T66I+N155H (n=1), Q148R (n=2), N155H (n=3) and T97A (n=1) (White 2014). Phenotypic resistance analysis of these isolates support the result of previous analysis that there is a significant cross-resistance between raltegravir and elvitegravir (Margot 2011, White 2014). In cases of therapy failure, sequencing these two agents should generally not be considered. Y143R unlikely occurs with the use of elvitegravir (Metifiot 2011). This mutation (associated with raltegravir), however, does particularly confer cross-resistance to elvitegravir due secondary mutations (Huang 2013).

Despite a high level of cross-resistance, the resistance patterns at time of virological failure differ in part, as shown in a Phase III trial (GS-US-183-0145) of pretreated patients receiving raltegravir or elvitegravir. The most frequently mutations on elvitegravir were T66I/A (12%) and E92Q (8%) which were not or only rarely seen with raltegravir (Molina 2011, Margot 2011). T66I confers phenotypic resistance to elvitegravir but not to raltegravir (mean fold-changes 6.6-15 and 0.5-1.4, respectively). In combination with E92Q, the fold-changes increase significantly for both (190 and 18) INSTIs (Kobayashi 2011, Van Wesenbeck 2011, Margot 2012a). According to current data, elvitegravir should not be used when a M184V or I mutation is or has been documented. On the other hand, the presence of the INSTI RAM T97A appears to have no effect on the treatment success (White 2014).

Dolutegravir has a higher genetic barrier than raltegravir and elvitegravir. RAMs occur after several months in cell culture (Canducci 2011, Abram 2012). Depending on the laboratory strain used, various mutations were selected for that could not be detected in clinical study in conjunction with therapy failure. This holds true, for example, for S153Y and S153S, which decrease the susceptibility of dolutegravir by two- to four-fold *in vitro* (Kobayashi 2011). In other experiments a three-fold decrease in susceptibility was due to the mutations E92Q and G193E. E92Q is the primary mutations selected for with elvitegravir. It is important to note that the clinical threshold for dolutegravir has not been definitively set regarding resistance. The VIKING-3 study defined a lower cut-off of 9.45 (Vavro 2013). However the calculation of this value was criticized. The actual cut-off may to be lower and needs to be calculated through further analysis.

Using five clinical HIV-1 subtype B isolates and low-dose dolutegravir concentrations, the mutation R263K was selected for after 20 weeks. Selection experiments

were also conducted using two HIV-1 subtype CRF02_AG isolates and one subtype C isolate. Either the mutation R263K or G118R were selected for in the subtype AG isolates; the latter was also detected with subtype C (Quashie 2012). G118R has also been detected with raltegravir in subtype CDR02_AG and causes a 3-fold reduction in susceptibility of dolutegravir. However, this mutation has not been observed in Phase III trials, which included mostly patients with subtype B from North America and Europe. Despite R263K confers only weak resistance (resistance factor 1.5-2.1), it can be considered a specific dolutegravir RAM, possibly even defining a specific resistance pathway (Mesplede 2013, Underwood 2013b).

Targeted *in vitro* mutagenesis analysis showed that single primary and secondary INSTI RAMs have no effect on dolutegravir efficacy. Only combinations of mutations lead to an increase in resistance factor. Highest values were seen with Q148 combinations. The level of resistance depends on the amino acid substituted at the Q148 position. The combination of E138K with Q148K causes high dolutegravir resistance with a resistance factor of 19±8, while other dual Q148 combinations had lower factors of 2 to 5 in the *in vitro* mutagenesis analysis. Though N155H has no effect on susceptibility, once combined with E92Q the resistance factor increases to 2.5±1.2 compared to wild-type (Kobayashi 2011). The clinical relevance of the N155H/E92Q combination remains to be determined.

In all Phase III trials in therapy-naïve patients treated with dolutegravir plus ABC+3TC (SPRING-2, SINGLE, FLAMINGO), no RAMs were detected. Of note this was also the case for the NRTI backbone (Raffi 2013, Clotet 2013). In the raltegravir arm of the SPRING-2 study, resistance to INSTIs or to NRTIs was documented for one and four persons with therapy failure (Raffi 2013). This supports the observation that dolutegravir has a high genetic resistance barrier. Whether or not this is comparable to that of a boosted PI remains to be determined.

In the SAILING trial on INSTI-naïve patients with prior treatment failure, R263K was once observed alone and once in combination with V260I. R263K was detected once with each HIV-1 subtype B and C infection and led to a resistance factor of 1.93 (Cahn 2013, Pozniak 2013, Undewood 2013). This mutation has been termed a "dead-end" by some study groups, as it strongly impacts viral fitness and no substitutions have been identified to date which may offset this. *In vitro* experiments selected for further mutations which impact viral fitness even more. Further, the development of NRTI- and NNRTI-RAMs was slower in viruses with the R263K mutation as shown by targeted mutagenesis experiments (Oliveira 2014). Yet one needs to consider, that both *in vitro* and *in vivo* replicating viruses with this resistance mutation have been observed. Hence, in the presence of the R263K, dolutegravir has been rated intermediate resistant in the HIV-GRADE algorithm despite a low resistance factor. The SAILING Study identified two more patients with resistant virus. One had a documented Q148 mutation at therapy start and developed the mutations T97A and E138T/A with failing therapy. In another patient, viruses with a mutation V151I were selected for, which alone confers no phenotypic resistance (RF=0.92) (Cahn 2103). After unblinding the study and further follow-up, three more patients had resistant virus. Suboptimal adherence was assumed for all three. The mutation R263K combined with additional secondary mutations was observed. The resistance factor was 5.8-fold higher than determined by previous experiments of clinical R263K samples. Furthermore, N155H was detected in two isolates (Underwood 2015).

In the single-arm VIKING Phase IIb Study, 27 patients with a history of or current raltegravir-specific RAMs were treated with dolutegravir 50 mg QD. At day 11, 21/27 patients had a viral load of <400 copies/ml or a viral load reduction of at least 0.7 log. In contrast to resistance mutations at positions 143 and 155, those at position in combination with two other secondary mutations were associated with

reduced efficacy (Soriano 2011). In the VIKING 3 Study, 183 patients with a INSTI resistance received dolutegravir 50 mg BID as a functional monotherapy for 7 days. Again, efficacy differed based on detected mutations. In patients without a Q148 mutation, viral load declined by -1.43 log HIV-1 RNA copies/ml. In patients with Q148 and one secondary mutation, decrease was -1.15 log, while with two secondary mutations, it was only -0.92 log. The following substitutions were considered as secondary mutations: G140A/C/S, E138A/K/T, L74I. After functional monotherapy, treatment could be optimized. 69% and 63% of patients had a viral load of <50 HIV-1 RNA copies/ml at week 24 and 48 respectively. In isolates without Q148HKR this rate was 63%, with Q148HKR and one secondary mutation 56% and with Q148HKR and two secondary mutations 29% at week 48. The following mutations were additionally detected at virological failure by week 48: L74L/M/I (n=3), E92Q (n=2), T97A (n=10), E138K/A (n=9), G140S (n=4), Y143H (n=1), S147G (n=1), Q148H/K/R (n=6), N155H (n=4) and E157E/Q (n=1) (Castagna 2014, Vavro 2014). Based on the Monogram data base, the level of resistance of viruses with the Q148 mutation in combination with other dolutegravir mutations can be ranked in the following simplified manner: Q148+G140+E138+L74 > Q148+G140+E138 > Q148+G140+L74 > Q148+G140 > Q148+E138 (Underwood 2013a).

Fusion inhibitors

This section describes resistance mutations seen with the use of enfuvirtide (T-20). The gp41 genome consisting of 351 codons has positions of high variability and well-conserved regions. Polymorphic sites are observed in all regions of gp41. The heptad repeat 2 (HR2) region has the highest variability. Primary resistance to **T-20**, the only fusion inhibitor thus far approved, is a rare phenomenon (Wiese 2005).
A loss of efficacy is generally accompanied by the appearance of mutations at the T-20 binding site which is the heptad repeat 1 (HR1) region of gp41. Especially affected are the HR1 positions 36 to 45, such as G36D/E/S, 38A/M/E, Q40H/K/P/R/T, N42T/D/S, N43D/K, or L45M/L. The decrease in susceptibility is greater for double mutations than for a single mutation. Additional mutations in HR2 also contribute to T-20 resistance (Sista 2004, Mink 2005).
The replication capacity (RC) in the presence of HR1 mutations is markedly reduced when compared to wild-type virus with a relative order of RC wild-type > N42T > V38A > N42T, N43K > N42T, N43S > V38A, N42D > V38A, N42T. Viral fitness and T-20 susceptibility are inversely correlated (r=0.99, p<0.001) (Lu 2004).

CCR5 antagonists

CCR5 antagonists are to be used in patients with exclusively R5-tropic virus. In the presence of X4- or dual-tropic virus, their use is not recommended. R5-tropic virus is detected in about 80% of treatment-naïve patients and 50-60% of treatment-experienced patients. Solely X4-tropic virus is unlikely but possible (Brumme 2005, Moyle 2005, Hunt 2006). X4-tropic virus populations are more frequent with reduced CD4 T cell counts, both in naïve and treatment-experienced patients (Brumme 2005, Hunt 2006). Only 62% of treatment-naïve patients with a CD4 T cell count of less than 200/µl harbored an R5-tropic virus population (Simon 2010).
There are two ways to build up resistance to CCR5 antagonists: a receptor switch from R5- to X4- or dual-tropic viruses or the emergence of mutations that enable the virus to use the CCR5 molecules for entry in the presence of CCR5 antagonists.
In approximately one third of patients on a failing regimen with **maraviroc**, a shift from R5- to X4-tropic virus was reported (Heera 2008). In individual cases, a recep-

tor-shift was observed also in the control arm not receiving maraviroc. Retrospective studies using more sensitive methods have shown that some patients had already harbored minor X4 variants at baseline (Mori 2007, Lewis 2007).

Samples of 360 patients from the MERIT-study with R5-tropic virus were reanalyzed using Trofile® ES, population-based sequencing and ultra-deep sequencing (454-method). Genotypic interpretation was performed using the co-receptor tool of geno2pheno and a FPR-limit of 5.75%. The tropism determined with these three methods was predictive of therapy success at week 48 and 96 irrespective of subtype (Sierra-Madero 2010).

Since not every minor X4 virus population necessarily leads to therapy failure, it remains unclear at which point the higher sensitivity of ultra-deep sequencing (UDS) becomes clinically relevant, or what proportion of X4-tropic viruses increases the risk for failure. Samples from the A0041029 and Motivate studies were reanalyzed using UDS; these were classified as non-R5 if at least 2% were non-R5 virus variants. Low comparable efficacy rates were observed in patients with 2 to 20% non-R5 virus as well as in those with greater than 20% non-R5 (Swenson 2011). Prior to their use in routine clinical care, additional analysis will be necessary to determine the clinical relevant limits of the more sensitive tests.

On failing treatment with maraviroc or vicriviroc without a switch in tropism, different mutations in the V3 loop of the HIV-1 envelope protein gp120 were detected. Resistance patterns were not uniform and included mutations outside the V3 loop. The frequency and clinical relevance of these *env* mutations still require further investigation before any conclusions on resistance can be made. Some of the detected mutations were not associated with an increase in IC_{50}. Instead, phenotypic resistance was characterized by dose-response curves that display a reduction in the maximal inhibition (Mori 2008, McNicholas 2009). Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptors for entry (Westby 2007). Cross-resistance between maraviroc and vicriviroc has been described after several *in vitro* passages, but cross-resistance to other CCR5 antagonists or complete class resistance including TBR-652 remains to be determined (Palleja 2010).

It remains unclear if R5-tropic virus with resistance to maraviroc may be suppressed by using monoclonal antibodies such as PRO 140. In contrast to maraviroc or vicriviroc, PRO 140 binds extracellularly to the CCR5 coreceptor. Therefore, cross-resistance between PRO 140 and maraviroc is unlikely (Jacobson 2009).

Summary

Resistance and tropism tests are standard diagnostic tools in the management of HIV infection and are recommended by treatment guidelines. Primary resistant viral variants can be observed in about 10% of treatment-naïve patients in regions that have access to antiretroviral drugs. Resistance testing prior to initiating ART results in significantly better response rates. The emergence of viral mutants is one of the main causes of virological treatment failure. Pharmacoeconomic studies have shown that genotypic resistance tests concerning reverse transcriptase and protease are cost-effective both in treatment-experienced and in ART-naïve patients (Weinstein 2001, Corzillius 2004, Sax 2005). Sequencing of the genomic regions of integrase and gp41 should be included in the evaluation of resistance – at least at time of treatment failure and when a treatment change is needed.

Genotypic and phenotypic resistance/tropism tests show good intra- and inter-assay reliability. The interpretation of genotypic resistance profiles has become very complex and requires constant updating of respective guidelines. The determination

of the thresholds associated with clinically relevant phenotypic drug resistance is crucial for the effective use of (virtual) phenotypic testing. As for of resistance testing, genotyping has become the preferred method of tropism testing in clinical practice. With the co-receptor tool of geno2pheno, viral tropism can be predicted.

Even though treatment failure requires the consideration of all causal factors such as patient adherence, metabolism of drugs and drug levels, resistance testing and measurement of viral tropism are of great importance in antiretroviral therapy. Finally, it needs to be emphasized that even with the benefit of well-interpreted resistance and tropism tests only experienced HIV practitioners should start, stop or change antiretroviral therapy keeping in mind the clinical and the psychosocial situation of the patient.

Resistance tables

All tables are based on rules-based interpretation systems such as HIV-GRADE (www.hiv-grade.de), the ANRS-AC11 (www.hivfrenchresistance.org/) and the Drug Resistance Mutations Group of the IAS (Johnson 2013) as well as the references mentioned in the text. These tables should not replace interpretation tools and communication between the practitioner and the laboratory experts.

Table 8: Mutations on the reverse transcriptase gene leading to NRTI resistance

RTI	Resistance mutations
Zidovudine (AZT)	T215 Y/F (esp. with other TAMs) ≥3 of the following: M41L, D67N, K70R, L210W, K219Q/E Q151M (esp. with A62V/F77L/F116Y) or T69SSX (insertion)* (Potential resensitizing effect ass. with K65R, L74V, Y181C and M184V)
Stavudine (d4T)	V75M/S/A/T T215Y/F (usually in combination with other TAMs) ≥3 TAMs* Q151M (esp. with A62V/F77L/F116Y) or K65R or T69SSX (insertion)* (Potential resensitizing effect associated with L74V, Y181C and M184V)
Abacavir (ABC)	M184V + 3 of the following: M41L, D67N, L74I, L210W, T215Y/F, 219Q/E ≥5 of the following: M41L, D67N, L74I, L210W, T215Y/F, 219Q/E K65R or Y115F or L74V Q151M (esp. with A62V, F77L, F116Y) or T69SSX (insertion)*
Lamivudine (3TC)	M184V/I or T69SSX (insertion)* or K65R (resistance possible)
Emtricitabine (FTC)	M184V/I or T69SSX (insertion)* or K65R (resistance possible)
Didanosine (ddI)	L74V, esp. with T69D/N or TAMs Q151M (esp. with A62V/F77L/F116Y) or T69SSX (insertion)* K65R T215Y/F and ≥2 of the following: M41L, D67N, K70R, L210W, K219Q/E
Tenofovir DF (TDF)	T69SSX (insertion)* ≥3 TAMs with M41L or L210W (only partial resistance) ≥3-5 of: M41L, E44D, D67N, T69D/N/S, L210W, T215Y/F, K219Q/E K65R or K70E/G (Potential resensitizing effect ass- with L74V and M184V)

TAMs = thymidine analog mutations   * T69SSX in combination with T215Y/F and other TAMs leads to a high degree of resistance to all NRTIs and tenofovir

Table 9: Mutations on the reverse transcriptase gene leading to NNRTI resistance

NNRTIs	Relevant resistance mutations and patterns	Further mutations associated with resistance
Efavirenz	K101P, K103N/H/S/T,V106M, Y188L, G190C/E/Q/S, M230L	L100I, V108I, Y181C/(I), Y188C, G190A, P225H, K103R+V179D
Nevirapine	K101P, K103N/H/S/T, V106A/M, Y181C/I/V,Y188C/L/H, G190A/C/E/Q/S/T/V	L100I, V108I, K103R+V179D
Etravirine	L100I, K101P, Y181C/I/V, M230L	V90I, A98G,K101E/H,V106I, E138A/G/K/Q, V179D/F/T, G190A/S, F227C
Rilpivirine	K101E/P, E138K/R/A/G/Q/S, Y181I/V, M230I/L	V90I, L100I, Y181C, Y188L, V189I, H221Y, F227C, M230V, L100I+K103N

Table 10: Mutations on the protease gene leading to PI resistance

PIs	Relevant resistance mutations and patterns	Further mutations associated with resistance
Saquinavir/r	I84V/A or 48V/M ≥3 of the following: L10F/I/M/R/V, K20I/M/ R/T, L24I, I62V, G73CST, 82A/F/S/T and L90M or ≥4 of the following: L10I/R/V, I54V/L, A71V/T, V77I, V82A/ F/S/T and L90M Possible L76V-associated resensitizing effect	≥2 PRAMs*
Nelfinavir	D30N I84A/V N88S/D L90M	V82A/F/S/T and at least 2 of: L10I, M36I, M46I/L, I54V/L/M/T, A71V/T, V77I ≥2 PRAMs*
Fosampre-navir/r	I50V L76V plus other PI mutations V32I plus I47V ≥6 of: L10F/I/V, K20M/R, E35D, R41K, I54V/L/M, L63P, V82A/F/T/S, I84V or ≥3 of: L10I/F/R/ V, L33F, M36I, M46I/L, I54L/ M/T/V, I62V, L63P, A71I/L/V/T, G73A/C/F/T, V82A/F/S/T, I84V and L90M or ≥3 of: L10F/I/V, L33F, M46I/L, I47V,I54L/M/V/A/T/S, A71V, G73S/A /C/T, V82A/F/C/G and L90M	≥2 PRAMs*
Lopinavir/r	I47A+V32I ≥3 of the following: M46I, I47A/V, L50V, I54A/M/V, L76V, V82FATS, I84V L76V plus other PI mutations	5-7 of the following: L10F/I/R/V, K20M/R, L24I, V32I, L33F, M46I/L, I47V/A, I50V, F53L, I54L/T/V, L63P, A71I/L/V/T, G73S, V82A/F/T, I84V, L90M ≥2 PRAMs*

Table 10: (continued)

PIs	Relevant resistance mutations and patterns	Further mutations associated with resistance
Atazanavir/r	I50L – frequently plus A71V ≥4 of the following: L10I/F, K20R/ M/I, L24I, V32I, L33I/F/V, M46I, M48V, I54V/M/A, A71V, G73C/S/ T/A, V82A/F/S/T, I84V, N88S and L90M (L76V possibly resensitizing)	N88S ≥2 PRAMs*
Tipranavir	≥7 mutations/points of the following: K20M/R/V, L33F, E35G, N43T, M46L, I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D and I84V; V82L/T and I84V with twofold points score Score >10 of the following: I10V (+1), L24I (-2), M36I (+2), N43T (+2), M46L (+1), I47V (+6), I50L/V (-4) I54A/M/V (+3), I54L (-7) Q58E (+5), T74P (+6), L76V (-2), V82L/T (+5), N83D (+4), I84V (+ 2) (L76V possibly resensitizing) Further resistance-associated mutations: I54S, I84C	6 mutations/points of the following: K20M/R/V, L33F, E35G, N43T, M46L, I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D and I84V; V82L/T and I84V with twofold points score Score 3-10 from I10V (+1), L24I (-2), M36I (+2), N43T (+2), M46L (+1), I47V (+6), I50L/V (-4) I54A/M/V (+3), I54L (-7) Q58E (+5), T74P (+6), L76V (-2), V82L/T (+5), N83D (+4), I84V (+ 2)
Darunavir/r	≥4 of the following: V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V (with V32I, I50V, I54M, L76V and I84V having a higher impact) Further resistance-associated mutations: L10F, E35N, I47A, V82L, G48M, V82F	≥3 of the following: V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V (with I50V, I54M, L76V and I84V having a higher impact)

* PRAMs (protease inhibitor resistance-associated mutations) include the following mutations: L33I/F/V, V82A/F/S/T, I84V and L90M. They lead to high PI cross-resistance

Table 11: Mutations leading to entry inhibitor resistance

Entry inhibitor	Resistance mutations
T-20	G36A/D/E/S/V or I37V or 38A/M/E/K/V or Q39R Q40H/K/P/R/T or N42T/D/S or N42T+(N43S/N43K) N43D/KH/S or L44M or L44M+ G36S or L45M/L/Q
Maraviroc	Individual RAMs described; no consistent pattern

The reduction in susceptibility is generally higher for double than for single mutations

Table 12: Mutations on the integrase gene leading to INSTI resistance

Integrase inhibitors	Resistance mutations (Resistance pathways and key mutations)	Other mutation and resistance profiles conferring or increasing resistance
Raltegravir	Q148H/G/K/R/E N155H Y143R/C T66I + E92Q The appearance of additional mutations produces an increase in the level of resistance	L74M, E92Q/V, T66I, T97A G118R, E138A/K, G140A/S Y143H, V151I/A/L, E157Q G163R/K, S230R
Elvitegravir	T66I/A/K E92Q T97A S147G Q148R N155H	H51Y, T66Q, L68V, V72I, E92V/G, Q95K, G118R, E138K, G140AS, Y143R, E157Q, S230R
Dolutegravir	Q148R + G140S G148H/K/R plus 2 additional mutations out of G140A/C/S, L74I, E138A/K/T R263K	H51Y, L74IM, E92Q, E138A/K/T, G140S/A N155H+E92Q G118R

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SECTION 3

AIDS

9. Opportunistic Infections (OIs)

CHRISTIAN HOFFMANN

In Western industrialized countries, many opportunistic infections (OIs) that in previous years were considered prevalent are now quite rare. This is particularly true for infections associated with severe immunodeficiency such as CMV and MAC. The incidence of these OIs has been reduced to less than one-tenth of their frequency in the pre-HAART era (Brooks 2009, Buchacz 2010). ART has not only decreased the incidence of OIs, but it has also changed the course of OIs considerably. In the early years of the AIDS epidemic, the life expectancy of individuals diagnosed with their first AIDS-defining illness was at most two to three years. Today, however, many patients live with AIDS for 15 years or longer. In our own clinical study of 144 patients with cerebral toxoplasmosis, data from 1990–1993 indicated a 5-year survival rate of 8%; it climbed to 30% by 1994–1996, and to 80% since 1997 (Hoffmann 2007).

Up to 90% of patients who develop AIDS or severe opportunistic infections are unaware of their HIV status. Typically, these patients seek medical attention late, when their overall health condition is serious. Since AIDS remains life-threatening, every HIV clinician should be familiar with the diagnosis of OIs and their respective therapies. Even with recent improvements, many challenges still exist. First, there is still no adequate treatment available for diseases such as PML or cryptosporidiosis. Second, resistance to treatment has become an increasing problem in OIs such as PCP. Even today, OIs like PML have a mortality rate comparable to that of non-Hodgkin lymphoma (ART-CC 2009). Third, ART does not always lead to immediate improvement. ART may even complicate things, given the atypical course of a variety of diseases with ART (see the separate section on "Immune Reconstitution Inflammatory Syndrome", IRIS). Fourth, in small HIV centers or regions with low HIV prevalence, diagnostic problems for many OIs may occur, due to a lack of familiarity with and inability to recognize these rarer pathogens. Therefore, it is highly recommended that specimens be sent to specialized reference laboratories. If needed, further advice can be sought from a specialized clinician or a clinical HIV center.

The predominant rule for nearly all OIs is that the poorer the immune status of the patient, the earlier the invasive diagnostic procedures should begin. The primary aim should not be to spare patients the unpleasant procedures associated with extensive diagnostic testing. And if the results are inconclusive and nothing is identified the first time, diagnostic tests must be repeated. Treatment should be initiated rapidly. The second rule is that many OIs can be excluded if the immune status is known. Table 1 indicates the CD4 cut-off values and the rates of certain OIs.

The third OI rule is that if ART is not already in place, it should be started as quickly as possible. Immune reconstitution is the best protection against relapses or other OIs. For patients with OIs such as PML or cryptosporidiosis, which have no specific therapy, starting ART is the best hope. Especially in these cases there is no time to waste. ART should also be started rapidly in cases of PCP or toxoplasmosis.

Table 1: Important cut-offs for CD4 T cells, above which particular AIDS-related illnesses are unlikely. However, exceptions are always possible

No cut-off	Kaposi's sarcoma, pulmonary tuberculosis, HZV, bacterial pneumonia, NHL
<250/µl	PCP, esophageal candidiasis, PML, HSV
<100/µl	Cerebral toxoplasmosis, cryptococcosis, miliary tuberculosis, HAND
<50/µl	CMV retinitis, atypical mycobacteriosis

Although OI therapy is not without toxicity and there are problems regarding inter-actions, the options of antiretroviral drugs has increased, making it easier to react to these issues. In ACTG A5164, a total of 282 subjects with an acute OI (63% PCP) were randomized to initiate ART immediately or after OI treatment (Zolopa 2009). At 48 weeks significantly less mortality and AIDS-related infections occurred in the group starting ART immediately. CD4 T cell counts also increased more rapidly. The risk of changing ART was slightly higher in the immediate group, although not the number of adverse events, hospitalizations or cases of IRIS. ACTG A5164 provides clear arguments for immediate initiation of ART when PCP is diagnosed. However, this does not necessarily apply to all OIs (Lawn 2011). Two randomized studies in patients with cryptococcal meningitis (Makadzange 2010) and tuberculous menin-gitis (Torok 2011) showed unfavorable effects when starting ART too early (see chapter on *Late Presenters*).

The next chapter is intended to be a practical overview and does not include clinical rarities. The literature cited refers to interesting reviews and almost exclusively to controlled studies, and when applicable, randomized studies. For more information on OIs see the detailed (more than 400 pages) US Guidelines https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf

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Pneumocystis pneumonia (PCP)

This interstitial pneumonia caused the majority of AIDS deaths in the early years of the HIV epidemic. In the last 20 years, there has been significant progress made in understanding this organism, especially through DNA analysis (Review: Thomas 2004). Although pneumocystis was previously classified as a protozoan, it was established in 1988 that it is in fact an unusual type of fungus (Edman 1988). In the 1990s, it was recognized that every host, whether rat, mouse, monkey or human, has its own specific pneumocysts. It also became clear that *Pneumocystis carinii* (*P. carinii*), which was first described in 1910, does not occur in humans at all, but only in rats. The Pneumocystis species that affects humans is now referred to as *Pneumocystis jiroveci*, and "carinii" has now been taken out of the name, although the abbreviation PCP remains (Stringer 2002).

Today, the majority of patients diagnosed with PCP are not on antiretroviral drugs, because many of them either do not know their HIV infection status. In Europe between 1997–2004, among 760 cases of so-called "late presenters" who were diagnosed with HIV infection and AIDS at the same time, PCP (35%) was the most frequent OI (Mussini 2008). In many cases with known HIV infection, adherence to antiretroviral therapy was poor prior to PCP (Denis 2014).

PCP is a life-threatening disease, which should be treated by an HIV specialist. It often requires mechanical ventilation and still continues to have a high fatality rate of up to 10% (Walzer 2008, Llibre 2013). Factors associated with mortality are older age, low hemoglobin level, and low partial pressure of oxygen at hospital admission (Walzer 2008, Miller 2010). Relapses seen frequently in the past have become rare, thanks to ART and prophylaxis. Scar tissue formation may result in susceptibility to recurring pneumothoraces. PCP may rarely occur in relation to immune reconstitution inflammatory syndrome (see below). Extrapulmonary manifestations of pneumocystis infections are also considerably rare. They may affect the liver, but many other organs may be involved.

Signs and symptoms

Every clinician should be familiar with the classic triad of PCP symptoms that include dry cough, subfebrile temperatures, and dyspnea on exertion; should ask patients specifically about their symptoms; and should measure the patients' respiratory rates. A subacute course that allows differentiation from the productive cough, acutely high fever, pain and less common dyspnea-associated bacterial pneumonia is typical. Oral thrush is a frequent symptom in patients with PCP. Also, substantial weight loss of several kilos in the weeks before PCP diagnosis is common. These and other symptoms may be more subtle in cases with suboptimal prophylaxis (rare).

Weeks and sometimes even months may go by before the diagnosis of PCP is made. It is noteworthy to state that decompensation – as with all interstitial pneumonias – often occurs much faster than expected. It is not rare for a patient to suddenly require ventilation after weeks of antibiotic therapy prescribed by the primary health care provider, especially when even "broad spectrum" antibiotics do not help. A patient with significant exertional dyspnea or even resting dyspnea should be directed immediately to hospital.

Diagnosis

If there is clinical suspicion of PCP determined by a physical examination with attention given to respiratory rate, oral thrush, and significant findings on auscultation, then a chest x-ray should follow without delay and, if possible, a high resolution

computed tomography (HRCT) of the lungs. The chest x-ray often shows relatively characteristic findings with a butterfly-shaped (perihilar) interstitial infiltrate. In the early stages, focus is on the mid- and lower fields. Indistinct, diffuse changes are more easily visible on HRCT than on a chest x-ray. A CT scan also allows a fairly certain distinction from other pulmonary infections (Hidalgo 2003). However, in cases where nothing pathological is visible on CT scan to an experienced radiologist, then rapid initiation of treatment is still justified even without a definitive diagnosis – particularly in the presence of the classic triad of symptoms, low CD4 T cell count and no previous PCP prophylaxis. Almost always present is partial respiratory insufficiency, which should be confirmed by arterial blood gas analysis. Lactate dehydrogenase (LDH) is often elevated and may have limited use as a predictive parameter for the course of disease. A high LDH is an unfavorable sign and may reflect the severity of the PCP. In contrast, CRP is often normal, provided there are no other concurrent infections.

Sputum specimens are generally not useful (Review: Cruciani 2002), so that a bronchoalveolar lavage (BAL) is usually necessary. This can lead to detection of pneumocysts even after several days of treatment. Therefore, it is not essential to wait for the BAL to start treatment. The lab should be specifically alerted to possible PCP. The routine test for detecting Pneumocystis in the BAL is direct immunofluorescence assay (DFA). A real-time PCR assay also seems to be an accurate diagnosis method and could replace the DFA (Fillaux 2008).

Performing the BAL as early as possible also allows for the timely diagnosis of co-infections (CMV, pneumococci). It should be noted that respiratory insufficiency can deteriorate with BAL. Full blood count, transaminases and kidney function must be monitored during treatment and baseline values should be determined at this point. Newer diagnostic approaches include antibody testing (Bishop 2003) and measurement of S-adenosylmethionine, an agent that pneumocysts require but cannot produce. S-adenosylmethionine levels are significantly reduced in patients with PCP (Skelly 2008). It is currently not foreseeable, whether these tests, which spare patients the discomfort of bronchoscopy, will be available for routine diagnostic testing in the future. This also applies to other serum markers such as beta-glucan or antibody tests (Desmet 2009, Watanabe 2009, Djawe 2010, Gingo 2011, Sax 2011). Elevated plasma beta-glucan has an especially high predictive value for diagnosing PCP in AIDS patients with respiratory symptoms (Wood 2013).

Treatment

General

Treatment should be initiated immediately if there is clinical suspicion. In cases of mild PCP (PO_2 >70–80 mm Hg), ambulatory treatment can be attempted. In very mild cases, even oral medication can be considered. This may well be possible in cooperation with a competent HIV nursing service. If such monitoring is not possible, if respiratory deterioration occurs, and in all cases with resting dyspnea, immediate hospitalization is advised. If ventilation becomes necessary, patients have a poor prognosis, even today (Crothers 2005, Walzer 2008). Non-invasive methods (like CPAP) may be beneficial if used from an early stage. This helps particularly in prevention of pneumothoraces (Confalonieri 2002).

The ACTG study above shows the advantages of starting ART with PCP treatment (Zolopa 2009). Another retrospective study showed improved survival in patients who began ART while hospitalized (Morris 2003). Possible cumulative toxicities and allergies which may require discontinuation of both PCP and HIV (Watson 2002), can be largely avoided with newer antiretroviral therapies such as integrase inhibitors.

Drugs

Acute therapy should last for 21 days. The drug of choice is cotrimoxazole. The dose of three 960 mg tablets three times daily is possible in milder cases. However, these higher oral doses are also associated with poor gastrointestinal tolerability. Some case reports have observed positive effects with lower doses, but controlled studies are lacking (Thomas 2009). All severe cases should be treated intravenously in hospital. Due to possible clinical deterioration, which is probably a result of the bursting of pneumocysts in the alveoli, 1 mg/kilo prednisone BID should always be simultaneously co-administered with the PCP therapy for 5-10 days. There should be no hesitation to use steroids, especially in patients with poor blood gases. There is evidence from a Cochrane review for a beneficial effect of steroids in adult patients with substantial hypoxaemia (Ewald 2015). On steroids, significantly less patients need intubation (Briel 2006). Important: clinical deterioration during the first week of treatment is still not uncommon. Initial treatment should be re-evaluated after one week at the earliest, and only after exclusion of coinfections such as CMV.

The high doses of cotrimoxazole require monitoring of full blood count, electrolytes, renal function parameters and transaminases at least three times weekly. The main problems in addition to myelotoxicity as well as liver and kidney problems include a rash that usually occurs after the middle of the second week of treatment and is often accompanied by drug fever. The rash is seen in up to 30% of patients (Fisk 2009) – patients should be checked daily for skin changes! If an exanthema occurs, one can attempt to interrupt treatment for one or two days, and then continue with half-dose steroids. Otherwise, cotrimoxazole must be discontinued and replaced with alternative treatments.

All alternatives to cotrimoxazole are less effective. In cases of intolerability or history of sulfonamide allergy, intravenous pentamidine is the drug of second choice. An induction therapy is administered over the first few days (200–300 mg in 500 ml 5% glucose or 0.9% NaCl), and half the dose can be given from day 6. This treatment is very toxic, which is why we have not used it for many years. Severe decompensations of electrolyte and blood glucose levels (both hyper- and hypoglycemia) are possible, as well as pancreatitis, arrhythmia and renal failure. Initially, daily monitoring of blood glucose, electrolytes and renal parameters is necessary.

In very mild cases of PCP, treatment with daily pentamidine inhalations (300–600 mg daily for three weeks) can be attempted (Arasteh 1990, Montgomery 1995). However, experiences have not been all positive (Conte 1990, Soo 1990), and the current US guidelines advise against inhalatory acute therapy (Benson 2004). Instead of pentamidine, treatment with atovaquone suspension or a combination of clindamycin and primaquine is possible. However, data on these therapies is only available for mild to moderately severe cases of PCP (Hughes 1993, Dohn 1994, Toma 1998). According to a meta-analysis, clindamycin-primaquine seems very promising as second-line treatment in patients who fail treatment with cotrimoxazole (Benfield 2008) and is superior to pentamidine (Helweg-Larsen 2009). Primaquine should not be administered to anyone with G6PD deficiency because of a high risk for hemolytic anemia.

In the past few years, these alternative agents have been used only in exceptional cases. It should be mentioned that a 10-day initial therapy of a high dose cotrimoxazole is achievable in almost all patients, most of whom are then already significantly better. If exanthema or toxicity forces the interruption of cotrimoxazole between day 10 and 14, daily pentamidine inhalation can be administered in the third and last week of acute therapy. As this is not toxic, it can usually be started in parallel to ART. However, a study on this strategy has yet to be published.

Prophylaxis

Patients with less than 200 CD4 T cells/µl (<14%) are at high risk of PCP. Above these values, the occurrence of PCP is rare. Therefore, these patients are treated prophylactically, ideally with cotrimoxazole. Daily dosages may be slightly more effective than three times weekly (El Sadr 1999). The gradual lead-in administration over a period of 14 days is supposed to prevent allergic reactions, but is cumbersome (Para 2000). In cases of a mild or moderate allergy to co-trimoxazole, desensitization after several weeks is possible (Leoung 2001), and should definitely be attempted. Although dapsone and pentamidine inhalations are almost equally effective (Bozzette 1995, Bucher 1997), co-trimoxazole prophylaxis is better for preventing bacterial infections such as enteritis, sinusitis and pneumonia (DiRienzo 2002). More importantly, co-trimoxazole simultaneously provides reliable protection for cerebral toxoplasmosis. Pediatric co-trimoxazole suspension can be used for desensitization, by slowly increasing exposure over six days from 12.5, 25, 37.5, 50 and 75 to 100% of the dose in the 480 mg tablet. In a study of almost 200 patients, no cases of severe allergy occurred, and there was a reduction of fever and headaches. Approximately three quarters of all patients are thus able to tolerate co-trimoxazole again. However, re-exposure should only be attempted after an interval of eight weeks (Leoung 2001). Monthly inhalation of pentamidine is a well-tolerated alternative. However, coughing may occur. Asthma attacks are rare, and pneumothoraces are even rarer. A suitable inhalation system should be used, after administration of a beta-sympathomimetic agent to dilate the bronchi. The loading dose (300 mg TID for the first 5 days) frequently used in the past is no longer a universal standard. In patients with severe pulmonary disease, inhalation is probably less effective.

Further options are problematic. Dapsone has poor gastrointestinal tolerability, is quite myelotoxic and often leads to elevation of LDH. LDH, an important diagnostic parameter, can therefore not be utilized during treatment with dapsone (Ioannidis 1996). Atovaquone was proven to be of comparable efficacy to co-trimoxazole, dapsone and pentamidine in two multicenter studies (El-Sadr 1998, Chan 1999), and since then, is considered to be a good alternative for PCP prophylaxis. The oral suspension has better tolerability than the tablet formulation (Rosenberg 2001). A significant disadvantage of atovaquone for long-term prophylaxis is the disproportionately high cost (in some European countries approx. 1000 euro/month).

PCP prophylaxis regimens can be discontinued fairly safely with sufficient immune reconstitution: a recent meta-analysis showed that more than 200 CD4 T cells/µl for three months is required (Costiniuk 2011). PCP has only rarely been described in cases with CD4 T cell counts greater than 200 cells after stopping prophylaxis (Degen 2002, Mussini 2003). If the viral load is suppressed, even lower CD4 cells are possible. In an analysis of 23,412 patients from 12 European cohorts who started taking ART after 1997, the incidence of primary PCP was very low among patients who had virologically suppressed HIV infection, were receiving ART, and who had CD4 cell counts of between 101–200 (COHERE 2010). However, there are no controlled studies addressing this issue. Stopping prophylaxis not only reduces side effects and costs, but also avoids other negative developments: the proportion of co-trimoxazole-resistant bacteria is constantly increasing among HIV+ patients (Martin 1999).

Treatment/Prophylaxis of PCP (daily doses, if not otherwise specified)

Acute therapy		Duration: always at least three weeks
Severe to moderately severe PCP	Co-trimoxazole	Co-trimoxazole 5–6 amp. at 480 mg TID **plus** prednisolone 2–2–0 tbl. at 20 mg (5–10 days)
Mild PCP	Co-trimoxazole	Co-trimoxazole 3 tbl. at 960 mg TID
Alternatives	Pentamidine	Pentamidine 200–300 mg IV for 5 days (4 mg/kg), then halve dose In very mild cases: daily inhalations with 300 mg
Prophylaxis		Below 200 CD4 T cells/µl; after PCP episode
First choice	Co-trimoxazole	Co-trimoxazole 1 tbl. at 480 mg QD or Co-trimoxazole 1 tbl. at 960 mg 3 x/week
Alternatives	Pentamidine Dapsone Dapsone + Pyrimethamine Atovaquone	Pentamidine inhalation 300 mg 1–2 x/month Dapsone 2 tbl. at 50 mg QD Dapsone 1 tbl. at 50 mg QD **plus** pyrimethamine 2 tbl. at 25 mg/week **plus** leucovorin 2 tbl. at 15 mg/week Atovaquone suspension 5 ml BID (750 mg BID)

Resistance issues, current controversies

The worldwide use of co-trimoxazole has also affected pneumocysts. Resistance analyses were previously difficult since this particular organism, even almost 100 years after its discovery, can not be easily cultured. However, it is now possible to sequence sections of the genome encoding for dihydropteroate synthetase (DHPS). DHPS is an important enzyme involved in the folate metabolism of many organisms, and is targeted by sulfonamides such as sulfamethoxazole (SMX) and dapsone. The first mutations in the DHPS gene in pneumocysts were discovered in 1997. A further study showed DHPS mutations in 43%, while the gene region for dihydrofolate reductase (DHFR), targeted by trimethoprim (TMP) and pyrimethamine, did not show a single relevant mutation. In contrast to SMX, there seems to be no selective pressure associated with TMP – a suspicion that has to be analyzed, that TMP is not effective against pneumocysts (Ma 1999). Recently, however, even DHFR mutations have been proven (Nahimana 2004). In addition, studies in large groups of patients have demonstrated that the frequency of sulfa resistance mutations has significantly increased in recent years. Resistance correlated significantly with the duration of prior prophylaxis and its failure (Helweg-Larsen 1999). However, it remains unclear whether DHPS mutations should affect decisions on PCP therapy or lead to a change in treatment (Review: Matos 2010).

The sequencing of the Pneumocystis genome has uncovered other possibly relevant findings: it seems highly likely that PCP is caused by a new infection, rather than the reactivation of an existing infection as previously assumed (Wakefield 2003). Asymptomatic HIV+ patients with frequent detection of pneumocysts may have reservoirs (Wakefield 2003), as well as HIV-negative patients on corticosteroid therapy (Maskell 2003) and patients with active PCP. Several reports also exist on nosocomial outbreaks (Schmoldt 2008, Le Gral 2012, Sassi 2012). However, other authors doubt patient-to-patient transmission (Wohl 2002), and isolation of PCP patients is still not generally recommended (Thomas 2004).

Pneumocysts do not always cause a manifest pneumonia: in healthy patients pneumocystis colonization has been observed (Ponce 2010, Vargas 2010). These patients may represent a potential infectious source (Le Gral 2012). Pneumocysts may also play a role in chronic obstructive lung diseases (Morris 2008).

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Cerebral toxoplasmosis

Although the incidence in Europe has been drastically reduced as a result of ART (Abgrall 2001), cerebral toxoplasmosis (or toxoplasmic encephalitis, TE) remains the most important neurological OI in HIV-infected patients. TE almost always results from the reactivation of a latent infection with *Toxoplasma gondii*, an intracellular parasite that infects birds, mammals and humans. Prevalence rates vary considerably worldwide (Porter 1992). Whereas *Toxoplasma gondii* is relatively rare in the US, seroprevalence rates in some regions within central Europe are as high as 90%. *Toxoplasma gondii* has an affinity to the CNS. Extracerebral organ manifestations (heart, muscle, liver, intestine, lung) are rare and often only detected at autopsy.

Altough prognosis has been markedly improved with antiretroviral therapy, cerebral toxoplasmosis is potentially life-threatening, especially during the first weeks. Treatment remains complicated. In severe cases, there may be residual neurological syndromes with significant disabilities, like hemiparesis. It is not rare to see a life-long susceptibility to seizures as a result of defective healing. It should be noted that relapses may occur even after long periods of time due to intracerebral persistence. In Western countries, there is some evidence that the situation of an HIV+ patient developing TE in recent years differs from TE patients seen during the early years of the HIV epidemic (Hoffmann 2007). Patients with TE today usually are not taking antiretroviral therapy or prophylaxis of any sort. They are likely to be diagnosed with HIV at the time of TE diagnosis, and TE is much more frequently the AIDS-defining illness in these patients than in the pre-HAART era.

Signs and symptoms

Clinical symptoms depend on the localization of lesions with acute or peracute onset within a few days. The major signs include focal neurological deficits such as paresis, speech problems or sensory loss (Porter 1992). A febrile psychosyndrome with confusion is also a frequent early sign. It is not unusual to see epileptic seizure as the initial presentation, in the absence of other symptoms. Headaches with fever or subfebrile temperatures are always suspicious. Meningitic signs, however, are less typical. Atypical manifestations in patients with immune reconstitution on ART have been described (Ghosn 2003).

A fairly rare, but important manifestation is Toxoplasma chorioretinitis. It causes impairment of vision, is an important differential diagnosis to CMV retinitis and may occur on its own (Rodgers 1996). Toxoplasma chorioretinitis should be treated in exactly the same way as cerebral toxoplasmosis.

Diagnosis

Cerebral toxoplasmosis seldomly occurs above a CD4 T cell count of 100 cells/µl; over 200 CD4 T cells it is very rare (Bossi 1998). In contrast, it should be expected below 100 CD4 T cells/µl. A CT or MRI scan of the head should be performed promptly within a week in every case of focal neurological deficit, but also if seizures occur in significantly immunocompromised patients. In this instance, an MRI is superior to a CT scan and almost always shows more visible lesions. A third of cases have solitary lesions, a third have several (2–5) and a third have multiple lesions. In approximately nine out of ten cases, ring enhancement is found around the lesions, often accompanied by edema. Hemorrhage may occasionally occur.

For all radiologically detected lesions, the most likely diagnosis is cerebral toxoplasmosis. The most important differential diagnosis is an "atypical" cerebral toxoplasmosis. The more lesions there are, the more likely the diagnosis of toxoplasmosis. However,

the distinction between toxoplasmosis and a bacterial abscess or a cerebral lymphoma may be difficult. Other rare differential diagnoses include PML, infarcts, tuberculomas and cryptococcomas. "HIV-unrelated" diseases such as brain tumors or vascular diseases should also be considered.

A brain biopsy is not obligatory. Suspicion of toxoplasmosis (clinically and radiologically) justifies a treatment attempt before biopsy. Response to therapy then confirms the diagnosis. However, if the patient does not improve clinically within one week, or even worsens, then stereotactical brain biopsy cannot be avoided, and in this case, should not be postponed. The cerebrospinal fluid (CSF), which also does not necessarily have to be analyzed if there are clear radiological findings (several lesions with contrast enhancement), usually shows moderate pleocytosis and slightly elevated total protein. Our experience with toxoplasma PCR from CSF has not been good. A negative result never excludes toxoplasmosis.

An updated serology should be available for every patient. Up to 97% of patients with cerebral toxoplasmosis have IgG antibodies, and so a negative result, which should be repeated in another lab if there is any doubt, makes toxoplasmosis unlikely. Some clinicians use levels of IgG titers or increased titers as indicators (Derouin 1996), but this approach has not been properly validated. IgM is only rarely positive, and therefore usually does not help. PCR from the blood has little relevance (Review: Bretagne 2003).

Treatment

Treatment of cerebral toxoplasmosis is difficult. The most frequently used combinations are usually effective (resistance has not yet been convincingly described), but require modification in at least half of patients due to side effects – particularly allergies. Sulfadiazine and clindamycin are presumably equally effective in combination with pyrimethamine (Dannemann 1992). One large European study demonstrated a trend, though not significant, in favor of sulfadiazine (Katlama 1996). Co-trimoxazole may also be an option. According to a Cochrane analysis, the available evidence fails to identify a best regimen that can be considered the gold standard (Dedicoat 2006).

We recommend that sulfadiazine and pyrimethamine be used for an initial attempt as oral treatment. In cases of sulfonamide allergy, sulfadiazine should be substituted with oral or intravenous clindamycin from the beginning. In addition, all disoriented patients should receive clindamycin infusions, at least for adherence reasons. Because of the high rate of allergies with sulfadiazine, some clinicians oppose clindamycin. We do not share this perspective, since clindamycin is also allergenic. Moreover, clindamycin can cause pseudomembranous colitis.

A loading dose for pyrimethamine during the first few days has been propagated since the first published study (Leport 1988). However, it has not been proven necessary. Even the dosages vary. For example, in the US, 200 mg is recommended for the first day (followed by 50-75 mg depending on body weight); in many European countries, 100 mg is often given for three days, followed by 50 mg. It should be noted that, in contrast to clindamycin, pyrimethamine is also active in the presence of an intact blood-brain barrier, and therefore, is sometimes the only effective agent. Due to the myelotoxicity of sulfonamides and pyrimethamine, which inhibits transformation of folic acid to folinic acid, it is imperative to substitute sufficiently with folinic acid, which unfortunately is expensive. Folic acid, which is much cheaper, is ineffective since it cannot be converted in the presence of pyrimethamine (Luft 2000). Good results have also been reported with intravenous co-trimoxazole, with administration of the same dosages as for PCP (Canessa 1992, Béraud 2009). In two

randomized studies in patients with ocular or cerebral toxoplasmosis, co-trimoxazole was as effective as sulfadiazine/pyrimethamine (Torre 1998, Soheilian 2005). If allergies or intolerance to both sulfonamides and clindamycin occur, then a combination of atovaquone and pyrimethamine is an alternative (Chirgwin 2002). A combination of azithromycin plus pyrimethamine could be another alternative (Bosch-Driessen 2002).

Acute therapy lasts for a period of four to six weeks, or longer for the less effective reserve therapies. Treatment success can be assessed clinically in the first 14 days. While an improvement in the symptoms can often be observed within a few days, a patient who has not improved after two weeks of therapy or has even deteriorated, probably does not have toxoplasmosis. If this occurs, the diagnosis has to be reviewed and a brain biopsy must be performed. Changing the TE therapy is not useful in such cases and just expends valuable time. Antiretroviral therapy should be initiated as soon as possible. Drugs with the potential of allergic reactions (abacavir, when HLA testing is not possible, NNRTIs, fosamprenavir, darunavir) should be avoided. A control MRI is recommended for stable patients after two weeks at the earliest. Significant resolution of lesions is often only visible after four weeks. In cases of increased intracranial pressure or extensive edema, steroids are given (8 mg dexamethasone q 6–8 h). Steroids should be given for a limited time, as there is a significantly increased risk of aspergillosis. All treatment combinations require initial monitoring of blood count, glucose, transaminases and renal parameters at least three times weekly. Maintenance therapy with the reduced dose should only be initiated if lesions have shrunk by at least 75%.

Prophylaxis

Exposure prophylaxis: IgG-negative patients can protect themselves from primary infection by not eating raw or undercooked meat (lamb, beef, pork, game, etc). It has not been proven, despite widespread opinion, that infection occurs by mere contact with cats, the definitive hosts of *Toxoplasma gondii*. To date, the only study that has seriously investigated this conjecture could not prove endangerment as a result of proximity to cats (Wallace 1993). Nevertheless, stricter measures of hygiene should be followed (e.g., gloves should be used when handling the litter box).

Primary prophylaxis: All IgG-positive patients with less than 100 CD4 T cells/µl require primary prophylaxis. The drug of choice is co-trimoxazole. In cases of co-trimoxazole allergy, desensitization may be considered (see PCP). An alternative is dapsone plus pyrimethamine or high-dose dapsone. Primary prophylaxes can be discontinued safely if CD4 T cells are above 200/µl for at least three months.

Maintenance therapy/secondary prophylaxis: In the absence of immune reconstitution, patients with cerebral toxoplasmosis require lifelong maintenance therapy or secondary prophylaxis, as there are otherwise recurrences in nearly all cases. It usually consists of half the dose of the acute therapy (Podzamcer 2000). Clindamycin is presumably less suitable as it cannot cross the blood-brain barrier (Luft 2000). Co-trimoxazole seems to be not as effective for secondary prophylaxis, but should be considered because it is simple. However, it definitely requires higher doses than those used to treat PCP (Ribera 1999, Duval 2004). With immune reconstitution (at least six months above 200 CD4 T cells), secondary prophylaxis can probably be stopped (Benson 2004, Miro 2006). When possible, an updated MRI scan should be available beforehand. If there is enhancement, then it may mean that lesions have become active even after years – and there is a risk of a recurrence. A recurrence even after five years has been observed, despite CD4 T cells being around 200/µl.

Treatment/prophylaxis of cerebral toxoplasmosis
(daily doses, if not otherwise specified)

Acute therapy		Duration: always at least four weeks
First choice	Sulfadiazine + Pyrimethamine	Sulfadiazine 2–3 tbl. at 500 mg QID **plus** pyrimethamine 2 tbl. at 25 mg BID (for 3 days, then halve dose) **plus** leucovorin 3 x 1 tbl. at 15 mg/week
First choice	Clindamycin + Pyrimethamine	Clindamycin 1 amp. at 600 mg IV QID or 1 tbl. at 600 mg QID **plus** pyrimethamine 2 tbl. at 25 mg BID (for 3 days, then half dose) **plus** leucovorin 3 x 1 tbl. at 15 mg/week
Alternative	Atovaquone + Pyrimethamine	Atovaquone suspension 10 ml bid (1500 mg BID) **plus** pyrimethamine 2 tbl. at 25 mg BID (for 3 days, then half dose) **plus** leucovorin 3 x 1 tbl. at 15 mg/week
Maintenance therapy		
	As for acute therapy	As for acute therapy, but half dose. Discontinue if >200 CD4 T cells/µl for >6 months (if MRI is normal or without contrast enhancement)
Possibly	Co-trimoxazole	Co-trimoxazole 1 tbl. at 960 mg QD
Primary prophylaxis		
First choice	Co-trimoxazole	Co-trimoxazole 1 tbl. at 480 mg QD
Alternative	Dapsone	Dapsone 2 tbl. at 50 mg QD
Alternative	Dapsone + Pyrimethamine	Dapsone 1 tbl. at 50 mg QD **plus** pyrimethamine 2 tbl. at 25 mg/week **plus** leucovorin 2 tbl. at 15 mg/week

This and other cases (Stout 2002, Ghosn 2003) have shown that quantitative measurement of CD4 T cells on ART does not always reflect the quality of the TG-specific immune response. As a result, there have been increasing efforts in recent years to improve the characterization of this specific immune response via ELISPOT. Studies have shown that the Toxoplasma-specific immune response remains poor in approximately 10–30% of patients on ART, despite good CD4 T cell counts (Fournier 2001, Miro 2003, Furco 2008). In the future, ELISPOT testing may allow identification of patients who are at risk of recurrence despite good CD4 counts who should continue with secondary prophylaxis.

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CMV retinitis

Infections with cytomegalovirus are widespread. In many countries, seroprevalence is around 50–70%, and above 90% in MSM. In severely immunocompromised individuals (CD4 count below 50 cells/µl), reactivation of CMV infection can lead to retinitis. In the past, CMV retinitis was a common AIDS-associated illness, leading to blindness in up to 30% of patients. It occurs mainly in untreated patients, who are often first diagnosed with HIV infection on presentation (Jacobson 2000). An inflammatory CMV retinitis, usually with severe vitritis, is also possible in the course of IRIS (see below). If CMV retinitis is not diagnosed and treated promptly, then the patient's sight is at risk. Impairment of vision is almost always associated with lesions, which are no longer reversible even with adequate treatment. This is why CMV retinitis remains a dangerous illness, although the prognosis has significantly improved with ART (Salzberger 2005, Thorne 2006).

Other manifestations of disseminated CMV infection are rare (15%), and can affect every organ. The lung (pneumonia), esophagus (ulcers), colon (colitis) and CNS (encephalitis) are most frequently involved. Sinusitis may also occur (Jutte 2000). The clinical signs of these CMV diseases depend on the organ affected. Diagnosis is often difficult and may only be possible on histology (Goodgame 1993). There is insufficient data on the treatment of these manifestations, so systemic therapies are usually chosen along with treatment for CMV retinitis (Whitley 1998).

Newer studies have suggested that CMV infection plays a role in the pathogenesis of artery disease and that CMV-induced T cell immunopathology could contribute to HIV-associated atherosclerosis (Parrinello 2012, Sacre 2012). There is also an association between CMV infection and altered immune reconstitution (Appay 2011, Wittkop 2013).

Signs and symptoms

Any visual impairment occurring peracutely or acutely, such as blurred vision or floaters – especially unilaterally – should prompt an immediate (same day, if possible) ophthalmological examination of the patient. Symptomatic CMV retinitis is an emergency. Once there is a black spot in the visual field, it will be permanent. Involvement of the posterior pole (zone 1 retinitis) accounts for approximately one half of incident visual acuity loss. Cataract and retinitis-related retinal detachment are also common causes of vision loss (Thorne 2006).

All CMV treatment regimens can prevent progression of lesions, but not reverse them. Eye pain, burning, increased production of tears, and conjunctival irritation are not typical. However, many patients suffer from systemic symptoms such as fever and weight loss.

Diagnosis

Diagnosis is made by fundoscopy. Assessment of the usually peripheral, whitish exudates is dependent on the experience of the ophthalmologist. However, this can frequently be a problem, due to the rare occurrence of CMV retinitis nowadays. Unfortunately, incorrect diagnoses do happen and retina are lost. Therefore, if the primary ophthalmologist remains undecided, it is best to start with oral valgancyclovir and transport the patient to a larger clinical center with ophthalmologists who are experienced in HIV. Furthermore, it is essential that the ophthalmologists receive information about the patient's immune status. In cases of poor immune status and CD4 count less than 100/µl, chorioretinitis caused by *Toxoplasma gondii* is the most important differential diagnosis. CMV retinitis can almost be excluded at CD4 T cell

counts above 100/µl; other viral infections (HSV, VZV) or even neurosyphilis should then be considered. CMV lesions may also be confused with cotton wool spots, which are not rare in patients with high HIV viral load. Multiple small lesions without hemorrhage or exudates are almost always cotton wool spots, and almost never CMV retinitis. Bilateral involvement is also usually the exception. Vitritis is rare, except with immune reconstitution syndrome.

CMV serology (IgG almost always positive, IgM variable) is seldom helpful for diagnosis. CMV PCR or a blood test for pp65 antigen to detect antibodies against a CMV-specific phosphoprotein may be more useful. CMV retinitis or a recurrence is unlikely with a negative PCR or pp65 result. The higher the levels of CMV viremia, the higher the risk of CMV disease. Patients with positive CMV PCR have a 3-5-fold elevated mortality risk (Casado 1999, Nokta 2002). Positive CMV PCR is also independently associated with a poor prognosis for the patient (Deayton 2004, Jabs 2005, Wohl 2005). As with *Toxoplasma gondii*, there have been efforts to determine the antigen-specific immune response more precisely (Jacobsen 2004), although such testing is not yet routine.

Treatment

CMV treatment should always be initiated promptly and strictly monitored by fundoscopy at least once a week in the beginning. Photodocumentation is advisable. Initially, an intensive induction therapy is administered for two to three weeks, until there is scar formation of the lesions. HIV clinicians and ophthalmologists should work closely together, particularly during the induction therapy, and when possible, communicate several times a week. Induction therapy is followed by maintenance therapy at a reduced dose.

ART in particular has dramatically improved the prognosis of patients. That said, all diagnosed patients should start ART without delay. This can restore CMV-specific immune responses (Komandouri 1998), so that CMV viremia may disappear even without specific therapy after a few weeks (Deayton 1999, O'Sullivan 1999). However, if retinitis is present, CMV-specific treatment should also be started, as immune reconstitution may take several months. Treating asymptomatic CMV patients with CMV agents remains controversial. There is some evidence that preemptive therapy lowers the incidence of CMV end-organ disease in some patients with CMV viremia (Mizushima 2013). However, monitoring of potential treatment-related side effects is required. Treating a positive CMV IgM serology (without any further diagnosis) is not only expensive, but also usually an unnecessary risk.

Systemic treatment

Valganciclovir, a prodrug of ganciclovir with good oral absorption, is the first choice in CMV treatment. In a randomized study (Martin 2002) on 160 patients with retinitis valganciclovir tablets were just as effective as ganciclovir infusions. However, the toxicity profile of both agents was comparable. This means that the blood count has to be as frequently monitored as for infusions and that the indication has to be equally carefully set. However, there are some experts in the field who prefer intravenous CMV treatment to oral treatment in advanced cases.

Other options for systemic treatment have become less important, and are only used in cases of recurrence. If there is intolerability or more rarely (Martin 2007) resistance to valganciclovir (Drew 1999), then foscarnet remains an option. This, however, requires daily infusions. Further problems with this drug include nephrotoxicity, and very painful penile ulcers. Very intensive hydration of the patient is therefore necessary in all circumstances.

There are no direct comparative studies available for cidofovir, which is also used occasionally. The benefit of the long half-life (once weekly dosing possible) is outweighed by the considerable renal toxicity of this drug (Plosker 1999). We observed creatinine elevations in every second patient treated, despite the fact that a strict infusion plan was closely followed (see Drugs section).

Newer anti-CMV drugs are under investigation. Maribavir failed to show a benefit in Phase III studies (Snydman 2011). Letermovir is a new agent with a novel mechanism of action targeting the CMV terminase. In a Phase II trial, letermovir was effective in reducing the incidence of CMV infection in recipients of allogeneic hematopoietic-cell transplants, with an acceptable safety profile (Chemaly 2014). The drug has been granted fast track status by FDA and orphan drug status by EMA. Additional treatment with G-CSF (filgrastim) improved survival in one analysis of three large studies enrolling patients with CMV retinitis in the years 1990–1997. In particular, there was a reduction of bacterial infections. However, the reason for this positive effect remains unclear. Thus, administration of filgrastim is presently not generally recommended (Davidson 2002).

Local treatment

Several options for local treatment of CMV retinitis have been tested (Review: Smith 1998). Although such treatments can be safely administered by experienced ophthalmologists and are associated with few complications (infections, hemorrhage), disadvantages remain. Weekly intravitreal injections of ganciclovir or foscarnet, or pellet implantation (Vitrasert®, must be replaced every 6–9 months) do not protect from infection of the contralateral eye or from extraocular manifestations (Martin 1999). The same is true for fomivirsen (Vitravene®), an antisense-oligonucleotide for intravitreal injection, which is astonishingly effective even with multiresistant CMV strains (Perry 1999). These local treatments have become less important since ART and valganciclovir and some have been taken off the market.

Treatment/prophylaxis of CMV retinitis
(daily doses, if not otherwise specified)

Acute therapy		Duration: always at least three weeks
Treatment of choice	Valganciclovir	Valganciclovir (Valcyte®) 2 tbl. at 450 mg BID (some experts prefer intravenous therapy in advanced cases!)
Alternative	Ganciclovir	Ganciclovir 5 mg/kg IV BID
Alternative	Foscarnet	Foscarnet 90 mg/kg IV BID
Alternative	Ganciclovir + Foscarnet	Half of the doses above
Maintenance therapy		Discontinue when >100–150 CD4 cells/µl >6 months
Treatment of choice	Valganciclovir	Valganciclovir (Valcyte®) 1 tbl. at 450 mg BID
Alternative	Foscarnet	Foscarnet 120 mg/kg IV QD on 5 days/week
Alternative	Cidofovir	Cidofovir 5 mg/kg IV QD every 14 days (plus probe-necid, hydration see Drugs section)
Primary prophylaxis		Not recommended

Prophylaxis

Primary prophylaxis: In the prospective studies that have been performed, no primary prophylaxis regimen has been convincing. There is also no effective vaccine. Therefore, the most important method for prevention in patients with CD4 counts below 200 cells/µl is still fundoscopy every three months. With good immune reconstitution, intervals between examinations can be extended. It is important to perform a fundoscopy in severely immunocompromised patients prior to starting ART. This allows detection of smaller lesions, which may later present with severe inflammation during the course of immune reconstitution.

Secondary prophylaxis: After approximately three weeks of acute therapy, but at the earliest with scar formation of lesions, a reduced dose secondary prophylaxis (maintenance therapy) should begin, preferably with oral valganciclovir (Lalezari 2002). However, the drug is not only very expensive but also just as myelotoxic as ganciclovir infusions. Discontinuation of secondary prophylaxis as quickly as possible is desirable (Tural 1998, Jouan 2001), but it also requires strict ophthalmologic monitoring. According to US guidelines, discontinuation should occur at the earliest after six months of maintenance therapy and with an immune reconstitution above 100–150 CD4 T cells/µl. However, we have successfully stopped ganciclovir at lower CD4 counts, if both HIV and CMV PCR in blood were below detection. One study showed that stopping after 18 months of ART, maintenance therapy can be safe above 75 CD4 T cells/µl (Jouan 2001). After stopping maintenance therapy, fundoscopy should be performed every four weeks over the first months.

The previously required life-long daily infusions of ganciclovir or foscarnet via port, pumps and nursing service are luckily now a thing of the past. If there are relapses during oral valganciclovir, re-induction and maintenance therapy with foscarnet or possibly with cidofovir can be considered.

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Candidiasis

Candidiasis is an infection with yeast-forming fungi. Of the 150 Candida species known to date, only approximately 20 cause disease. By far the most frequent species is *C. albicans*. Other species such as *C. tropicalis*, *C. glabrata* and *C. krusei* are rare, but may respond less readily to treatment with azoles. Although it was commonly assumed that azole resistance is a problem particularly with albicans strains, this has not been the case to date (Sanglard 2002).

Candidiasis is an important indicator of immunodeficiency and should be seen as a reason to consider starting ART, even with good immune status. Esophageal candidiasis and even oral thrush often occur following other OIs. Fever, not a classic symptom of candidiasis, is a particular indication to be on the alert for. If immune status is good, it must be remembered that there are also other reasons for thrush – alcoholism and steroid treatment are only two of many possibilities. In addition to candidiasis of the oropharynx and esophagus, vaginitis is a frequent problem in women (also occurring in healthy individuals). Candidemia occurs only rarely in HIV+ patients, even with severe immunodeficiency.

Signs and symptoms

The oropharynx is usually affected, with taste disturbances and sometimes a burning sensation on the tongue. White, non-adherent plaques on the buccal mucosa, tonsillar ring and tongue confirm the diagnosis. Involvement of the tongue alone is rare. Occasionally, there may be atrophic candidiasis, which presents only with an erythematous mucosa.

Candida esophagitis usually occurs with oropharyngeal involvement, but in about one third of cases there is no oral thrush. It often presents with dysphagia ("drinking is ok, but food can't go down") and retrosternal pain. Some patients complain of nausea, although vomiting occurs only rarely.

Diagnosis

Diagnosis in the oropharynx can be made based on clinical appearance. A swab is not usually required. Characterization by culture or even determination of drug susceptibility (beware laboratory uncertainty!) is only advised if a treatment attempt with fluconazole or itraconazole has failed. Oral candidiasis is not to be confused with oral hairy leukoplakia (OHL). In contrast to candidiasis, the whitish, hairy plaques of OHL, on the sides of the tongue, cannot be scraped off. OHL is not caused by fungi but by EBV, and is an important disease marker for HIV, even if it is harmless and does not require treatment.

Candida esophagitis can also initially be diagnosed clinically. Dysphagia, retrosternal pain and oral candidiasis make the diagnosis very probable. Empiric fluconazole therapy reduces costs (Wilcox 1996). Upper GI endoscopy is only required if complaints persist. To distinguish fluconazole-resistant esophageal candidiasis from herpes or CMV esophagitis, samples of lesions should always be taken. In contrast, determination of serum antibodies or antigen is always unnecessary.

Treatment

With relatively good immune status at first presentation, treatment with topical antimycotics such as nystatin, amphotericin B or miconazole can be attempted. However, systemic treatment is usually necessary. This is more effective and prevents relapses for longer (Pons 1997).

Fluconazole is the treatment of choice, and one week of oral treatment is usually sufficient (Sangeorzan 1994). According to a recently published trial, shorter treatment duration with higher dosages may be an option. In this large randomized study, a single dose of 750 mg of fluconazole was safe, well tolerated, and as effective as the standard 14-day fluconazole therapy (Hamza 2008).

If symptoms last for more than a week, a swab should be taken and the daily fluconazole dose may be increased to 800 mg for the second attempt. Itraconazole should only be used if the second treatment attempt fails and non-albicans strains have been found. It will be effective in approximately two thirds of cases (Saag 1997). Although itraconazole suspension is as effective as fluconazole (Graybill 1998), we do not primarily use it as plasma levels are unreliable and there are problems due to numerous interactions.

A new alternative are miconazole mucoadhesive tablets. These tablets adhere to the oral mucosa and provide sustained local release of miconazole over a period of several hours with just one daily application. Miconazole has recently been approved in Europe (Loramyc®) and the USA (Oravig™) for the treatment of oropharyngeal candidiasis (Lalla 2011). In a large trial, miconazole was shown to be noninferior to treatment with clotrimazole 10 mg troches 5 times daily for 14 days in HIV+ patients (Vasquez 2010). Trials comparing miconazole with oral fluconazole are lacking.

Several new and promising antimycotics have been developed in recent years. However, these should only be used in clear cases of fluconazole resistance. There is insufficient evidence on the superiority of these drugs in the treatment of non-resistant candidiasis (Pienaar 2006). Voriconazole is expected to be as effective as fluconazole, but is possibly not tolerated as well (Ruhnke 1997, Ally 2001). This may be also true for posaconazole (Vasquez 2006). Like amphotericin B, these new azoles should only be used for treatment of multi-azole resistant mycoses.

The new antimycotic class of echinocandins has good efficacy, among them drugs such as caspofungin, micafungin or anidulafungin. These drugs showed similar efficacy and tolerability to intravenous fluconazole for treatment of candida esophagitis in randomized studies (Villaneuva 2001, de Wet 2004, Reboli 2007). However, these drugs can only be administered intravenously which restricts their use to azole-resistant candidiasis.

Antiretroviral therapy should be initiated when such mycoses occur, particularly with multiresistant strains, as these usually disappear with sufficient immune reconstitution (Ruhnke 2000).

Treatment/prophylaxis of candidiasis (daily doses)

Acute therapy		Duration: 5–10 days
In mild cases	Topical	e.g., amphotericin B 1 lozenge QID or nystatin suspension 1 ml QID
Treatment of choice	Fluconazole	Fluconazole 1 x 1 cap at 100 mg for oral candidiasis Fluconazole 1 x 1 cap at 200 mg for esophageal candidiasis (twice the dose on the first day in each case)
Alternative	Itraconazole	Itraconazole 1–2 cap. at 100 mg BID or Itraconazole suspension 10-20 ml BID (1 ml = 10 mg)
Prophylaxis		Not recommended

Prophylaxis

No survival benefit has been demonstrated for any Candida prophylaxis to date (McKinsey 1999, Rex 2000, Goldmann 2005). In a large randomized study, a reduction in oral candidiasis episodes as well as in invasive candidiasis was observed on long-term prophylaxis (Goldman 2005). The hypothesis that long-term prophylaxis will lead to the selection of resistant non-albicans strains (Vazquez 2001) was not confirmed in this study. Azole resistant infections were not seen more frequently in the long-term therapy group. Nonetheless, every immunocompromised patient should be screened for oral thrush at every visit. Chlorhexidine based gels and mouth rinses have a broad antimicrobial activity, with some antifungal properties.

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Tuberculosis (TB)

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Introduction

TB ranks among the leading causes of death worldwide. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from this disease, of whom 360,000 were HIV-positive (Figure 1, WHO 2014). TB-related deaths in people living with HIV have fallen by 33% in the last decade (UNAIDS 2014). Although it is recommended that every TB patient should be tested for HIV, only about 48% of TB patients had a documented HIV test globally. Mortality during treatment is more than three times higher among HIV-positive TB patients (11% versus 3.4%).

High rates of HIV and tuberculosis coinfection are found in Africa (e.g., Lesotho and Swaziland 74%) but also in some European countries (estimated numbers) like Latvia (20%), Portugal (18%) and Ukraine (16%) (ECDC 2015).

The TB epidemic is closely related to the HIV prevalence in the general population with an >8 times higher risk for the development of TB in HIV+ individuals (Corbett 2006). The incidence of TB has continuously declined in countries where ART is available (Liu 2015). Nevertheless, patients with advanced immunodeficiency remain at high risk of developing TB. Clinical management of coinfected patients is complicated due to a long duration of dual therapy, a wide range of drug-drug interactions, adverse events of medications and high demands on a patient's compliance due to a substantial pill burden.

Additional information can be found in recent reviews (Swaminathan 2010, Curran 2012, Dierberg 2013, Lawn 2013, Lee 2013).

Interaction of HIV and *M. tuberculosis*

HIV and *Mycobacterium tuberculosis* (MTB) infections have synergic influence on host immune regulation. HIV infection impairs cell-mediated immunity. In part, this occurs through depletion of CD4 T cells (Geldmacher 2012). Other hypotheses including functional T cell exhaustion due to chronic inflammation, HIV-mediated immunosenescence of T cells or downregulation of lysosomal autophagy have been proposed to unravel the molecular mechanisms of the immunological impairment seen in HIV-MTB coinfection (Shankar 2014). Even at early stages of HIV infection there is a higher susceptibility to MTB infection. While most opportunistic infec-
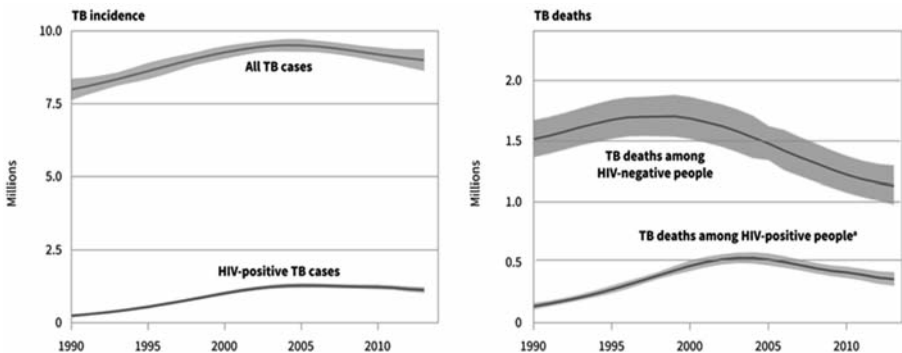
Figure 1: Global TB incidence and TB deaths (in millions per year) 1990-2013 (WHO 2014)

tions including non-tuberculosis mycobacterial infections (NTM) occur almost exclusively in advanced stages of HIV infection, TB is prevalent at any stage regardless of the CD4 T cell counts (Ackah 1995). A large part of pulmonary TB cases occurs in patients with more than 200 cells/µl (Badri 2001). However, the incidence of disseminated TB is much higher in patients with advanced immunodeficiency (Wood 2000).

The incidence rate ratio for TB in HIV+ persons versus uninfected persons has been estimated to range from 20.6 (95% confidence interval, 15.4–27.5) to 36.7 (11.6–116) (Getahun 2010). The risk of TB is already enhanced in the first year after HIV-seroconversion (Sonnenberg 2005). Low CD4 T cell counts, late presentation, low body mass index, anemia, ongoing viral replication and diabetes mellitus are known TB risk factors (Van Rie 2011, Ronacher 2015, Sester 2015). In turn, it is likely that TB enhances the immunodeficiency related to HIV infection (Toossi 2003).

Clinical manifestations

In the early stages of HIV infection the clinical symptoms of TB are similar to those in HIV-negative patients. Fever, fatigue, night sweats and weight loss are common.

Pulmonary TB: As in HIV-negative cases, typical lesions of pulmonary TB in HIV+ patients with more than 200 CD4 T cells/µl are upper lobe lung infiltrates (with or without cavities). Opacities are often present on chest radiography as well as enlarged mediastinal lymph nodes. As immunodeficiency progresses, atypical pulmonary presentations or TB pleuritis become more frequent. Bronchopulmonary symptoms are often absent when TB occurs in the advanced stages of HIV infection. Because CD4 T lymphocytes are required for granuloma formation their cellular structure changes with increasing immunodeficiency (Diedrich 2011). With the progression of immunodeficiency, hematogenous and lymphatic spread of mycobacteria is more common leading to miliary or disseminated TB or localized extrapulmonary TB (Elliott 1993).

Miliary or disseminated TB: Clinical manifestations depend on multiple small granular lesions (lat. milium effusum) and their localization. Lungs may be involved and micro-nodular opacities are evident on chest x-ray. On radiological criteria alone, these lesions cannot be distinguished from pulmonary cryptococcosis. Miliary dissemination of TB can also involve the abdomen. In febrile patients with abdominal pain and ascites, peritoneal TB must be included in the differential diagnosis.

Extrapulmonary TB occurs predominantly in patients with CD4 T cells below 200/µl, most commonly affecting the cervical lymph nodes (Schutz 2010). Lymph nodes are enlarged, hard and generally not painful on palpation. The formation of abscesses and draining fistulas as well as fever and malaise are common.

Tuberculosis meningitis often emerges with ambiguous prodromal symptoms such as headache, nausea and vomiting followed by elevated temperature and clinical signs of meningeal irritation. The basal meninges are usually involved and cranial palsies of the III and VI nerves are common. Mono-, hemi- or paraparesis as well as loss of consciousness and seizures can occur. In any patient with symptoms and signs of meningitis, a lumbar puncture should be performed without delay.

Other extrapulmonary localizations include pericarditis, osteoarthritis, the urogenital tract and the skin. Tuberculosis lesions may involve adrenal glands causing Addison's disease. Practically, any organ can be involved.

Diagnosis of active TB

The diagnosis is established based on clinical, radiological and microbiological findings. Diagnostic steps in the management of an HIV+ patient with suspected TB do not differ from those with HIV-negative cases (Lange 2004). The differential diagnosis includes other infections such as NTM (e.g., *M. avium complex*), aspergillosis, cryptococcosis, histoplasmosis, bacterial pneumonia and lung abscess but also sarcoidosis, lymphoproliferative diseases, in particular non-Hodgkin lymphoma, and solid malignant neoplasia.

Radiology: Radiographic images of pulmonary TB can vary substantially. Pulmonary TB can mimic a variety of other pulmonary diseases and can be present without evident changes on chest radiography. However, typical chest radiographic findings are ill-defined single or multiple opacities in the upper lobe, with or without cavities inside the opacities, and enlarged mediastinal lymph nodes. Calcifications and fibrotic scar formation may be either a sign of healed pulmonary TB or a clue of re-activated disease. In miliary TB, the chest radiography shows disseminated micronodular opacities. Patients with low CD4 T cell counts are less likely to present with typical radiographic changes but may have a normal chest X-ray, no cavities or a pleural effusion (Chamie 2010). In case of doubt, a chest CT scan is recommended whenever possible. If extrapulmonary TB is diagnosed, lung radiographic imaging as well as abdominal ultrasound should be performed to identify possible pulmonary disease, liver and spleen abscesses, thickening of the intestinal mucosa or ascites.

Respiratory samples: When pulmonary TB is suspected, 2-3 sputum samples should be collected on consecutive days for mycobacterial culture and direct sputum smear examination for acid-fast bacilli (AFB). Sputum quantity (>3-5 ml) and its origin from the lower respiratory tract are essential.

If patients are unable to cough deeply or cannot produce sputum, induced sputum should be provoked by 10–15 minutes inhalation of hypertonic sodium (3%) chloride. The collection of early morning gastric aspirate is an alternative if bronchoscopy is not available. The aspirate should be buffered in phosphate solution immediately. Bronchoscopy is indicated when the clinical findings remain highly suspicious for TB. Bronchial secretions or bronchoalveolar lavage obtained by bronchoscopy do not allow a more sensitive or specific diagnosis of TB than sputum smear in patients with HIV infection (Conde 2000). However, bronchoscopy is very helpful in the differential diagnosis of TB and other diseases particularly since co-existence of several pulmonary diseases is frequent in HIV+ patients (Narayanswami 2003). Furthermore, histopathological examination of transbronchial biopsies may show typical tuberculosis granulomas. On the day after bronchoscopy, sputum should be collected as the microscopic yield is higher following the intervention even if no mycobacteria is detected in lavage fluid.

Mycobacterial culture: Sputum and all other materials (including heparinised blood, urine, fluids, biopsies) should always be sent for culture that detects *M. tuberculosis* with a high sensitivity and specificity. The gold standard is culture identification of *M. tuberculosis* after incubation of biological samples preferentially in liquid or in solid media. Liquid media takes less time (2–4 weeks) than solid media (3-5 weeks) to obtain a positive result. A culture is only considered negative if no mycobacteria are identified after 6–8 weeks of incubation. Non-tuberculous mycobacteria (NTM) usually grow much faster and can often be identified within two weeks. All new clinical isolates of *M. tuberculosis* should undergo drug susceptibility testing for first-line and in case of MDR-TB for second-line antibiotics.

Microscopy: Sputum and all other biological materials should be evaluated for the presence of AFBs. The sensitivity of fluorescence microscopy (49%) is superior to conventional light microscopy (38%) (Cattamanchi 2009). At least 5,000–10,000 mycobacteria per slide are necessary to achieve a positive result in a routine setting. Approximately 50% of all patients with culture positive pulmonary TB have undetectable AFB on three consecutive sputum samples. AFB positive smears are present in approximately 5% of cases where pulmonary lesions are not visible on standard chest radiography (Ackah 1995). Specificity of direct sputum microscopy is poor. Discrimination between *M. tuberculosis* and other acid-fast bacteria is not possible by microscopy. A confirmatory test for MTB is required. The differential diagnosis includes infections with NTM, *Nocardia* spec. and *Rhodococcus* spec. Microscopy in HIV+ patients with more than 200 CD4 T cells/µl and typical radiographic changes has the same yield as in negative patients. With advanced immunodeficiency, the likelihood of an AFB positive smear decreases (Chamie 2010).

Biopsies of the lymph nodes, pleura, peritoneum, synovia and pericardium and diagnostic fluid aspirates from all anatomic compartments are suitable for AFB microscopy and histological examination for typical granulomas.

Nucleic acid amplification (NAAT): Mycobacterial nucleic acid can be detected in biological samples by a routine automated NAAT (e.g., Xpert® MTB-Rif, Cepheid – recommended by WHO). M. tuberculosis NAAT is faster than culture and more sensitive and specific than sputum smear microscopy. NAAT is especially helpful for differentiation of mycobacterial species when AFB are visible on microscopy. Under these circumstances, the sensitivity of MTB PCR is >95% (Boehme 2010). Unfortunately, the sensitivity decreases when smear negative morning sputa are analysed directly (Rachow 2011, Boehme 2010) or in paucibacillary disease (Jafari 2013, Threon 2011). The Xpert® MTB-Rif allows the detection of mutations in the rpoB gene resulting in rapid identification of rifampicin resistance as a proxy for MDR-TB (Boehme 2011). Because Xpert® MTB-Rif can yield false positive results for rifampicin resistance, especially in countries with low MDR-TB prevalence, reports should always be interpreted within the clinical context. Line probe assays (Hain-Lifescience, AID – recommended by WHO) allow rapid molecular detection of additional mutations in the genome of M. tuberculosis, e.g., in the katG, inhA, rrs, gyrA/B and emb genes, that correlate to phenotypic drug resistance. In the future, it will be possible to evaluate the whole bacterial genome for such mutations in order to tailor the initial choice of drugs in M/XDR-TB according to the results of molecular drug-resistance analysis (Walker 2015).

The diagnostic accuracy of *M. tuberculosis* NAAT in formalin fixed material is not known. For NAAT analysis biopsy samples should be preserved in normal saline or in "HOPE" (HEPES-glutamic acid buffer mediated organic solvent protection effect) media (Olert 2001), not in formalin.

Diagnosis of latent infection and preventive therapy

In the absence of active TB, a positive *M. tuberculosis* adaptive immune response identified by the tuberculin skin test (TST) or an interferon-γ release assay (IGRA) defines latent infection with *M. tuberculosis* (LTBI) (Mack 2009). The WHO suggest to screen all HIV+ persons by TST or IGRA and to provide preventive chemotherapy for all with a positive test result (Getahun 2015). Neither TST nor IGRA can distinguish latent infection from active or past disease. Immunodiagnostic testing is part of the TB prevention strategy. Once active TB has been ruled out (as much as possible) TST and IGRAs should be used to identify individuals with the highest risk for future disease development among individuals from TB risk groups.

As positive TST results may also be found in individuals who were BCG-vaccinated or who had contact with NTMs, IGRAs are more specific for the diagnosis of LTBI than the TST. Some authors have suggested that the T-Spot.TB® test results are less dependent on the level of CD4 T cells (Rangaka 2007a, Hammond 2008, Stephan 2008), while the IFN-γ responses in the QuantiFERON®-TB Gold In-Tube Test strongly correlate to the CD4 cell count (Leidl 2009).

Better specificity does not automatically translate into a higher positive predictive value for disease development. Only a few studies have evaluated TB progression rates in relation to TST and IGRA testing in HIV-infected patients (Aichelburg 2009, Sester 2014). According to a recent study from Europe, HIV+ patients with a viral load >50 copies/ml have the highest risk for progression to TB among immuno-compromised hosts (Sester 2014).

Disease progression can be effectively prevented by chemotherapy (Bucher 1999, Elzi 2007, Sester 2014). In HIV+ persons with ongoing viral replication from Western Europe the number needed to treat to prevent one case of TB was less than 10 when TST was used for screening. A 6-month prophylactic course of isoniazid (INH) reduced the incidence of TB among HIV+ subjects from about 11.5 to 4.9 per 100 person years (Grant 2005, Charalambous 2010). Therefore, a 6–9 month course of INH (300 mg daily) and pyridoxine is usually recommended for the treatment of LTBI in low incidence countries. A treatment regimen consisting of rifapentine 600 mg and INH 900 mg once weekly for 12 weeks was shown to be non-inferior to a 9 month daily treatment regimen, but few HIV+ individuals were included in this trial (Sterling 2011). In high burden TB countries INH preventive chemotherapy was shown to be more effective when administered for 36 months vs. 6 months in TST positive individuals with HIV infection (Samandari 2011). However, the effect wears off when preventive treatment is discontinued (Churchyard 2014).

Infection control

Most patients develop disease after recent transmission, emphasising the need for patient-to-patient infection control measures (Horsburgh 2010, Houben 2011, Sonnenberg 2001). Isolation is generally indicated to prevent the spread of the infection. Effective treatment according to the drug resistance profile of the TB case seems most relevant to control infectiousness also in HIV+ TB patients (Escombe 2008). Recent evidence suggests that effectively treated, although smear and culture-positive, cases might not be infectious any more (Dharmadhikari 2014). Until further evidence is gathered, culture negativity seems the safest marker of non-infectiousness for drug-susceptible and drug-resistant cases. In pulmonary TB sputum should be regularly collected (weekly in the initial phase, later monthly), evaluated for AFB by direct microscopy and for viable *M. tuberculosis* by culture until the end of treatment (Lange 2014).

TB therapy

Drug susceptible TB is treated with the first-line drugs rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB). INH and especially RIF are the most potent first-line drugs. Streptomycin (SM) is only rarely used, e.g., in some countries recommended as part of a TB-meningitis treatment regimen. To prevent the development of drug resistance, active TB should always be treated with a combination of four drugs in the initial phase. The standard therapy consists of a 2-month course of daily RIF, INH, EMB and PZA, followed by a continuation phase of 4 months with daily RIF and INH. The four drugs of the initial treatment phase

should be administered until a culture-based drug susceptibility result of *M. tuberculosis* isolates is available. Anti-tuberculosis drug doses, side effects and drug interactions are shown in Table 1.

Table 1: Anti-tuberculosis drug doses, side effects and drug interactions

Drug	Recommended daily dose	Common adverse events	Drug interactions and comments
Rifampicin (RIF) Also available as IV injection	10 mg/kg >50 kg: 600 mg <50 kg: 450 mg	Toxic hepatitis; allergy, fever; gastrointestinal disorders; discoloration (orange) of body fluids; thrombopenia	Many drug interactions: (see Drug Chapter), liver monitoring, safe up to 35 mg/kg (Boeree 2015)
Isoniazid (INH) Also available as IV or IM injection	5 mg/kg maximum 300 mg/day Administered with vitamin B6	Peripheral neuropathy; elevated liver enzymes, toxic hepatitis; CNS side effects: psychosis, seizures	Avoid d4T, ddI Avoid INH if pre-existing liver damage; avoid
Ethambutol (EMB) Also available as IV injection	15 mg/kg (15–20 mg/kg)	Optic neuritis (contra-indicated in case of pre-existing lesions of optic nerve); peripheral neuropathy (rare)	Baseline/monthly screen for visual acuity and colour perception Antacids decrease absorption
Pyrazinamide (PZA)	25 mg/kg (20–30 mg/kg)	Arthralgia, hyperuricemia, toxic hepatitis, gastro-intestinal discomfort	Hyperuricemia: uricosuric drug (allopurinol); monitor LFTs
Streptomycin (S) IV/IM administration only	15 mg/kg maximum cumula-tive dose 50 g!	Auditory and vestibular nerve damage; renal damage; allergies, nausea, skin rash, pancytopenia	Audiometry; monitor renal function; do not use in pregnancy
Amikacin (Am) IV/IM administration only	15 mg/kg	Auditory and vestibular nerve damage	Audiometry; monitor renal function; do not use in pregnancy
Capreomycin (CM) IV/IM administration only	15 mg/kg max 1 g/day	Renal damage, Bartter-like syndrome, auditory nerve damage	Audiometry; monitor renal function; do not use in pregnancy
Cycloserine (CS)	10–15 mg/kg maximum 1,000 mg/day	CNS disorders (within the first 2 weeks): anxiety, confusion, dizziness, psychosis, seizures, headache	Aggravates CNS side effects of INH and prothionamide Contraindicated in epileptics
Levofloxacin (LFX) Also available as IV injection	500 or 1,000 mg/day	Gastrointestinal discomfort, CNS disorders, tendon rupture (rare) QT prolongation	Not approved in children; (in adults use moxifloxacin first)
Linezolid (LZD) Also available as IV injection	600 mg /day	Thrombopenia, anemia, CNS disorders	Expensive, optimal dose to be defined

Table 1: (continued)

Drug	Recommended daily dose	Common adverse events	Drug interactions and comments
Moxifloxacin (MFX) Also available as IV injection	400 mg/ day	Gastrointestinal discomfort, headache, dizziness, hallucinations QT prolongation	Similar activity as levofloxacin
Prothionamide (PTO)	0.75–1 g/day	CNS disorders; liver damage, gastrointestinal discomfort	Slowly increase dosage; monitor LFTs
Rifabutin (RFB)	300 mg/day	Gastrointestinal discomfort; discoloration (orange or brown) of body fluids; uveitis; elevated liver enzymes; arthralgia	Weaker CYP450 inducer than RIF (see text), thus preferred in patients on ARVs (see table 2), monitor liver
Bedaquiline (BDQ)	400 mg QD for 2 weeks, then 200 mg 3 x /week for 22 weeks	Gastrointestinal symptoms, arthralgia, headaches, QT-prolongation (increased mortality! Diacon 2014)	Rifampicin halves plasma concentration, EFV reduces plasma level, drugs with QT prolongation should be avoided ECG at 2,4,8, 12 and 24 weeks, LFT monthly
Delamanid (DLM)	100 mg BID for 24 weeks	Nausea, vomiting, dizziness, QT prolongation	Avoid strong C3A4 inducers, drugs with QT-prolongation. ECG at 2, 4, 8, 12 and 24 weeks
Clofazimine (CFZ)	100–200 mg/d	Red skin discoloration, dry skin, pruritus, gastro-intestinal intolerance, photosensitivity, QT prolongation	Avoid use with QT-prolonging drugs Avoid direct sunlight
Meropenem/ Clavulanic acid (MPM; AMX/CLV)	1000 mg TDS IV plus 125 mg clavulanic acid (amoxicillin/ clavulanic acid)	Nausea, vomiting diarrhea	Only active in combination. Very limited data

WHO recommends that HIV+ TB patients should receive at least the same duration of TB treatment as HIV-negative TB patients. Data from a systematic review and meta-analysis suggest that a minimum of 8 months duration of rifamycin (rifampicin or rifabutin) therapy and concurrent ART might be associated with better outcomes (Khan 2010). When there is initially a high bacterial load, and sputum smear conversion is not achieved by two months of therapy or when PZA is not part of the induction regimen, continuation phase treatment with RIF and INH should be prolonged for a total treatment of 9 months (BHIVA 2012).

Treatment of MDR-TB

Bacillary resistance to RIF and INH is defined as multidrug-resistance (MDR) although mono-resistance to RIF is *de facto* comparable to MDR-TB. Patients with MDR-TB can be successfully treated with second-line drugs. Second-line injectable drugs (SLID) are amikacin, capreomycin and kanamycin. Second-line oral drugs include levofloxacin, moxifloxacin, prothionamide, cycloserine, para-aminosalicylic acid. When there is bacillary resistance to any fluoroquinolone or any SLID, the resistance level is termed extensively drug-resistant (XDR). In advanced MDR-TB or XDR-TB alternative drugs like clofazimine, linezolid and/or meropenem in combination with amoxicillin/clavulanic acid are frequently included in a drug regimen. Treatment of M/XDR-TB should always be guided by an experienced physician (Lange 2014). Drug resistant tuberculosis should be treated with at least four drugs to which the bacilli are susceptible by drug testing. WHO recommends including an SLID in the first 8 months of treatment. The total duration of MDR-TB treatment recommended by the WHO is 20 months (WHO 2011). Treatment recommendations are currently based on a retrospective cohort analysis (Ahuja 2011) and are likely inadequate for individual patients (Heyckendorf 2014). Clinical trials with new and shorter regimens including HIV+ patients are ongoing.

Since 2013, two new TB-specific drugs have been introduced into the market. The diarylquinoline bedaquiline (Situro®, FDA/EMA conditional approval), and the nitroimidazole delamanid (Deltyba®, EMA conditional approval) are recommended for the treatment of MDR-TB, if an adequate regimen cannot be otherwise put together. Clinical data on the combined use of bedaquiline and delamanid are not yet available.

Timing of ART and TB therapy

Optimal timing of ART in HIV/TB patients was investigated in three randomized clinical trials, namely SAPIT, CAMELIA and STRIDE (Abdool Karim 2011, Blanc 2011, Havlir 2011). Patients with particularly low CD4 T cells benefit from early ART initiation. It is recommended to start ART in patients with CD4 T cells below 100 cells/µl latest after two weeks of TB treatment. At higher CD4 T cells, ART can be initiated between 8 and 12 weeks post-start of TB treatment (EACS 2014). US guidelines recommend that ART should be initiated between 2–4 weeks in patients who have a CD4 T cell count of 50-200 cells/µl and evidence of clinical disease of major severity, low Karnofsky score, low BMI, low hemoglobin, low albumin, or organ system dysfunction (OARAC 2015). A recent trial suggests that ART can be delayed until 6 months after TB treatment initiation in patients with CD4 T cells above 200 cells/µl (Mfinanga 2014). Another recent trial tested empiric TB treatment versus IPT in patients with advanced HIV (median CD4 T cell counts 18 cells/µl) in high incidence settings. There was no difference in mortality after 24 weeks (Hosseinipour 2015).

In cases of drug-resistant TB, controlled data on ART initiation are not available. According to expert opinion, ART should be started 2–4 weeks post TB treatment initiation (OARAC 2015). Pregnant women should also start ART as soon as possible in order to prevent mother-to-child transmission.

Drug interactions and ART regimen during TB treatment

There are many pharmacological interactions between ART and anti-tuberculosis drugs. The enzyme induction of cytochrome P450 3A4/5 by RIF requires particular attention. As rifampicin and PIs are both metabolized by cytochrome P450 3A, concomitant therapy is generally not recommended (OARAC 2015, EACS 2014). In low resource settings, rifampicin can be combined with double dose lopinavir/r or super-boosted ritonavir (400 mg BID) plus lopinavir, if there is no better alternative.

The preferred antiretroviral regimen is efavirenz (<60kg: 600 mg QD; >60kg 800 mg QD) in combination with TDF+FTC or ABC+3TC or raltegravir in combination with TDF+FTC while the patient is receiving rifampicin. In high resource settings, HIV drug resistance testing is generally recommended before initiation (EACS 2014). Drug dosages of ARVs when coadministered with rifampicin are listed in Table 2.

Table 2: Recommendations for coadministering ART with rifampicin*

Drug	ARVs dosage adjustment	Comment
Lopinavir/r	800/200 mg BID (double dose) or 400/400 mg BID (super-boosting)	Can be used if no other alternative available (low resource settings), hepatotoxicity, GI intolerance
Other boosted PIs	No coadministration	
Efavirenz	600 mg (<60 kg weight) or 800 mg (>60 kg weight) QD	Recommended for coadministration with rifampicin
Nevirapine	200 mg BID	
Other NNRTIs	No coadministration	
Maraviroc	No coadministration	If required: use maraviroc 600 mg BID
Raltegravir	400 or 800 mg BID	RAL levels decrease by 61% (TDM), further evidence on dosing required (see text)
Elvitegravir	No coadministration	
Dolutegravir	50 mg BID	
NRTIs	Standard dose	Triple NRTI therapy not recommended

*EACS 2014, OARAC 2012, CDC 2013 (modified). Comment: There is no recommendation for any dose adjustment for rifampicin

In contrast to rifampicin, dose-adjusted rifabutin can be coadministered with boosted PIs. One trial reported increased rates of neutropenia when combined with atazanavir/r (Table 4) (Zhang 2011). The recommended dosage is 150 mg rifabutin 3x/week (EACS 2014), while recent data suggest under-dosing of rifabutin with this regime. US guidelines recommend daily rifabutin 150 mg with monitoring for neutropenia and uveitis (OARAC 2015).

Efavirenz (standard dose) can also be combined with rifabutin (450 mg once daily), which has less cytochrome P450 3A-inducing potential (EACS 2014). Nevirapine, rilpivirine and etravirine are not recommended in combination with rifamycins.

Raltegravir, metabolized via the UDP-glucuronosyltransferase, is a good and safe alternative. Raltegravir 400 mg BID and 800 mg BID proved to be a safe and efficacious treatment option for HIV-TB coinfection in a small Phase II study, the best available evidence for its use with rifampicin pending a Phase III trial (Grinszteijn 2014).

A combination of 3–4 NRTIs (AZT, ABC, 3TC ± TDF) could represent a short-term option for patients with viral load <100,000 copies/ml until TB treatment with RIF is completed. With rare exceptions, other regimens may include T-20 as it has no interactions with rifamycins (Boyd 2003).

There are limited data about the combination of rifampicin and some other antiretroviral agents like elvitegravir, cobicistat, rilpivirine, etravirine, tipranavir and maraviroc. Maraviroc should only be given under close observation. No significant interactions were reported with tenofovir (Droste 2005). A Phase I pharmacokinetic study in healthy volunteers suggested that dolutegravir should be dosed twice daily (50 mg BID) in combination with rifampicin (600 mg QD), while the standard dose (50 mg QD) can be combined with rifabutin (300 mg QD) (Dooley 2013).

Bedaquiline use with CYP3A4 inducers and inhibitors is not recommended (van

Heeswijk 2014). Clinical data on interaction of delamanid with antiretroviral drugs are not yet available.

Adherence to therapy is difficult due to the large number of ART and anti-tuberculosis drugs administered simultaneously and their overlapping toxicities. The most decisive determinant for the success of TB treatment is good drug adherence for the entire duration of therapy. When compliance is impaired, the development of drug resistance and relapses are common. Therefore, WHO recommends that all patients with TB should be enrolled in directly observed therapy programs.

Immune reconstitution inflammatory syndrome (IRIS)

A critical question is the timing of ART initiation in coinfected patients as the timing of ART is closely related to the risk of occurrence of TB associated immune reconstitution inflammatory syndrome (IRIS). TB-associated IRIS has been reported to occur on average in 15% of severely immunocompromised patients although incidence data are highly variable (Müller 2010).

In paradoxical TB associated IRIS patients are diagnosed with active TB and initially show a positive treatment response. However, within three months of initiation of ART there is clinical worsening (i.e., lymphadenopathy, infiltrates, effusions, CNS symptoms). Other causes of clinical worsening (e.g., drug resistance, drug toxicity, other opportunistic infections or poor adherence) must be excluded (Meintjes 2008). It has been suggested that an acute exacerbation of a TH1 immune response against mycobacterial antigens is responsible for the paradoxical reaction in ART experienced HIV/MTB coinfected patients (Bourgarit 2006).

In the so-called unmasking TB-IRIS active TB is not diagnosed at the initiation of ART, but is diagnosed within 3 months of initiation (Meintjes 2008). It is thought that the recovery of pathogen-specific immune responses during the initial months of ART trigger the unmasking of a subclinical disease. Screening strategies for underlying TB need to be carefully emphasized in order to prevent severe unmasking manifestations.

The only randomized controlled trial for the management of paradoxical TB associated IRIS used 1.5 mg/kg prednisolone for 2 weeks and 0.75 mg/kg for a further 2 weeks. It showed a significant reduction in paradoxical IRIS-related hospital days and outpatient procedures (Meintjes 2010). During TB-associated IRIS, both ART and TB therapy should be continued (OARAC 2015). Two trials investigating meloxicam and prednisolone for the prevention of TB-IRIS are ongoing. As in patients with TB meningitis and HIV infection the risk of IRIS-related mortality is high, and it is recommended to start ART in TB meningitis patients 8 weeks after starting the TB therapy (Török 2011).

Adverse events

The most frequent and significant adverse events of TB drugs are listed in Table 1. INH should routinely be coadministered with prophylactic pyridoxine (vitamin B6) to prevent peripheral polyneuropathy.

Before and during therapy with EMB, colour vision should be examined and monitored as this drug may affect the optic nerve. Dosages of EMB and PZA need to be adjusted in patients with impaired renal function. Drug-induced liver injury (DILI) is a common problem in the management of HIV/TB coinfection. A consensus statement of the South African HIV Society gives helpful advice in the management of DILI (Jong 2013).

Monthly audiometric monitoring should be performed when streptomycin or second-line injectables are used. Following the start of TB therapy, liver enzymes,

serum creatinine, electrolytes and full blood count should be monitored on a regular basis (e.g., in the initial phase every week, then every 4 weeks). Hyperuricemia is common when PZA is used. A mild polyarthralgia can be treated with allopurinol and non-steroidal antiphlogistic drugs. Arthralgia can also be induced by RIF and rifabutin. Therapy of drug resistant TB with nephrotoxic injectables (amikacin, kanamycin, capreomycin, streptomycin) in combination with tenofovir should be avoided (Kenyon 2011).

Data on use of bedaquiline and delamanid in patients on ART are rare. Both lead to QTc interval prolongation. QTc interval monitoring is essential for both drugs, particular in combination with flouroquinolones, clofazime and clarithromycin (WHO 2013, WHO 2014b).

Patients who exhibit severe adverse events should always be hospitalized for diagnosis and treatment. Drugs thought to be responsible for a given adverse event ought to be discontinued. If visual disturbance occurs on EMB, renal failure or shock or thrombocytopenia on RIF and vestibular dysfunction on SM/SLID therapy, re-exposure to these agents must be avoided. Other drugs can be reintroduced one by one when symptoms resolve, beginning with the drug that is least likely to cause the adverse event. All drugs should be restarted at low doses and doses should be increased stepwise (Table 3). When no adverse effects occur after 3 days, additional drugs can be added. The drug that is most likely to be responsible for an adverse effect should be the last one to be restarted if no alternative is available. When second-line drugs are used it is usually necessary to prolong the standard treatment duration (WHO 2014c).

Table 3: Re-introduction of TB drugs following drug-related adverse event(s)

Drug	Day 1	Day 2	Day 3
INH	50 mg	300 mg	5 mg/kg/day (max 300 mg/day)
RIF	75 mg	300 mg	10 mg/kg/day (max 600 mg/day)
PZA	250 mg	1,000 mg	25 mg/kg/day (max 2 g/day)
EMB	100 mg	500 mg	25 mg/kg/day for 2 months then 15 mg/kg/day
Streptomycin	125 mg	500 mg	15 mg/kg/day (max 1 g/day)

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Atypical mycobacteriosis

Atypical mycobacterioses are usually synonymous with infections with *Mycobacterium avium complex* (MAC). Although MAC is by far the most frequent pathogen, numerous other atypical mycobacterioses exist that cause a similar disease pattern, such as *M. celatum*, *M. kansasii*, *M. xenopi* or *M. genavense*. MAC bacteria are ubiquitous and can be found in diverse animal species, on land, in water and in food. Exposure prophylaxis is therefore not possible. Consequently, isolation of infected patients is not necessary. While MAC may be detectable in the sputum or stool of asymptomatic patients (colonization), only patients with massive immunodeficiency and less than 50 CD4 T cells/µl develop disease (Horsburgh 1999). This used to include up to 40% of AIDS patients in the pre-HAART era (Nightingale 1992).

The infection has now become very rare in industrialized countries (Karakousis 2004). However, it remains important, as it has developed into a completely new disease in the ART era. It previously occurred mainly with a chronic, disseminated course of disease, often in patients with wasting syndrome. MAC infections under ART are now almost always localized and related to immune reconstitution inflammatory syndrome (IRIS). The disease now occurs with manifestations that were previously never seen (see below).

Signs and symptoms

The symptoms of *disseminated* MAC infection are unspecific. When the CD4 count is less than 100 cells/µl, fever, weight loss and diarrhea should always lead to consideration of atypical mycobacteriosis. Abdominal pain may also occur. As described above, disseminated MAC infection has now become rare.

Localized forms of atypical mycobacterioses are far more frequent. These include, above all, lymph node abscesses, which may occur practically everywhere. We have seen abscesses in cervical, inguinal and also abdominal lymph nodes, some of which developed fistulae and resolved only slowly even after surgical intervention. Any abscess appearing whilst on ART (with severe immunosuppression) is highly indicative of MAC! In addition to skin lesions, localized forms include osteomyelitis, particularly of the vertebrae, and septic arthritis (observed: knee, hand, fingers).

Diagnosis

Diagnosis of the disseminated form is difficult. Blood cultures (heparinized blood) should always be sent to a reference laboratory. Although atypical mycobacteria usually grow more rapidly than TB bacteria, the culture and differentiation from TB may take weeks. In cases presenting with anemia, bone marrow aspiration is often successful. If atypical mycobacteria are detected in the stool, sputum or even bronchoalveolar lavage BAL, it is often difficult to distinguish between infection requiring treatment and mere colonization. In such cases, treatment should not be initiated if general symptoms are absent. This is also true for *Mycobacterium kansasii* (Kerbiriou 2003).

Laboratory evaluations typically show elevated alkaline phosphatase (AP) – a raised AP in severely immunosuppressed patients should make us think of MAC. Similarly, MAC infection should be considered in any cases of anemia and constitutional symptoms. Cytopenia, particularly anemia, often indicates bone marrow involvement. Ultrasound reveals enlargement of the liver and spleen. Lymph nodes are often enlarged, but become apparent due to their number rather than their size (Gordin 1997). Here, differential diagnoses should always include TB or malignant lymphoma.

Direct specimens should always be obtained for localized forms, as identification of the organism from material drained from the abscess is usually successful.

Treatment

Treatment of MAC infection detected from culture is complex. Similar to TB, monotherapy does not suffice. Since 1996, many clinicians prefer the combination of a macrolide (clarithromycin or azithromycin) with ethambutol and rifabutin (Shafran 1996). In the past, this treatment was given lifelong; today it is generally considered sufficient to treat for at least six months and until a ART-induced increase in the CD4 T cell count to above 100 cells/µl has been achieved. After publication of data indicating that rifabutin may be omitted from the regimen (Dunne 2000), the multicenter randomized ACTG 223 study demonstrated a survival benefit with the triple combination C+R+E compared to C+E and C+R – mortality rates were halved in the triple combination arm (Benson 2003).

Due to the high potential for interactions, rifabutin can be discontinued after several weeks when clinical improvement is observed. The clarithromycin dose should not exceed 500 mg BID. In at least two randomized studies, there was a significantly higher number of deaths in the treatment arms with a higher clarithromycin dose, for reasons that remain unclear (Chaisson 1994, Cohn 1999). Instead of clarithromycin, azithromycin can also be given, which is cheaper and interacts less with cytochrome P450 enzymes. Azithromycin and clarithromycin have comparable efficacy in combination with ethambutol (Ward 1998).

In disseminated illnesses, treatment should be monitored through regular blood cultures. Cultures must be negative by eight weeks at the latest. In the localized form, the response can be assessed better clinically. Every MAC therapy has a high potential for side effects and drug interactions. Concomitant medications, including ART, should be carefully examined – dose adjustments are frequently required and there may be contraindications (see Drugs section).

Reserve drugs such as amikacin, quinolones or clofazimine are only required in rare cases today. It is important to perform resistance testing for all atypical mycobacterial infections with species other than *M. avium complex*.

We have generally stopped treatment of localized MAC infections when the abscess has healed – which usually takes several months. In individual cases, steroids may be helpful temporarily. However, there are no specific guidelines for treatment of local MAC infections.

Prophylaxis

In the US, large placebo-controlled trials have shown that the macrolides, clarithromycin and azithromycin, as well as rifabutin, significantly reduce MAC morbidity and mortality when used for primary prophylaxis in severely immunocompromised patients (Havlir 1996, Nightingale 1992, Pierce 1996, Oldfield 1998). Prophylaxis also saves costs (Sendi 1999). However, MAC infections are more rare in Europe. As a result, and because of concerns over compliance and development of resistance, few patients in Europe receive primary MAC prophylaxis (Lundgren 1997).

For patients failing currently available ART regimens and without new treatment options, prophylaxis with a macrolide can be considered at low CD4 T cell counts (<50 cells/µl). Weekly dosing with azithromycin is convenient for patients and has comparable efficacy to daily rifabutin (Havlir 1996). According to a recent meta-analysis, azithromycin or clarithromycin appeared to be the prophylactic agent of choice for MAC infection (Uthman 2013).

Primary prophylaxis and maintenance therapies can be discontinued quite safely at CD4 T cell counts above 100/µl (Currier 2000, El Sadr 2000, Shafran 2002, Aberg 2003). It is possible that even partial viral suppression suffices for MAC-specific immune reconstitution (Havlir 2000). Complete recovery as a result of immune reconstitution is possible (Aberg 1998).

Treatment/prophylaxis of MAC
(daily doses, if not specified otherwise)

Acute therapy		
Treatment of choice	Clarithromycin + ethambutol + possibly rifabutin	Clarithromycin 1 tab. at 500 mg BID **plus** ethambutol 3 tab. at 400 mg QD **plus** rifabutin 2 tab. at 150 mg QD
Alternative	Azithromycin + ethambutol + possibly rifabutin	Azithromycin 1 tab. at 600 mg QD **plus** ethambutol 3 tab. at 400 mg QD **plus** rifabutin 2 tab. at 150 mg QD
Maintenance therapy		As for acute therapy, but without rifabutin Discontinue if >100 CD4 T cells/µl >6 months
Primary prophylaxis		Consider for CD4 cells remaining below 50/µl Discontinue if >100 CD4 T cells /µl >3 months
Treatment of choice	Azithromycin	Azithromycin 2 tab. at 600 mg/week
Alternative	Clarithromycin	Clarithromycin 1 tab. at 500 mg BID

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Herpes simplex

Infections with herpes simplex viruses are a frequent problem for HIV+ patients (Chang 1995). Chronic disease is frequent, particularly with severe immunodeficiency (below 100 CD4 T cells/µl). Two viruses should be distinguished.

HSV-1 is transmitted by direct contact with mucosal membranes such as kissing, and causes the typical, itchy perioral blisters on the lips, tongue, gums, or buccal mucosa.
HSV-2 is sexually transmitted and leads to lesions on the penis, vagina, vulva and anus. HSV-2–associated lesions significantly increase the risk of HIV transmission (Freeman 2006, see Prevention).

In severe cases, other organs may be affected. These include mainly the esophagus (ulcers), CNS (encephalitis), eyes (keratitis, keratoconjunctivitis, uveitis) and respiratory tract (pneumonitis, bronchitis). In such cases and with persistence of lesions for a period of more than four weeks, herpes simplex infection is an AIDS-defining illness.

Signs and symptoms

The typical blisters itch and burn. Oral involvement may impair food intake. In cases of genital or anal herpes (proctitis), urination and defecation can be very painful. Extensive lesions may occur with severe immunosuppression. Regional lymph nodes are often enlarged. The clinical symptoms of disseminated disease depend on the organs affected.

Diagnosis

Diagnosis of oral, genital or perianal herpes can often be made clinically. If there is doubt, then swabs should be taken, placed in viral culture media, and quickly transported to the laboratory. The diagnosis of organ manifestations usually requires histology. Diagnosis is particularly difficult for HSV encephalitis, as cerebrospinal fluid often does not help. Serologies are only useful if they are negative, therefore making HSV infection improbable.

Treatment

Every treatment, whether topical, oral or systemic, is more effective when started early. For patients with a good immune status and only discrete lesions, topical treatment with acyclovir cream or ointment is adequate. Penciclovir cream is probably as effective as acyclovir (Chen 2000) and allegedly less irritant, although significantly more expensive.

The nucleoside analog acyclovir remains the treatment of choice for systemic treatment. Acyclovir inhibits the DNA polymerase of herpes viruses. Resistance is rare, despite the fact that this agent has been used since 1977 and numerous generics are now available (Levin 2004). Acyclovir is usually well tolerated and effective against both HSV-1 and HSV-2. Severe cases with mucocutaneous or organ involvement should be treated immediately intravenously. As CNS levels are lower than in plasma, the dose should be increased to treat encephalitis. If acyclovir is to be given intravenously, renal blood values should be checked.

Valacyclovir and famcyclovir are equally effective alternatives to acyclovir (Ormrod 2000, Conant 2002), though substantially more expensive. The main advantage is their improved oral bioavailability; they require less frequent dosages. In cases of recurrent genital herpes lesions shorter therapeutic regimens (i.e., two days of famciclovir) may be as effective as standard 5-day courses (Vinh 2006, Bodsworth 2008). Brivudine remains a good alternative for HSV-1 and HZV (zoster). However, it is pos-

sible that this dihydropyrimidine dehydrogenase inhibitor causes mitotoxicity and reduces the efficacy of HIV drugs (U. Walker 2005, personal communication). Foscarnet should only be used in exceptional cases due to its toxicity. However, it may be helpful in extensive, refractory cases.

Newer drugs such as pritelivir, that do not inhibit DNA polymerase but rather helicase, another herpes virus enzyme, have been effective in clinical trials (Tyring 2011, Wald 2014). However, additional studies are warranted to define the potential of helicase inhibitors.

A local anesthetic that can be produced by the pharmacist can be prescribed in addition for painful mucocutaneous lesions. Unfortunately, the approved tetracaine solution (Herviros™) has been taken off the market. Some pharmacists can, however, confect something similar in-house.

Prophylaxis

Primary prophylaxis is not recommended. However, a meta-analysis of almost 2000 patients in eight randomized studies showed that acyclovir can reduce the risk of both HSV and HZV disease by more than 70%. Even mortality was reduced by 22% (Ioannidis 1998). The introduction of ART has changed the relevance of this data. Nevertheless, it can still make sense, even today, to treat persistent recurrences with long-term low-dose acyclovir or valacyclovir (DeJesus 2003, Warren 2004). However, short bursts of subclinical genital HSV reactivation are frequent, even during high-dose acyclovir therapy (Johnston 2012). Herpes simplex vaccines are still in early stages of development (Belshe 2012).

Treatment/prophylaxis of HSV infection (daily doses)

Acute therapy		Duration: 7–14 days
Treatment of choice	Acyclovir	Acyclovir 1 tab. at 400 mg 5x/day
Severe cases		Acyclovir ½–1 amp. at 500 mg TID (5–10 mg/kg tid) IV
Alternatives	Valacyclovir	Valacyclovir 2 tab. at 500 mg TID
Alternatives	Famciclovir	Famciclovir 1 tab. at 250 mg TID
Alternatives	Brivudin	Brivudin 1 tab. at 125 mg QD
Prophylaxis		Not recommended

Interactions between HIV and herpes simplex

Prevalent HSV-2 infection is associated with a three-fold increased risk of HIV transmission among both men and women (Freeman 2006, see the Prevention section in the *ART* chapter). Large randomized studies demonstrated that during anti-HSV therapy, HIV replication is also inhibited. During treatment with acyclovir, HIV plasma viremia is decreased by 0.33 log (Ludema 2011). High dose valacyclovir resulted in a slightly greater reduction of HIV replication (Mugwanya 2011, Perti 2013). Even the rate of disease progression can be reduced. In a large randomized trial in Uganda, acyclovir showed a significant clinical benefit, with the greatest effect in individuals with a high baseline viral load (Reynolds 2012).

Despite the fact that acyclovir does not prevent the transmission of HIV (Celum 2008+2010, Watson-Jones 2008), these results have recently revived the interest in acyclovir therapy (Vanpouille 2009). This old drug has become interesting again. Possibly new derivatives will be developed that are better tolerated and more effective in terms of HIV antiviral potency.

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Herpes zoster

Herpes zoster is the reactivation of an earlier infection with varicella virus, which subsequently maintains a lifelong residence in the spinal ganglia. Herpes zoster episodes can occur even in HIV+ patients with relatively good immune status, and are also seen during immune reconstitution (Martinez 1998). Given the still high incidence of zoster episodes in HIV+ patients, herpes zoster can be regarded as an indicator disease for HIV infection (Søgaard 2012, Moanna 2013). With more advanced immunodeficiency, herpes zoster tends to become generalized. In addition to involvement of one or more dermatomes, dangerous involvement of the eye (affecting the ophthalmic branch of the trigeminal nerve, "herpes zoster ophthalmicus", with corneal involvement) and ear (herpes zoster oticus) may occur. Most feared is involvement of the retina with necrotizing retinitis. The neurological complications include meningoencephalitis, myelitis and also involvement of other cranial nerves (Brown 2001).

Signs and symptoms

There are often prodromal signs with headache, malaise, and photophobia, accompanied only rarely by fever. The affected areas are initially hypersensitive, and then become pruritic and/or painful within hours or days. Pain can precede lesions by several days. Lesions often show segmental, yet always unilateral, erythema with herpetiform blisters within one or more dermatomes. Lesions ulcerate, are often hemorrhagic, and gradually dry up. They should be kept dry and clean to avoid bacterial superinfection. Involvement of several dermatomes often leaves treatment-resistant pain syndromes with zoster neuralgia. Post-herpetic neuralgia can be assumed if pain persists for more than a month (Gnann 2002).

Diagnosis

Cutaneous involvement usually allows clinical diagnosis of herpes zoster. However, diagnosis may be difficult especially on the extremities and in complicated zoster cases. Typical cases do not require further diagnostic tests. If there is uncertainty, a swab may be taken from a blister and sent to the laboratory in viral culture media. An immunofluorescence assay is presumably more reliable. HZV encephalitis is only detectable through analysis of CSF by PCR. Herpes zoster oticus should be considered in cases of unilateral, peracute hearing loss, which is not always visible from the outside. Either examine the ear yourself or consult an ENT specialist! For visual impairment the same rules apply as for CMV retinitis – refer the patient to the ophthalmologist as quickly as possible.

Treatment

Monosegmental zoster can be treated on an outpatient basis with oral acyclovir. Rapid initiation of treatment is important. Systemic therapy is always necessary, and doses are higher than for HSV. Lesions dry up more rapidly if calamine lotion is used, which also relieves pain. Gloves should be worn, given that the lesions are highly infectious initially. Likewise, unvaccinated individuals without a history of chickenpox should not come into close contact with a case of herpes zoster.

Analgesics (novaminsulfone, or better still tramadole) should be given generously. Any complicated, multi-segmental or facial herpes zoster should always be treated with intravenous therapy.

As with HSV, several alternatives for treatment include valacyclovir, famcyclovir and brivudine (see HSV). There is still controversy if the unpleasant post-herpetic neuralgia allegedly occurs less frequently under these drugs than under acyclovir (Li

2009, McDonald 2011). Valacyclovir, famcyclovir and brivudine have not been tested widely in HIV+ patients, and are not licensed for treatment of immunocompromised patients. They are also substantially more expensive than the numerous acyclovir formulations. Acyclovir resistance may occur in the thymidine kinase gene, but is rare (Gershon 2001, Saint-Leger 2001). In these cases, foscarnet can be given. Novel anti-HZV drugs have recently been evaluated in clinical trials but are still in early phases of development (Review: Andrei 2011).

Pain management of post-herpetic neuralgia is problematic. Carbamazepine or gabapentine only partially help. Steroids are generally not advised (Gnann 2002). Since November 2007 lidocaine medicated plasters (Versatis®) are licensed in Europe which can be pasted to painful areas. Side effects are local skin irritation. Herpetic lesions should be healed before use (Garnock-Jones 2009). In 2009, the FDA approved Qutenza® 8% patch for the management of neuropathic pain due to postherpetic neuralgia. Qutenza® delivers a synthetic form of capsaicin, the substance in chili peppers that gives them their heat sensation, through a dermal delivery system. The patch is applied by a physician or a healthcare professional.

Prophylaxis

Varicella vaccination, previously contraindicated in HIV patients, seems to be fairly safe and effective for patients with more than 400 CD4 T cells/µl (Gershon 2001, Weinberg 2010). It should be considered if HZV serology is negative. In individuals with negative serology and exposure to highly infectious HZV, administration of hyperimmunoglobulin (2 mg/kg IV) may be attempted in individual cases. Long-term primary prophylaxis is not advised. Some dermatologists prefer long-term low dose therapy if there are persistently recurring episodes.

Treatment/prophylaxis of HZV infection (daily doses)

Acute therapy		Duration: at least 7 days
Treatment of choice	Acyclovir	Acyclovir 1 tab. at 800 mg 5x/day
Severe cases		Acyclovir 1–2 amp. at 500 mg TID (10 mg/kg tid) IV
Alternatives	Valacyclovir	Valacyclovir 2 tab. at 500 mg TID
Alternatives	Famciclovir	Famciclovir 2 tab. at 250 mg TID
Alternatives	Brivudin	Brivudin 1 tab. at 125 mg QD
Prophylaxis		Not recommended

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Progressive multifocal leukoencephalopathy (PML)

PML is a severe demyelinating disease of the central nervous system. It is caused by JC virus (JCV), a polyoma virus found worldwide. JCV was named after the initials of the first patient John Cunningham, from which this simple DNA virus was first isolated in 1971 (Major 1992). Therefore, JC has no connection, as is often wrongly assumed, with Jakob-Creutzfeld syndrome. As seroprevalence is high, at up to 80%, latent persistent infection is assumed. Kidneys and bones seem to be important reservoirs. Only impaired cellular immunity leads to reactivation of JCV and manifestation of disease. It seems certain that JCV reaches the CNS via leukocytes, and then affects mainly oligodendrocytes and consequently the cells which comprise the myelin sheaths. Destruction of these is as macroscopically apparent as multifocal demyelination. The main focus of the disease is the white matter of the cerebral hemispheres, but in some cases, the cerebellum, the grey matter may also be affected. PML is a classic opportunistic infection and can occur in patients with hematological diseases or during therapy with monoclonal antibodies such as rituximab, natalizumab or efalizumab (Yousry 2006, Carson 2009, Major 2010). However, HIV+ patients are by far the largest patient group. Severe immunodeficiency is frequently seen, but not obligatory for development of PML. In contrast to CMV or MAC infection, PML does not always indicate the final stages of HIV infection. Although CD4 T cells are usually below 100/µl at manifestation of disease, PML may also occur at above 200 CD4 T cells/µl (Gasnault 2008). The decrease in incidence is not as marked as with other OIs (Engsing 2009). After cerebral toxoplasmosis, it is now probably the second most common neurological OI (Antinori 2001).

Prognosis was poor in the pre-HAART era. The median interval between the onset of the first symptoms and death was between 3 and 6 months. Patients usually died of secondary complications after being bedridden for many weeks. Disease progression seems to be much slower on ART, and even complete remission seems possible (Albrecht 1998). However, these effects are not as impressive as for other OIs (Falco 2008, Engsing 2009) and PML remains the OI with the highest mortality (ART-CC 2009). Complete remission is rarely seen, even with sufficient ART. They mainly occur in cases of inflammatory PML, which occurs in the course of immune reconstitution inflammatory syndrome (IRIS) (Du Pasquier 2003, Hoffmann 2003, Tan 2009). The number of CD4 T cells and the JC virus-specific immune response seem to be relevant as prognostic markers. In contrast, JCV viral load does not seem to have any impact on the course of the disease (Khanna 2009, Marzocchetti 2009).

Signs and symptoms

Although there is a broad spectrum of PML symptoms due to the variety of localized areas of demyelination, the clinical signs and course of the disease have several common characteristics. In addition to cognitive disorders, which may range from mild impairment of concentration to dementia, focal neurological deficits are very typical of PML. Mono- and hemiparesis are observed most frequently, as well as speech and even visual deficits. We have seen several blind patients with PML. These deficits may be isolated and initially present as discrete changes in coordination, rapidly leading to considerable disabilities. Epileptic seizures may also occur. Loss of sensibility, fever, and headache are rare and are usually more typical of cerebral toxoplasmosis.

Diagnosis

Clinical suspicion of PML should be rapidly confirmed radiologically. But beware: a CCT scan is not helpful – it does not clearly reveal hypodense lesions. An MRI is much more sensitive to detecting both the number and size of lesions than a CCT

and usually shows high signal intensity lesions in T2 weighted imaging and in FLAIR sequence, which are hypointense in T1W and often do not show gadolinium enhancement or mass effect. ART may result in inflammatory courses that involve significant enhancement (see IRIS). Exclusion of grey matter is typical – since this is a leukoencephalopathy. Furthermore, it should be noted that the lesions are almost always asymmetrical.

An MRI often allows clarification between cerebral toxoplasmosis or lymphoma. However, the huge, extensive lesions covering an entire hemisphere that are often shown in the literature are not always present. Every PML starts small – very discrete, localized, solitary lesions can occur and certainly do not exclude the diagnosis. PML can occur anywhere in the brain, and there are no typically susceptible areas. Lesions are often parieto-occipital or periventricular, but the cerebellum may also be involved. It is important that the images are assessed by a radiologist or clinician familiar with PML. Even then, it is difficult to distinguish PML from HHV-6 infection (Caserta 2004) or HIV leukoencephalopathy (Langford 2002).

Clinicoradiological diagnosis is therefore not definitive. Examination of cerebrospinal fluid is essential. Generally, if there is no other coinfection, unspecific inflammatory signs are absent although the total protein content is usually slightly elevated. Pleocytosis is rarely seen, and more than 100/3 cells make PML unlikely. CSF should always be tested for JCV. Newer PCR methods have a sensitivity of around 80% and a specificity of over 90%. A CSF sample should be sent to a JCV-experienced laboratory.

PML is very probable in cases of clinicoradiological suspicion and positive JCV PCR. In such cases, brain biopsy is not necessary. Nevertheless, a negative PCR does not exclude the diagnosis. Levels of JCV viral load may vary significantly and do not correlate with the extent of lesions (Eggers 1999, Garcia 2002, Bossolasco 2005). Unfortunately, JCV PCR is even less useful – many patients with PML have a low or undetectable JCV CSF viral load while on ART (Bossolasco 2005). Stereotactic brain biopsy may become necessary in individual cases. Recently, a consensus statement has been published which establishes detailed criteria for PML diagnosis (Berger 2013).

Treatment

A specific PML treatment is not available. Foscarnet, interferon, immune stimulants, steroids, camptothecin/topotecan or cytosine arabinoside are not effective (Hall 1998). Unfortunately, this is also the case for the nucleotide analog cidofovir, which is licensed for CMV retinitis. According to an analysis of 370 patients from numerous studies (De Luca 2008), a real benefit has not been proven for cidofovir. Our experiences have been rather disappointing and, in a retrospective analysis of 35 patients, cidofovir was even associated with a poorer prognosis. However, this chiefly reflects the frustration of patients and clinicians – cidofovir was mainly used in cases of progressive disease (Wyen 2004). There may no longer be an argument for the use of cidofovir in PML patients.

In recent years, 5-HT2a inhibitors and/or serotonin receptor antagonists have been proposed for PML treatment. It has been shown that the serotonergic receptor 5HT2AR could act as the cellular receptor for JCV on human glial cells (Elphick 2004); the blockade could represent a therapeutic goal. Case studies for some agents such as risperidone and mirtazapine, which block serotonergic receptors, exist already (Verma 2007, Focosi 2007+2008, Cettomai 2009). However, controlled studies are missing. On the basis of *in vitro* efficacy (Brickelmeier 2009), mefloquine (a drug that has been used extensively for prophylaxis and treatment of malaria) was tested in a

clinical trial. In this study on 37 patients with PML, no evidence of anti-JCV activity by mefloquine was found (Clifford 2013).

Thus, it should be an absolute priority to optimize ART in cases of PML. Improvement of the JC virus-specific immune response which is often observed within immune reconstitution determines the patient's further progress to a large extent (Khanna 2009, Marzocchetti 2007+2009, Gasnault 2011). Our early observation that prognosis significantly improved on ART (Albrecht 1998) was confirmed by several other groups (Clifford 1999, Dworkin 1999, Gasnault 1999+2008, Berenguer 2003, Khanna 2009). Since synergy between HIV and JCV has been demonstrated *in vitro*, maximal HIV suppression should be the goal. Although progression of disease has been described with sufficient antiretroviral therapy, ART often remains the only real hope for patients. There is also some evidence that intracerebral penetrating antiretroviral agents such as AZT, FTC, abacavir, nevirapine and lopinavir are more efficient regarding survival of patients with PML (Gasnault 2008). There is one small pilot trial suggesting that an intensive 5-drug ART may improve survival of patients with PML (Gasnault 2011).

Treatment/prophylaxis of PML

Acute therapy		
Treatment of choice	ART	The most important goal is maximal HIV suppression and immune reconstitution! Use intracerebral penetrating agents such as AZT, FTC, abacavir, nevirapine and lopinavir
Experimental		Only within clinical trials (risperidone? mirtazapine?)
Prophylaxis		Not available

Prophylaxis

There is none. Exposure prophylaxis is also not possible.

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Bacterial pneumonia

Bacterial pneumonia occurs even with a relatively good immune status (>200 CD4 T cells/µl). It is not as closely associated with immunodeficiency. Furthermore, the decrease in incidence since the HAART era has been more moderate than for other opportunistic infections. Only recurring, radiologically <u>and</u> culturally detected acute pneumonia (more than one episode in the last 12 months) is considered AIDS-defining. As with HIV-negative patients, community-acquired pneumonia should be distinguished from nosocomial pneumonia. Travel history is important, particularly for community-acquired pneumonia.

The bacteria that are most frequently found to cause community-acquired pneumonia in HIV+ patients are Pneumococcus and *Hemophilus influenza*. Mycoplasma is important to consider, particularly in younger patients. Klebsiella, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are other common pathogens. Legionella are rare.

Intravenous drug users develop community-acquired pneumonia significantly more often than other patient groups. Comorbidity, alcohol over-use and current smoking are other risk factors (Grau 2006, De 2013). Therapy interruption and cigarette smoking were also major risk factors in SMART (Gordin 2008). Abstinence from smoking significantly reduces the risks of bacterial pneumonia (Bénard 2010). Earlier reports about increased incidence of bacterial pneumonia on a T-20 containing regimen have not been confirmed (Kousignian 2010). Low CD4 T cell counts and an existing liver cirrhosis are major risk factors for severe cases (Manno 2009, Madeddu 2010).

Nosocomial pneumonia is often caused by hospital germs such as klebsiella, staphylococcus, pseudomonas (Franzetti 2006). In such cases, treatment depends on local resistance patterns and experience (Gant 2000, Vogel 2000).

Signs and symptoms/diagnosis

Acute, usually high, fever and productive cough are typical. Breathing may be painful because of accompanying pleuritis, but real dyspnea is rare. Auscultation almost always allows distinction from PCP. If something can be heard, then PCP is unlikely. Chest radiography secures the diagnosis. CRP is significantly elevated, LDH usually normal. It is essential to take several blood cultures at body temperatures above 38.5°C before starting treatment. A major problem regarding the blood culture is that diagnosis takes time (24–48 hours) and is not so sensitive. However it is the only procedure that allows a resistance test. Sputum culture is a simple method allowing determination of etiology in approximately half of all cases – however, its overall utilization remains controversial and results strongly depend on the clinician's experience (Cordero 2002). This also applies to the pneumococcal antigen determination in urine and the diagnosis of other specific viruses which are not recommended in current guidelines (Tessmer 2010).

Treatment

General

Treatment of bacterial pneumonia in HIV+ patients is similar to that in HIV-negative patients. Therapy should always begin empirically, without waiting for sputum or blood culture results. Many HIV+ patients with bacterial pneumonia can be treated as outpatients. Patients with poor immune status below 200 CD4 T cells should be hospitalized (Madeddu 2010), as well as patients with high fever (above 39.5°C), poor compliance, signs of organ failure, CNS disorders (confusion) or poor vital signs

(tachypnea, tachycardia, hypotonia) and older patients (above 65 years). In a prospective trial on 835 patients with pneumonia in Uganda, a four-point clinical predictor score was identified and included heart rate >120 beats/minute, respiratory rate >30 breaths/minute, oxygen saturation <90%, and CD4 cell count <50 cells/µl (Koss 2015). The 30-day mortality, stratified by score, was 13% (0-1), 23% (2-3) and 54% (4). Sufficient hydration is important in all patients. If patients remain in ambulatory care, then this is an indication that they should drink a lot (more than 2 liters of water daily). The use of supportive therapy with expectorants or mucolytics such as N-acetylcysteine or antitussives is controversial. On adequate therapy, improvement can be expected within 48–72 hours. If patients, especially the severely immunocompromised, have a persistent fever, then the treatment must be reconsidered after 72 hours, at the latest. It should be noted that the current first-line therapies are not effective against *Pseudomonas aeruginosa*.

Medication

Different drugs are possible for ambulatory treatment. Even an attempt with penicillin may be justified in some circumstances – depending on local rates of Pneumococcus and *Hemophilus influenzae* resistance. It should be noted that HIV+ patients frequently develop allergies.

Empiric treatment/prophylaxis of community-acquired bacterial pneumonia
(daily doses) – there may be significant differences in prices!

Outpatient		Duration: 7–10 days
Mild	Amoxicillin + clavulanic acid	1 tab. at 875/125 mg TID
Mild	Clarithromycin	1 tab. at 500 mg BID
Mild	Roxithromycin	1 tab. at 300 mg QD
Mild	Cefuroxime	1 tab. at 500 mg BID
Mild	Cefpodoxime	1 tab. at 200 mg BID
Inpatient		
Severe	Piperacillin (+ tazobactam) + macrolide	Tazobac® 1 bottle at 4.5 g IV TID **plus** roxithromycin 1 tab. at 300 mg QD **or** clarithromycin 1 tab. at 500 mg BID
Severe	Ceftriaxone + macrolide	Ceftriaxone 1 infusion at 2 g QD IV **plus** roxithromycin 1 tab. at 300 mg QD **or** clarithromycin 1 tab. at 500 mg BID
Severe	Cefuroxim + macrolide	Cefuroxim 1 infusion at 1.5 g TID IV **plus** roxithromycin 1 tab. at 300 mg QD **or** clarithryomcin 1 tab. at 500 mg BID
Prophylaxis	Vaccination (pneumococcal polysaccharide)	Pneumovax 23™ pre-filled syringe IM

Aminopenicillins are effective against *Hemophilus influenza* and various gram-negatives. However, when combined with clavulanic acid, active against beta-lactamase-producing bacteria, they are associated with more gastrointestinal complaints. Newer oral cephalosporins have a broader spectrum against gram-negatives, while at the same time have good efficacy against Pneumococcus and Hemophilus. They are, however, expensive.

Macrolides are advantageous for atypical bacteria such as Mycoplasma, Chlamydia and Legionella – but the proportion of macrolide-resistant Pneumococcus is increasing (14% in Germany). Efficacy is also limited in some Hemophilus strains.

For quinolones, it should be noted that ciprofloxacin has no or only weak efficacy against many important pathogens. Therefore only newer quinolones should be used. However, in 2009, a 'Dear Doctor' letter was sent to European health care professionals, describing the rare occurrence of fulminant hepatitis and the Stevens-Johnson syndrome or toxic epidermal necrolysis in patients using moxifloxacin. These side effects must be placed in the overall balance of pros and cons of moxifloxacin as compared to the alternatives.

If patients are hospitalized, then intravenous administration is possible initially. In this case, at least two antibiotics should be combined.

Targeted treatment after isolation of the pathogen, and, in particular, treatment of nosocomial pneumonia, should depend on local resistance patterns and the recommendations of the in-house microbiologist.

Prophylaxis

The Pneumovax® vaccine provides effective protection. It should be utilized in all HIV+ patients with >200 CD4 T cells/µl. However, newer data suggest that Pneumovax® has a significant, independent protective effect against pneumococcal disease, regardless of CD4 lymphocyte count (Peñaranda 2007). Although it does not avert pneumonia in all cases it seems to have a positive effect on the further course of the treatment (Imaz 2009).

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Cryptosporidiosis

Cryptosporidiosis is a parasitic intestinal disease with fecal-oral transmission. It is mainly caused by the protozoon *Cryptosporidium parvum* (two genotypes exist, genotype 1 is now also known as *C. hominis*) and may affect both immunocompetent and immunocompromised hosts (Review: Chen 2002). First described in 1976, cryptosporidia are among the most important and most frequent causes of diarrhea worldwide. Important sources of infection for this intracellular parasite include animals, contaminated water and food. The incubation period lasts approximately 10 days. While diarrhea almost always resolves within a few days in otherwise healthy hosts or in HIV+ patients with CD4 counts greater than 200 cells/µl, cryptosporidiosis is often chronic in AIDS patients. Particularly in severely immunocompromised patients (<50 CD4 T cells/µl), diarrhea may become life-threatening due to water and electrolyte loss (Colford 1996). Only chronic, and not acute, cryptosporidiosis is AIDS-defining.

Signs and symptoms

The typical watery diarrhea can be so severe that it leads to death as a result of electrolyte loss and dehydration. Up to twenty bowel movements a day are not uncommon. Tenesmus is frequent, along with nausea and vomiting. However, the symptoms are highly variable. Fever is usually absent. Additionally, the biliary ducts may occasionally be affected with the elevation of biliary enzymes. Pancreatitis is also possible.

Diagnosis

When submitting stool samples, the laboratory should be informed of the clinical suspicion. Otherwise, cryptosporidia are often overlooked. If the lab is experienced and receives the correct information, usually just one stool sample is sufficient for detection. In contrast, antibodies or other diagnostic tests are not helpful. The differential diagnosis should include all diarrhea-causing pathogens.

Treatment

No specific treatment has been established to date. Diarrhea is self-limiting with a good immune status; therefore, poor immune status should always be improved with ART – and this often leads to resolution (Carr 1998, Miao 2000). To ensure absorption of antiretroviral drugs, symptomatic treatment with loperamide and/or opium tincture, a controlled drug prescription, at its maximum dosage, is advised. If this is unsuccessful, then treatment with other anti-diarrheal medications, perhaps even sandostatin, can be attempted. Sufficient hydration is necessary and infusions may even be required.

Recent reviews confirm the absence of evidence for effective agents in the management of cryptosporidiosis (Abubakar 2007, Pantenberg 2009). We have observed good results with the antihelminthic agent nitazoxanide (Cryptaz®). Nitazoxanide proved to be effective in a small, randomized study (Rossignol 2001). In 2005 it was licensed in the US for treatment of cryptosporidia-associated diarrhea in immunocompetent patients. Nitazoxanide is not approved for AIDS patients and showed no effects in a double-blind randomized study in HIV+ children with cryptosporidia (Amadi 2009). Rifaximine (Xifaxan®, 200 mg) is a nonabsorbed rifampicin derivative, already licensed in the US as an anti-diarrheal. The first data with AIDS patients are very promising (Gathe 2008).

Paromomycin (Humatin®) is a nonabsorbed aminoglycoside antibiotic and has shown favorable effects on diarrhea in small uncontrolled studies (White 2001). In one double-blind randomized study, however, there was no advantage over placebo (Hewitt 2000). Potentially, there is an effect in combination with azithromycin (Smith 1998).

Treatment/prophylaxis of cryptosporidiosis (daily doses)

Acute therapy		
Symptomatic	Loperamide + opium tincture	Loperamide 1 cap. at 2 mg 2–6 times daily **or** loperamide solution 10 ml (10 ml = 2 mg) 2–6 times daily **and/or** Opium tincture 1% = 5–15 drops QD
Symptomatic	Octreotide	Sandostatin solution for injection 1 amp. at 50 µg SC BID or TID (increase dose slowly)
Curative attempt	Nitazoxanide	Nitazoxanide 1 tab. at 500 mg BID
Curative attempt	Rifaximin	Rifaximin 2 tab. at 200 mg BID
Prophylaxis		Exposure prophylaxis: no tap water

Prophylaxis

There is no generally accepted prophylaxis, although retrospective analyses have reported a protective effect of rifabutin and clarithromycin (Holmberg 1998). The importance of good hygiene and not drinking tap water should be emphasized to patients, at least in countries with limited access to clean, adequate drinking water. Contact with human and animal feces should be avoided. The tendency for patients to become ill during the summer months can often be linked to swimming in rivers or lakes. Cryptosporidia are resistant to most disinfectants. In hospitals and other medical facilities, the usual hygienic measures, such as wearing gloves, are adequate. Moreover, patients do not need to be isolated. However, they should not be put in the same room with other significantly immunocompromised patients.

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Cryptococcosis

Infection with the yeast *Cryptococcus neoformans* is a rare AIDS-defining illness in Europe. In the US and especially in Southeast Asia, cryptococcosis occurs much more frequently and is considerably one of the more prominent AIDS-defining illnesses worldwide. Presumably transmitted via inhalation, bird droppings are a key reservoir for *C. neoformans*. This pulmonary infection may remain subclinical in immunocompetent patients, but is almost always followed by disseminated disease in HIV+ patients. Apart from the lungs, the main manifestation after hematogenic spread is in the CNS. For this reason, a CSF examination is obligatory in every suspected case. However, isolated skin manifestations and lymphadenitis can also occur. Organ involvement, such as in the urogenital or gastrointestinal tract, is rare.

Cryptococcosis almost always occurs with severe immunodeficiency. In a collection of 114 cases, 87% had less than 100 CD4 T cells/µl; the median CD4 count was 30 (Weitzel 1999). Many cases seen today occur in the setting of an immune reconstitution inflammatory syndrome. Cryptococcosis is fatal if untreated. Treatment is lengthy, complicated and should be managed only on an inpatient basis. Relapses were frequent in the pre-HAART era and occurred in at least 15% of cases. In addition, cryptococcosis occurs relatively frequently in the presence of an immune reconstitution inflammatory syndrome. Prognosis has much improved over the last years. In one study from France, the mortality rate per 100 person-years was 15 in 1996–2000, compared with 64 in the pre-HAART era although early mortality did not differ between the two periods (Lortholary 2006).

Signs and symptoms

The CNS manifestation with encephalitis is the most frequent manifestation (ca. 80%). Patients complain mainly of headaches, fever and confusion or clouding of consciousness which progresses rapidly over a few days. Disorders of gait, hearing, and vision may occur, as well as paresis, particularly of the cranial nerves. In such cases intracranial pressure is almost always increased. However, meningeal symptoms are usually absent. In the course of an immune reconstitution syndrome, clinical symptoms are often atypical and characterized by extensive abscesses (Manfredi 1999).

Pulmonary disease leads to symptoms of atypical pneumonia with unproductive cough and chest pain. Skin lesions can initially resemble molluscum contagiosum, and later become confluent in the form of larger, ulcerative lesions.

Diagnosis

Cryptococcosis is life-threatening, and the mortality rate in larger studies is between 6 and 25% (Saag 2000). There is no time to lose during diagnostic testing. Rapid examination of the lungs (HRCT) and CNS in particular (MRI) should be initiated in every suspected case (e.g., positive cryptococcal antigen test).

The chest x-ray usually does not reveal much; therefore, an HRCT scan must be performed if pulmonary involvement is suspected. The spectrum of morphology on the image is very variable. Diffuse, small lesions similar to tuberculosis may occur, but there can also be sharply defined infiltrates reminiscent of bronchopneumonia. Cavitation and bronchiectasis may also be present. Every attempt should therefore be made to clearly identify the causative organism by BAL.

An MRI scan of the head should always be performed if there are neurological symptoms. However, in contrast to toxoplasmosis and primary CNS lymphoma, it usually

does not reveal much, and isolated or multiple mass lesions (cryptococcomas) are very rare. Nevertheless, intracranial pressure is often increased and a fundoscopy (papillary edema) should be performed.

The most important test for cryptococcosis is lumbar puncture after a fundoscopy and/or an MRI. Diagnosis can be made via India ink stain in almost all cases. CSF must be examined even in cases with pulmonary or other manifestations to exclude CNS involvement. Cryptococcal antigen (CrAg) in the blood (titer >1:8) is a good parameter and should always be determined, especially in patients with low CD4 T cell counts (Jarvis 2011). Blood cultures are also often positive. With cutaneous involvement, the diagnosis is usually made from a biopsy.

Treatment

In cases of CNS involvement an immediate combination of antimycotics is urgently recommended followed by maintenance therapy with fluconazole (Saag 2000). Fluconazole alone is not sufficient, even in high doses, as shown by two randomized trials from Africa. In these trials, mortality of cryptococcal meningitis was unacceptably high. Within the first weeks, 54–59% of the patients died (Longley 2008, Makadzange 2009).

Combination prevents resistance and allows reduction of acute therapy to 4-6 weeks. The choice of combination is not clearly defined. In some countries, combination therapy with the three antimycotics amphotericin B, flucytosine and fluconazole is often used for meningitis. The triple therapy leads to complete remission of meningitis in around 80% of cases (Weitzel 1999), and consequently the possibility of a slightly higher rate than under dual therapy with amphotericin B and flucytosine as favored in the US (van der Horst 1997).

However, other data raises questions as to the superiority of triple therapy. According to the measurements of cryptococcal clearance in the CSF, two small randomized studies in Thailand and Vietnam, the combination of amphotericin B and flucytosine was the most effective treatment (Brouwer 2004, Day 2013). It was even significantly better than triple therapy and also amphotericin B and fluconazole.

Amphotericin B at a dosage of 1 mg/kg plus is possibly more rapidly fungicidal than is standard-dose amphotericin B (Bicanic 2008). If amphotericin B is not available, the combination of flucytosine and fluconazole is better than fluconazole alone (Nussbaum 2010).

Nevertheless, in view of the toxicity of flucytosine, available in many countries only in infusion and not in tablet form, the combination of amphotericin B and fluconazole is preferable. In a Phase II study the high doses of 800 mg fluconazole daily was most effective (Pappas 2009). A newer study showed that the efficacy of high dose fluconazole is equivalent to flucytosine (Loyse 2012).

In addition to having significantly lower toxicity, liposomal amphotericin (Ambisome®) is slightly more effective than conventional amphotericin B (Leenders 1997, Hamill 1999). However even Ambisome®-containing combinations are highly toxic. Daily monitoring of kidney and liver enzymes, blood count and electrolytes are recommended. Fluconazole should be administered as an infusion, particularly if patients seem confused.

In untreated patients, ART is typically started during the acute phase of treatment. Caution should be taken with tenofovir, given an observed case of renal failure requiring dialysis after treatment with tenofovir and amphotericin B. Since there is also a higher risk for the development of IRIS, the optimal time for initiation of ART is still under debate. In ACTG 5164, early ART was an advantage (Zolopa 2009). In a small African study on seriously ill patients, however, mortality was increased in patients

starting ART immediately after diagnosis (Makadzange 2010). In a study on 177 HIV+ adults in Uganda and South Africa who had cryptococcal meningitis and had not previously received ART, deferring ART for 5 weeks after the diagnosis of meningitis was associated with significantly improved survival, as compared with initiating ART at 1 to 2 weeks, especially among patients with a paucity of white cells in cerebrospinal fluid (Boulware 2014).

In cases of isolated pulmonary involvement (CSF-negative) or other extracerebral manifestations, treatment without flucytosine can be completed (acute therapy with amphotericin B and fluconazole) within two instead of four weeks. If there is a positive cryptococcal antigen test without evidence of CNS, pulmonary or other infection, then treatment can consist of fluconazole alone.

Treatment success is monitored based on the clinical course and repeated lumbar punctures. CSF is negative in approximately 60% of cases after two weeks (Saag 2000). When this is the case, maintenance therapy or secondary prophylaxis can be started, though not sooner than after four weeks of acute therapy. The quicker the CSF shows to be negative, the better the prognosis (Bicanic 2009, Chang 2012). If there is increased intracranial pressure, then CSF drainage may become necessary (Graybill 2000). There is some evidence that therapeutic lumbar punctures are beneficial (Rolfes 2014). Steroids are ineffective (Saag 2000).

Treatment/prophylaxis of cryptococcosis (daily doses, unless specified otherwise), see also Drugs section for further details

Acute therapy		Duration: always at least six weeks
Treatment of choice	Amphotericin B + fluconazole + flucytosine*	Amphotericin B 0.5–0.75 mg/kg QD **or** liposomal amphotericin B 3 mg/kg QD (preparation by pharmacy) **plus** fluconazole 1 bottle at 200 mg IV BID **or** fluconazole 1 cap. at 200 mg BID **plus** flucytosine 1 bottle at 250 ml (2.5 g) IV QD (= 100–150 mg/kg distributed in four separate doses)
Maintenance therapy		Discontinuation possible from >200 CD4 cells/µl >3-6 months
Treatment of choice	Fluconazole	Fluconazole 1–2 cap. at 200 mg QD
Alternative	Itraconazole	Itraconazole 2 cap. at 100 mg BID
Primary prophylaxis		Not recommended

*Note: We usually omit flucytosine. In this case the daily doses of fluconazole should be 800 mg. Instead, we begin with ART in the acute therapy phase in these patients who are almost always ART-naïve.

Prophylaxis

Pre-exposure prophylaxis does not seem to exist. A survival benefit has not not demonstrated and primary prophylaxis against *Cryptococcus neoformans* is not recommended even in endemic areas such as Thailand (McKinsey 1999, Chariyalertsak 2002). Recently, in a large double-blind randomized placebo-controlled trial in Uganda on 1,719 patients with negative CrAg on screening, fluconazole prophylaxis was shown to prevent cryptococcal disease while waiting for and in the early weeks of antiretroviral therapy, particularly in those with CD4 counts of less than 100 cells/µl. However, all-cause mortality was not reduced (Parkes-Ratanshi 2011).

Fluconazole is given as secondary prophylaxis or maintenance therapy. It is significantly more effective than itraconazole. For example, in a large randomized study, the relapse rate in the fluconazole arm was only 4% compared to 23% in the itraconazole arm, resulting in discontinuation of the study before completion (Saag 1999). Fluconazole can probably be discontinued with sufficient immune reconstitution (above 200 CD4 cells/μl, undetectable viral load for three to six months), as demonstrated in several studies (Aberg 2002, Kirk 2002, Vibhagool 2003, Mussini 2004), and after at least six months of maintenance therapy. It is prudent to check for cryptococcal antigen before stopping (Mussini 2004). Positive antigen tests require continuation of treatment as the risk of relapse is high, especially in patients with high antigen titres (Lortholary 2006).

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Salmonella septicemia

Infection with non-typhoid Salmonella, which typically only causes enteritis in healthy individuals, can lead to severe septicemia in immunocompromised patients (Jacobs 1985). A newer study indicates that impaired immunity against nontyphoidal Salmonella bacteremia in HIV infection results from excess inhibitory antibodies against Salmonella lipopolysaccharides, whereas serum killing of Salmonella is induced by antibodies against outer membrane proteins (MacLennan 2010).

In Central Europe, Salmonella septicemia is rare in HIV+ patients, and accounts for less than 1% of AIDS cases. In the Swiss cohort of over 9000 patients, only 22 cases of recurring salmonellosis were documented over a period of nine years (Burkhardt 1999).

In Southern Europe as well as Africa, salmonellosis is much more frequent. Infected food, particularly poultry, is most widely recognized as a reservoir for Salmonella. In most cases, relapses are frequent. In addition to septicemia, atypical infections with osteomyelitis, empyema, pulmonary abscesses, pyelonephritis or meningitis have been described (Albrecht 1992, Nadelman 1985). Recurring, non-typhoid Salmonella septicemia is considered an AIDS-defining illness. The risk of recurrent septicemia decreased significantly in the ART era (Hung 2007).

Signs and symptoms/diagnosis

Patients are often severely ill. Chills and high fever are usually present. If treatment is delayed, there is always a danger of septic shock. Diarrhea may be absent.

Blood cultures mainly lead to isolation of enteritis-causing Salmonella strains such as *S. enteritidis* and Y. The pathogens causing typhoid or paratyphoid fever, *S. typhi* and *S. paratyphi*, are rare.

Treatment

Ciprofloxacin is the treatment of choice (Jacobson 1989). Although oral bioavailability is good, intravenous dosing is preferable. In the US the resistance situation is relatively good (Forrest 2009). In contrast to Asia, where rates of ciprofloxacin resistance have clearly increased and risen to up to 30% (Hung 2007). In these cases, cephalosporins such as cefotaxime or ceftriaxone have proven to be effective.

Treatment/prophylaxis of Salmonella sepsis (daily doses)

Acute therapy		7–14 days
Treatment of choice	Ciprofloxacin	Ciprofloxacin 1 bottle at 200 mg IV BID
Alternative	Ceftriaxone	Ceftriaxone 1 bottle at 2 g IV QD
Prophylaxis		For relapses
		Ciprofloxacin 1 tab. at 500 mg BID (6–8 months)

One week of treatment with ciprofloxacin or ceftriaxone is usually enough. Maintenance therapy should continue for 6–8 months and not be stopped too early (Hung 2001). However, lifelong secondary prophylaxis, which was propagated in the past (Nelson 1992), no longer seems necessary.

Prophylaxis

Drug prophylaxis is not recommended. However, HIV+ patients should generally be advised to pay attention to food hygiene, especially in warmer countries.

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Immune reconstitution inflammatory syndrome (IRIS)

For the first time, in mid-1997 and early 1998, two groups described atypical manifestations of CMV retinitis (Jacobsen 1997) and MAC disease with abscess formation (Race 1998) in HIV+ patients within a few weeks of initiation of ART. Although the pathogens, pathogenesis and localization were very different, all these illnesses had a distinct inflammatory component and were associated with significant immune reconstitution in these patients. Consequently, it was suspected early on that these presentations could constitute a syndrome during which a latent infection present at initiation of therapy is fought more effectively by the recovering immune system (Overview: French 2009). Infections are not the only cause of IRIS. Malignancies and other diseases have also been decribed as IRIS-related (see below). The International Network for the Study of HIV-associated IRIS (INSHI, http://www.med.umn.edu/inshi/) has established the following consensus criteria for diagnosis of IRIS:

1. Response to ART (at least one $\log_{10}$ copies/mL decrease in HIV RNA)
2. Clinical deterioration of an infectious or inflammatory condition temporally related to ART initiation.
3. Symptoms cannot be explained by expected clinical course of a previously recognized and successfully treated infection, medication side effect or toxicity, treatment failure or complete non-adherence.

One must differentiate between subclinical infections first appearing on ART ("unmasking IRIS") and clinically evident infections already existing at therapy initiation, which often paradoxically become worse during therapy ("paradoxical IRIS"). IRIS in many publications today is often a collection of bizarre, sometimes grotesque case reports, which have actually only one thing in common: an unexpected, usually clinically impressive infection, differing significantly from the course of disease seen during the pre-HAART era. Nevertheless, IRIS has three rules:

• Anything is possible.
• Nothing is as it was in the pre-HAART era.
• IRIS does not mean that ART has failed. In fact, patients usually have a good prognosis.

How frequently does IRIS occur? Due to the lack of a definition in the early years of ART, the data vary substantially. In our experience, a frequency of 5–10% in patients with less than 200 CD4 T cells/µl is realistic. Very low CD4 T cells, a high viral load before initiation of therapy or a rapid drop of HIV RNA on ART seem to be important predictive factors for IRIS. If one focuses on patients who were already infected with mycobacteria or cryptococcus neoformans before ART was started, IRIS rates of 30% can be reached (Müller 2010).

Mycobacterial IRIS. For MAC, the number of published cases with grotesque, fistular lymphadenitis, cutaneous or muscular abscesses, osteomyelitis, nephritis or meningitis is too large to be cited here. After a total of 83 patients started ART with a CD4 T cell count of less than 200/µl, only six mycobacterioses, among these four MAC infections, were observed within the first weeks of therapy (Hoffmann 1999). Lymph node abscesses usually occur during the first weeks of ART. IRIS cases with *Mycobacterium xenopi* or *kansasii* have also been described (Chen 2004, Phillips 2005).

There are now numerous reports on tuberculosis (John 1998, Chien 1998), reminiscent of the "paradoxical" reactions to TB treatment known in the 1950s. All of these patients suffered an initial deterioration while on correct tuberculostatic treatment and ART-induced immune reconstitution. By the same token, meningitis as well as

marked lymphadenopathy with unspecific histology can complicate the course of disease, yet both respond astonishingly rapidly and well to steroids. Prednisolone was effective in a placebo-controlled trial (Meintjes 2010).

An early or immediate start of ART in therapy-naïve patients facilitates the occurrance of IRIS. In large randomized trials the risk of IRIS increased when ART was started immediately in patients with TB, especially in those with low CD4 T cells (Abdool 2011, Blanc 2011, Havlir 2011, Wondvossen 2012, Naidoo 2013). In all studies, however, the increased risk did not lead to increased mortality. This may be different in patients with tuberculous meningitis, in which at least one randomized trial showed a less favorable outcome with early ART (Torok 2009). In cases of meningitis, steroids should be given (Meintjes 2012).

CMV IRIS. In addition to mycobacteriosis, numerous cases of unusual CMV infections under ART have been published. In patients with previously diagnosed CMV retiniotis, IRIS developed in 38% (Müller 2010). Inflammatory CMV retinitis with vitritis that may lead to visual impairment, papillitis and macular edema, can now be described as a distinct syndrome, differing significantly from the course of CMV retinitis seen in the pre-HAART era (Jacobson 1997, Karavellas 1999). Neovascularization endangers vision even after resolution (Wright 2003). As with MAC disease, *in vitro* studies have shown that the CMV-specific immune response is improved most significantly in those patients developing vitritis (Mutimer 2002, Stone 2002). Inflammatory CMV manifestations are not limited to the retina and may involve other organs.

PML IRIS. The course of inflammatory PML that occurs during IRIS is different from the infaust prognosis seen during the pre-HAART era (Collazos 1999, Cinque 2001, Miralles 2001). Clinical symptoms are often more fulminant initially, and on radiology, there is a contrast enhancement which is otherwise atypical for PML, that may resolve over time. Patients have a better prognosis, and PML seems to resolve completely (Hoffmann 2003, Du Pasquier 2003). It appears that a number of patients with inflammatory PML, who have been asymptomatic for years, live without any residual symptoms. However, fatal cases of inflammatory PML have also been reported (Safdar 2002). Previously documented experiences indicate that steroids are ineffective, although there have been accounts of positive results (Nuttall 2004, Tan 2009).

Cryptococcal IRIS. Numerous cases with inflammatory courses of disease have been described (Overview: Haddow 2010). Together with MAC/TBC and CMV, cryptococci are probably the most influential pathogens that contribute to IRIS. In particular, severely immunocompromised patients who start with ART after cryptococcal therapy should be watched closely for the first few weeks and months. Deferring ART for five weeks after the diagnosis of meningitis may be associated with improved survival (Boulware 2014). Studies show that 10–20% of patients with coinfection develop a cryptococcal IRIS (Sungkanuparph 2009, Müller 2010). The MRI usually shows choriomeningitis with significant enhancement in the choroid plexus. Cryptococcal antigen in the CSF is positive, although culture remains negative (Boelaert 2004). The intracranial pressure is often particularly high (Shelbourne 2005). As well as meningitis, lymphadenitis can also occur (Skiest 2005).

IRIS induced by other infections. A large variety of case reports have been published: leishmaniasis (Jiménez-Expósito 1999), penicillosis (Ho 2010), histoplasmosis (De Lavaissiere 2008), pneumocystosis (Barry 2002, Koval 2002, Godoy 2008, Jagannathan 2009, Mori 2009), toxoplasmosis (Martin-Bondel 2011) or herpes (Tobian 2014). Herpes zoster and hepatitis B or C episodes also seem to occur on ART, particularly during the first weeks (Behrens 2000, Chung 2002, Manegold 2001,

Martinez 1998, Domingo 2001). HHV-8-associated Kaposi's sarcoma can worsen significantly on ART in the presence of IRIS (Bower 2005, Leidner 2005, Feller 2008). Increasing dermatological problems such as exacerbation of pre-existing folliculitis or skin disease have also been reported (Handa 2001, Lehloenyia 2006, Pereira 2007, Iarikov 2008). There are even reports about parvovirus and leprosy (Nolan 2003, Couppie 2004, Bussone 2010, Watanabe 2011).

IRIS and other diseases. Diseases other than OIs are now recognized to occur as IRIS. These include autoimmune diseases such as Graves' disease, lupus, Sweet's and Reiter's syndromes, Guillain-Barré syndrome, acute porphyria, gout and sarcoidosis, to name but a few (Bevilacqua 1999, Behrens 1998, Fox 1999, Gilquin 1998, Makela 2002, Mirmirani 1999, Neumann 2003, Piliero 2003, Sebeny 2010, Rasul 2011). Even two cases of Peyronie's disease, a fibrosis of the penis, were reported (Rogers 2004). These reports raise the question of whether all of these manifestations are truly induced by immune reconstitution or perhaps merely chance occurrences. While most reports initially offered little information on the etiology beyond purely hypothetical discussions, it has recently become apparent that changes in the cytokine profile are involved in the pathogenesis of IRIS, together with an activation of the cellular immune response. However, it seems that the mechanisms differ according to disease and genetic profile (Price 2001, Shelbourne 2005).

Consequences

Patients starting ART with less than 200 CD4 T cells/µl and particularly those who have a high viral load require close clinical monitoring during the first weeks. Close attention should be given especially in cases where very immunocompromised patients suddenly feel physically "affected," express subfebrile conditions, and want to start ART "after thinking about it for a long time." Latent infections are often present in such cases and rapidly become apparent as immune reconstitution occurs – the poorer the immune status and the longer its duration, the greater the danger of IRIS. Although newer studies prove that infection parameters such as CROP, D-dimer or cytokines such as IL-6 or IP-7 are predictive of IRIS or OI (Rodger 2009, Antonelli 2010, Porter 2010) it is not generally practiced in routine diagnosis.

However chest radiography, abdominal ultrasound and fundoscopy should be included in routine investigations of such patients before beginning treatment. Moreover, clinical examination which nowadays are often gladly overlooked should be taken seriously. Some authors suggest that MAC prophylaxis start even before ART in severely immunocompromised patients seems problematic, even though prophylaxis cannot prevent MAC IRIS (Phillips 2002+2005). Still, prospective clinical studies have yet to prove whether administration of IL-2 or GM-CSF is worthwhile, as was recently postulated (Pires 2005).

Mycobacterioses in particular should be treated generously with steroids. This has been confirmed in a randomized trial (Meintjes 2010). However, the beneficial effects of steroids are not seen in viral IRIS (Meintjes 2012).

In conclusion, one should always be prepared for atypical localizations, findings and disease courses of opportunistic infections. The prognosis of IRIS is usually good. Mortality of patients developing IRIS is reportedly not higher than that of patients without IRIS (Park 2006).

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Wasting syndrome

Wasting syndrome is defined as involuntary weight loss of at least 10% of original body weight accompanied by persistent diarrhea (at least two bowel movements daily for more than 30 days) or extreme fatigue and/or fever without apparent infectious etiology. With thorough and competent work-up, a specific causative agent can usually be found for wasting syndrome because it is essentially a classical exclusion diagnosis and really more of an epidemiological instrument than a specific disease. Although previously a very frequent condition, wasting syndrome has become rare today. For example, in a large study conducted in the year 2000, only 14% of patients indicated having lost more than 10% of their original body weight (Wanke 2000). Rates are higher in intravenous drug users (Campa 2005). Weight loss remains an independent risk factor for mortality, even in the HAART era, and every patient should be weighed regularly. In one large study, mortality risk in patients with a loss greater than 10% of body weight was more than four to six times higher than that of patients with stable body weight (Tang 2002). Patients with classic wasting syndrome are often extremely weak and the risk for opportunistic infections is significantly elevated (Dworkin 2003). There is also cognitive impairment in these patients (Dolan 2003).

Diagnosis

The causes of wasting syndrome are complex. First, it is necessary to exclude or treat opportunistic infections (TB, MAC, cryptosporidiosis and microsporidiosis). If none are found, then several reasons remain that may contribute, even in combination, to wasting syndrome. These include: metabolic disorders, hypogonadism, poor nutrition and malabsorption syndrome (Overview: Grinspoon 2003).

Consequently, a thorough patient history is extremely beneficial. Does the patient have a nutritional diet? How are meals distributed throughout the day? Is the patient depressed? Which drugs (ART) are being taken? Distinction from antiretroviral-induced lipoatrophy (d4T/ddI) is often difficult. Significant weight loss also occurs frequently on interferon (Garcia-Benayas 2002), but rapidly resolves after finishing treatment. In addition, hypogonadism should be ruled out with the measurement of testosterone. While there are several simple tests for malabsorption syndromes, it is prudent to start with testing albumin as well as TSH and cholesterol levels.

Further tests such as D-xylose absorption or biopsies of the small intestine should only be initiated after consulting with a gastroenterologist. Other tests, such as DEXA, densitometry, bioelectrical impedance analysis, etc, should be conducted in centers experienced with AIDS wasting syndrome to determine the patient's body composition.

Therapy

Wasting syndrome always requires competent diet counseling. Exercise, if possible, is also good. Of course, they only have limited success. Supportive parenteral nutrition only helps if there are problems with absorption (Kotler 1990, Melchior 1996). Effective ART, ideally without drugs that cause lipoatrophy such as d4T or ddI, and possibly even omitting nucleoside analogs completely, is ideal. Severe lipoatrophy may require complete omission of nucleoside analogs (see Chapter on *Nuke-sparing*). Beyond this, many kinds of drug treatment have been attempted. However, these have limited success and are often problematic.

Megestrol acetate, a synthetic gestagenic hormone, shows some benefit as an appetite stimulant in wasting syndrome, as demonstrated in some studies (Von Roenn 1994,

Mulligan 2006). Its side effects are those typically associated with steroids, including induced hypogonadism, which should always be avoided, especially in cases of wasting syndrome. As a result, it is not widely nor currently recommended that this drug be used.

Dronabinol, the main active ingredient in marijuana, has been licensed in the US since 1985 as Marinol®, and may be prescribed for pharmacy formulation as drops or hard gel capsules. This drug is certainly attractive for many patients and sometimes actively demanded. Prescription should be carefully considered, particularly in view of the significant cost associated with the medication. In some European countries, dronabinol costs approximately 600 euros per month for the usual dose of 5 mg TID. Without a clear diagnosis of wasting syndrome, communication with the insurance company may minimize substantial payment problems. Some health insurances and other payors reject the request. The effect on wasting syndrome is moderate at best, if detectable at all (Beal 1995). It tends to be even weaker than megestrol acetate (Timpone 1997).

Hypogonadism, a frequent condition of patients with wasting syndrome, calls for the measurement of testosterone levels. If the age-dependent levels are low, then testosterone substitution has proven itselffuseful, both for weight gain and quality of life (Grinspoon 1998). A dose of 250 mg testosterone is given IM every 3-4 weeks, and there are a variety of less expensive generic names. The effect is sustained, even with long-term use (Grinspoon 1999). If testosterone levels are normal, substitution is not indicated. In women, one should exercise caution when administering androgenic hormones. Other anabolic steroids are available in addition to testosterone, such as oxandrolone or nandrolone (Gold 2006, Sardar 2010). Although possibly more effective than testosterone, these drugs are commonly associated with other side effects, particularly those related to the liver (Corcoran 1999). Positive effects have been demonstrated with the anabolic steroid oxymetholone in a small, double-blind, randomized study (Hengge 2003). However, extremely high elevation of transaminases have been observed.

High costs and side effects have limited the use of recombinant human growth hormones (rHGH), for which long-term data is still not available (Mulligan 1993, Schambelan 1996). However, the results of a recent metaanalysis suggest that growth hormones may be more effective than anabolic steroids or testosterone in wasting syndrome (Moyle 2004). Common adverse events with rHGH therapy include blood glucose elevations, arthralgia, myalgia, and peripheral edema, but these usually respond to dose reduction or drug discontinuation (Review: Gelato 2007).

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Rare OIs

CHRISTIAN HOFFMANN AND GERD FÄTKENHEUER

Several opportunistic infections that rarely occur in central Europe or have become increasing rare due to the introduction of ART include: aspergillosis, bacillary angiomatosis, histoplasmosis, isosporiasis, coccidioidomycosis (*Coccidioides immitis*), visceral leishmaniasis, microsporidiosis, *Penicillium marneffei* mycosis, and rhodococcosis. In addition to affecting HIV+ patients more frequently than immunocompetent individuals, these infections are also considered to have more severe courses of disease and more frequent recurrences in HIV+ patients than in HIV-negative patients. Despite this, according to the current CDC/WHO classification, only histoplasmosis, isosporiasis, and coccidioidomycosis are AIDS-defining.

Aspergillosis

Aspergillosis occurs almost exclusively in severely immunocompromised patients but is not classified as AIDS-defining. In the largest cohort described worldwide to date, in a study of 342 cases of invasive aspergillosis, almost all of the patients had less than 50 CD4 T cells/µl (Mylonakis 1998). Although the lungs are largely susceptible to pneumonia or tracheobronchitis, almost all other organs can be compromised, particularly the CNS. Sinusitis or abcesses in kidney or liver are other manifestations (Hunt 2000, Myolonakis 2000).

For the most part, aspergillosis occurs in HIV+ patients on long-term and in some cases excessively long steroid treatment for another OI. Severe neutropenia (<1000 leucocytes) is another risk factor. Found in over 90% of invasive aspergillosis cases, *Aspergillus fumigatus* is by far the most frequent pathogen. Other important aspergillus pathogens are *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*. The severely ill patients complain of fever, cough, dyspnea and chest pain. Hemoptysis frequently occurs. The only way to reach a reliable diagnosis is biopsy. A serum antigen test on Galactomannan, a component of the cell wall of Aspergillus (not exclusively, also other mycoses) may support the diagnosis. Chest x-rays often remain inconspicuous. In the HRCT, bilateral, multifocal and nodular lesions may be the most common radiological characteristic, while Halo and crescentic signs occur occasionally.

Treatment should be initiated immediately. Suspicion of aspergillosis justifies a treatment attempt without definitive diagnosis, i.e., biopsy results. Each delay worsens a potentially unfavorable prognosis substantially. At present voriconazole is considered treatment of choice (Schwartz 2005). In contrast to other antifungal drugs, voriconazole penetrates well into the CNS. In patients with invasive aspergillosis, initial therapy with voriconazole led to better responses and improved survival and resulted in fewer severe side effects than the standard approach of initial therapy with amphotericin B (Herbrecht 2002). Voriconazole is given at a dosage of 4 mg IV/kg BID (loading dose: 6 mg/kg BID on day 1, oral therapy with 200 mg BID starting from day 7). Main adverse events are visual disturbances (20%) and (reversible) increases of liver enzymes.

An alternative approach is amphotericin B, whose inferiority to voriconazole is questioned by some (Jorgensen 2006). The effect of combinations is not proven (Garbati 2012). Salvage therapy includes lipid-based formulations of amphotericin B, caspofungin, high-dose itraconazole or posaconazole (Dockrell 2008). A systematic steroid therapy should be stopped if possible and every patient should receive antiretroviral treatment immediately. Some case reports describe that permanent therapy can be dropped if immune reconstitution is sufficient (Yoganathan 2009).

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Bacillary angiomatosis

Bacillary angiomatosis in HIV+ patients was first described in the 1980s (Review: Maguina 2000). Bacillary angiomatosis is caused by the rickettsial species *Bartonella henselae* and *Bartonella quintana* ("Rochalimaea" until the beginning of the 1990s). While Bartonella henselae is typically associated with cats, its primary host, and cat fleas, its vector; Bartonella quintana frequently affects homeless patients and is associated with poor hygiene and social-economic conditions. Several possible reservoirs have been discussed for such cases (Gasquet 1998). In a Spanish study of 340 HIV+ patients, 22% patients reacted to one or more Bartonella antigens. Of all the studied seroprevalence factors, only age was statistically significant (Pons 2008). Reportedly, Bartonella occurs more often in North and South America than in Europe. In Brasil, the seroprevalence was 38% (Lamas 2010). In a study of 382 febrile HIV+ patients in San Francisco, Bartonella was found to be the causative organism in 18% (Koehler 2003).

Bacillary angiomatosis remains a significant differential diagnosis in all cases with skin lesions of unknown etiology. The pseudoneoplastic, vascular skin proliferation is quite often clinically and histologically mistaken for Kaposi's sarcoma or hemangioma. The vascular nodules or tumors may be isolated, but are usually multiple and reminiscent of fresh Kaposi's sarcoma, with cherry red or purple nodules. One quarter of the cases may have bone involvement with painful osteolytic foci (AP elevation). Here, the skin lesions sometimes resemble dry hyperkeratotic changes such as those seen in psoriasis. Different organs may be affected. In a collection of 21 cases, 19 patients had skin, 5 bone and 4 liver involvement (Plettenberg 2000). Manifestations in lymph nodes, muscle, CNS, eye, gingiva and gastrointestinal tract have also been reported.

Diagnosis of BA is difficult. The gram-negative bacteria are only visible on biopsy samples stained with Warthin-Starry silver stain. If this stain method is not applied, then bacillary angiomatosis will not be found. Moreover, pathologists should be informed of the suspected diagnosis, as the Warthin-Starry silver stain is not routinely performed. PCR is also possible. Reference labs should be contacted for further diagnostic details.

Treatment of bacillary angiomatosis is with erythromycin (at least four weeks with 500 mg QID) or clarithromycin. Relapses are common, which is why some physicians favor therapy for at least three months. Supposedly effective, doxycyclin is the

therapy of choice for CNS involvement. Since transmission is generally via cats, US guidelines recommend not having cats as pets. Preferably, cats should be healthy and older than one year; and scratches should be avoided.

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Histoplasmosis

Histoplasma capsulatum is a dimorphic mold, found largely in moist soil and without a capsule despite its name. The Southern and Midwestern of regions of the US as well as Central America and Africa are endemic areas. Even in the HAART era, morbidity and mortality due to histoplasmosis remains a public heatlh problem in low-income and high-income countries (Review: Adenis 2014).

Inhalation of microconidia, the spores of *H. capsulatum*, can cause granulomatous disease in the lungs of immunocompetent individuals. In HIV patients with impaired immunity (85% have less than 100 CD4 T cells/µl), infection leads to an acute, life-threatening disease with dry cough, fever, dyspnea and malaise (Gutierrez 2005, Mora 2008). Miliary TB and PCP are important differential diagnoses. Disseminated courses of disease may also occur, in which the fungus can be detected in bone marrow or by liver biopsy (Albrecht 1994). Skin ulcerations, oropharynx or CNS involvement may also occur (Scheinfeld 2003, Wheat 2005, Antonello 2011). Hepatosplenomegaly is common, occurring in almost 90% of the patients (Mora 2008).

Histoplasmosis is an AIDS-defining illness whose pathogen like that of cryptococcal antigen can be reliably detected in the blood with an antigen test. Laboratory evaluations often reveal significantly elevated LDH and alkaline phosphatase as well as transaminases.

Amphotericin B should be given as initial treatment. Liposomal amphotericin B (3 mg/kg daily for 14 days) is not only less toxic, but possibly also more effective (Johnson 2002). In milder cases, itraconazole (200 mg BID or TID) is effective, and can also be used as a secondary prophylaxis. It is significantly more effective than fluconazole (Wheat 2002), but is associated with a high risk of interactions, particularly with ritonavir, but also with efavirenz (Crommentuyn 2004, Andrade 2009, Hills-Nieminen 2009). In such cases a modification of the doses is often necessary. With regard to other OIs, secondary prophylaxis for histoplasmosis can be discontinued if immune reconstitution is sufficient (Goldman 2004). Initiation of ART and the subsequent immune reconstitution may reveal undiagnosed latent disseminated histoplasmosis (Nacher 2006).

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Isosporiasis

Isospora belli is an ubiquitous intestinal parasite. While rare in Europe, it is a issue of great concern in the developing world, especially in the tropics and subtropics (Lagrange-Xelot 2008). In India *Isospora belli* was the most frequent diarrhea infection after cryptosporidiosis in HIV+ patients (Kulkarni 2009). Similar to cryptosporidiosis, this microbe may cause epidemic-type outbreaks in immunocompetent hosts. Patients suffer at a minimum with enteritis-like complaints and occasionally, also experience very severe watery diarrhea, abdominal pain, cramps and nausea. In immunocompromised patients, chronic diarrhea and malnutrition may occur (Review: Goodgame 1996). Fever is seldom seen. Median CD4 T cell count in patients with *Isoporiasis* is 150, slightly higher than in cases of cryptosporidiosis and microsporidia. Chronic isosporiasis with diarrhea lasting for more than four weeks is AIDS-defining. Detection of the relatively large oocysts is possible via normal stool sampling for parasites, as well as in acid-fast stains. Blood tests usually reveal eosinophilia (Certad 2003). Treatment is co-trimoxazole (960 mg daily for one week). Ciprofloxacin is slightly less effective (Verdier 2000). Relapse is common despite co-trimoxazole maintenance therapy (Lagrange-Xelot 2008).

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Coccidioidomycosis

Infection with the mold *Coccidioides immitis* is endemic in the Southwestern US and therefore, needs to be considered when presented with patients who have been in this region (Reviews: Ampel 2007, Nguyen 2013). Laboratory personnel also should also be informed of the high risk of infection, even in suspected cases.

After inhalation of spores, the primary manifestation begins in the lungs (Pappagianis 1993). Approximately 1–3 weeks after exposure, a pneumonia-like illness develops with fever, cough, chest pain and general malaise. The infection, although often symptomatic, usually resolves in immunocompetent patients without sequelae. Occasionally, there is residual cavitation which in some cases require surgical intervention (Jaroszewski 2009). Disseminated coccidioidomycosis beyond the lung and Hilar lymph nodes (for example chronic meningoencephalitis) occurs only in significantly immunocompromised patients with CD4 counts of less than 250 cells/µl (Ampel 2007, Drake 2009). Disseminated coccidioidomycosis is an AIDS-defining illness. Prognosis was poor in the pre-HAART era. In an analysis of 602 patients with disseminated coccidioidomycosis, mortality after one year was 63% (Jones 1995). With ART the course of this illness is mostly less severe (Massannat 2010).

Serology is not very helpful in immunodeficient patients. Diagnosis is mostly made by cultures or histological materials (Adam 2009). Due to high infection risks, laboratory staff should be informed when in doubt of coccidioidomycosis.

Amphotericin as well as azoles are effective (Hernandez 1997), and should be, if necessary, combined (Ampel 2007). Detailed recommendations for the different situations (meningeal or disseminated cases must be treated more intensively) can be found (Galgiani 2005). Fluconazole should be given as maintenance therapy at high doses (400 mg). In cases of chronic refractory meningitis, posaconazole is also an option (Schein 2011).

In the past few years, it seems that the disease has become rarer as a result of ART, and that maintenance therapy can be discontinued when CD4 cells are greater than 250/µl with only initial pulmonary involvement. However, lifelong treatment is still recommended for cases of meningeal involvement (Woods 2000, Galgiani 2005, Ampel 2007).

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Leishmaniasis (visceral)

Leishmaniasis is an infectious disease that is caused by 20 species pathogenic for humans belonging to the genus Leishmania, a protozoa transmitted by sand flies. One must differentiate between the cutaneous and the visceral forms of leishmaniasis (Kalar Azar), the manifestation form depends on the species (*L. donovani, L. infantum, L. chagasi*). According to WHO, there are 12 million people infected with leishmania worldwide, with approximately 350 million living in risk areas. With such numbers, leishmaniasis is one of the most important parasitosis. In Europe, leishmaniasis is common and countries around the Mediterranean, such as Spain, Portugal, France and Italy are affected the most. The following link provides a global overview: www.who.int/leishmaniasis/leishmaniasis_maps/en/index.html.

Visceral leishmaniasis appears more frequently in HIV+ patients. In Spain, on third of all patients with visceral leishmaniasis have HIV (Gil-Prieto 2011). While important, leishmaniasis is still not an AIDS-defining illness. A review of 15 cases in Germany showed that all HIV patients were significantly immunosuppressed (usually less than 100 CD4 T cells/µl). A few patients had not been in endemic areas for several years (Albrecht 1998). Bone marrow involvement is reflected by the almost obligatory pancytopenia, which may be particularly severe in HIV patients (Pintado 2001). Other symptoms include fever, hepatosplenomegaly, and mucocutaneous lesions. The diagnosis is usually made from bone marrow aspirate.

Treatment of visceral leishmaniasis is difficult (Review: Olliaro 2005). Pentavalent antimony compounds such as sodium stibogluconate (Pentostam®) or or meglumine antimoniate (Glucantime®) have been used for about 60 years (usual dosage: 20 mg/kg IV or IM daily for 28 days). However, these drugs are extremely toxic. Myalgia, arthralgia, cardiotoxicity and chemical pancreatitis often lead to discontinuation (Laguna 1999). Combination therapies are possibly more effective and allow for shorter therapy (van Griensven 2010, Sundar 2011).

According to a recent meta-analysis, available evidence suggests that amphotericin is superior to antimony treatment in HIV+ patients (Cota 2013). Many guidelines recommend liposomal amphotericin B (AmBisome®) as the treatment of choice (2–5 mg/kg daily). However, recent trials have suggested that effectiveness of liposomal amphotericin is limited in HIV-coinfected patients (Rijtmeier 2011, Sinha 2011). Classic amphotericin B is also effective (Lachaud 2009).

The only orally bioavailable leishmaniasis drug and a promising new drug, due to its good tolerability and efficacy, is miltefosine (Impavido®), an alkylphosphocholine analog that was licensed in Europe in 2004. Although clarity is still needed as to how miltefosine inhibits leishmania metabolism, a Phase III study in India demonstrated it as highly effective (Sundar 2002). Another randomized study in Ethiopia showed that among HIV+ patients with leishmaniasis, miltefosine was less effective than sodium stibogluconate, but tolerability was better (Ritmeijer 2006). The dose was 100 mg daily (~2300 euros/month). We have successfully treated some patients with miltefosine to date. Another option may be paromomycin, an aminoglycoside which seems to be effective as at least two randomized studies from India have shown (Sundar 2007+2011). In Europe paramomycin (Humatin®) has so far only been licensed as a gastrointestinal drug for local use.

As a secondary prophylaxis pentamidine may be effective (Patel 2009). In contrast, fluconazole seems to show no effects (Rybniker 2009). Relapses are frequent and occur in almost half of all cases. ART seems to change this – another argument for inclusion in the AIDS classification (de La Rosa 2002, Fernandez-Cotarelo 2003). Interestingly, *in vitro* studies have consistently documented an inhibitory effect of protease inhibitors on leishmania parasites (van Griensven 2013).

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Microsporidiosis

Microsporidiosis is an important cause of diarrhea in HIV+ patients. Microsporidia are obligate intracellular protozoa. At least four genera, with *Enterocytozoon bieneusi* considered the most noteworthy, are described as pathogenic in humans.

Even in Germany, microsporidia were previously among the most recurrent diarrhea-causing microbes. Furthermore, in the pre-HAART era, microsporidia could be found in approximately one-third of all patients. Some studies documented up to two-thirds of all HIV+ patients with chronic diarrhea (Sobottka 1998). The incidence of microsporidiosis has been reduced significantly due to ART; consequently, it is now only diagnosed occasionally. Although microsporidiosis is not AIDS-defining, chronic microsporidiosis almost always occurs in severely immunocompromised patients with CD4 T cell counts of less than 50 cells/µl.

Diarrhea may be very severe; watery, though not bloody; and accompanied by abdominal pain, nausea and vomiting. Fever is almost always absent. While myositis, keratoconjunctivitis and sinusitis have rarely been described, infections of the biliary ducts are considered common.

In light of the fact that microsporidia, like cryptosporidia, are very small, an experienced lab is desirable for detection. Those who have never seen them or who are

not asked to explicitly test for them will probably not detect them. Culture has not generally been established. Direct detection is most successful with specialized staining methods. Special transport or preparation is not necessary.

Although effective, albendazole (1–2 tab. at 400 mg BID for 4 weeks) is not most advantageous in every case. For example, *Enterocytozoon bieneusi* is often resistant to albendazole. Repeated positive reports in such cases, especially from France, give an account of treatment with fumagillin (watch for thrombocytopenia), but these case numbers remain low (Molina 2002). Case reports (Bicart-See 2000) are also available for niazoxanide (see cryptosporidiosis). There have also been positive reports of symptomatic treatment with thalidomide. ART-induced immune reconstitution, however, seems to have the greatest effect (Carr 1998+2002, Maggi 2000).

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Nocardia

Nocardia are aerobic bacteria or actinomycetes that occur worldwide. Several species exist that cause pneumonia as well as systemic disease. In a survey of 30 cases of HIV+ patients with nocardiosis, pulmonary manifestation occurred in 21 cases (Uttamchandani 1994). Pulmonary manifestation of nocardiosis is often confused with tuberculosis. Extrapulmonary manifestation may occur in the skin, brain, nerves, muscle and bone. The immune response to Nocardia is cellular. As a result, there is generally an increased risk of pulmonary or systemic disease in immunosuppressed patients. In HIV+ patients, however, opportunistic infections with Nocardia are rare. Patients are usually significantly immunocompromised (Javaly 1992, Uttamchandani 1994). Nocardia respond well to sulfonamides such as sulfadiazine even in HIV+ patients (Pintado 2003). In cases of suspected nocardiosis, an experienced laboratory should be consulted.

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Penicillium marneffei

Most fungi belonging to the Penicillium species are not pathogenic. One exception is *Penicillium marneffei*, which is a problem mainly for HIV+ patients in Southeast Asia (Le 2011). In these areas, it is the most frequent fungal infection in AIDS beside cryptococcosis, and is considered AIDS-defining by many clinicians (but is not included in the CDC classification). The known reservoirs for *Penicillium marneffei* are humans, rats and dogs.

Lungs and skin are the organs most frequently affected (Ma 2005). The clinical symptoms consist of prolonged high fever, lymphadenopathy, weight loss, malaise, cough and hemoptysis, diverse cutaneous and mucocutaneous lesions (reminiscent of molluscum contagiosum) and abnormal liver enzymes. There is often hepato-splenomegaly. Disseminated cases also occur (Ma 2005).

Definitive diagnosis relies upon the identification or isolation of *P. marneffei* in clinical specimens (blood, bone marrow, sputum). However, conventional culture usually takes at least three days. The use of the Galaktomannan antigen assay may facilitate earlier diagnosis of *Penicillium marneffei* infection for HIV+ patients in endemic areas (Huang 2007).

There are no randomized studies which have evaluated different treatment options for *P. marneffei* infections. Amphotericin B, voriconazole and itraconazole are effective treatments (Supparatpinyo 2007, Ustianowski 2008). To prevent relapses, however, patients who have had the disease should take itraconazole as a perma-nent prophylaxis (Supparatpinyo 1998). Primary prophylaxis is not recommended even with longer stays in endemic areas (Chariyalertsak 2002). The only patient we have seen with *Penicillium marneffei* had spent several months on vacation in Thailand (Sobottka 1996).

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Rhodococcus

Rhodococcus equi (previously *Corynebacterium equi*) is a sporeless, gram-positive intra-cellular pathogen, which is ubiquitous in air, water and soil. *R. equi* has been found on all continents, and was first identified as a pathogen in young horses. For half a century, only veterinarians were interested in this microorganism, but in the last two decades, it has been found more and more frequently in humans, primarily in significantly immunocompromised patients. In these patients, it causes severe gran-ulomatous or abscess forming pneumonia, and sometimes also disseminated infec-tion. The coryneform bacteria seen in sputum cultures are often confused with normal diphtheroid flora found in the mouth and therefore not diagnosed.

In 1986, the first case with respect to an AIDS patient was described (Samies 1986). In a collection of 78 cases, mostly AIDS patients with less than 50 CD4 T cells/µl were affected. The main symptoms were fever, dyspnea and unproductive cough (Capdevila 1997). Cavitation, mainly in the upper lobes, is frequently seen radio-logically (Capdevila 1997, Marchiori 2005). Rhodococci are best detected in sputum and blood cultures (Torres-Tortosa 2003).

Erythromycin, azithromycin, ciprofloxacin, rifampin and vancomycin are effective, and some of these drugs can be combined. However, treatment is difficult and com-

plete recovery is rare, even with ART (Plum 1997, Sanz-Moreno 2002, Ferretti 2011). Surgical measures may also be necessary if there is extensive cavitation.

Survival of patients treated with ART is much higher than that of patients who did not receive ART (Torres-Tortosa 2003, Topino 2010).

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Trypanosoma cruzi

Trypanosoma cruzi is a protozoan that is transmitted via contaminated feces of triatomid bugs (assassin bugs), found almost exclusively on the American continent. It causes Chagas disease, one of the most frequent causes of cardiomyopathy in South America.

HIV+ patients are more frequently affected and have higher levels of parasitemia (Sartori 2002), probably due to the fact that the Trypanosoma-specific immune response is mainly cellular in nature. In addition, a more frequent occurrence in HIV+ patients is meningoencephalitis, which is usually severe and radiologically not distinguishable from cerebral toxoplasmosis or primary cerebral lymphoma. Most probably it is a reactivation (Diazgranados 2009, de Almeida 2011). In HIV+ patients from South America, Trypanosoma infection should therefore be considered in the differential diagnosis (Silva 1999, Cordova 2008, Llenas-García 2012). Whenever possible, lumbar puncture should be performed because of the high accuracy for early diagnosis. However, treatment (for example benznidazole) is rarely successful and mortality is high (Sartori 2007, Cordova 2008). Possibly itraconazole or ketoconazole are also effective (de Almeida 2009).

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10. Kaposi's Sarcoma

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Kaposi's sarcoma (KS) is the most common malignancy in patients with HIV infection. In 1981, the simultaneous occurrence of KS with pneumocystis pneumonias in young gay men led to the first descriptions of AIDS. This entity is designated after the Hungarian dermatologist Moritz Kaposi who first described the "classical" KS 100 years earlier. Classical KS predominantly occurs in elderly, but otherwise healthy people from the Eastern Mediterranean area. It affects often only the skin at the lower extremities and thereby, clearly differs from HIV-associated KS which will be the focus of the following chapter.

In contrast to classical KS, HIV-associated KS may affect all skin and mucous membranes. Lymph nodes and internal organs such as stomach, gut, lung or liver may also be involved. The progression of HIV-associated KS is very variable and reaches from small lesions, remaining stable for years, to extremely aggressive courses, in which progression may lead to death within a few months.

Compared to the 1980s and early 1990s, when KS was one of the most common AIDS illnesses, prevalence of KS today is relatively low (Francesci 2010) and the incidence has fallen to less than a tenth of what it was (Grabar 2006, Simard 2011). In addition, the clinical course of KS has changed. The refractory variants with an aggressive, devastating and often fatal course which were seen in the pre-HAART era have become a rarity today. However, mortality of KS patients remains elevated even after initiation of ART, especially during the first year (Maskew 2013). Moreover, there are still some very aggressive cases occurring today, typically only a few weeks or months after ART initiation. This so-called IRIS-associated KS often comes with rapid visceral lesions and high mortality (Crane 2005, Achenbach 2012, Letang 2013). High HHV-8 and HIV viremia seem to be risk factors for this IRIS-associated KS (Letang 2013).

Pathogenesis

The cellular origin of the spindle cells (considered the KS tumor cells) is still controversial. Newer investigations suggest lymphatic, endothelial cells (Dupin 2006). Since 1994, it is well known that KS is induced by an infection with the human herpesvirus-8 (HHV-8) or Kaposi's sarcoma-associated herpesvirus (KSHV). HHV-8 can be always detected in the tumor tissue, and the level of HHV-8 plasma viremia correlates quite well with KS progression (Laney 2007). In HIV+ patients with KS, a significant HHV-8 viremia is frequently found (Marshall 2010). Transmission of HHV-8 occurs predominantly via saliva (Pauk 2000), but also sexually, vertically and via blood products (Pica 2008). In some regions, particularly in Italy and Central Africa, HHV-8 can be found in up to 50% of the general population. The exact role of HHV-8 in the pathogenesis of KS is not clear. However, HHV-8 is able to exploit the normal differentiation pathway of endothelial cells (EC). Through manipulation of EC-specific transcriptional regulators this may contribute to viral persistence and KS sarcomagenesis (Review: Cancian 2013).

Infection with HHV-8 does not lead inevitably to KS. Interactions particularly with HIV-1 (Aoki 2004), possibly also with other viruses such as HHV-6 and HSV-1, changed signal transduction chains, an increased production of growth factors as well as cytokine dysregulation, all may play a role (McCormack 2005).

Among the HIV+ population, gay men are almost the only ones affected by KS; in HIV+ women, children or hemophiliacs, KS is a rare disease. An immune defect and/or low CD4 T cells promote emergence and growth of KS. However, severe

immunodeficiency is not a prerequisite for the development of KS which is one of the few AIDS illnesses occurring in patients with a relatively preserved immune status. Approximately 29% of all patients who participated in the US in the years 1996–2007 in KS studies, had more than 300 CD4 T cells/µl and an HIV plasma viremia below detection (Krown 2008). In one study, the activation of the CD8 T cells correlated more strongly with the progression than the number of CD4 T cells (Stebbing 2006). Interestingly, some cohorts with HIV-negative MSM developing KS have been reported (Rashidghamat 2014).

Signs, symptoms and diagnosis

HIV-associated KS does not have a preferential pattern of localization. It can begin on any area of the skin, but may also appear on oral, genital, or ocular mucous membranes. Typical findings at manifestation are a few asymptomatic purple macules or nodules. These lesions have a predilection for distribution along relaxed skin tension lines. As mentioned above, the disease progression is very variable: the tumors can remain unchanged for months to years, or grow rapidly within a few weeks and disseminate. Rapid growth can lead to localized pain and a yellow-green discoloration of the area around the tumor as a result of hemorrhage. Further progression of the tumor can lead to central necrosis and ulceration. The tumors may bleed easily. Plaque-like and nodular KS lesions often become confluent and can be accompanied by massive lymphoedema. In the oral cavity, the hard palate is frequently affected. Lesions begin with purplish erythema and progress to plaques and nodules that ulcerate easily. KS lesions may also involve the external genitalia including the foreskin and glans penis.

Regression of KS during treatment is not only indicated by reduction of the size of the lesions but also by change in color from dark to bright red. However, some lesions may persist lifelong. These often dirty-grey-brown to light brown hyperpigmentations are caused by hemosiderin deposits and, possibly, increased stimulation of melanocytes due to inflammation. Lymphoedema can also persist for years.

Diagnosis

Diagnosis of cutaneous KS is usually made based on clinical findings. However, in all questionable cases a histologic diagnosis is recommended. Differential diagnosis includes other neoplasia such as cutaneous lymphomas or angiosarcoma, but also infectious diseases such as syphilis and bacillary angiomatosis. Histological findings include spindle-shaped cells with vascular channels lined by abnormal endothelial cells. Extravasated erythrocytes, hemosiderin, and fibrosis can often be seen.

In all cases of KS, clinical staging procedures are recommended, including:

1. Complete inspection (oral and genital mucous membranes!)
2. Abdominal ultrasound
3. Gastroduodenoscopy and colposcopy (both procedures obligatory when mucous membranes are involved)
4. Chest radiography (exclusion of a pulmonary KS)

Treatment

If KS is newly diagnosed in an HIV+ patient naïve to antiretroviral therapy, ART should be initiated: in early KS, additional chemotherapy is only required in 20% of cases (Bower 2009). In patients on ART without complete suppression of HIV plasma viremia, ART should be optimized. With decreasing HIV plasma viremia and immune

reconstitution, many KS lesions stabilize or even resolve completely without any specific treatment. Among 213 ART-naïve patients with early KS stages who were treated with ART alone, overall survival at five years was 95%, while progression-free survival was 77% (Bower 2014). In one Italian study in 22 ART-naïve KS patients, the overall clinical response rate to ART alone was 91%: 18 complete and 2 partial responses were achieved, and only two patients experienced disease progression. Complete remission was sustained in all 18 patients with complete response (Cattelan 2005).

Animal and *in vitro* experiments have suggested a direct anti-proliferative effect of PIs (Sgadari 2002, Gantt 2011). There is some evidence that PIs may reduce oral shedding of HHV-8 (Gantt 2014) and that KS incidence is reduced with longer PI use (Kowalkowski 2015). However, there is no ART combination of choice for KS patients. PIs are not required necessarily as NNRTI-based regimens are also effective with regard to KS regression (Grabar 2006, Martinez 2006).

With ART, there is also an improvement of the humoral response against HHV-8 (Sullivan 2010) and HHV-8 viremia rapidly decreases (Cattamanchi 2011). ART interruptions should be avoided in patients with current or previous KS. In the SMART study, KS was among the most frequent AIDS-defining illnesses during treatment interruptions, in particular among patients with a history of KS (Silverberg 2007).

ART as the only therapy is not recommended in all cases. In patients with rapidly progressive disease (especially in the setting of IRIS), with KS-related symptoms, or with visceral disease or lymphoedema, ART should be combined with cytotoxic chemotherapy (Grabar 2006). There are different options:

Chemotherapy: Pegylated liposomal doxorubicin hydrochloride (Caelyx® or Doxil®) at a dosage of 20 mg/m² body surface is the treatment of choice (Di Trolio 2006). With Caelyx® complete remission rates of up to 80% are possible (Lichterfeld 2005). The infusions for 30–60 min. every 2–3 weeks are feasible on an outpatient basis and usually well tolerated. An antiemetic therapy is not necessary. Usually 6-8 cycles are required to achieve a good clinical response. Relapses during Caelyx® therapy occur rarely and particularly during the first year (Martin-Carbonero 2008). During treatment, myelotoxicity and cardiotoxicity of doxorubicin should be considered. Although the latter is rare and occurs only above cumulative doses of 450 mg, echocardiography (ejection fraction?) is recommend at the beginning of therapy as well as controls after six cycles. Another important side effect of Caelyx® is palmoplantar erythrodysesthesia (PPE, "hand-foot-syndrome"), which becomes apparent as painful erythemas at hands and feet (Lorusso 2007).

In August 2011, Janssen-Cilag reported a shortage of Caelyx® (Doxil®) due to production delays at a contract manufacturer. Intermittent capacity constraints were seen during the following months. In the setting of this shortage, liposomal daunorubicine (DaunoXome®) is an alternative. However, DaunoXome® appears to be less effective than Caelyx® (Cooley 2007). Of note, non-liposomal and non-pegylated forms of doxorubicin are not bioequivalent.

Beside doxorubicin and daunorubicin, paclitaxel (Taxol®) is also effective in KS (Tulpule 2002, Dhillon 2005, Stebbing 2006, Cianfrocca 2010). However, paclitaxel is more myelotoxic and leads almost always to complete alopecia, often during the very first cycle (patients must be informed!). Paclitaxel should be used only if KS lesions show progression during therapy with Caelyx® or when Caelyx® or DaunoXome® are not available. Docetaxel (Taxotere®) is also effective according to uncontrolled studies (Autier 2005, Lim 2005). It should be mentioned that significant interactions may exist between the taxanes and ART. Paclitaxel levels may increase significantly when combined with PIs (Bundow 2004, Cianfrocca 2011).

For the treatment cases refractory to doxorubicin, beside taxanes, oral etoposide (Evans 2002), irinotecan (Vaccher 2005) and the ABV regimen, a combination of adriamycin, bleomycine and vincristine, may be considered. According to a retrospective study from Kenya, even gemcitabine has promising activity in KS (Strother 2010).

Immunotherapy: With interferons (IFN) acceptable remission rates are reached. However, CR rates seem to be lower than with pegylated liposomal doxorubicin (Kreuter 2005). The effect mechanism of IFN on KS is not fully clarified. Apart from an immune modulating effect, IFN probably induces the apoptosis in KS cells. It is important to note that the effectiveness depends on the immune status. In patients with more than 400 CD4 T cells/µl, remission rates during IFN are at least 45%, compared with only 7% in patients with less than 200 CD4 T cells/µl. There may be other factors to predict response to IFN such as endogenous IFN levels, which are increased in the advanced stages of HIV infection.

Table 1: Specific therapies for KS when ART is not sufficient

Therapy	Dosage	Comments
Pegylated liposomal doxorubicine (Caelyx™ or Doxil™)	20 mg/m² IV every 2 weeks	Treatment of choice, beware of myelotoxicity, cardiotoxicity, hand-foot syndrome
Liposomal daunorubicin (DaunoXone™)	40 mg/m² IV every 2–3 weeks	Slightly less effective than Caelyx™, seldom used during the past decade. However, as capacity constraints for Caelyx™ are expected for 2012, an important alternative
Interferon-α 2a (Roferon™)	3–6 x 10⁶ I.E. SC or IM 3x/week	Considerable side effects, less efficacy than with doxorubicin. Use only when CD4 T cells are >200/µl and limited disease
Pegylated Interferon-α 2b (PegIntron™)	50 µg SC weekly	Tolerability improved compared to conventional IFN-α (2a,b), but lack of data in AIDS KS, off-label use!
Paclitaxel (Taxol™)	100 mg/m² IV every 2 weeks or 135 mg/m² IV every 3 weeks	Beware of neutropenia, peripheral neuropathy, allergic reactions, alopecia Off-label Use! Caution with ART interactions

There are currently no standardized IFN treatment regimens. Due to the considerable side effects, a high-dose treatment (up to 30 million IU/day) is not commonly administered. Daily doses of 3-6 million IU subcutaneously are usually given. After remission (tumour growth stopped, tumours flattened, loss of purple color, change to brownish color), interferon dosing can be reduced to 3x/week. Remission can be expected after 6–8 weeks of treatment (often significantly later). There is not sufficient data on the use of the pegylated IFN for HIV-associated KS. It is not licensed for KS and optimal dosage is unknown. However, there are some promising case reports in AIDS patients (Rokx 2013) and in patients with classical KS (Di Lorenzo 2008).

Local therapy: is well-tolerated and less costly. Many different methods are used depending on the size and location of tumors: cosmetic camouflage, cryosurgery, intralesional injections of Vinca alkaloids or interferons, soft x-ray radiation, electron beam therapy, cobalt radiation (fractionated) or Imiquimod (Celestin Schartz

2008). Compressive therapy with elastic stockings is an important strategy for the treatment of KS associated lymphoedema (Brambilla 2006).

KS is a strikingly radiosensitive tumor (Becker 2006, Donato 2013). Superficial macular or plaque-like KS lesions respond well to daily doses of 4–5 Gy (total dose 20–30 Gy, fractionated 3x/week) of soft x-ray radiation. In the case of large KS lesions with edema, radiation with fast electron beams (5 x 2 Gy per week, total dose 40 Gy) is recommended.

As KS is a multifocal systemic disease, surgical treatment is limited to excisional biopsies for diagnosis and palliative removal of small tumors in cosmetically disturbing areas. Since tumors often extend further into the surroundings than is clinically visible and local trauma can lead to new tumors (Koebner phenomenon), local and regional recurrences can be expected. These can be prevented by radiation therapy: in order to reach the tumor cells spreading along the vascular channels, the field of radiation should be extended 0.5-1.0 cm beyond the edges of the tumor.

New therapeutic approaches: With regards to the KS pathogenesis, several new therapies have been suggested such as virustatic agents, cytokines and inhibitors of angiogenesis. They are described here briefly:

• Valganciclovir –a promising approach; this antiviral agent significantly reduces HHV-8 replication, shown in a randomized trial (Casper 2008). Antiviral efficacy is higher than with valacyclovir or famciclovir (Cattamanchi 2011). However, there are no data on clinical efficacy in AIDS-related KS published to date. As HHV-8 is involved in the early steps of KS pathogenesis, it is questionable if valganciclovir has any effect on manifest lesions. In patients with classical KS, the drug remained inefficient (Krown 2011).

• Interleukin-12 – high response rates in a Phase II study, in which this cytokine was combined with liposomal doxorubicin (Little 2007). No randomized studies.

• Sirolimus (and everolimus) – new immunosuppressive agents used in the transplant and rare diseases (tuberous sclerosis, LAM) settings. Good response rates in uncontrolled studies on HIV-negative renal transplant recipients with KS (Stallone 2005, Campistol 2007). It is postulated that these drugs inhibit tumour angiogenesis through impaired vascular endothelium growth factor production.

• Lenalidomide – immunomodulatory drug with antiangiogenic effects, encouraging case reports, an ongoing US Phase I/II trial to evaluate the efficacy and tolerance of lenalidomide in HIV-related KS with and without visceral involvement (Martinez 2011, Steff 2013).

• Bevacizumab – an early study of this VEGF antibody showed moderate response rates in 31% of 17 HIV+ patients with KS progression on ART (Uldrick 2010). A study of combination with liposomal doxorubicine is ongoing.

• Imatinib (Glivec®) – activation of the platelet-derived growth factor (PDGF) and c-Kit receptors has been proposed as important in mediating the growth of AIDS-related KS. In a Phase II study, treatment with imatinib mesylate yielded to partial regression in 33% of AIDS/KS cases (Koon 2013).

• Sorafenib (Nexavar®) – an oral Raf kinase inhibitor, approved for advanced renal cancer. Case reports on KS (Ardavanis 2008). Phase I studies are underway.

• Matrix metalloproteinases (MMPs) – are involved in tumour metastasis and are over-expressed in KS cells. MMP inhibitors such as COL-3 have shown activity in a Phase II study on patients with advanced KS (Dezube 2006). However, clinical response rates were at best moderate. The most common adverse events were photosensitivity and rash. Encouraging Phase II study with topical halofuginone (Koon 2011).

• Retinoid compounds (tretinoin, isotretinoin, acitretin) – may inhibit the prolifer-

ation of KS cells. Many studies on different formulations have been conducted (Duvic 2000, Bodsworth 2001, Bernstein 2002, Aboulafia 2003). However, efficacy is only moderate. Retinoids will probably face a difficult path in attaining approval for KS.

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11. Malignant Lymphomas

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Malignant lymphomas are neoplastic diseases of the lymphatic system that grow rapidly and aggressively, and lead to death within a few weeks or months if left untreated. Hodgkin lymphoma (HL) is distinguished from the large group of non-Hodgkin lymphomas (NHL). In comparison to the general population, HIV+ patients are affected significantly more frequently by all types of lymphoma. Aggressive non-Hodgkin lymphomas of B cell origin are particularly frequent. The incidence of lymphomas has been markedly reduced by the introduction of antiretroviral therapy. Nonetheless, risk remains greatly elevated relative to the general population (Gibson 2014, Table 1) and there is evidence that this reduction overall was not as impressive as with KS or most other opportunistic infections (COHERE 2009, Franceschi 2010). Thus, the relative proportion of lymphoma among all AIDS-associated illnesses is increasing. The decline of incidence seems to be greater for lymphoma subtypes that mainly occur in severe immunodeficiency (Kirk 2001, Polesel 2008).

In some HIV cohorts, malignant lymphomas have already overtaken KS as the most frequent malignancy. In the EuroSIDA study, the proportion of AIDS-defined illnesses that were malignant lymphomas increased from less than 4% in 1994 to 16% in 1998 (Mocroft 2000). Among the AIDS-related deaths, lymphoma is by far the most frequent disease involved. In France, lymphomas accounted for 24% of all AIDS-related deaths in HIV patients (Morlat 2012).

Table 1: Relative risk of different lymphomas in HIV+ patients in comparison to a non-HIV+ population in the HAART era (adapted from Gibson 2014)

Overall NHL	10.6
Diffuse Large B cell lymphoma	17.6
Burkitt NHL	33.7
Not classifiable	19.9
Primary CNS lymphoma (PCNSL)	47.7
Low-grade Non-AIDS-defining NHL	
Follicular	1.3
Peripheral T cell	3.6

Malignant lymphomas in HIV+ patients are also biologically very heterogeneous and differ in several aspects. The frequency and extent of oncogenic mutations or cytokine dysregulation differ, as does the histogenetic origin of the malignant cells (Porcu 2000). In addition, the association with EBV and other oncogenic viruses such as HHV-8 or SV40 is very variable. The extent of immunodeficiency also varies significantly. Burkitt's lymphoma and Hodgkin's lymphoma (HL) frequently occur even when immune status is good. In contrast, immunoblastic and especially primary CNS lymphoma (PCNSL) are almost always associated with severe immunodeficiency. There is now some evidence that some subtypes of malignant lymphoma can be considered to be "opportunistic" as severe immunodeficiency is required for the development of these entities. For other lymphoma subtypes, chronic B cell activation, possibly induced by even low HIV viremia, is a prerequisite (Epeldegui 2007, Zoufaly 2009, Regidor 2011).

However, HIV-associated lymphomas – both NHL and HL – have numerous common clinical features. Characteristics include the usually aggressive growth, diagnosis in the advanced stages with frequent extranodal manifestations, poorer response to

treatment, high relapse rates and an overall poor prognosis (Levine 2000). Despite a better prognosis during recent years (see below), HIV+ patients with NHL continue to endure substantially higher mortality compared with HIV-uninfected patients with NHL (Chao 2010).

The treatment of malignant lymphoma remains problematic. Although aggressive chemotherapy is possible in many patients with existing immunodeficiency, it is complicated and requires a close cooperation between HIV clinicians and physicians with experience in hematology/oncology.

We discuss systemic NHL, PCNSL and HL separately; multicentric Castleman's disease will also be mentioned as a distinct entity, although it is not considered a malignant lymphoma. Low-grade (indolent) NHLs are very rare in HIV+ patients, and will therefore not be discussed here. As there are no recommendations available, the treatment of such cases should follow the recommendations for HIV-negative patients.

Systemic non-Hodgkin lymphomas (NHL)

A close association between systemic NHL and AIDS has been described for a long time – the first cases were published only about a year after the first description of AIDS and even before the discovery of HIV (Ziegler 1982). High-grade BNHLs have been AIDS-defining since 1985.

More than 90% of HIV-associated NHLs are of B cell origin. They are almost always of high-grade malignancy. Two main histological types dominate: according to the WHO classification, these are Burkitt's lymphomas, which comprise 30–40% of cases, and diffuse large-cell B cell lymphomas, comprising 40–60%. However, a relatively large proportion of HIV-associated lymphomas (up to 30%) cannot be classified even by reference laboratories. A small proportion of NHLs (1-3%) are primary effusion or body cavity-based lymphomas and are considered a distinct entity (see below).

The prognosis of patients with NHL was poor in the pre-HAART era, being between 6 and 9 months (Levine 2000). Due to the introduction of combination that compared the variable impact on mortality of AIDS-defining events diagnosed during combination antiretroviral therapy, NHL was the AIDS-defining event with the greatest mortality hazard ratio (ART-CC 2009). Whether the clinical and pathological spectrum of lymphoma subtypes is also changing remains unclear. A French study showed no differences in lymphoma features in antiretrovirally treated patients compared to treatment-naïve patients (Gérard 2009). However, it seems possible that, compared to HL or Burkitt's lymphoma, the percentage of "opportunistic" NHL such as immunoblastic lymphoma will decrease.

Prevention and early detection

There is no data supporting specific therapies or diagnostic procedures (such as periodical ultrasound controls, etc) for prevention or for early detection of malignant lymphomas. Antiretroviral therapy seems to be the best protection against lymphoma. ART not only improves the immune status but it also reduces the chronic B cell stimulation, another risk factor for the development of lymphoma (Grulich 2008). HIV plasma viremia should be as low as possible as cumulative HIV viremia is an independent and strong predictor of AIDS-related lymphoma among patients receiving ART (Zoufaly 2009). Blood EBV DNA load also represents a risk factor (Leruez-Ville 2012).

Besides ART, there have been numerous studies evaluating factors (so called "biomarkers") that may precede the development of AIDS-associated lymphoma. For example, it has been shown that the levels of serum globulins (Grulich 2000), inter-

leukin-6 or -10 (Breen 2003), soluble CD33 (Pordue 2009, Breen 2012), activity of activation-induced cytidine deaminase (Epeldegui 2007) or circulating immuno-globulin-free light chains (Landgren 2009, Bibas 2013) may predict the risk of NHL. These activation markers were markedly elevated in those who developed AIDS-related NHL, when compared to AIDS patients and HIV-negative controls. These findings may help us understand the pathogenesis of lymphomas in HIV+ patients. However, a routine diagnostics measure has not been found.

Signs and symptoms

The main symptom is lymph node enlargement. Lymphomas are firm, immobile or barely mobile and painless. A large proportion of patients have advanced-stage lymphoma at the time of diagnosis. Ann Arbor stages III-IV are almost always the rule, and B symptoms with fever, night sweats and/or weight loss are found in the majority of cases (60–80%). General asthenia, significant malaise and rapid physical deterioration are also frequently seen. Extra-nodal involvement is common, and may be to a grotesque extent. In our own cohort of 203 patients, 81% had at least one extra-nodal focus (Hoffmann 2003). Every conceivable region of the body can be affected—the orbital cavity, testes, heart, breasts, bladder, kidneys, muscles, bones, etc. The gastrointestinal tract, liver, and bone marrow are affected particularly frequently. Secondary CNS involvement can also occur. With extra-nodal disease, additional symptoms arise depending on the localization. These include, for example, abdominal pain from hepatosplenomegaly, hemorrhage or ileus symptoms due to intestinal involvement, bone pain with skeletal infiltration, or headache caused by brain disease.

Diagnosis

Rapid histological diagnosis is essential. If bone marrow biopsy cannot secure the diagnosis, then a lymph node (e.g., cervical, axillary or inguinal) should be extir-pated. Mere puncture biopsy of a lymph node is often not sufficient to secure a rep-resentative specimen. It is imperative to send the material to a specialized pathol-ogy laboratory with extensive experience in lymph node morphology. Every case should be discussed with the pathologist and caution taken to avoid a misdiagno-sis. A typical yet mostly wrong diagnosis is that of a high- or low-grade T cell lym-phoma in an AIDS patient. T cell lymphomas are very rare in AIDS patients and in most cases, T cell infiltrates indicate several infectious diseases like malignant syphilis rather than lymphoma.

The basic pathological diagnosis should include information about the subtype of lymphoma (Burkitt?), the proliferation rate and the expression profile (definitely CD20, and probably CD10, CD138, MUM-1) as these can influence the therapy (see below). All patients with suspected NHL should be staged according to the Ann Arbor classification (Tables 2a, b).

Basic diagnostic tests for staging include chest radiography; abdominal ultrasound; CT scans of the neck, thorax and abdomen; and bone marrow biopsy; aspiration alone is not enough. In addition to an updated immune status and viral load, the following should be determined at the very least: blood count, ESR, CRP, uric acid, LDH, liver and kidney parameters and electrolytes. ECG and echocardiography are also important right away. The possible cardiotoxicity of chemotherapy (anthracy-clines) during the course of treatment can only be evaluated if these tests have been performed at the start. Pulmonary function should be tested before treatment with regimens containing bleomycine is initiated.

Table 2a: Staging according to the updated Ann Arbor classification

I	Involvement of a single lymph node region (I) or involvement of a single extralymphatic organ or site (IE)
II	Involvement of 2 or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site plus its regional lymph nodes, with or without involvement of other lymph node regions on the same side of the diaphragm (IIE)
III	Involvement of lymph node regions on both sides of the diaphragm (III) can be accompanied by localized extralymphatic organ involvement (IIIE) or spleen involvement (IIIS) or both (IIIE+S)
IV	Diffuse or disseminated involvement of one or more extralymphatic organs with or without associated lymph node involvement; or isolated involvement of an extralymphatic organ with involvement of distal (non-regional) lymph nodes.

Table 2b: Every stage is divided into categories A and B

A	Asymptomatic
B	General symptoms: a) unexplained weight loss of more than 10% in the last six months, and/or b) unexplained persistent or recurring fever with temperatures above 38°C, and/or c) drenching night sweats

After two cycles of chemotherapy, a re-staging should be performed to evaluate treatment success. This restaging should be oriented according to the original localization of lymphoma. After completion of the protocol, a complete restaging with bone marrow biopsy (if there was initial involvement) and all CT scans are necessary. With a complete remission, restaging is recommended initially at three-monthly intervals. These intervals can be prolonged to six months after one year and to twelve months after two years. Relapses after more than three years are rare.

In advanced stages (Ann Arbor III-IV), and particularly with ENT involvement, a diagnostic lumbar puncture is necessary before initiating systemic chemotherapy to exclude meningeal involvement. In such cases, 15 mg of methotrexate can be administered intrathecally as prophylaxis. Whether this action, generally accepted by oncologists, actually has benefit or not, has never been shown in controlled studies. However, newer data suggest that there may be a benefit (Spina 2010).

Therapy

Due to extremely rapid generalization, even "early stages" move quickly. Every aggressive HIV-associated lymphoma should be treated with systemic chemotherapy with a curative intent. Surgery or radiation therapy alone are not sufficient. Treatment should be started rapidly due to the aggressive nature of these lymphomas. In particular, time should not be wasted on staging. The necessary tests should be completed within a week.

In Europe, diffuse large cell NHLs have been treated for many years with CHOP-based regimens (usually 4–6 cycles, see Table 3). CHOP is the abbreviation for the combination chemotherapy of the cytostatics **c**yclophosphamide, adriamycin (**h**ydroxydoxorubicin), vincristine (**O**ncovin®) and **p**rednisolone. To date, no other chemotherapy regimen has been shown to have better efficacy. There are no randomized controlled trials comparing CHOP with other regimens such as CDE or EPOCH which have been proposed by several working groups.

In contrast to CDE or EPOCH, CHOP can be administered in ambulatory care and is fairly well tolerated. At least 4-6 cycles should be administered, and – as far as possible – 2 cycles after reaching complete remission (CR).

The standard three-week CHOP regimen (CHOP-21) is shown in Table 3. Following the success of CHOP-14 (one cycle every two weeks) in older HIV-negative patients (Pfreundschuh 2004), CHOP-21 can also be "intensified". In CHOP-14 the use of the growth hormone G-CSF (e.g., Filgastrim 30-48 million units or Neupogen® 300/480 µg SC daily on days 4 to 13) reduces the duration of neutropenia. This approach not only decreases the phase of increased susceptibility to infections, but also increases the dose intensity of chemotherapy. However, there is no comparative data on this for HIV+ patients. So far, we have had fairly positive experiences – in most HIV+ patients, it is possible to shorten the interval.

Recently, a study from East Africa reported on a dose-modified oral chemotherapy, consisting of lomustine, etoposide and cyclophosphamide/procarbazine. This pragmatic approach had acceptable remission rates in 49 patients with AIDS-related NHL and could be considered an alternative in resource-poor countries (Mwanda 2009).

Table 3: CHOP regimen (4–6 cycles of 3 weeks each, repeat on day 22)*

Cyclophosphamide	Endoxan®	750 mg/m² IV day 1
Doxorubicin	Doxo-Cell®, Adriblastin®	50 mg/m² IV day 1
Vincristine	Vincristin®	1.4 mg/m² (maximum 2 mg) IV day 1
Prednisolone	Decortin H®	2 tab. at 50 mg QD, days 1–5
Mesna	Uromitexan®	20% of cyclophosphamide dose at hours 0, 4, 8 (with reference to cyclophosphamide IV given as a short infusion or orally)

*Standard CHOP regimen ("CHOP 21")

We recommend the administration of co-trimoxazole as an adjuvant therapy, up until one month after completion of chemotherapy (960 mg three times weekly), independent of CD4 T cell count. Oral mucous membranes should be treated with mouthwashes and topical amphotericin. Good compliance from the patients is an important factor. During chemotherapy, at least twice weekly monitoring of the patient's condition, blood count, liver and kidney parameters is necessary. Treatment is usually continued with the full dose according to protocol if leukocytes are above 3000/mm³ and platelets above 80,000/mm³ on the planned day of treatment. Patients should be advised to carry out daily temperature monitoring and be told to present immediately in case of fever.

Rituximab in HIV+ patients

The introduction of the monoclonal CD20 antibody rituximab (MabThera® or Rituxan®) was one of the biggest advances in oncology in recent years. In numerous lymphomas, this antibody, which binds highly specifically to CD20-positive B cells (CD20 is expressed by most lymphoma cells), has markedly improved the effectiveness and length of response of conventional chemotherapy. A combination of CHOP and rituximab (R-CHOP) is now standard in many lymphomas. Rituximab is usually well tolerated, but often leads to a longer lasting B cell depletion, and occasionally to severe neutropenia (Voog 2003).

It is not clear whether rituximab has a similarly large clinical benefit for HIV+ patients as it has for HIV-negative B cell lymphoma. The results from AMC 010, a multicenter prospective US study, have at least raised doubts (Kaplan 2005). In this study,

143 patients with CD20-positive AIDS-related NHL were randomized (1:2) to CHOP or R-CHOP (rituximab in the usual dose of 375 mg/m² on day 1 with a monthly maintenance therapy for 3 months following chemotherapy). In addition to the chemotherapy, all patients also received G-CSF, a co-trimoxazole prophylaxis and an AZT-free ART. Both groups were well matched. The planned CHOP cycles were carried out at the same intensity in both groups.

The main results were disappointing. There was only a trend towards a better response in the R-CHOP arm (complete response rate 58% versus 47%, p=0.15). No differences were found with respect to the length of response, disease-free or total survival. However, neutropenia and incidence of (especially severe) infection were significantly higher in the rituximab group. Out of a total of 15 patients who died from an infection during the study, 14 had received rituximab (14% versus 2%, p=0.035). The cause of death was usually septicemia from various bacteria – both gram-negative and gram-positive were identified. Death occurred in the majority (8/15) during the first two cycles, although six cases happened during the rituximab treatment at the end of the chemotherapy. Fatalities occurred in all centers and were therefore not due to a possible lack of expertise in any one location. A further risk factor for "death from infection" was a low baseline CD4 count – 8/13 patients had less than 50 CD4 T cells/µl. The cause of the high rate of severe infections is still unclear. Pathophysiologically, it is at least possible that in pre-existing T cell defects present in HIV+ patients, a long-lasting rituximab-induced B cell depletion or hypoglobulinemia has particularly negative effects (Miles 2005).

There are also some case reports on HIV-negative patients developing PML after rituximab (Carson 2009), raising concerns about an additive or even exponential risk for PML in HIV+ patients exposed to rituximab. However, up to now, our preliminary data suggest no evidence for an early excess incidence of PML in HIV+ patients treated with rituximab (Hoffmann 2012).

In contrast to the results of AMC 010, there are numerous mostly uncontrolled studies which did not find an elevated risk for serious infection with the use of rituximab (Spina 2005, Boue 2006, Ribera 2008, Sparano 2009). In our own prospective cohort study of 164 patients with NHL since 2005, treatment with rituximab was beneficial even in severely immunosuppressed patients (Wyen 2012). Moreover, we did not find evidence for a high incidence of PML (Hoffmann 2012). Recent meta-analyses show a moderate benefit with regard to CR rates and survival for HIV+ patients treated with rituximab (Castillo 2012, Barta 2013).

Following the current data, the use of rituximab can be considered in all HIV+ patients with CD20-positive NHL. Even a severe immune deficiency (less than 200 CD4 T cells/µl) is not a contraindication. However, intensive monitoring and the prophylactic use of co-trimoxazole (and possibly quinolones) may be advisable. In addition, it is imperative that more data is obtained.

More intensive chemotherapy as standard CHOP

After earlier studies showed that intensive chemotherapy led to a disproportionately high risk of infection and toxic complications (Kaplan 1997), the tendency for a long time was to withhold HIV+ patients from therapy and often to treat them with reduced-dose regimens. This seems to be changing in the age of combination ART. Several prospective studies have shown that the tolerability of chemotherapy is improved through ART (Powles 2002, Sparano 2004, Bower 2008).

In the past few years, small pilot studies have been repeatedly published in which HIV+ patients have been treated with CHOP. There are also studies in which doxorubicin has been given as liposomal Caelyx® (Levine 2004+2013) or where the

dose of cyclophosphamide was increased (Costello 2004). In addition, CDE, a regimen which, when given for several days as infusion is supposed to overcome the potential chemotherapy resistance of lymphoma cells, is propagated again and again (Sparano 2004, Spina 2005). This is also the case for the EPOCH regimen (Little 2003, Barta 2012). The CR rates in these studies were between 50 and 75%. In our experience, CR rates up to 70% are also possible with ART and standard CHOP. Whether these new attempts, which always cause a stir, are really better than CHOP, remains speculative. In our view, they are not ready for use outside of trials.

Even stem cell transplantations are now possible in HIV+ patients – a scenario that was unthinkable just a few years ago. Very high doses of myeloablative chemotherapy in combination with ART are well tolerated (see below). In patients with Burkitt's lymphoma, intensive protocols that were originally developed for HIV-negative patients are also being successfully employed (see below). Today, the decisive question regarding more intensive chemotherapy in HIV+ patients is, therefore, not whether it can be used, but who actually needs it or will benefit from an increased dose.

What ART when?

In early studies, the effect of combination ART on the prognosis of HIV-associated NHL was only modest (Levine 2000). However, many studies clearly demonstrated that prognosis of patients with NHL is markedly improved with ART (Antinori 2001, Besson 2001, Ratner 2001, Hoffmann 2003). In addition to survival, some studies also showed improved disease-free survival, response rates and even improved tolerability of chemotherapy. Even cases in which ART alone led to a complete remission of lymphoma have been published (Amengual 2008, Baraboutis 2009, Teng 2011). There is no doubt that every patient with AIDS-associated lymphoma should start an antiretroviral therapy, even in the setting of a relatively preserved immune function.

In most cases, an already existing, virologically effective ART can be continued during chemotherapy. However, a switch from AZT (myelotoxic) and from d4T/ddI (high risk of polyneuropathy, in particular when given with vinca alkaloids) to other nucleoside analogs or to a nuke-free regimen should be considered. Before switching to abacavir, an HLA-B*5701 genetic screening is recommended. When switching to tenofovir, intensive monitoring of renal function parameters is required.

In naïve patients, the first one or two CHOP cycles can be completed before starting ART. Some clinicians prefer to complete all six cycles out of concern for interactions and cumulative toxicities (Little 2003). In our opinion, this is not necessary, even though data on possible interactions between ART and chemotherapy is limited (Review: Mounier 2008). For example, the effect of PIs and NNRTIs on doxorubicin levels seems to be only moderate (Toffoli 2004) and in many studies, the concomitant use of ART and chemotherapy was feasible and safe (Powles 2002, Weiss 2006, Simcock 2007, Bower 2008). However, there have been some reports of patients who experienced severe vinblastine-associated neurotoxicity during concomitant treatment with ritonavir-boosted PIs (Cheung 2010). If PI-containing combinations are used, TDM is recommended. However, due several reports on an enhanced toxicity risk with PIs (Levêque 2009, Cingolani 2011, Corona 2013, Ezzat 2013), we would recommend avoiding PI-based regimens in patients receiving chemotherapy.

Thus, in ART-naïve patients without pre-existing renal damage, we would favor a combination of tenofovir, FTC and raltegravir. The integrase inhibitor raltegravir has a low risk for interactions and side effects. Moreover, many studies suggest a faster viral decay with this agent compared to other antiretrovirals. During tenofovir, renal function should be monitored carefully.

Special entities of lymphoma

Burkitt's or Burkitt-like lymphomas: the particularly high proliferative capacity and aggressiveness of Burkitt's or Burkitt-like lymphomas is a problem even in HIV-negative patients. In this case, the CHOP regimen is insufficient (Trümper 2001). Although it is still unclear whether this is also true for HIV+ patients with Burkitt's lymphomas, many clinicians have in recent years tended to treat such patients more intensively. A modified dose-adapted protocol of the German multicenter study group for adult acute lymphoblastic leukemia (GMALL) is usually used for the treatment of HIV-negative cases of Burkitt-NHL/B-ALL, and consists of four to six short, intensive 5-day polychemotherapy cycles, alternating A and B cycles. A cytoreductive pretreatment with cyclophosphamide and prednisone, each for 5 days, was given before the first cycle. During cycle A, fractionated doses of ifosfamide for 5 days, intermediate- or high-dose methotrexate 500–3000 mg/m², VM26, cytarabine (ara-C), vincristine, and dexamethasone are given. During cycle B, ara-C, VM26 and ifosfamide are replaced by doxorubicin and cyclophosphamide (Hoelzer 1996). Preliminary data show better responses than with CHOP (Hoffmann 2006) and rates comparative to those of HIV-negative patients (Oriol 2008, Xicoy 2014). However, the GMALL protocol is very intensive and cannot be administered on an outpatient basis. Strict monitoring of patients in hospital for several weeks is very important. Centers without experience should not administer it to HIV+ patients.

Other intensive therapies have been also reported (Ferreri 2013, Alwan 2015, Noy 2015). A significant problem with most studies is that there is no control group. There is no randomized study. However, there is increasing evidence that conventionally treated patients with Burkitt's lymphoma continue to have a worse prognosis even in the age of combination ART (Lim 2005, Spina 2005). Although this has not been confirmed by all study teams (Bower 2005), intensive therapy should be considered for every patient with Burkitt's lymphoma. A poor immune status or the existence of a concurrent opportunistic infection does not necessarily have to be an obstruction (Lehmann 2005). Interestingly, a new low-density approach with the R-EPOCH regime seems to be very effective, according to a small case series (Dunleavy 2013).

Plasmablastic lymphomas: are a relatively "new" entity in HIV+ patients. Plasmablastic lymphomas probably belong to the diffuse large cell NHLs, but display a completely characteristic immune phenotype, which usually correlates to a post-germinal center cell – markers for the B cell antigen CD20 are negative, whereas the plasma-cell reactive antibodies VS38c and CD138 are positive (Brown 1998, Teruya-Feldstein 2004). The oral cavity is the site of involvement (Gaidano 2002), although extra-oral manifestations do occur (Chetty 2003). There is a close association with an HHV-8 infection but also EBV (Castillo 2008, Riedel 2008). Like Burkitt's lymphoma, plasmablastic lymphomas have a very high rate of proliferation and are extremely aggressive. Prognosis remains poor (Castillo 2012, Schommers 2013). In a study on 89 people with NHL, we were able to show that a post-germinal center profile, as often occurs in plasmablastic lymphomas, is independently associated with a worse prognosis (Hoffmann 2005). This observation was confirmed by other groups (Dunleavy 2010). Intensive chemotherapy regimens do not seem to increase survival (Castillo 2012). New options are urgently needed. These could include bortezomib, which is a selective potent proteasome inhibitor that has been approved for clinical treatment of multiple myeloma and mantel cell lymphoma (Bibas 2010, Saga 2013, Castillo 2015, Fernandez-Alvarez 2015).

Primary effusion lymphoma (PEL): a further therapeutic problem is the relatively rare entity of the so-called primary effusion lymphoma or body cavity lymphoma (Carbone 1997+2000). These lymphomas are often very difficult to diagnose histologically. A visible tumor mass is usually absent, so malignant cells can only be found in body cavities (e.g., pleural, pericardial, peritoneal). There are histological similarities to immunoblastic and anaplastic cells with a non-B-, non-T phenotype. Every pleural or pericardial effusion occurring in an HIV+ patient and containing malignant cells, is suspicious of PEL. The involved pathologist should always be informed about this suspicion. There is a characteristic close association with the herpes virus HHV-8, which can be detected in malignant cells, and which provides a relatively typical gene expression profile (Simonelli 2005, Fan 2005). Recently, a solitary variant has been reported, which is neither morphologically nor immunophenotypically distinguishable from the classical PEL types (Chadburn 2004).

The response to the CHOP regimen is usually poor and poorer than that of centroblastic NHL (Simonelli 2003). Case studies with complete remission on ART alone have been described (Boulanger 2001, Hocqueloux 2001). We have, however, seen two PEL patients who have also died of progression despite CHOP and ART after only a few months. A small study reported encouraging results with a combined chemotherapy with high-dose methotrexate. In at least 3/7 patients a complete remission was achieved – a notable achievement in view of the otherwise poor prognosis, and an approach that should be followed up (Boulanger 2003). On the other hand, there are reports in which even intensive treatment regimens were unsuccessful (Waddington 2004). A new option may be bortezomib, which is a selective potent proteasome inhibitor. Xenograft models have shown that bortezomib induces PEL remission, providing a rational basis for clinical evaluation (Sarosiek 2010).

Relapse therapy, stem cell transplantation

At the moment, no general recommendations for relapse therapy of NHL can be given. The prognosis of NHL relapse is poor. A team from the US reported their positive experiences using the ESHAP protocol (etoposide, methylprednisolone, ara-C and cisplatin). DHAP appears to have no effect here (Bi 2001). The EPOCH regimen may also be effective. Other salvage monotherapies with mitoguazone or liposomal daunorubicin are purely palliative (Levine 1997, Tulpule 2001).

It should always be checked whether the affected patient with a relapse of lymphoma qualifies in principle for an autologous stem cell transplant (ASCT). In ASCT, the intensity of the chemotherapy can be markedly increased by the preceding gain of pluripotent stem cells (own cells: autologous; foreign cells: allogenic). Following the myeloablative chemotherapy, the patients are re-infused with the stem cells. Several hundred cases of SCT in HIV+ patients have been described so far worldwide. They have clearly shown that efficacy is comparable to HIV-negative patients (Simonelli 2010, Krishnan 2010, Re 2013). Even a few allogenic SCT have been reported (Kang 2002, Bryant 2008, Gupta 2009, Oka 2010).

In 2009, one of these cases attracted much intention. German researchers from Berlin transplanted stem cells from a donor who was homozygous for CCR5 delta32 in a patient with acute myeloid leukemia and HIV-1 infection. The patient remained without viral rebound for years after transplantation and discontinuation of ART (Huetter 2009, Allers 2011). There is no doubt that this case offers great hope for potential gene therapies.

The critical problem of autologous SCT in many hematological centers is above all a logistical one, namely the complicated storage of stem cells, which has to conform to strict safety regulations.

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Primary CNS lymphoma

Primary CNS lymphomas (PCNSL) are a late complication of HIV infection and used to occur in up to 10% of AIDS patients. The incidence of PCNSL seems to have decreased significantly in the last years in comparison to systemic lymphomas (Polesel 2008). PCNSL are EBV-associated in almost 100% of cases (Camilleri-Broet 1997). Histologically, findings are almost always consistent with diffuse large cell non-Hodgkin's lymphomas. The CD4 T cells are almost always below 50/µl at the time of diagnosis. In the pre-HAART era, PCNSL had the poorest prognosis of all the AIDS-defining illnesses, with a median survival of less than three months (Fine 1993). In more recent years, this bleak picture, often characterized by therapeutic nihilism, has changed significantly. In the HAART era, survival may be several years and complete remission has become possible (Hoffmann 2001).

Signs and symptoms

Different neurological deficits occur depending on the localization. Epileptic seizures may be the first manifestation of disease. Personality changes, changes in awareness, headaches and focal deficits such as paresis are also frequent. Fever is usually absent. As patients are almost always severely immunocompromised, constitutional symptoms may mask the real problem.

Diagnosis

Cranial CT or (better) MRT scan should be performed rapidly. The most important differential diagnosis is cerebral toxoplasmosis. A solitary mass is usually more indicative of PCNSL. However, 2–4 lesions may be present, which are usually fairly large (more than 2 cm in diameter). More than four lesions are rarely found.

In addition to an updated toxoplasmosis serology, which – if negative – makes toxoplasmosis very unlikely, a recent CD4 T cell count should be available. The better the immune status, the less likely the diagnosis of PCNSL. In our own cohort, less than 20% of patients had more than 50 CD4 T cells/µl at the time of diagnosis. At over 100 CD4 T cells/µl, however, cerebral toxoplasmosis is also less likely.

In addition to the physical examination, a minimal diagnostic program (chest radiography, abdominal ultrasound) should clarify whether the CNS involvement is secondary to systemic lymphoma. This should always include fundoscopy to exclude ocular involvement (up to 20%).

Besides cerebral toxoplasmosis, differential diagnoses include abscesses, glioblastoma and cerebral metastasis of solid tumors. In the absence of increased intracranial pressure, lumbar puncture is advised. If steroids have already been administered, however, the probability of finding malignant cells is diminished. EBV DNA is commonly detected in the CSF of HIV+ patients. Quantitative EBV PCR in the CSF improves the diagnostic specificity, although the predictive value remains too low for it to be used as an isolated marker for PCNSL (Corcoran 2008). In EBV positive cases, the possibility of primary CNS lymphomatoid granulomatosis should be considered in any differential diagnosis (Wyen 2006, Patsalides 2006).

In most cases, a treatment attempt for toxoplasmosis can be made initially. If this is unsuccessful, PCNSL is more likely. In such cases, stereotactic brain biopsy is essential to secure the diagnosis.

Treatment

For many years, cranial radiation therapy has been the only option for patients with PCNSL. In HIV-negative patients, using the combination of radiation therapy and steroids, a remission of 12–18 months duration is usually achieved. In HIV+ patients in the pre-HAART era, radiation only improved survival from 0.9 to 3.0 months (Fine 1993). Survival of more than one year was rare.

The prognosis for HIV-negative patients has improved in the last years due to the introduction of methotrexate-based (MTX) chemotherapies (Carraba 2010) and of rituximab (Korfel 2013). Whether these results will be applicable in HIV+ patients is not clear. In addition, the incidence of PCNSL is now diminishing to such an extent that convincing data on therapy efficacy can hardly be expected in the near future. A clear recommendation for treatment cannot be made at this time.

Some clinicians still favor cranial radiation therapy alone in HIV+ patients (fractionated, 40 Gy total dose). In our experience, before radiation a treatment attempt with intravenous MTX is justified (3 g/m^2 every 14 days with leucovorin rescue) – also in order to avoid possible neurological damage from radiation. A small study in HIV+ patients has shown that this approach is practical (Jacomet 1997).

However, the decisive factor in all cases – independent of the specific therapy chosen – is the best possible immune reconstitution. With ART, survival of several years has become realistic. Complete remissions have even been described after treatment with ART alone (McGowan 1998, Aboufila 2007, Travi 2012). In our own cohort of 29 patients with histologically diagnosed PCNSL, all four patients who experienced an increase in CD4 T cells survived longer than 18 months. Three out of four patients reached complete remission. One patient has now lived for over eight years without evidence of relapse (Hoffmann 2001). In a multivariate analysis, combination ART was shown to be the only factor associated with a prolonged survival in addition to cranial radiation therapy. Two of these patients, however, died after about three years of a progressive neurological syndrome, which was probably a long-term sequela of radiation therapy in both cases. In view of the better prognosis for patients today, radiation toxicity should be considered more than in the past. Three further studies from France, the US and Australia have since shown a survival of several years thanks to ART (Rigolet 2001, Skiest 2003, Newell 2004).

All patients with PCNSL should therefore be treated intensively with antiretroviral therapy, to achieve the best possible immune reconstitution. If only a moderate immune reconstitution is possible, additional immunomodulatory or antiviral therapies should be evaluated. The partially very positive reports about ganciclovir, foscarnet and interleukin-2 (Raez 1999, Aboulafia 2002, Marretta 2011) or hydroxyurea (Slobod 2000) should, however, be interpreted with caution. "Between the lines" of these publications, in which either individual or hardly more than 2-4 patients were described, combination ART was almost always a factor.

In all cases with signs of raised intracranial pressure, rapid administration of steroids (e.g., dexamethasone 8 mg TID, decreasing the dose rapidly after resolution of edema) is indicated, even if diagnostic testing is more difficult as a result. In the case of recurrent PCNSL, use of rituximab should be considered (Ferro 2012).

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Hodgkin lymphoma (HL)

The incidence of HL is elevated in HIV+ patients by a factor of 5-15 compared to the HIV-negative population. For particular subtypes, such as lymphocyte-depleted and mixed-cellularity HL,the relative risk is presumably much higher (Frisch 2001). Despite this and the growing realization that these subtypes at least are clearly associated with immunodeficiency, HIV-related HL is not included as an AIDS-defining illness.

There is growing evidence that the incidence of HIV-related HL is increasing in the setting of improved immunity. Several studies reported on an increased incidence during the last years (Clifford 2005, Biggar 2006, Engels 2008, Bohlius 2011). In our own cohort we found significant differences between NHL and HL (Wyen 2008). Whereas the majority of NHL cases is diagnosed in ART-naïve patients, HL mainly occurred in subjects receiving a virologically effective ART. For example, in our own cohort of 415 cases of systemic high-grade NHL and HL, significantly more patients with HL were treated with ART and had a viral load below 50 HIV RNA copies/ml at lymphoma diagnosis than patients with NHL (57.3% vs. 27.9%, p<0.001), The proportion of HL in the whole cohort was 20.7%. In the subgroup of ART-naïve patients it was only 7% but increased to 35% in patients with current viral load below 50 HIV RNA copies/ml (Hoffmann 2014).

The reason for this phenomenon is still not clear. As CD4 T cells usually predominate in the tumor microenvironment of HL, it is speculated that immune reconstitution

induced by ART provides an appropriate micro-environment allowing adequate growth signals for proliferation and survival of the neoplastic Reed-Sternberg (RS) cells in HL (Gloghini 2007). In addition, CD40/CD40L interactions and EBV infection may contribute to constitutive activation of NFkB which is an antiapoptotic factor in RS cells. Interestingly, patients whose CD4 T cell counts decline despite suppression of HIV-1 replication, are at risk for HL (Bohlius 2011).

An advanced stage of disease at diagnosis is typical, as is frequent extranodal involvement and a trend towards prognostically poorer subtypes (Tirelli 1995, Rapezzi 2001, Thompson 2004). Mediastinal disease is significantly less frequent than in HIV-negative patients. A further difference to HL in seronegative patients is the predominance of cases with RS cells, as well as the clear association with EBV infection, which is 80–100%, depending on the study. EBV infection is therefore seen as an important etiologic factor for development of HIV-related HL.

In comparison to HIV-negative HL, which is a highly treatable tumor, the prognosis of HIV-related HL was poor in the pre-HAART era. In nearly all cohorts with more than 20 patients, the median survival was only between 15–20 months, respectively (Tirelli 1995, Levine 2000). The response to chemotherapy was also moderate compared to the normal population. Complete remission rates were between 40 and 80%, and hematological and infectious complications were frequent. This gloomy scenario has clearly changed since the introduction of combination ART. In our own multicenter cohort of 56 patients, the median survival was 40 months. In patients with adequate ART, the two-year survival rate was 84%, which was very encouraging (Hoffmann 2004). In the meantime, other groups have also reported better prognoses with ART (Ribera 2002, Gérard 2003, Berenguer 2008). There is now overwhelming evidence that HIV status no longer influences outcome in patients with classical HL in the HAART era (Montoto 2013).

Signs and symptoms

B symptoms occur in the majority of cases. Extranodal and advanced stages are almost always the rule. Lymphomas are firm, immobile or hardly mobile and painless, and the distinction from HIV-related lymphadenopathy or tuberculous lymphadenitis is not always possible.

Diagnosis

Staging is necessary as for non-Hodgkin lymphomas (see NHL above). Diagnostic lymph node extirpation is even more important here than with NHL, as puncture only rarely allows diagnosis of Hodgkin's disease. Single accurate diagnostics are better than half-heartedly bothering the patient with repeated punctures and losing time unnecessarily. Surgical extirpation is possible as an outpatient in many centers. As with NHL, specimens should be sent to reference laboratories if possible. Since bleomycine will be administered, a lung function test should always precede the first chemotherapy.

Treatment

Risk-adapted treatment strategy in patients with HIV-related HL in accordance with standard treatment procedures established for HIV-negative patients with HL is recommended. The achievement of complete remission (CR) is important. In one larger cohort, the only variable independently associated with overall survival was the achievement of CR (Berenguer 2008).

In limited (Ann Arbor I-II, no risk factors) and intermediate (I-II with risk factors) stages, many clinicians still favor the classical ABVD regimen (four double cycles, see Table 4) for HIV+ patients. ABVD is the abbreviation for the combination chemotherapy with the cytostatics adriamycine, bleomycine, vinblastine and DTIC (dacarbazine). Ambulatory treatment is possible.

Table 4: ABVD regimen (4 double cycles, repeat on day 29)*

Adriamycine (doxorubicin)	Doxo-Cell®, Adriblastin®	25 mg/m² IV days 1 + 15
Bleomycine	Bleomycin Hexal®, Bleo-Cell®	10 mg/m² IV days 1 + 15
Vinblastine	Velbe®, Vinblastin Hexal®	6 mg/m² IV days 1 + 15
Dacarbazine (DTIC)	Detimedac®	375 mg/m² IV days 1 + 15

*ABVD regimen. Due to strong emetogenicity of dacarbazine, 5HT3 receptor blocker anti-emetics should always be administered, e.g., granisetron, tropisetron or ondansetron

In HIV-negative patients in advanced stages (as is almost always the case for HIV-related HL) the BEACOPP regimen of the German Hodgkin Study Group has been used recently, mainly with escalated dosing. This has proven to be significantly more effective, both with regard to response rates and long-term survival. However, the BEACOPP regimen is more toxic. Whether these positive results can be seen in HIV-related HL is still not clear. However, based on initial reports and our own experience, BEACOPP seems to be possible (Hartmann 2003, Hentrich 2012). There is also growing experience to date with the Stanford V protocol, for which there have recently been promising reports (Spina 2002).

In all patients with HIV, HL should immediately be treated with ART. With regard to toxicity and interactions, PI-based regimens should be avoided (Levêque 2009, Cheung 2010, Ezzat 2012, Corona 2013).

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Multicentric Castleman's disease (MCD)

Although rare, multicentric Castleman's disease is a highly problematic illness for patients – not only due to the poor prognosis in HIV infection, but also because many clinicians and pathologists are not very familiar with this entity. The usually severely ill patients are often subjected to diverse diagnostic and therapeutic procedures.

In comparison to the benign, localized hyperplasia of lymphatic tissue, first described by Castleman in 1956, HHV-8-associated multicentric Castleman's disease, as it occurs in HIV infection, is a malignant lymphoproliferative disease (Oksenhendler 1996, Talat 2011). Although HIV-related MCD is not classified as a lymphoma or AIDS-defining illness, prognosis is poor. In a prospective study, the median survival was 14 months (Oksenhendler 1996). According to a review on 84 cases with HIV-related MCD, life expectancy of the patients seems to have significantly improved in the era of combination ART with a mortality rate of only 29% (Mylona 2008). During recent years, prognosis further improved, mainly due to the increased use of the monoclonal antibody rituximab (Bower 2011, Hoffmann 2011, Gérard 2012).

Pathogenesis

The pathogenesis of the disease is not completely understood. There is a close association to HHV-8, and as a result about half of the patients also have KS. Lymph nodes involved in HIV-related MCD are often involved coincidentally with KS (Naresh 2008). HHV-8 viremia in patients with MCD is often higher than in patients with KS (Sayer 2011). HHV-8 encodes a homologue of IL-6 (viral IL-6) that has been shown to be biologically active in several assays and whose activities mirror those of its mammalian counterparts. In particular IL-6 and IL-10 are elevated with close association to the HHV-8 viral load (Oksenhendler 2000).

Viral IL-6 mediates its effects through the gp130 signal transducer, but signaling is not dependent on the structurally related IL-6 receptor subunit of the receptor-signal transducer complex (Moore 1996, Li 2001, Suthaus 2010). It is thus postulated that viral IL-6 has a broader spectrum of potential target cells than human IL-6. This may be reflected by the clinically impressive "cytokine storms" which are observed peri-

odically in patients suffering from HIV-related MCD. However, newer studies have shown that both viral and human IL-6 can independently or together lead to MCD flares, suggesting that they may jointly contribute to disease severity (Polizotto 2013). HHV8-infected plasmablasts localize to the mantle zones of the lymphoid follicles (El Daly 2010). Of note, there are reports of MCD cases negative for HHV-8 infection (Seo 2009) but also of an IL-6-related systemic inflammatory syndrome in HIV+ patients with HHV-8 but without MMCD (Uldrick 2010). In these cases, the pathogenesis remains unclear.

It remains also unclear why only a small proportion of patients with active HIV/HHV-8 coinfection develops HIV-related MCD. It should be noted that the extent of immunodeficiency varies significantly in these patients. We and others have seen MCD patients with a normal immune status and low viral load (Powles 2009). Moreover, ART does not appear to protect against HIV-related MCD. In our own cohort of 52 patients, the majority of the patients with HIV-related MCD were already on ART and had a viral load of less than 400 copies/ml at the time of diagnosis (Hoffmann 2011). It is also of note that HIV-related MCD, unlike KS, is not associated with a lack of HHV-8-specific CD8 T cells or limitation of their functional profile (Guihot 2008). There is also evidence that the incidence of HIV-related MCD is increasing. It appears to occur more frequently in older HIV-positive individuals with well-preserved immune function (Powles 2009).

Progression to malignant lymphoma (often HHV-8-associated entities such as PEL or plasmablastic subtypes) is frequent. In by far the largest prospective study to date with 60 MCD cases, 14 patients developed malignant lymphoma after a median observation period of 20 months (Oksenhendler 2002). Subtypes mainly include rare entities associated with HHV-8 infection, such as plasmablastic or primary effusion lymphomas. In patients treated with rituximab, the lymphoma risk appears to be significant lower than in patients treated with conventional chemotherapies (Hoffmann 2011, Michot 2011).

Signs and symptoms

The main signs are the often significant lymph node enlargements, which are almost always combined with considerable B symptoms including fever, night sweats and weight loss. Almost all patients complain of weakness and severe malaise. There is always massive splenomegaly. Hepatomegaly (70%), respiratory symptoms (65%) and edema with hypoalbuminemia (55%) are also seen in the majority of cases. Lymph nodes, which may be anything from very soft (as with tuberculosis) to rock hard (as with lymphoma) on palpation, can normalize or relapse within weeks without any intervention.

The extent of symptoms is very variable and may fluctuate considerably. Many patients report on "Castleman episodes", lasting from a few days to one or two weeks. Between these episodes, most patients do again relatively well for weeks or even months. In most patients who leave HIV-related MC untreated, the frequency of the episodic flares increases over time.

Diagnosis

The diagnosis is made histologically after lymph node extirpation – providing that the pathologist knows what HIV-related MCD looks like. Hyaline-vascular and plasma cell types of Castleman's disease can be distinguished. The classical pathological features include angio-follicular hyperplasia and hypocellular germinal centers with hyalinization and mantle zone hyperplasia. In this mantle zone, concentric layers

of small lymphocytes generate the so-called "onion-skin" feature, associated with an intense interfollicular plasmacytic hyperplasia. Only a subset of mature, CD20-positive B cells ("plasmablasts") within the mantle zone is HHV-8-infected.

Clinicians should explicitly indicate their suspicion. It is possible that a significant proportion of cases are never correctly diagnosed. In every case of episodic flares of B-symptoms, splenomegaly, lymphadenopathy and elevated CRP, the diagnosis of HIV-related MCD must be considered. HIV alone rarely causes such severe illness! In the case of the symptoms described above, the pathological diagnosis of HIV-associated lymphadenopathy should not be accepted too easily.

Ultrasound reveals hepatosplenomegaly. Positron Emission Tomography (PET) findings correlate well with activity, severity and inflammatory parameters in MCD (Polizzotto 2015). Laboratory tests show hypoalbuminemia and hypergammaglobulinemia. There is often significant anemia which may be hemolytic, often reflecting pancytopenia or hemophagocytic syndrome (Stebbing 2009).

In our experience, CRP is a useful parameter for monitoring the activity of HIV-related MCD and observing the effectiveness of MCD treatment. During an episodic flare, CRP levels of more than 100 mg/l can be seen. Between the episodes, however, CRP is often within normal ranges. In some patients, clinical symptoms are preceded by elevated CRP levels. Treatment success is reflected by sustained decrease of CRP. Determining the HHV-8 DNA level may also be useful in diagnosis and for follow-up (Marcelin 2007, Sayer 2011, Stebbing 2011).

Treatment

In patients with HIV+ MCD, something has to be done quickly as the course of disease can be extremely fulminant. According to newer data, we believe that the use of rituximab is the treatment of choice in HIV+ patients with MCD (see below). Some experts advocate rituximab monotherapy for good performance in patients without organ involvement and rituximab with chemotherapy for more aggressive disease (Bower 2010). However, there is no widely accepted recommendation for a specific treatment for MCD. A wide variety of strategies has been reported, including cytotoxic elimination of cells responsible for hypercytokinemia, anti-herpesvirus therapies and anti-inflammatory and immunosuppressive therapies. More recently, blockade of IL-6 signaling with monoclonal antibodies (mAb) have been discussed. ART should always be given, although it does not always help (Dupin 1997, Lanzafame 2000, Aaron 2002, de Jong 2003, Sprinz 2004). Some cases have even been described to occur after starting ART, leading to the suspicion that the inflammatory component of MCD may be increased by immune reconstitution (Zietz 1999).

Rituximab: this monoclonal antibody against CD20-expressing cells is also used in B cell lymphomas (see above). It has been speculated that rituximab is effective in HIV-related MCD by eliminating or reducing the pool of HHV-8 infected B cells which are localized mainly in the mantle zone of lymph nodes. Rituximab has been tried with success in several patients with HIV-related MCD (Corbellino 2001, Marcelin 2003, Casquero 2006). At least two larger studies showed encouraging results. In a French study, 16/24 patients with HIV-related MCD reached a complete remission of clinical symptoms after four cycles of rituximab (Gérard 2006). The overall survival (OS) after one year was 92% and the disease-free survival (DFS) was 74%. In a British study, 20/21 patients achieved a clinical remission with rituximab, and 14/21 patients showed a radiological response (Bower 2007). After two years, OS and DFS were 95% and 79%, respectively. CRP, immunoglobulins, cytokines such as IL-5, IL-6 or IL-10 and HHV-8 viremia decreased after treatment (Bower 2009). In our cohort, rituximab markedly improved prognosis in HIV-infected patients with MCD,

compared to patients receiving chemotherapy only (Hoffmann 2011). There is also evidence that rituximab decreases the risk of lymphoma (Bower 2011, Gérard 2012). Rituximab is usually given at a dose of 375 mg / m² body surface, once weekly over four weeks. Attention should be paid to good hydration. Rituximab is usually well tolerated. The main adverse event seems to be a reactivation of KS, which is seen in up to a third of the cases (Bower 2007). A recent study suggested that KS progression can be prevented by combination of rituximab with liposomal doxorubicin (Uldrick 2014). Rituximab is also effective as retreatment for rituximab-pretreated HIV-related MCD (Powles 2007). Based on the data published to date and on our own experience, we would consider rituximab to be the first option in patients with HIV-related MCD. However, there also some case reports in which rituximab was not successful (Neuville 2005, Buchler 2008). For these cases, other therapeutical approaches are briefly discussed here.

Valganciclovir: promising, as this antiviral agent may act against HHV-8. As shown by a randomized trial, valganciclovir significantly reduces the frequency and quantity of HHV-8 replication (Casper 2008). More recently, preliminary data suggest that valgancyclovir (combined with high-dose AZT) is active in HIV-related MCD. Of 14 patients, 12 had "clinical improvement", showing a decline of inflammatory markers such as CRP, IL-6 and HHV-8 viremia (Uldrick 2011). However, in our own cohort we were unable to confirm these findings (Hoffmann 2011). According to some experts, valganciclovir may have a role as maintenance therapy in the future (Bower 2010). In contrast, antiviral therapy with foscarnet or cidofovir had no benefit (Coty 2003, Senanayake 2003, Berezne 2004).

Chemotherapy: well-tolerated chemotherapies such as vincristine (2 mg IV as a bolus at 14-day intervals) or oral etoposide (50 mg daily) have proven effective according to several reports as well as our own experience (Scott 2001, Kotb 2006). CHOP standard chemotherapy can help, but does not seem to significantly prolong survival.

Splenectomy: may be appropriate in severe cases. It is speculated that IL-6 production is reduced and that a large reservoir of HHV-8 is removed through the splenectomy. In a series of 40 patients, the median survival following splenectomy was 28 versus 12 months (Oksenhendler 2002). According to a US study, the symptoms were improved in 10/10 patients following splenectomy (Coty 2003).

Anti-IL-6 antibodies: In HIV-negative patients, very optimistic data from Japan have been published, in which patients were successfully treated with anti-IL-6 receptor antibodies such as tocilizumab (Nishimoto 2005, Matsuyama 2007). In Europe, tocilizumab was approved in 2009 for treatment of rheumatoid arthritis. However, there only case reports for HIV-related MCD (Nagao 2014). Data is also lacking for siltuximab, a new IL-6 antibody. In a randomized trial of 53 patients with idiopathic MCD (negative for HHV-8 and HIV), 34% achieved a durable response (van Rhee 2014).

Thalidomide: This drug is believed to inhibit cytokine dysregulation as well as the inflammatory component of MCD. Case reports in HIV-related MCD exist (Lee 2003, Jung 2004). It should be noted that thalidomide has been associated with venous thromboembolic events, including deep venous thrombosis and pulmonary emboli. Anticoagulation during thalidomide administration is mandatory. We have seen two patients developing pulmonary emboli despite anticoagulation. Therefore we would not recommend the use of thalidomide in HIV-related MCD.

Other immune therapies: For interferon, there are positive as well as negative examples (Coty 2003, Nord 2003). Steroids have no effect on MCD.

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12. Non-AIDS-defining Malignancies

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HIV+ patients have an increased risk of cancer. This applies not only to the three AIDS-defining malignancies (ADMs), namely Kaposi's sarcoma, non-Hodgkin lymphoma and cervix carcinoma, but also to different non-AIDS-defining malignancies (non-ADMs). The risk for non-ADMs is approximately two to three times higher in HIV+ patients than in the non-infected population (Frisch 2001, Franceschi 2010). Incidence of some diseases such as Hodgkin lymphoma (see *Malignant lymphomas*) and anal carcinoma are so high that there is, according to many experts, a demand to classify them as ADM. In contrast, breast cancer incidence is either same or less compared to the general population (Latif 2011).

One-third of all malignancies in HIV+ patients today are non-ADMs. They are, therefore, as frequent as malignant lymphomas and Kaposi's sarcoma (Engels 2006). Over the last years, incidence has remained relatively stable (Worm 2013). As a result, non-ADMs are a significant mortality factor within the HIV+ population. In industrial countries, more deaths are attributed to non-ADMs than to ADMs, hepatitis C or cardiovascular diseases. Non-AIDS cancer is now the leading non-AIDS cause of death and without any evidence of improvement. In the D:A:D cohort, the proportion among specific causes of death in people with HIV increased from 9% in 1999–2000 to 23% in 2009–2014 (Smith 2014). The following diagram shows the percentage of malignant diseases relative to total causes of death in HIV+ patients in France in 2000 and 2005 (Bonnet 2009).

Figure 1 clearly shows that the percentage of AIDS-defining tumors, NHL and KS, are slightly on the decline, whereas the proportion of non-ADM are rising slightly. Different reasons may explain this. In the D:A:D study, the main risks of non-ADMs resulting in death were advanced age and acute smoking, and interestingly also CD4 T cell counts. Risk of non-ADMs increased, the lower the CD4 T cell counts were. Patients with CD4 T cells <50/µl had a 15-fold higher risk than patients with
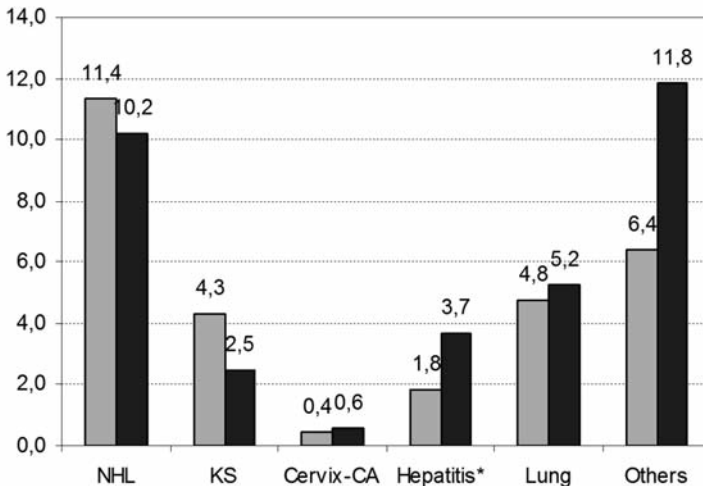
Figure 1: Proportion of malignancies among all causes of death in HIV+ patients in France in 2000 (grey, n=924) and in 2005 (black, n=1013). Numbers according to Bonnet 2009. *Hepatitis-associated tumours

>500 CD4 T cells/µl (Monforte 2008). The high risk persists if CD4 nadir was low (Worm 2012). This correlation between non-ADM and severe immune deficiency is from the EuroSIDA study (Reekie 2010). In a US databank analysis which included 300,000 AIDS patients (Frisch 2001), some malignomas cases were associated with immunodeficiency: Hodgkin's lymphoma, lung cancer, penile carcinoma, soft tissue sarcomas, testical and lip cancer.

Apart from immunodeficiency, other factors certainly play a role. Mainly smoking but also life-style (alcohol, UV exposure) or coinfections (HPV, HBV, HCV) contribute to the risk. In the absence of smoking, however, the increase in risk is confined to cancers related to viral infections, whereas the risk of other cancers is not elevated and does not seem to be associated with immune deficiency (Helleberg 2014). Given the fact that HIV+ patients are aging, an increase of incidences of malignancies is to be expected (Shiels 2011). ART seems to have little influence on the occurrence of non-ADMs since therapy interruption does not increase the risk for non-ADMs, in contrast to ADMs (Silverberg 2007).

Early diagnosis and prevention

It remains unclear whether HIV+ patients require cancer screening and preventive medical checkups more frequently than negative patients. There are some indications for a benefit regarding anal carcinomas (see below). Regarding colon carcinoma the situation is not clear; however, there is evidence that neoplastic changes are found more frequently in colorectal cancer screening with HIV+ patients (Bini 2009, Boesecke 2012). This examination, however, is not so popular with HIV+ patients or with treating physicians. Compared to the HIV-negative population, colorectal cancer screening is utilized to a lesser degree (Reinhold 2005). With respect to PSA screening, which is discussed controversially in general, there is no specific recommendation for HIV+ patients (Tyerman 2012). Gynaecological examinations are discussed in the chapter *HIV and Gynaecology*. In patients coinfected with HCV, bi-annual ultrasound sonographies can have a benefit, as a recent study with 70 patients showed: hepatocellular carcinomas were less progressed at diagnosis in regularly screened patients resulting in a slightly better survival (Nunez 2010).

Finally, physicians should inform patients about the advantages of not smoking and support smoking cessation. Smoking contributes to substantial morbidity and mortality in the HIV+ population (Lifson 2010). Patients often request and insist upon more medical checkups, but it is repeatedly forgotten that abstinence from smoking is still the most important preventive measure for malignant diseases.

In a setting where care is well organized and antiretroviral therapy is free of charge, HIV+ smokers lose more life-years to smoking than to HIV. The population-attributable risk of death associated with smoking is doubled compared to the background population (Helleberg 2013). In the absence of smoking, the increase of many cancers is not elevated and does not seem to be associated with immune deficiency (Helleberg 2014).

Thus, smoking cessation, avoidance of obesity and a healthy lifestyle are more helpful than expensive medical examinations.

Treatment

One problem in the therapy of non-ADMs is that too little is known about chemotherapeutic substances and their interactions with ART. Especially since the new targeted substances have mostly not been investigated in HIV+ patients. There are no prospective studies and very little data on imatinib, erlotinib, sunitinib, bortezomib,

sorafenib or temsirolimus (Review: Rudek 2011). Few case reports exist for many malignant diseases. In most cases patients are younger compared to the HIV-negative population which may be due to better monitoring (Shiels 2010). Publications over the last years on different entities such as glioblastoma (Hall 2009) or colon carcinoma (Chapman 2009, Alfa-Wali 2011), bladder cancer (Gaughan 2009), prostate cancer (Pantanowitz 2008) or esophageal cancer (Stebbing 2010) show that HIV+ patients prosper from the recent and amazing progress made in the oncological field. There should be no difference in treatment of HIV+ and non-infected patients – however, oncologists often need to be properly informed in order to avoid adhering to an outdated and pessimistic concept of HIV treatment.

Anal carcinoma

Anal cancer (AC) is probably the most frequent non-ADM. There is a close association to infections with human papilloma virus (HPV). An overwhelming number of studies and reviews has been published over the last decade, including several reports on dramatic increases of the AC incidence in HIV+ MSM. Moreover, there is a high prevalence of pre-stage AC, the so-called anal intraepithelial neoplasias (AINs). High-grade AINs (HGAINs), the precursors for anal cancer, are present in about 30% of cases. This has led to considerable concerns and uncertainty in patients and physicians. Unfortunately, large, good-quality prospective studies are lacking and there is still controversy about whether to routinely screen for AC in HIV+ patients. Here we will carefully review the available data.

Epidemiology, HPV association

HPV infections are among the most frequently sexually transmitted virus infections. HPV belongs to the family of *papoviridae* and infect the basal cells of the epithelium of the skin and mucous membranes. HIV+ patients have a 2 to 6-fold higher risk for anal HPV infection, independent of sex and sexual practices (Palefsky 1998, Piketty 2003). Risk of persistent HPV infection is 7-fold and inversely correlated with CD4 T cell counts (Piketty 2003). By now almost 100 different HPV types are known, among them 20 that are associated with anal or cervix carcinomas. In particular, HPV-16 and -18 have a high oncogenic potential.

HIV+ patients commonly have coinfections with several HPV subtypes (Machalek 2012). In a German study (Kreuter 2005), anal HPV infection was found in 86% of 103 male patients, among them especially HPV-16 (53%) and HPV-18 (27%), but also HPV-58 (22%) and HPV-83 (22%). Persistent HPV infection may lead to precancerous preliminary stages, the anal intraepithelial neoplasia (AIN).

There is no doubt that anal cancer rates are substantially higher for HIV+ patients. In a large study, the adjusted rate ratios were 80 for MSM and 27 for other men compared with HIV-uninfected men (Silverberg 2012). The risk is also elevated in HIV+ women in whom high grade AINs are frequently found (Hou 2012). When discussing the high relative risk of HIV+ patients, one should consider, however, that anal cancer is very rare in the general population. This means that a "substantially" or "dramatically" higher risk compared to the general population does not inevitably mean a high absolute risk. According to one systematic review (Machalek 2012), the pooled anal cancer incidence was 46 per 100,000 patient years in MSM. In HIV+ patients, the incidence increased from 22 to 78 in the HAART era. Incidence differs regionally and is highest in the US at 147 (Chiao 2013). In the D:A:D cohort, mainly including HIV+ patients from Europe, the incidence per 100,000 patient years is only 45 (Worm 2013), in the Suisse Cohort even lower at 25 (Francesci 2010).

The routinely repeated thesis of a worldwide dramatic increase of incidence over the last years has not been clearly verified. Moreover, the risk elevation is not the same for all HIV+ patients. In particular, AC incidence appears to be higher in patients with a low CD4 T cell nadir (Piketty 2012, Bertisch 2013, Chiao 2013, Duncan 2015). Cumulative HIV viremia and smoking are further risk factors (Bertisch 2013, Chiao 2013). There seems to be no strong protective effect of ART. In our own cohort of 121 patients with anal carcinoma, the vast majority of patients were on ART, with a well suppressed viremia and a median CD4 T cell count of 400/µl (Hoffmann 2011). It seems possible that cumulative use of PIs may be associated with a higher risk of anal cancer (Bruyand 2015).

Anal cancer screening, treatment of pre-stages

AIN is histologically graded depending on the degree of dysplasia in grade 1 (mild), grade 2 (moderate) or grade 3 (severe). In the latter, the whole epidermis is affected. AINs II/III or high grade AINs (HGAINs) are very common and are found in around one third of all HIV+ patients. It is important to remember that even these HGAINs do not inevitably lead to invasive cancer. According to a large systematic review the risk of anal cancer is relatively low (Machalek 2013). In total, the theoretical progression rate from HGAIN to anal cancer was calculated to be one in 633 patients (one in 377 in the HAART era) per year in HIV+ men, and one in 4,196 patients per year in HIV-negative men. Thus, the majority of HGAIN will never progress to anal cancer, and progression might occur less often than it does for cervical intraepithelial neoplasias (CINs). Even HGAINs have a high potential for spontaneous regression (Tong 2013, Grulich 2014).

At first glance, early detection and treatment of precursors seems to be important, since often many years can pass between AIN and AC manifestation. However, given the low progression rates as shown above, treatment of AIN bears a considerable risk for overtreatment. There is only limited data supporting current guidelines insisting that digital anorectal examinations as well as perianal and intra-anal smears should be taken yearly (Review: Ong 2014). The substantial differences in the natural history of anal HPV infection to those of cervical HPV infection suggest that one cannot simply transfer cervical cancer screening strategies to anal cancer screening. Until evidence from large prospective studies is available, screening for anal cancer should be done only in research settings. More data is needed on progression and regression rates of HGAIN and on biomarkers that predict HGAIN or anal cancer. To date, many HIV physicians remain ambivalent regarding screening for anal cancer (Ong 2015).

This applies also to treatment of HGAINs. The absence of reliable evidence for any of the interventions used in AIN precludes any definitive guidance or recommendations for clinical practice (Macaya 2012).

So what do do? In case of AIN 1, a topical therapy with imiquimod (or podophyllotoxin) may be adequate, AIN 2+3 can be removed either surgically (electrocaustic therapy) or via laser ablation. Infrared coagulation is also possible (Stier 2008). In a randomized study on 148 HIV+ MSM with AIN, three procedures were compared, including 16 weeks of imiquimod (3 x / week), 5-FU (twice a week), and monthly electrocautery for 4 months. This study showed that regarding both efficacy and side effects electrocautery is superior to imiquimod and 5-FU in treatment of AIN, but recurrence rates were substantial (Richel 2012).

Condyloma should be dissected by a proctologist (electrocoagulation, cryotherapy). A topical therapy alone with the immune modulator imiquimod (Aldara® cream) is possible, however the effects are often less than with non-infected patients. Still,

imiquimod clearly reduces the risk of a relapse in follow-up treatment. The mechanism of imiquimod is not directly antiviral, instead it almost certainly destroys tumor cells via cytokine induction. The most significant side effect is a local erythema (which means it is working!), more seldom may be burning and pruritis. Severe skin irritations are rare.

Diagnosis of anal cancer

The most common symptom in cases of anal carcinoma is rectal bleeding. A patient reporting blood in stool absolutely must visit a proctologist! Patients usually attribute the bleeding to hemorrhoids; however, this self-made diagnosis should not be trusted. Other symptoms are burning and pain during stool or pruritus. If an anal carcinoma has already developed squamous cell carcinoma and more seldom transitory epithelial carcinoma are histologically present. Anal canal and sphincter can already be infiltrated at an early stage. Regional lymph nodes are affected depending upon where the anal carcinoma is localized. Deep-seated anal carcinomas infiltrate inguinal, central pelvic, high lying mesentery. Distant metastases are rare. In addition to proctoscopy, if possible, an endosonography, a CT of the abdomen and the pelvis should be done.

Treatment of anal cancer

If anal carcinoma manifests and the lesion is smaller than 2 cm, a continence preserving operation is preferable. In these cases, an adjuvant chemo- or radiotherapy is not necessary. Larger lesions are treated with combined radio-chemotherapy (mitomycin 10 mg/m² on days 1 and 29 and 5-FU 1000 mg/m² on days 1–5 and days 29–33, with subsequent radiation therapy of up to 50 Gray in fractions). Other more intensive therapies are possible (Blazy 2005). Complications can occur under such regimens. If something can go wrong, it will: we have experienced a patient who had first developed severe extravasation under mitomycin, followed by myocardial infarction under 5-FU and then a perforating, feculent radiation colitis.
Additionally patients should always be treated in oncological departments. Following radiotherapy, a proctoscopy should take place every six months. Although positive effects are not certain (Bowler 2005), HIV+ patients with anal carcinoma should receive ART. Overall prognosis is not worse than with HIV-negative patients (Chiao 2008, Hoffmann 2011, Alfa-Wali 2012).

HPV vaccines

HPV vaccines have proven to be protective for intraepithelial neoplasia and persistent HPV infections in cases of cervix carcinoma (Harper 2006). In 2011, a large study confirmed that use of the qHPV vaccine also reduces the rates of anal intraepithelial neoplasia, including grade 2 or 3, among men who have sex with men (Palefsky 2011). The vaccine had a favorable safety profile and may help to reduce the risk of anal cancer. There is also some evidence for a therapeutic effect of the vaccine in HPV-infected patients (Anderson 2009, Wilkin 2010). The approved vaccines induce a sufficient immune response (Toft 2014).

Testicular tumors

Testicular tumors are the most frequently occurring cancer in men between 20 and 35. The relative risk factor for HIV+ patients compared to normal population in the same age group is 2.5-fold (Frisch 2001, Powles 2003). This especially applies to semi-

noma, not so much to non-seminoma (Goedert 2007). So far, the largest analyses report of 34 and 35 patients (Powles 2003, Fizazi 2001). The median CD4 T cell counts were between 300 and 350/µl at time of diagnosis, although with great variation. Overall prognosis was good and a matched-pair analysis did not prove worse with HIV+ patients (Powles 2004). Other studies confirm the positive course (Fizazi 2001). Patients should be treated with the standard regimens that are also recommended for negative patients. Depending on histology and stage of cancer, the regimen consists of orchiectomy, lymph node extirpation or radiation, and or a platinum-based chemotherapy. High dose therapies are also possible (Hentrich 2009). Treatment should be performed in cooperation with a urologist experienced in oncology and an HIV specialist.

Lung cancer

In the general population, lung cancer is the most frequent cancer disease that leads to death in male patients. This tendency is increasing in women and already ranks third. The risk seems to be rising with HIV+ patients. More recent studies from France show that lung carcinoma accounts for 5% of all causes of death and leads more frequently to death than Kaposi's sarcoma (Bonnet 2009). In a British cohort, the relative risk in the early years of the HIV epidemic was similar to that of the normal population and has now risen by a factor of 8 (Bower 2003). In other cohorts, relative risk remained constant between 3–10 (Engels 2006, Cadranel 2006, Dal Maso 2009). Overall risk seems to rise as immunodeficiency increases (Guiguet 2009, Reekie 2011). In our own retrospective study of 72 patients developing lung cancer during the last decade, most cases occurred in the setting of limited immune deficiency and a long-lasting sufficient viral suppression (Hoffmann 2011).

This increase can partly be explained by simple reasons: first, HIV+ patients live longer and have more time to develop lung cancer and second, HIV+ patients smoke more than non-infected patients. In some HIV outpatient clinics, up to 60–70% of the patients are smokers. Smoking remains the main risk factor for developing lung cancer (Hoffmann 2011, Clifford 2012). Thus, one should discuss the issue of smoking: "It's time to quit" – there are possibilities to cease smoking (Niaura 2000). Apart from age and nicotine abuse, other factors also seem determine an increased risk (Kirk 2007, Chaturvedi 2007). This is underlined by the fact that the most frequent subtype found in HIV+ patients, adenocarcinoma, is the subtype that is least associated with nicotine consumption (Cadranel 2006). Because often immune deficiency is not present, other factors, such as specific lung infections and a resulting scarring, are assumed, but also increased proinflammatory cytokines in the lungs or reduced glutathione levels are found frequently in HIV+ individuals. These factors can worsen the damage caused by smoking. Generally, HIV+ patients seem to be more sensitive towards carcinogenesis (Engels 2006, Kirk 2007, Chaturvedi 2007). In the US veterans cohort, an increased risk for HIV+ patients remained significant, even after adjusting for smoking, age, ethnicity and COPD (Sigel 2010). There is also some evidence for a genetic predisposition (Engsig 2011).

From a diagnostic-therapeutic view, patients always stand a better chance when the lung cancer has been diagnosed early. Symptoms are unspecific and when they present, it is often too late. In the case of HIV+ patients, diagnosis is seldom early enough. In our own cohort of 72 cases of lung cancer diagnosed 2000-2010, only 34% of the patients were in stages I-IIIa which are considered to be curable (Hoffmann 2011). Patients in early tumor stages should undergo surgery with curative intention since chemotherapy only suspends further progression for a few months (Cadranel 2006, Lavolé 2009). In our own cohort, median estimated overall survival (OS) was

1.12 years with a total 2-year OS of 24%. Clinical stage was highly predictive and long-term OS could only be achieved in very limited disease stages (Hoffmann 2011). If chemotherapy is indicated, patients with non-small cell lung carcinoma (NSCLC) in otherwise good condition should receive standard therapy beginning with cis- or carboplatin plus either taxane (paclitaxel), gemcitabine or navelbine. Carboplatin/ gemcitabine seem to be tolerated well (Bridges 2008). A second choice is pemetrexed or erlotinib, an inhibitor of epidermal growth factor receptor (EGFR) kinase. Preliminary data suggest an EGFR mutation status similar to that of the general population (Okuma 2015).

A large study recently found no significant difference in clinical outcome between HIV+ patients and uninfected controls with lung cancer. Survival after curative surgical resection in early-stage patients was similar. Thus, HIV status should not affect therapeutic decision making in lung cancer (Rengan 2012). HIV doctors should talk with and convince the oncologist not to expect the worst just because HIV-infection is involved and that HIV is not a contraindication for any drug. If general condition is poor, however, a well-tolerated combination of gemcitabine and navelbine can be given, which has been known to stop progression for a short time.

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Other Infections than HIV-1

13. HIV and HBV/HCV Coinfections

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HIV and HCV coinfection

Epidemiology and Transmission

Coinfection with HIV and HCV occurs frequently, due to the fact that they are transmitted via the same pathways (parenteral, sexual, vertical). In the US about 25% of HIV+ individuals are estimated to be infected with both viruses. Several European countries have even higher rates of coinfection (Rockstroh 2005). In Russia, about 70% of the 940,000 HIV+ patients are also HCV-positive as a result of the high incidence of IV drug users. Needle exchange programs have resulted in a marked decline in new infections of HCV in Western Europe. For example, in Barcelona the prevalence of HCV coinfection in persons with newly diagnosed HIV-infection has decreased from 24% during 2000–2002 to 10% in 2006–2008 (Trevino 2009).

HCV is ten times more infectious than HIV via blood-to-blood contact. Intravenous drug users and recipients of blood products are particularly susceptible to coinfection. Nevertheless, the probability of transmission from occupational needlestick injuries after exposure to HCV-contaminated blood is less than 2%, possibly even lower; i.e., 0.3% as after exposure to HIV-contaminated blood (Kubitschke 2007).

In contrast, sexual transmission of HCV occurs significantly less frequently than HBV or HIV (risk of transmission via heterosexual intercourse is <1%). About 4–8% of all HIV+ men who have sex with men (MSM) are also infected with HCV. The first cases of acute hepatitis C among HIV+ MSM were observed in London, Paris, Amsterdam and Berlin but have spread to a worldwide epidemic over the last decade (Boesecke 2015). The risk of transmission depends on concomitant sexually transmitted diseases such as syphilis or lymphogranuloma venereum, performance of sexual practices that are prone to injuries of the mucosal membranes like fisting or intensive repetitive anal sex, and intravenous use of recreational drugs ("Chem sex") (Vogel 2005, GMFA 2013).

Perinatal transmission of hepatitis C is rare in immunocompetent individuals (<1%). The transmission rate rises with increasing immunosuppression in HIV+ mothers, and is estimated to be as high as 20%. On the other hand, HIV+ mothers treated effectively with antiretroviral therapy do not appear to have an increased risk for materno-fetal transmission of the hepatitis C virus (<3% with cesarean section) (Pembrey 2005). Cesarean section did not reduce the risk of transmission to the newborn of HCV-monoinfected women putting the role of cesarean section into question (Indolfi 2009).

Clinical course and pathogenesis

The clinical course of hepatitis C and HIV coinfection is determined by HIV-associated immunosuppression. Progression of immunosuppression accelerates the course of hepatitis C. Conversely, there is no significant influence of hepatitis C on the course of HIV infection (Rockstroh 2005).

The latent period until development of liver failure or hepatocellular carcinoma in coinfected patients is estimated to be 10–20 years, whereas it is 30–40 years in HCV-monoinfected patients (Benhamou 1999). Improved treatment options for HIV infection have increased the likelihood of patients actually living to experience the development of liver failure which has become at least in some centers a frequent cause of death (Rosenthal 2007). ART can improve the unfavorable course of hepa-

titis C and delay the development of liver failure. This is particularly true for patients who achieve good immune recovery (Pineda 2007). Therefore and as a result of the START study (see *ART* chapter) initiation of ART regardless of CD4 T cell count is recommended in HCV-coinfected patients (EACS 2015).

On the other hand, hepatitis C infection can aggravate the potential hepatotoxicity of ART regimens. Up to 10% of patients have to discontinue ART due to severe hepatotoxicity. This risk is associated especially with the so-called "d drugs" (ddI, d4T). These agents should be avoided in coinfected patients. Nevirapine and tipranavir should be used with caution. In some coinfected patients, a temporary increase in transaminases is observed after initiation of ART. This most likely corresponds to an increased inflammatory activity of hepatitis C as a result of improved immune status. Nevertheless, long-term follow-up has shown that ART improves the course of hepatitis C.

Diagnosis

Diagnostic tests in coinfected patients are no different from those used in HCV monoinfection (see Table 1). Detection of HCV antibodies (anti-HCV) confirms exposure to HCV, but does not distinguish between resolved and chronic hepatitis C. Chronic hepatitis C is diagnosed by the detection of HCV viremia (HCV RNA). It should be noted that HCV antibodies might be lost during the course of HIV infection as a result of the underlying immunosuppression, although nowadays this phenomenon has become rare, probably due to improved test kits. It may therefore be useful to determine HCV RNA levels, even if the anti-HCV test is negative, if there is clinical suspicion or advanced immunodeficiency (it can also occur in patients undergoing chemotherapy). Similarly, determination of HCV RNA levels is indicated in cases of suspected acute HCV infection. HCV antibodies usually only become detectable one to five months after infection. In one study, they were still lacking in 37% of patients 3 months after first detecting HCV RNA (Thomson 2009).

Patients with HIV/HCV coinfection have significantly higher levels of HCV viremia than patients with HCV monoinfection (about 1 log). Based on current knowledge the level of viremia does not have a prognostic value for the course of hepatitis C. However, it should be noted that some patients might lose HCV RNA in parallel with progression of immune deficiency, but experience a flare up of hepatitis C together with clinical symptoms following immune reconstitution on ART (Kim 2006). Therefore, regular testing around the initiation of ART seems prudent.

When considering the treatment of hepatitis C, genotyping is necessary before starting. Six genotypes with numerous subtypes are known, and are seen to have different regional distributions: genotypes 1 and 3 are predominantly found in Europe, whereas genotypes 4 and 5 are found in Africa, and genotype 6 in Asia. Genotypes 2 and 3 in particular have been associated with significantly better responses to interferon-containing therapy. Coinfection with several genotypes is possible.

Another marker associated with response to interferon-containing treatment is determination of IL28B genotype. This is a T/C dimorphism close to a region coding for human interleukin 28B. Likelihood of response to interferon-containing, direct acting antiviral (DAA)-free treatment is about twofold higher in IL28B CC genotype than with the TT variant (Nattermann 2011). Spontaneous clearance in case of acute infection is better in CC genotype patients as well.

Assessment of liver fibrosis is very important to assess liver disease stage. Among several non-invasive methods available the Fibroscan® device is of special interest. This device measures liver stiffness directly correlated to the degree of fibrosis with a special technique (transient elastography).

Table 1: Diagnostic procedures for hepatitis C in HIV-coinfected patients (EACS 2015)

Diagnosis of HCV

HCV Ab (turn positive 1–6 months after infection as late seroconversions have been described, may rarely be lost due to immunosuppression)

HCV RNA levels[1] (in particular important for the prediction of response to IFN treatment)

Status of Liver Damage

Staging of fibrosis (e.g., FibroScan, liver biopsy, serum fibrosis markers[2])

Hepatic synthetic function (e.g., coagulation, albumin, cholinesterase)

Ultrasound every 6 months if cirrhosis (gastroscopy upon diagnosis of cirrhosis and every 2–3 years thereafter if negative for esophageal varices)

Before HCV Treatment

HCV genotype (GT), HCV RNA, renal and liver function tests

Autoantibodies (ANA, LKM1)[3]

TSH, thyroid autoantibodies (risk of hyperthyroidism under IFN-based therapy)

Monitoring of HCV Treatment

Differential blood count, creatinine, liver enzymes and, in persons with advanced fibrosis, bilirubin, albumin and INR every 2–4 weeks.

In persons treated with IFN-free regimens HCV RNA at 2–4 weeks and whenever needed in order to assess compliance and or breakthrough in patients experienced with oral DAAs.

HCV RNA at week 4 (to evaluate rapid virological response (RVR) under IFN-based HCV regimens) and on all treatments at end-of-treatment and at week 12 and 24 after treatment cessation (to assess SVR). In patients receiving all-oral DAA therapy no association between viral load at any given timepoint on therapy and SVR has yet been found.

CD4 cell count and HIV VL every 12 weeks

TSH and non-organ specific autoantibodies every 12 weeks on IFN-based therapy

[1] Low HCV RNA defined as <400,000-600,000 IU/mL when using PEG-IFN+RBV. There is no standard for converting the amount of HCV RNA reported in copies/mL to the amount reported in IU/mL. The conversion factor ranges from about one to five HCV RNA copies per IU/mL.
[2] Serum fibrosis markers include APRI, FIB-4, hyaluronic acid, Fibrometer, Fibrotest, Forns, Hepascore and other indices; complex tests such as Fibrometer, Fibrotest and Hepascore predict liver fibrosis more accurately than simple biochemical tests such as APRI, FIB-4 or Forns.
[3] Persons with positive anti-LKM or ANA with homogeneous pattern should be evaluated for concurrent autoimmune hepatitis especially in the presence of ALT elevation during INF-based treatment. Other causes of liver disease should be identified by blood tests and liver biopsy if needed.

There are several histological classifications used. In Europe the METAVIR score is most often used. It distinguishes five stages of fibrosis (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = some septa, 3 = significant septa without cirrhosis, 4 = cirrhosis). Hepatitis activity is graded according to the intensity of necroinflammatory lesions (A0 = no activity, A1 = mild activity, A2 = moderate activity, A3 = severe activity). As fibrosis progression is accelerated in HIV+ patients, monitoring of fibrosis in yearly intervals seems prudent. An increase of 2 or more stages in liver fibrosis after only 3 years was observed in 25% of all coinfected patients in one study (Sulkowski 2007).

If there is clinical suspicion requiring the detection or exclusion of extrahepatic manifestations (vasculitis, glomerulonephritis, systemic cryoglobulinemia), appropriate investigations may be necessary (skin biopsy, urine tests, kidney biopsy, detection of serum cryoglobulins).

The recommendations for diagnostic procedures in HCV/HIV-coinfected patients can be found in Table 1. For detailed information on TSH and autoantibody testing prior

to interferon-containing therapy please refer to the previous version of this book. As interferon-containing therapy is no longer recommended as first choice for treatment of chronic HCV following the licensing of various DAAs this chapter will only focus on DAA-based treatment of HCV coinfection. If HCV treatment is deferred, sonography of the liver should be performed every 6 months in cirrhotics in order to detect hepatocellular carcinoma (HCC). As the course of fibrosis is accelerated in HIV-coinfected patients and 10–30% of patients will develop HCC without preexisting cirrhosis, screening at regular intervals should be considered for patients with less advanced liver disease.

Therapy

Treatment of acute hepatitis C

Increasing numbers of acute hepatitis C have been observed in MSM. Mainly patients with high-risk sexual contacts are affected. These include "chem sex" (see above), unprotected anal intercourse, use of insertive sex toys and fisting. Diagnosis of acute hepatitis C is made according to anamnesis, elevated liver enzymes (usually 5-fold rise above the upper limit of normal; ideally to be documented as normal previously) and positive HCV RNA. HCV antibodies will be negative in many instances due to the long latency of the antibody response. Infection may possibly be missed, as it will be asymptomatic in about one half up to 2/3 of patients.

Up to 20% of HIV+ patients with acute hepatitis C clear the virus spontaneously (up to 40% in HCV monoinfection). Factors such as IL28B CC genotype, female sex, sexual transmission (versus intravenous drug abuse), or symptomatic course have been associated with a higher likelihood of clearance. In the absence of randomized, controlled data on the use of DAAs in acute HCV coinfection, treatment with pegylated interferon and ribavirin should be based on an individual decision. The known toxicities and longer treatment duration under dual therapy should be weighed against a potentially strong patient wish for early HCV cure, particularly in HIV+ MSM with a higher risk of HCV transmission and in countries where DAAs will only be reimbursed in chronic HCV infection with ≥F3 fibrosis. After diagnosis of acute infection, HCV RNA should be measured 4 weeks later. Treatment can be discussed in persons without a decrease of 2 logs of HCV RNA at 4 weeks compared with initial HCV RNA and in persons with persistent serum HCV RNA 12 weeks after diagnosis of acute HCV (NEAT 2010). With early treatment consisting of pegylated interferon and ribavirin response rates of about 70% (80% in genotype 2/3) can be achieved. Early discontinuation of dual therapy is justified in persons experiencing significant side effects. Enrollment of persons with acute HCV coinfection in ongoing trials using interferon-free DAA combination therapy is strongly encouraged.

Treatment of chronic hepatitis C

The goal of hepatitis C treatment is to achieve permanently negative HCV RNA levels. This is generally referred to as a "sustained virological response" (SVR). It is defined as a negative HCV RNA 12 to 24 weeks after completion of treatment.

Negative HCV RNA at the end of the treatment period is described as "end of treatment response" (ETR). If transaminases have normalized, this is referred to as a biochemical response. However, the latter does not correlate with the clinical course of hepatitis C. Failure to respond to treatment is referred to as a non-response. In the following text, response rates always refer to sustained responses. Only sustained responses have been clearly associated with resolution of liver fibrosis and extrahepatic manifestations, as well as with the prevention of further transmission. When HCV RNA becomes detectable again after having been negative, it is referred to as a

relapse. The probability of a relapse is highest within the first months following completion of treatment and decreases steadily afterwards. Therefore, the success of therapy is usually determined and evaluated 12–24 weeks after the end of treatment.

Treatment indication

Achieving SVR has been associated with an improved survival even in earlier fibrosis stages (F2) suggesting benefits of HCV therapy beyond cure of HCV and prevention of further liver disease progression. Therefore, every person with co-infection should be considered for treatment when the benefits of therapy outweigh the risks including patients pre- or post-liver transplantation particularly in the light of better HCV treatment outcomes with the use of DAAs in these persons. Thus HIV coinfection gives a high priority to anti-HCV treatment already at lower liver fibrosis stages (F0/F1). In case of the availability of a liver biopsy or FibroScan® demonstrating lack of or minimal liver fibrosis (F0-1), regardless of HCV GT, treatment can be deferred, however, in countries where no or only limited DAAs have become available so far or where cost reimbursement issues still have not been clarified. In these cases, fibrosis assessment should be carried out every 12 months periodically to monitor for fibrosis progression.

If chronic HCV and HIV infection are newly diagnosed at the same time and CD4 cell count is >500 cells/μl treatment of HCV in the presence of immediate HCV treatment indication (≥F2 fibrosis) can be considered prior to ART initiation to avoid potential drug-drug interactions between ART and HCV DAAs.

Treatment regimen

The combination of sofosbuvir 400 mg QD and a weight-adjusted dose of ribavirin of 1000 (<75 kg) to 1200 (>75 kg) mg/day BID for 12 weeks has become the new gold standard therapy for all HCV GT2 persons, promising cure in >90%. Persons with cirrhosis can be treated for an extended duration of 16 weeks. The approval of further DAAs have offered the opportunity of interferon- and partially also ribavirin-free DAA combination regimens which because of significantly improved tolerability and higher HCV cure rates can now be considered the gold standard in HCV therapy. In particular, the combinations of sofosbuvir and simeprevir (GT1/4), a fixed-dose combination of sofosbuvir/ledipasvir (GT1/4), sofosbuvir and daclatasvir (GT1/2/3/4) or a combination of ombitasvir/paritaprevir/r and dasabuvir (GT1, GT4 without dasabuvir) are recommended (see Table 2). Addition of ribavirin may be considered to reduce relapse rate and shorten treatment duration for some of the DAA combinations. Ribavirin should also be added to the ombitasvir/paritaprevir/r and dasabuvir combination when treating GT1a and ombitasvir/paritaprevir/r when treating GT4.

Use of older, first generation HCV PIs (boceprevir and telaprevir, only indicated in GT1) is no longer recommended because of increased toxicities. Simeprevir can cause hyperbilirubinemia and skin reactions/photosensibility.

Due to drug-drug interactions, in particular HIV and HCV PIs, careful checking for interactions is urgently recommended prior to starting HCV therapy, see www.hep-druginteractions.org or drug-drug interactions between ARVs and DAAs. During PEG-IFN+RBV therapy, ddI is contraindicated in persons with cirrhosis and should be avoided in persons with less severe liver disease. D4T and AZT should also be avoided where possible.

Table 2: Interferon-free HCV treatment options in HCV/HIV coinfection (EACS 2015)

HCV GT	Treatment Regimen	Treatment duration (weeks) and ribavirin (RBV) usage		
		Non-cirrhotic	Compensated Cirrhotic	Decompensated Cirrhotics CTP Class B/C
1 & 4	SOF + SMP ± RBV	12 without RBV	12 with RBV or 24 without RBV[1]	Not recommended
	SOF/LDV ± RBV	12 without RBV	12 with RBV or 24 without RBV in cirrhotics or pre-/post-transplant[1]	
	SOF + DCV ± RBV	12 without RBV	12 with RBV or 24 without RBV in cirrhotics or pre-/post-transplant[1]	
	OBV/PTV/r + DSV	12 in GT1b	Not recommended	
	OBV/PTV/r + DSV + RBV	12 in GT1a	12 in GT1b 24 in GT1a	Not recommended
	OBV/PTV/r + RBV	12 in GT4	24 in GT4	Not recommended
2	SOF + DCV ± RBV	12 without RBV	12 without RBV	12 weeks with RBV
	SOF + RBV	12	16–20[2]	
3	SOF + PEG-IFN/RBV	Not recommended	12 in pts eligible to pegylated IFN	Not recommended
	SOF + RBV	24	Not recommended	
	SOF + DCV ± RBV[3]	12 without RBV	24 with RBV	
5	SOF/LDV	12 without RBV	12 without RBV	
6	In the absence of clinical data on DAAs in HCV GT6 infection persons should be treated similarly to HCV GT1 and 4 infection			

RBV ribavirin, SOF sofosbuvir, SMP simeprevir, DCV daclatasvir, LDV ledipasvir, OBV ombitasvir, PTV/r paritaprevir/ritonavir, DSV dasabuvir
[1] Cirrhotic patients with negative predictors of response can be treated 24 weeks with ribavirin (negative predictors: treatment-experienced, platelet count < 75000/µl)
[2] Possible extension up to 16 weeks in treatment-naïve cirrhotics or relapsers; up to 20 weeks in treatment-experienced cirrhotics
[3] Based on expert opinion and preliminary data from studies in patients on pre-marketing expanded access programs

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HIV and HBV coinfection

Introduction

The hepatitis B virus is one of the most common human pathogens worldwide. Up to 95% of all HIV+ patients have been infected with hepatitis B, and approximately 10–15% have chronic hepatitis B, with considerable variation among geographical regions and risk groups (Alter 2006, Konopnicki 2005). It is estimated that around 100,000 HIV+ patients in the US suffer from chronic hepatitis B. Due to implementation of vaccination programs in many countries transmission rates decrease, especially in the younger population. Sexual transmission is the most frequent route of transmission. Transmission via the bloodstream is more likely than for HIV: following a needle stick injury contaminated with HBV-infected blood, the risk of infection is around 30% (HCV <2%; HIV approximately 0.3%).

Acute HBV infection leads to chronic hepatitis in 2-5% of immunocompetent adults, whereas HIV+ patients experience chronicity about five times more often. A possible reason for this is the HIV-associated immunodeficiency, whereas virus-specific factors such as hepatitis B viral load and genotype do not contribute significantly (Bodsworth 1991).

Hepatitis B and HIV share several features, although hepatitis B is a non-integrating, circular DNA virus ("closed circular supercoiled" DNA [cccDNA]). Hepatitis B is one of a few known non-retroviral viruses which uses reverse transcription as a part of its replication process. Therefore, replication can be inhibited with NRTIs. Although elimination can basically be achieved by cytotoxic T lymphocytes (CTL), it has to be assumed that hepatitis B virus DNA will persist life-long in most patients. Therefore reactivation can occur after many years, e.g., due to immunosuppression in advanced HIV infection or following chemotherapy, regardless of the pattern of antibodies found.

HBV diagnosis methods in HIV+ patients do not differ from negative patients. Table 1 summarizes the interpretation of serological test results. HBV screening of HIV+ patients starts with HBsAg, anti-HBs, and anti-HBc. If a positive HBsAg is found, testing for HBeAg, anti-HBe, and HBV DNA should follow.

The isolated presence of anti-HBc in the absence of HBsAg and anti-HBs (so called "anti-HBc only") is found quite frequently in HIV+ patients (less than 2% in healthy blood donors). Three situations should be considered: 1) early phase of an acute hepatitis B, 2) many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels, or 3) after many years of chronic HBV infection when the HBsAg titer has decreased to below the cutoff level for detection. Clinical significance of this state has not been clearly defined. In most instances, it will be a loss of anti-HBs without any clinical consequence.

Table 1: Interpretation of serological test results for HBV

Interpretation	HBsAg	anti-HBs	anti-HBc	HBeAg	anti-HBe	HBV DNA
No prior contact with HBV	–	–	–	–	–	–
Acute infection	+	–	+ (IgM)	+	–	+
Past infection with immunity	–	+	+ (IgG)	–	+	–
Chronic hepatitis B	+	–	+ (IgG)	+	–	+
Latent/occult infection[1]	–	–	+/– (IgG)	–	–	+
Pre-core mutant	+	–	+ (IgG)	–	+	+
Inactive carrier state	+	–	+ (IgG)	–	+	–
Immunity after vaccination	–	+	–	–	–	–

[1] Controversial. See text

A so-called occult infection means lack of HBsAg, but positive HBV DNA (with or without anti-HBc). Prevalence and clinical impact in coinfection is still unclear. Patients with chronic hepatitis B should be tested for hepatitis D infection also. In general, not only cirrhotic patients with chronic hepatitis B should be screened for hepatocellular carcinoma (HCC) but also HBV/HIV coinfected patients with high risk for HCC occurrence such as Asian or black decent, family history of HCC, NAFLD, and replicating HBV infection. Screening should be performed every 6 months by ultrasound of the liver as 10 to 30% of patients who develop HCC do not have pre-existing cirrhosis.

Course of hepatitis B with concurrent HIV infection

The course of hepatitis B is negatively influenced by HIV infection. Liver-associated mortality is about 15 times higher than in HIV-negative patients (Thio 2002, Konopnicki 2005). In addition, HIV coinfection accelerates the progression of hepatitis B and increases the risk of cirrhosis. Entanglement between the profibrogenic effect of HIV itself and the alteration by HIV of innate and adaptive immune responses to HBV has been elucidated further in recent years (e.g., direct cytopathic effect on liver tissue by CCR5-mediated activation of hepatic stellate cells and hepatocytes; indirect upregulation of pro-inflammatory and apoptotic factors). Despite these unfavorable effects, the clinical course appears initially usually more benign in HIV+ patients, although viral replication is increased. This seems contradictory at first, but can be explained by the impairment of cellular immunity, which may lead to an increase in viral replication, but at the same time also reduces hepatocyte damage. Therefore, transaminases in HBV/HIV-coinfected patients are frequently only mildly increased. In contrast, HBV DNA, as a marker for viral replication, is higher than in immunocompetent patients. Accordingly, despite less inflammatory activity, liver fibrosis and cirrhosis are more common. This phenomenon can be observed in other groups of patients with immunosuppression, e.g., organ transplant patients.

There is a direct correlation between the extent of immunosuppression and the control of viral replication of HBV. Patients with apparently resolved hepatitis B (anti-HBe-positive, HBV DNA-negative) and increasing deterioration of the immune system may result in a reactivation of the HBV infection (Soriano 2005). Notably, some cases of reactivation of hepatitis B have been described following immune reconstitution after initiation of ART.

HIV/HBV coinfection possibly has also a negative impact on the course of HIV infection. An increase of overall mortality and AIDS-defining events has been described (Nikolopoulos 2009, Chun 2012). Moreover, the risk for ART-related hepatotoxicity is about three times higher.

Whether or not the prognosis of coinfected patients is changed by effective therapies for ART and HBV remains to be seen. According to some studies, a reduction in HBV-associated mortality is associated with effective treatment of HBV (e.g., French GERMIVIC cohort) (Puoti 2007, Rosenthal 2009).

Prevention

All HIV+ patients with negative hepatitis B serology should be vaccinated. The vaccine may, however, be less effective due to immunosuppression. Approximately 30% of HIV+ patients have a primary non-response (only 2.5% in immunocompetent individuals). The response to the vaccine is influenced by the CD4 T cell count and level of HIV RNA. Patients with CD4 T cell counts of less than 200/µl who are

not on ART should receive ART first and HBV immunization thereafter. Vaccination is performed as recommended by the manufacturers (20 µg at months 0, 1, and 6). Revaccination can be considered in case of an insufficient response (anti-HBs <10 IU/ml 12 weeks after vaccination). Double dose revaccination (40 mg) at 3–4 vaccination timepoints (months 0, 1, 6 and 12) may help to improve vaccination response rates (Fonseca 2005, Launay 2011).

Loss of protective immunity is seen in up to 30% of patients each year following seroconversion. Therefore, anti-HBs should be monitored once a year and consideration given to booster doses if anti-HBs antibody levels are less than 100 IU/l. HIV patients, who are not adequately immunized against HBV should be screened yearly for newly acquired infection.

Of note, recent studies have demonstrated that HBV-active ART protects against the occurrence of *de novo* HBV infection, most strongly when tenofovir is used (Heuft 2014). This could therefore also be a strategy in patients who do not seem to respond to HBV vaccination.

HIV/HBV-coinfected patients who are seronegative for hepatitis A should be vaccinated (months 0 and 6), as there is an increased rate of severe or fulminant hepatitis in case of acute hepatitis A. Patients who are susceptible to both hepatitis A and B can be vaccinated with a bivalent vaccine (months 0, 1, and 6).

Following immunization, patients should be counseled about common measures to prevent further transmission and transmission of other viruses such as hepatitis C (safer sex practices, avoidance of needle sharing, etc). They should also be educated about strategies to prevent progression of liver disease such as avoidance of alcohol consumption, tobacco use (controversial), or herbal supplements, many of which are hepatotoxic. The application of hepatotoxic drugs (e.g., antituberculous agents) should be carried out cautiously.

Newborns of mothers with chronic hepatitis B should receive hepatitis B-immunoglobulin and active immunization.

Treatment

In HBV/HIV-coinfected patients, loss of HBsAg with development of protective anti-HBs antibodies is difficult to achieve because of impaired immune function. Realistic treatment goals are seroconversion from HBeAg to anti-HBe, a complete suppression of HBV DNA, normalization of transaminases, improvement of liver histology, and prevention of hepatocellular carcinoma. Other benefits of HBV therapy include a reduction in the risk of transmission and possibly in the risk of ART-induced hepatotoxicity. As mentioned above, HBV-associated mortality is likely to improve also.

Drugs with HBV activity

Possible treatment options for hepatitis B are nucleoside analogs, nucleotide analogs and interferon (see Table 2). Tenofovir, active against both HIV and HBV, is the most important drug. All other drugs play a less significant role today.

Besides tenofovir, 3TC, FTC, and entecavir are active against both HBV and HIV. Drugs with activity against HBV only are adefovir and telbivudine. Interferon – occasionally used in HBV monoinfection – does not play a relevant role in the setting of HIV/HBV coinfection.

Tenofovir is the drug with the best clinical activity. More than 95% of patients treated with tenofovir have viral suppression after 5 years. As a consequence, no distinct mutations have been described so far that are associated with phenotypic resistance (possibly A194T).

Table 2: Current therapeutic options for chronic hepatitis B in HIV/HBV coinfection

Drug	Dose
Adefovir	10 mg QD
Emtricitabine (FTC)	200 mg QD
Entecavir	0.5 mg (3TC naïve) QD or 1.0 mg (3TC experienced) QD
Lamivudine (3TC)	300 mg QD
Telbivudin	600 mg QD
Tenofovir (TDF)	300 mg QD
Interferon-α	5 MU per day or 10 MU 3 days per week
PEG-Interferon	Pegasys® 180 µg once a week PEG-Intron® 1.5 µg/kg body weight once a week

In contrast to tenofovir, most other agents are associated with significant development of resistance: monotherapy with 3TC selects a mutation in the YMDD motif in the HBV DNA polymerase gene (similar to a pre-core-mutant, HBeAg production may stop in case of mutations in this motif). The frequency of resistance development has been reported to be at least 20% of patients per year. Cross-resistance exists between 3TC, FTC, entecavir, and telbivudine. This can be overcome only partially with an increase in dose (e.g., entecavir has to be administered with a higher dose following treatment with 3TC). Although the nucleotide analog adefovir has a different mechanism of resistance development, selection of the A181T mutation with ongoing viral replication has been described.

It is reasonable to assume that combination of two drugs active against HBV will enhance antiviral activity and delay the selection of HBV resistance. No resistance mutation has been observed in small cohorts when a nucleoside and a nucleotide analog are combined. However, up to now there is no formal proof that combination therapy is indeed more efficacious. In light of the lessons learned from HIV, combination therapy of at least two drugs is recommended by some experts.

Treatment guidelines

HIV+ patients with HBV and/or HCV coinfection benefit from early ART because liver fibrosis progression is reduced with immune reconstitution and suppression of HIV viremia. Thus, ART initiation is recommended in all HIV+ patients with HBV coinfection (HBsAg-positive) irrespective of CD4 T cell count.

Liver biopsy is not mandatory in most cases. It may provide additional information on differential diagnosis (e.g., hepatotoxicity) and inflammatory activity. To assess liver fibrosis several non-invasive methods are available. Among these the Fibroscan® system has an outstanding role. Liver stiffness is measured as a surrogate of liver fibrosis by transelastography. It has to be taken into account that cut-off values differ in HIV/HBV coinfection from those in HBV monoinfection or HIV/HCV coinfection (Lacombe 2012).

Several histological classifications exist. In Europe, the METAVIR score is most often used. It distinguishes five stages of fibrosis (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = some septa, 3 = significant septa without cirrhosis, 4 = cirrhosis). Hepatitis activity is graded according to the intensity of necroinflammatory lesions (A0 = no activity, A1 = mild activity, A2 = moderate activity, A3 = severe activity). Treatment is recommended for grades F2-F4, it may be deferred for grades F0 and F1.

Current treatment guidelines are summarized in Figure 1.
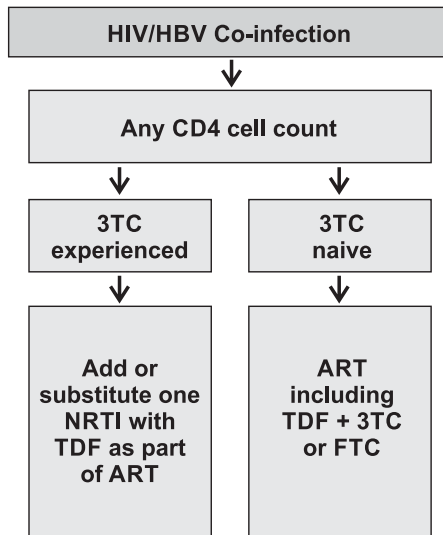
Figure 1: Treatment recommendations for HIV/HBV-coinfected patients (modified after EACS Guidelines October 2015)

All persons with HBV/HIV coinfection should receive ART including TDF+3TC or FTC unless history of TDF intolerance. For exclusive HBV treatment lower doses of TDF can be used due to the lower IC50 of HBV. This allows safer use in patients with more advanced renal disease. Lower dosages only apply when concomitant HIV is treated with an independent, self-sufficient ART regimen. If TDF is strictly contraindicated, entecavir plus adefovir can be considered. However, efficacy and renal toxicity need to be closely monitored, because of the proven renal toxicity of adefovir. In persons with no prior 3TC exposure, entecavir may be used alone. NRTI substitution should only be performed if feasible and appropriate from the perspective of maintaining HIV suppression. Caution is warranted when switching from a TDF-based regimen to drugs with a lower genetic barrier, e.g., FTC or 3TC, in particular in 3TC-pretreated cirrhotic persons as viral breakthrough due to archived YMDD mutations is likely to happen. This has also been described in individuals with previous 3TC HBV resistance who have switched from TDF to entecavir. The addition of entecavir to TDF in persons with low persistent HBV replication has not statistically proved to be efficient and should therefore be avoided.

A transient elevation of transaminases – which is usually moderate and soon resolves – may be observed after initiation of HBV therapy. It is caused by immune reconstitution and subsequent increased inflammatory activity. In case of marked and/or ongoing elevation of transaminases, other explanations have to be considered (e.g., increasing HBV replication and resistance, lactic acidosis, hepatotoxicity of antiretroviral drugs, superinfection with hepatitis viruses other than hepatitis B).

Initial normalization of ALT and significant reduction of HBV DNA will be achieved in most cases by any anti-HBV agent. ALT levels do not correlate well with inflammatory activity and are influenced by many other factors such as hepatotoxicity of ART or other drugs, alcohol consumption, and immune reconstitution. Therefore, their value for monitoring treatment is limited.

The optimal duration of HBV treatment is unclear. As eradication is most unlikely, continuous suppression of viral replication probably will be necessary as it is in HIV. Therefore, HBV active drugs are integrated into the ART combination permanently.

If drugs active against HBV are discontinued, an acute hepatitis may develop clinically. In rare cases even fatal liver failure may occur. Therefore any interruption of treatment has to be considered thoroughly in coinfected patients. In the setting of cirrhosis special consideration has to be given as hepatic decompensation may occur with interruption of HBV active drugs, therefore stopping effective anti-HBV treatment is not recommended. In case of resistance, treatment may be discontinued safely without any danger of clinical deterioration of hepatitis. All nucleos(t)side analogs have to be dose-adjusted in case of renal insufficiency.

HBeAg seroconversion will occur in as many as 40% of patients treated with tenofovir, a loss of HBsAg will occur in about 10% of patients after 5 years.

As most cases of *acute* hepatitis B even in HIV+ patients resolve spontaneously, only symptomatic treatment is recommended. In addition, data in this situation are sparse (e.g., danger of resistance in case of early therapy with no options afterwards).

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14. Human Pegivirus HPgV Infection
(formerly GB virus C/Hepatitis G virus)

MATTHIAS STOLL

Hippocrates (5th century BC) was the first to postulate that signs of inflammation represent both a symptom of disease and as well as a hint of its cure. Edward Jenner (18th century) demonstrated that an artificial infection with a harmless cowpox is able to prevent the dangerous smallpox. William Coley (19th century) prevented progress of malignancies by bacterial toxins (Coley 1893). In 1927 the Nobel Prize for Medicine was awarded to the Austrian neurologist Julius Wagner von Jauregg, who was able to obtain improvement in patients with late stage symptomatic neurosyphilis by infecting them with the malaria parasite. Thus, infectious diseases may result in harm reduction under certain conditions: Immunomodulatory effects and direct interactions between the causative microorganisms of infection and coinfection have been postulated to be the beneficial effector mechanisms.

Human pegivirus (HPgV) is a flavivirus closely related to hepatitis C virus (Stapleton 2011, Adams 2013). Its former name GB virus (GBV-C) stems from early experiments on the transmission of acute hepatitis from humans to marmosets. One of the first source patients had the initials "G.B." (Deinhardt 1967). Later on, two hepatotropic viruses, GB virus A (GBV-A) and GB virus B (GBV-B), were isolated from marmosets. Two research groups simultaneously discovered the related human pegivirus in humans with hepatitis in the mid-90s. Subsequently, HPgV has promoted a discussion as to whether the natural course of HIV infection might be modulated in a favorable way by this particular coinfection. In addition, because HPgV was first found in humans with hepatitis, and due to its close relationship to GBV-A and GBV-B in marmosets, human pegivirus/GBV-C was also named "hepatitis G virus (HGV)" by one research group. However, it has been shown that HPgV neither causes hepatitis nor worsens preexisting hepatitis (Berenguer 1996, Tillmann 1998, Rambusch 1998, Stark 1999). Whether viremia is increased in lymphoma patients without HIV infection, is debated (Krajden 2009, Ernst 2014).

Although the primary permissive cell type of HPgV has not yet been identified, the virus has been found in lymphocytes and peripheral blood mononuclear cells, bone marrow, liver, and spleen (Chivero 2015). HPgV has until yet not been shown to cause any known disease in humans but a couple of studies indicated a more favourable course of HIV-, HCV-, and more recently Ebolavirus-associated diseases (Lauck 2015) in those individuals chronically coinfected with HPgV.

Prevalence studies revealed HPgV viremia within the general population ranging from less than 5% in industrialized countries up to more than 15% in some developing countries. Although approximately 10% to 30% of blood donors have specific antibodies against HPgV, affected individuals are not excluded from the donation of blood, assuming that the virus is apathogenic. Consequently, serological diagnostics on HPgV are not routinely performed. An estimated 25% of HPgV-infections persist, and the other 75% clear viremia within two years of infection (Gutierrez 1997; Tanaka 1998). Two serological markers for HPgV infection exist: HPgV viremia can be determined using a PCR method; and antibodies to the envelope region E2 (anti-E2) are detected by ELISA (Table 1). As they are mutually exclusive, either HPgV viremia or the presence of anti-E2 is detectable in HPgV infected individuals (Gutierrez 1997; Tanaka 1998). HPgV viremia may persist for decades but in the majority HPgV viremia is transient and ends with seroconversion to anti-E2, resulting in immunity to new infections. However, this does not seem to be a lifelong immunity (Table 1).

Transmission of HPgV occurs in both ways: horizontally and vertically as well parenterally and mucosally, similar to HIV, HBV and HCV infections. Hence coinfection of human HPgV and HIV is common and persistence of HPgV viremia (HPgV RNA positivity) is prolonged in HIV infection.

Until now six genotypes and several subtypes of HPgV have been described with significant variation in their regional distribution and in virologic characteristics.

Table 1: Serological markers and stages of HPgV infection

Marker Method	Pegivirus-C-Viremia (RNA) PCR / b-DNA	Anti-E2-Antibodies ELISA
HPgV negative	negative	negative
Replicative HPgV-C Infection	positive	negative
Past HPgV-C Infection	negative	positive / (negative)*

* Anti-E2-antibodies may disappear over time

HIV and HPgV coinfection: Pas de deux

In 1998 the first cohort studies described a modulating impact of HPgV coinfection on HIV-infection (Toyoda 1998, Heringlake 1998): The HPgV viremic subgroup presented with lower HIV viremia, higher CD4 T cell counts, slower progression to AIDS and improved survival as compared to the HPgV non-viremic patients. These beneficial effects were confirmed by different research groups (Lefrère 1999, Yeo 2000, Tillmann 2001, Xiang 2001) and were also seen in antiretrovirally treated HIV+ individuals (Tillmann 2004+2006, Nunnari 2003, Williams 2004, Ernst 2011). The modulatory effects were associated to persisting HPgV viremia but were not present in those without or with cleared HPgV infection. A meta-analysis described an improved response to ART and clinical benefit for HIV/HPgV coinfected patients, which was more pronounced with longer follow-up (Zhang 2006).

Conflicting results came from some studies (review: Battharai 2012), which did not find an effect of HPgV viremia on HIV infection (Sabin 1998, Birk 2002, Bjorkman 2004, van der Bij 2005), including two studies in women (Kaye 2005, Williams 2005). One of these studies summarized viremic and anti-E2-positive patients as HPgV positive group (Sabin 1998). Another study focused on HPgV viremia at study entry (van der Bij 2005). In this study the subgroup with persistent HPgV RNA had a superior clinical outcome. A less pronounced potential gender-specific modulating effect of HPgV on HIV in women may exist (Kaye 2005, Williams 2005). Another study on HIV+ pregnant women found a lower HIV viral load in HPgV viremic mothers and less vertical HIV transmission in the HAART era but not in the pre-HAART era (Handelsman 2008). The lower risk for vertical transmission of HIV seems to be associated with replicative HPgV infection in the child rather than by HPgV status of the mother. Surprisingly the risk for vertical transmission of HPgV was found to be increased under HAART in HIV/HPgV coinfected pregnant women (Bhanich-Supapol 2009). In addition, there is evidence from a multicenter trial, that HPgV genotype 2 coinfection was associated with higher CD4 T cell counts (Schwarze-Zander 2006). This may explain regional differences and at least in part conflicting results from cohort studies from different regions.

In summary, most studies found more pronounced antiretroviral and immunological effects in ART-treated HPgV RNA positive patients. However, other studies did not find any difference. No study to date described a negative influence of HPgV viremia on the effect of ART.

Table 2: Potentially beneficial effects of replicative HPgV coinfection on HIV disease

Increase	Decrease
• CD4+ T cells	• HIV plasma viremia
• Response to ART	• Mother to child transmission of HIV
• Survival	• Progression to AIDS or death
• Quality of life	
• Proportion of naïve CD4+ and CD8+ T cells; number of double negative T cells (CD3+/CD4-/CD8-); immunosuppressive cytokines (TGF-ß, IL-10)	• T cell activation: T cell activation markers (CD25, CD38, and CD69), cytotoxic CD8+ T cell functions, T cell-receptor signaling

HPgV, HIV, and HCV: Ménage à trois

Triple infected patients with HIV, HCV and HPgV had less progressed liver disease as compared to those without HPgV infection (Barbosa 2009, Berzsenyi 2009), and an improved response to interferon/ribavirin therapy of HCV (Hofer 2011) indicating as well a potential interdependency of HPgV with HCV.

However, if harm reduction is exclusively seen in the chronically replicative HPgV/HIV coinfection – is it then necessary to keep HPgV viremia ongoing like a tamagotchi game? A couple of cases with HPgV seroconversion have been associated with a particularly worse prognosis (Williams 2004, Bjorkman 2004, van der Bij 2005). This raised concerns about interferon-based HCV-therapies, which are able to terminate HPgV replication and to induce anti-E2 seroconversion (Yu 2001, Hofer 2011). However, a negative impact of interferon was not seen in a large multicenter trial (Schwarze-Zander 2006).

Proposed pathomechanisms

Up to now, the fundamental chicken or egg dilemma remains unsolved: whether HPgV viremia is an epiphenomenon or the cause for an improved outcome of HIV infection. A major drawback of the first studies was the lack of any pathophysiologic concept. Meanwhile many different hypotheses have been postulated about direct inhibitory effects of HPgV on HIV replication, about competition of both viruses at certain steps of action during the replication cycle, and about immunomodulatory mechanisms in the host induced by HPgV. After more than two decades of research, it has been shown that more than one way leads to Rome. The knowledge about the pathophysiology of HPgV coinfection in HIV looks rather like a varied bunch of pleiotropic effects of numerous different modes of (inter-)action. The modulating effects of HPgV on HIV disease (Table 3) have be explained by attachment or entry inhibition, downregulation of CD4- and chemokine receptors including upregulation of their ligands, enhancement of innate immunity, downregulation of immune activation and apoptosis, and modulation of T cell responses.

The elucidation of the underlying molecular pathomechanisms is still fragmentary. However, HPgV treads several independent pathways, using E2-protein, NS5A-protein, and Anti-E2-antibodies. Hence it might be speculated, that mankind shares a long coevolution together with HPgV and retroviruses, which could explain why HIV – in contrast to SIV in other primates – until recently was not able to establish a stable endemic.

Table 3: Proposed mechanisms of interactions between HPgV, HIV and their host

Mechanism (Agent)	Pathway / Effector		References
0	HPgV-C E2-protein blocks the fusion peptide of HIV and modifies its conformation	Inhibition by a peptide sequence from E2 (269-286)	Jung 2007, Mohr 2009, Herrera 2009, Eissmann 2013
CXCR4- and CD4-downregulation (HPgV-NS5A protein)	Decreased CD4 and CXCR4 expression, increased release of the CXCR4 ligand SDF-1	Inhibition by a peptide sequence from NS5A (152-167)	Chang 2007, Ziang 2008, Schwarze-Zander 2010
CCR5-downregulation	Increase of CCR5 ligands (β-chemokines: RANTES, MIP-1α, MIP-1β)		Nattermann 2003, Xiang 2004, Tillmann 2002
Enhancement of innate immunity	HPgV induces PDCs and activation of interferon related genes	Activated PDCs, IFN-gamma- and RNA-dependent protein kinase R mRNA levels higher in HPgV viremic patients (exact mechanism is unknown)	Lalle 2008
Normalisation of apoptosis	Normalized levels of CD95 (Fas-ligand) in HPgV viremic HIV+ patients without HAART	Downregulation of T cell apoptosis in HPgV coinfected individuals	Mönkemeyer 2008
Modulation of T cell immunity (Effects are in part mediated by NS5A)	HPgV viremia resulted in a more stable Th1-cytokine profile (e.g. IL-2, IL-12) and less increase of Th2-cytokines (IL-4, IL-10)	In part mediated by activation of activated PDCs (CD80+ pDCs)	Nunnari 2003, Ryzde 2012, Stapleton 2009
Deceleration of increased T cellular immune activation	In HPgV viremic HIV+ pts less expression of CD38+, CCR5+, CD69, and CD25 on T cells	HPgV viremia reduces IL-2 production and IL-2 induced T cell proliferation	Maidana-Giret 2009 Bhattarai 2012a
Increase of double negative T cells (DNTC: CD3+/CD4-/CD8-)	DNTCs are associated with decreased immune activation	HPgV viremia associated with higher levels of immunosuppressive cytokines (TGF-ß, IL-10)	Bhattarai 2012c Petitjean 2012
Reduction of NK-, B cell and monocyte activation	Decreased expression of CD69 (NK-cells), CD86 (B cells) and CCR5 (monocytes)		Stapleton 2013
Inhibition of HIV-attachment (Anti-HPgV-E2-antibodies)	Anti-E2-antibodies precipitate and neutralise HIV particles in vitro and inhibit CCR5- and CXCR4-tropic HIV replication	Inhibition of HIV by attachment-inhibition to target cells but not by entry inhibition	Mohr 2010

PDCs, plasmacytoid dendritic cells

Hypothetically in the past spread of HIV could have been limited by two other viral diseases, both formerly highly prevalent in Africa where HIV had its origin: The chemokine receptor inhibition by HPgV might have prevented transmission, especially vertical transmission, which is a result of perinatal HPgV transmission in HIV+ mothers (Handelsmann 2007, Bhanich-Suparol 2009). On the other side periodical epidemics of pox might have killed efficiently any human host of HIV, because a fatal course in pox is common especially in cases with preexisting cellular immunodeficiency. The possible result of this two competing coinfections: HIV was – until recently – not able to establish a stable endemic in humans over a long time.

The story of HPgV coinfection in HIV started with observational epidemiology and revealed an unexpected clinical observation: HPgV presents as a non-pathogenic virus in humans and as a beneficial coinfection in HIV+ individuals. At this point science started at bedside and went to bench in the last two decades. A puzzling diversity of pathomechanisms had been described meanwhile and may have raised more questions than answers. For more detailed information to the complex role of HPgV in the pathophysiology of HIV-infection it is referred to recent reviews of the scientific literature in this evolving field of infectiology (Bhattarai 2012a, Chiveiro 2015, Maidana Giret 2012, Schwarze-Zander 2012, Shankar 2011).

How to deal with HPgV coinfection in clinical practice?

Beyond the tales of a potentially beneficial infection the impact of HPg-Virus may be in understanding the pathophysiology of virus to virus- and virus to host-interactions rather than in a hypothetical role in clinical practice:

1. Until now it is not recommended to test HIV+ patients for their HPgV serostatus nor for HPgV replication by PCR beyond clinical studies. But some authors claim such tools for clinical practice (Battharai 2012b).

2. HIV+ patients should be informed that – at least in adults (Tenckhoff 2012) – there is no evidence that (an artificial) HPgV infection, which happens after HIV seroconversion will be of benefit in the course of HIV infection. It cannot be predicted whether an infection with HPgV will remain chronically replicative. Coinfections with HPgV in an in vitro model show evidence for an inhibition of HIV replication when HPgV infection occurs before HIV infection but not later (Xiang 2001). In addition the substantial risks of any mucosal or parenteral inoculation with infectious materials from human sources should be considered carefully.

3. There is no evidence to support the deferral of HCV therapy in HIV/HCV/HPgV coinfected patients, although interferon therapy can terminate chronic HPgV replication. Whether DAAs may be as well active against the closely related flavivirus HPgV must be elucidated by further (*in vitro*)studies.

We are still in the early stages of HPgV history. Over the last two decades we have accrued some fascinating insights into possible mechanisms of HIV and HPgV interaction and the roles that individual host factors may play. At present, HPgV gives us the opportunity to obtain insight into clinically relevant regulation pathways of HIV. This may help us develop auxiliary therapeutic concepts. These concepts may be both clinically and therapeutically promising because an additional benefit of HPgV remains evident in several studies after the initiation of ART.

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15. HIV and Sexually Transmitted Diseases

STEFAN ESSER

Epidemiology

A sexually transmitted infection (STI) or disease (STD) seldom occurs in isolation, without other STIs. STIs can contribute to the transmission of HIV or other venereal infections. In the case of an STI, the sexual partners of the patient should be informed, examined, and treated, if necessary.

Rarely, HIV RNA and proviral DNA can also be detected in genito-anal secretions and fluids of successfully ART treated HIV+ patients especially when they are coinfected with other STIs. The influence of the STIs on genito-anal HIV shedding seems to be different (Potlich 2012, Storim 2015, Kelley 2015, Rosenberg 2015). Therefore, testing and treatment of STIs can reduce the risk of HIV transmissions.

The incidence and prevalence of STIs and STDs are on the increase. For example, the incidence of syphilis has been increasing in Western Europe and the US since the end of the 90s. In patients with newly diagnosed syphilis infections in Germany, the prevalence of HIV infection is approximately 45% (RKI 2010). In recent years, there have also been reports of regional epidemics of lymphogranuloma venereum (LGV) in Europe, which had been regarded as an STI mainly in the tropics and subtropics. Men having sex with men (MSM) are particularly affected (in more than 90% of cases of LGV, and more than 60% of cases of syphilis). HIV+ persons seem to be more vulnerable to some STDs (RKI 2004+2005).

Human papilloma virus (HPV) is among the most frequent sexually transmitted pathogens in woman as well as men. Usually self-limiting in otherwise healthy people, HPV infections may persist in HIV+ patients more often and can cause condylomata acuminata and precancerous intraepithelial neoplasia. Over time chronic high risk HPV-type infection can result in malignancies such as cervical or anal carcinoma. In spite of the use of antiretroviral treatment the incidence of HPV-associated cancers is much more frequent in HIV+ patients than in the general population. Also infections like hepatitis C (Larson 2011, Obermeier 2011) and shigella (RKI 2005, Aragón 2007, Daskalakis 2007), usually not preferentially sexually transmitted, have accumulated regionally in HIV+ MSM in some large German cities linked with certain sexual practices.

The incidence of STDs increases more rapidly in HIV+ persons. Screenings in different countries find a high prevalence of asymptomatic sexually transmitted coinfections in HIV+ cohorts (Heiligenberg 2012). MSM in cities still practice high-risk sexual behaviours frequently (Dirks 2011, Mayer 2012). STI screening in HIV+ persons should be performed routinely (RKI 2010, Esser 2011, Heiligenberg 2012, Mayer 2012). STDs are HIV indicator diseases (Sullivan 2011). All STD patients not known to be HIV-positive should be offered an HIV test. Condoms are still the best method to reduce the transmission risk of all STIs but the protection is not 100%.

Following is a detailed description of the most important STDs. Sexually transmitted diseases like hepatitis B as well as herpes simplex or bacterial vaginoses will be described in other sections of this book.

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Syphilis (Lues)

Syphilis is caused by *Treponema pallidum*, a bacterium belonging to the *Spirochaetaceae* family. The bacteria are mainly transmitted by direct sexual contact with infected persons, and penetrate into the organism through microlesions in the mucosa or the skin. Even kissing can cause an infection. In the case of unprotected sexual contact, the risk of transmission ranges from 30 to 60%. Hematogenous or congenital transmissions very seldomly occur in western countries.

Clinical course

The incubation period is usually 14 to 24 days. Approximately 40 to 50% of infections show no symptoms or are self-limiting. Persistent infections may affect various organ systems, going through stages of the course of the disease. However, these stages can be skipped or repeated. The highest risk of transmission is during the clinical symptomatic stages of early syphilis (primary and secondary syphilis), especially in case of a primary lesion in stage I. During the late latency period (>1–2 years after infection) and the clinically symptomatic late stages (tertiary syphilis: 2–50 years post infection) syphilis is considered to be non-infectious.

Primary syphilis: 2–3 weeks after infection the primary lesion with ulcus durum (hard chancre, erosive chancre) appears at the site of inoculation. This indolent, sturdy ulcer with infiltrated borders usually yields a clear treponema-rich exudate when compressed or squeezed. The chancre is accompanied by a usually strong one-sided lymphadenitis, swelling of the lymph nodes. This primary complex will spontaneously resolve after 4 to 6 weeks without treatment.

Secondary syphilis: Variable general symptoms occur after 4 weeks up to 6 months at varying intervals, among these generalized swelling of the lymph nodes and symptoms in various organs. Even an ocular involvement manifesting as episcleritis or iritis can be seen in secondary syphilis. The clinical variety of the frequent syphilids on the skin or the mucous membranes varies from exanthema (usually with palmoplantar participation) to roseola, alopecia syphilitica, moist papule, angina specifica, to condylomata lata (genital and perianal) as well as pigment changes (leukoderma specificum) and lues maligna. Headaches at night are a sign of an early syphilitic meningitis cerebrospinalis.

Latent syphilis: When the infection is brought under control by the immune system the clinical features usually disappear entirely. However, during this latency period, syphilis remains serologically detectable and a relapse or progression is possible. During the early latency period (<1–2 years after infection) the syphilis can still be transmitted by blood.

Tertiary syphilis: Years after primary infection, the so-called gummata may appear. These can affect any organ, showing tuberous or granulomatous changes with a tendency to ulceration and cicatricial healing. Major cardiovascular features of tertiary syphilis are asymptomatic aortitis, aortic insufficiency, coronary ostial stenosis and aortic aneurysm. Tertiary syphilis of the central nervous system (CNS) has many manifestations, involving the meninges and the arteries and parenchyma of the cerebral cortex. Meningovascular syphilis is characterized by an obliterative endarteritis of the meningeal vessels with subsequent arterial thrombosis and ischemic necrosis in the brain and spinal cord. Strokes are observed even in young patients with persistent untreated syphilis infection.

Quarternary syphilis: In untreated patients, a late neurosyphilis occurs in various forms after some years. In case of tabes dorsalis, a shooting and burning pain, sensory ataxia, reflective pupilloplegia (signs of Argyll Robertson syndrome) and optic atrophy are observed. Regarding syphilitic meningitis, cranial nerve paresis, an increase of intracranial pressure and other neurological symptoms are seen. In case of progressive paralysis symptoms like headache and a change in personality prevail followed by dysplasia (a speech disorder), cramps, dementia and apoplectic attacks. An untreated progressive paralysis will lead to death in 4 to 5 years.

Connatal/Congenital Syphilis: Diaplacental transmission usually happens in the 4th to 5th month of pregnancy. Depending on the stage of syphilis in a pregnant woman, it will lead to an abortion or a lues connata of the infant, progressing in the following ways: **Lues connata praecox**, rhinitis syphilitica, interstitial hepatitis, encephalomeningitis with hydrocephalus communicans hypersecretorius as well as Parrot's pseudoparalysis. The typical stigmata of **lues connata tarda** (from the age of 3) are saddle nose, Parrot's ulcer and Hutchinson's triad: Hutchinson's teeth, keratitis parenchymatosa and labyrinthine deafness.

In HIV+ patients, unusual manifestations and fulminant progress of syphilis are often observed (Gregory 1990). Reactivation of earlier infections as well as shorter latency periods and faster progression to the later stages including neurosyphilis occur in addition to symptoms of the coexistent stages. Neurosyphilis can be diagnosed in about 20% of syphilis/HIV-coinfected patients during the early syphilis stages (Esser 2011). Syphilis can lead to a temporary increase in HIV viral load and to an additional deterioration of the immune status even in patients on effective ART.

Diagnosis

The diagnosis of syphilis in HIV+ patients can be complicated because of a nonspecific clinical course and also due to unreliable screening tests and atypical syphilis serologies like a late IgM descent after treatment and fluctuating VDRL titers (Venereal Disease Research Laboratory test, detection of phosphatide antibodies).

Silvery-shining, spiral treponema are noticeable due to their typical rotating and bending movements when applying large-scale dark-field microscopy obtained from the stimulus secretion from the ulcus durum. A direct microscopic viral detection should be done when a primary syphilis lesion is suspected, particularly in the case of an initially prevailing seronegativity. As a first reaction, IgM antibodies will appear (diagnostic test and lipoid antibody detection will still be negative).

Due to a possible overlap of disease stages, each serologically syphilis-positive patient should be neurologically examined. As the risk for neurosyphilis is markedly increased in HIV+ patients, a lumbar puncture to collect cerebrospinal fluid (CSF) is recommended when the patient has low CD4 cells (<350 cells/µl) or high viral loads (HIV RNA >100,000 copies/ml) or is not on antiretroviral treatment or shows neurological symptoms or ocular involvement or the time of infection is not certain (DSTIG 2014, Ghanem 2008, Marra 2004).

Diagnostic findings in the CSF and neurological symptoms may have therapeutic consequences (see below). Interpretation of CSF results in HIV+ patients should be done by experts on the basis of the ITPA index (intrathecal-produced *Treponema pallidum* antibodies), parameters of a cerebrovascular barrier disorder and the detection of lymphomonocytic pleocytosis.

Interpretation of syphilis serology in HIV-infected patients

Syphilis serology is based in principle on treponema-specific diagnostic tests. These are TPHA (*Treponema pallidum* hemagglutination assay), TPPA (*Treponema pallidum* particle agglutination test), or ELISA (enzyme-linked immunosorbent assay). If positive, treponema-specific tests to confirm will follow, like IgM ELISA, IgM and IgG Western Blot or 19-S-IgM-FTA-ABS (treponemal antibody-absorption test). In the case of a reactive 19-s-IgM-FTA-ABS test in untreated patients or a reactivation of the test in treated patients (Lues non satis curate), there is always need for treatment.

False-negative test results can be explained by inadequate production of antibodies or by suppression of IgM production due to high IgG levels. When in doubt, specific tests such as FTA-ABS or cardiolipin tests should be carried out, even though false-negative results may occur again. Should a syphilis infection be serologically confirmed, a quantitative evaluation of the non-treponema specific activity parameters (lipoid antibodies, e.g., VDRL test or KBR) is required. The prozone phenomenon refers to a false-negative response resulting from disproportionately high antibody titers that interfere with the formation of antigen-antibody lattice necessary to visualize a positive flocculation test. This effect can be expected during secondary syphilis and in syphilis/HIV-coinfected patients (Smith 2004). HIV-associated unspecific activation of B lymphocytes can also cause false positive VDRL tests. Possibly quantitative *Treponema pallidum* PCR may facilitate the diagnosis and monitoring of the course in syphilis patients.

The longer a patient has untreated syphilis the longer the normalisation of the syphilis activity parameters will take even after a successful syphilis therapy in HIV+ patients. The IgM test may remain reactive for years. A successful therapy during this IgM-reactive period is indicated by a clear titer decrease of the non-treponema-specific activity parameters (reduction of VDRL by at least 2 titer levels within 3 months).

Diagnostic algorithm for assuming a syphilis infection

On clinical suspicion or as screening of probands

Syphilis screentest
(TPHA,VDRL)

positive or
doubtful positive

negative   in case of persistent clinical course

differential diagnostic
considerations

repeated testing after 2 weeks

confirming test
(IgG-FTA-Test, FTA-abs-Test)

positive   again negative

specific IgM AK-test
(19S-IgM-FTA, IgM-Westemblot

differential diagnostic considerations

pres. CNS involvement

lumbar puncture
(VDRL,TPHA,ITpA-Index)

follow-up after therapy by
TPHA,VDRL after 3, 6, 9, 12, 24 months

Repeated increase of titer > 2 levels VDRL
or insuff. TPHA decrease

Re-puncture and/or
further treatment, if necessary

Due to an increase of the previously decreased activity parameters, a re-infection or a re-activation may happen during this time. A re-infection or re-activation is assumed when the serological titers increase by more than two titer-levels by the end of therapy compared to the initial value. A serological differentiation between re-infection and re-activation is not possible. As the activity parameters are not treponema-specific they often vary in HIV+ patients, mainly when contracting additional infections. Repeated syphilis re-activations are an indication for liquor cerebrospinalis punctuation to exclude an untreated neurosyphilis.

Therapy

The generation period of *Treponema pallidum* is between 30 to 33 hours. Therefore, the therapy period should not be less than 10 to 11 days. A parenteral dose of penicillin is the therapy of choice at all stages. Resistance to penicillin has not been seen for *Treponema pallidum*. Recommendations for the early stages of syphilis include intramuscular injections of benzathine penicillin 2.4 MU (e.g., 1 ampule Pendysin® or Tardocillin® of 1.2 MU IM in each buttock) weekly for 1 week during early syphilis and in later stages of syphilis for at least 3 weeks. When the infection date is uncertain, syphilis should be treated like late-stage syphilis.

In cases of penicillin intolerance, doxycycline 100 mg BID orally, erythromycin 2 g/day orally for at least 2 weeks, azithromycin or ceftriaxone (intramuscular, intravenous) is recommended. Apart from ceftriaxone these alternatives are considered less effective than the intramuscular injection with penicillin.

Neurosyphilis is usually treated with 3 x 10 MU or 5 x 5 MU or 6 x 4 MU penicillin G, administered intravenously for 10–21 days. Current guidelines recommend an initial dose of 4 g ceftriaxone followed by 2 g intravenously daily for 10–14 days as an alternative treatment option (Deutsche STD-Gesellschaft 2014).

Cross-reacting allergies (<10%) between penicillin and cephalosporin are possible. Alternative treatment options are doxycycline 100 mg BID or erythromycin 500 mg QD for at least 3 weeks. When treating with macrolides the possible development of resistance to *Treponema pallidum* should be considered (Lukehart 2004). Therefore, despite suspecting a penicillin allergy a controlled penicillin hardening under stationary conditions in reanimation readiness until the required full therapeutic dosage is administered is performed in specialized centers.

When starting syphilis therapy – irrespective of the stage – a Jarisch-Herxheimer reaction should be differentiated from a penicillin allergy. Depending on the stage of syphilis, the Jarisch-Herxheimer reaction is observed in just 20% of patients within 48 hours after the first administered dose of antibiotics. It is caused by a release of pyrogenic, a vasoactive endotoxin, the result of a fast decomposition of bacteria, showing exanthema and influenza-like symptoms such as shivering, fever, arthralgia or myalgia. The Jarisch-Herxheimer reaction can be avoided or at least reduced by administering a single dose of 1 mg/kg prednisolone orally or intravenously prior to the first dose of antibiotics.

A successful therapy should have a clinical and serological follow-up 3, 6, 12, 18 and 24 months after treatment. A successful therapy is reflected by the disappearance of clinical symptoms and a clear titer decrease of the non-treponema-specific activity parameters (reduction of VDRL by at least 2 titer levels within 3 months). A repeated increase of the previously decreased activity parameters may mean a re-infection or a re-activation requiring treatment. This is assumed when the serological titer increases by more than two titer levels after the end of therapy in comparison to the initial result. Even in HIV+ patients, the IgM test should not be reactive 2 years after a sufficiently administered syphilis therapy. In case the IgM test is no longer reactive, a repeated reactivity means a re-infection or re-activation, requiring further treatment (see above, interpretation of syphilis serology).

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Gonorrhea (the clap)

Gonorrhea, also called the clap, is caused by the *Neisseria gonorrhoeae* bacteria. The bacterium can be found worldwide and depending on the region shows a varying and changing resistance profile. Gonorrhea is typically localized in the genitourinary mucosa and transmission is almost exclusively through sexual activity (exception: neonatal conjunctivitis); the incubation period lasts from 2 to 10 days.

Clinical course

The primary symptoms in men are urethritis, frequent strangury, a burning pain when urinating, and urethral pain. A typical symptom is the *bonjour drop*, a purulent discharge from the urethra after several hours of restricted micturition. It is often accompanied by a strangury. Without treatment, gonorrhea can cause prostatitis. Symptoms are a burning after miction, pain in the intestinal area and an enlargement of the prostate. Furthermore, it can cause an epididymitis with pain and swelling.

In women, the course of gonorrhea is often asymptomatic, although urethritis may occur. Only in pre-pubescent girls is a vaginal colonization possible. Involvement of the cervix and adnexa of the uterus may cause complications like peritonitis and pelvic inflammatory disease.

Extra-genital manifestations of gonorrhea occasionally cause pharyngitis or proctitis. Perinatal transmission of gonococcal conjunctiva is rare. Which is why Credé's prophylaxis for newborns (temporary treatment with eye drops: originally 1% silver nitrate solution; later, erythromycin-containing eye drops or ointments) was stopped in Germany. Systemic infections with general symptoms like fever, arthritis and endocarditis including gonococcal sepsis are rare (Rompalo 1987). Coinfections with other STI are frequent in patients with gonorrhea (Abraham 2013).

Diagnosis

The most sensitive and specific detection method of *Neisseria gonorrhoeae* is the PCR or nucleic acid amplification test. Usually genitourethral infections are diagnosed by PCR detection in the urine. The PCR gives no information about the resistance status of the infections agent. Microscopic preparations are taken urethrally, anally, pha-

ryngeally and in women also endocervically. When pus does not discharge spontaneously out of the urethra the patient should not urinate for four hours before the urethral smear is taken. The diagnosis can be confirmed by microscopy of preparations from intracellular, gram-negative diplococci using methylene-blue or gram stain. It is almost never necessary to do serological tests or immunofluorescence microscopy. Laboratory culture should be performed mainly to confirm resistance. Currently other molecular biologic methods for the detection and monitoring of resistance are being tested.

Worldwide development of resistance to *Neisseria gonorrhoeae* is increasing with different regional characteristics. *Neisseria gonorrhoeae* was found in sex workers in Indonesia (Joesef 1994), 89% of whom were penicillinase-producing and 98% of whom were resistant to tetracycline, but responded well to cephalosporins and fluoroquinolones. At the same time, a reduced response to quinolones by up to 24% was detected in the US (CDC 1998). Penicillinase-producing (resistant) gonococcal stains are seen in the US in 25%, in Asia 30%, and in Africa up to 90%. Also an increase of resistance to 3rd generation cephalosporins has been observed in many regions (Bala 2010, Ison 2010, Chisholm 2011). Resistance to ceftriaxone has been reported (Carnicer-Pont 2012, Unemo 2011) as well as to macrolides like azithromycin (Chisholm 2009, Ison 2010). Systematic evaluation of antibiotic resistance in Germany has not been performed. Gonorrhea is often treated without laboratory culture and resistance testing. A small German study in the Heidelberg and Stuttgart regions with 65 smears from patients with uncomplicated gonorrhea during the years 2004/2005 (Enders 2006) found resistance to penicillin in 21.5%, to tetracyclins in 29.2%, to ciprofloxacin in 47.7% and to azithromycin in 7.7%. All isolates were fully susceptible to ceftriaxone, cefixime and spectinomycin, which are no longer available. Comparable results were published in Berlin from 1995 until 1997 and from northern parts of Germany from 1997–2000 (Wagner 2001, Ungeheuer 2001) looking at 85 isolates. Examinations from 2001 until 2010 in Dresden found in *Neisseria gonorrhoeae*-positive cultures 46% ciprofloxacin-resistant isolates but no resistance against cefotaxim or ceftriaxone (Abraham 2013). About 30% of patients with symptomatic gonorrhea are coinfected with chlamydia serotypes D-K.

Therapy

Therapy depends on geographical resistance profiles. With respect to fluoroquinolone-resistant bacteria strains in Germany, a one-time IM or IV dose of 1000 mg ceftriaxone (Rocephin®) (DSTIG 2013) is the treatment of choice in Germany and coadministration of a one-time dose of 1500 mg azithromycin or doxycycline 200 mg daily for seven days is recommended due to resistance of *Neisseria gonorrhoeae* and frequent chlamydia coinfections.

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Chlamydia infection, lymphogranuloma venereum

Genital infections with *Chlamydia trachomatis* are nearly twice as prevalent as gonococcal infections. There are several serotypes that can cause different diseases.
Serotypes D-K are broadly distributed in Europe and cause urogenital infections, which can be sexually transmitted as well as conjunctivitis or pneumonia after perinatal transmission. Serotypes L1, L2 and L3 cause lymphogranuloma venereum (LGV). LGV used to be known strictly as a tropical disease but has undergone a renaissance in Europe and the US (Gotz 2004, Krosigk 2004).

Clinical course

In men, symptomatic genital chlamydia, serotypes D-K, may be present as urethritis. As in gonorrhea, epididymitis, prostatitis or proctitis may occur. Reiter's syndrome with conjunctivitis and reactive arthritis is also possible.
A chlamydial infection in about 20% of the female patients may manifest as urethritis, cervicitis, salpingitis, endometritis, proctitis and arthritis. Cervicitis mainly contains purulent fluorine. Possible consequences of a salpingitis are sterility by tubal occlusion or ectopic pregnancy.
In lymphogranuloma venereum caused by serotypes L1-3, a primary lesion occurs at the entry location. Some weeks later a painful swelling of the regional lymph nodes develops that tends to exulcerate. After healing this may lead to scars, which may cause discharge disorders and fistula due to a blocking in the lymphatic vessels. Especially in HIV+ MSM, extremely painful and therapy refractory proctitis as well as preanal and intra-anal ulcerations by chlamydial infections with serotypes L1-3 may occur (RKI 2004+2005, Peerenboom 2006).

Diagnosis

The best methods to confirm the diagnosis of infection with *Chlamydia trachomatis* are amplification tests (PCR). This is more sensitive, and at least as specific as the results obtained by cell cultures (Morre 2005) used in the past. Urethral *Chlamydia trachomatis* infections can be detected by PCR from urine. Using dry cotton wool, apply with pressure for a few seconds to collect epithelioid cells, which should be sent in dry storage to the lab (routine in most labs). The samples should be tested

for serotypes D-K. Due to the fact that especially young women are frequently infected with *Chlamydia trachomatis* serotypes D-K (RKI 2013) screening in pregnant women and woman under 26 years old is recommended in Germany.

More than 90% of the LGV cases in Germany were HIV+ MSM with proctitis symptoms. Only 10% of these patients complain of an urethritis without proctitis (Martin-Iguacel 2010, Mohrmann 2011). PCR tests of serotypes L1, L2 and L3 are only done on request in specialized labs.

A positive test result for all chlamydial serotypes described above leads to a therapy indication. Antigenic tests by ELISA or direct immunofluorescence tests are possible as well, but there is a lack of sensitivity in 75% and a lack of specificity in 97-99% in patients with low chlamydial prevalence resulting in a high number of false-positive test results.

Therapy

The therapy of choice is doxycycline (Supracyclin®) 100 mg BID for 7 to 10 days. Alternatively, ofloxacin (Tarivid®) 200 mg BID or erythromycin (e.g., Erythrocin®) 500 mg QID for 7 days can be given. Even a single dose of 1000 mg azithromycin (Zithromax®) has proven effective in uncomplicated cases. Lymphogranuloma venereum requires longer treatment: doxycycline should be given for at least 3 weeks.

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Genital ulcers (ulcus molle, soft chancre, chancroid)

Genital ulcers are caused by an infection by *Haemophilus ducreyi*. It is an endemic infection found primarily in tropical or subtropical regions. Officially, less than 100 cases per year were diagnosed in Germany in the years 1999 to 2004 (Health Report of the Federal Government, 2006). However, the estimate for unknown cases may be higher.

Clinical course

Usually, the incubation period is 2 to 7 days causing one or more frayed-looking ulcers at the entry location, mostly in genitourinary or perianal locations. These ulcers are not indurated (soft chancre) but characteristically cause severe pain. In about half of the patients the regional lymph nodes are swollen resembling lymphogranuloma venereum, mainly unilateral and very painful. Balanitis, phimosis or paraphimosis occur less frequently.

Diagnosis

Due to the manifold symptoms partly resembling other ulcer-causing genital infections such as syphilis or even herpes simplex, a clinical diagnosis is difficult. Microscopy of ulcer smears may demonstrate gram-negative bacteria. But a purulent punctate from affected inguinal lymph nodes offers more reliable results. Sometimes a biopsy from the ulcer is necessary to distinguish it from a malignancy.

Therapy

Asingle dose of 1000 mg azithromycin (Zithromax®) is recommended (Martin 1995). Alternative therapies are erythromycin (e.g., Erythrocin®) 500 mg QD for 4 to 7 days, or ciprofloxacin (e.g., Ciprobay®) 500 mg BID for 3 days. Lymph nodes that are severely swollen or might burst open should not be split but punctured for relief.

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Condylomata acuminata (fig warts)

Human papillomavirus (HPV) exclusively infects epithelial cells and is one of the most frequently transmitted viral infections in men as well as in women. It takes at least 3 weeks from the incubation period to clinical manifestation, but may also take months or years. Even a transmission via smear infection or contaminated objects is possible. Besides frequent casual sex and smoking, immune deficiency and other diseases in the genito-anal region are the main risk factors for an HPV infection. In general, HPV infections are seen more often in HIV+ patients. HPV infections tend

to persist longer resulting more often in the development of clinical symptoms. Patients who have anogenital warts should be offered HIV testing. The numerous different HPV subtypes may cause infections in the anogenital region in patients older than 20. HIV+ patients suffer very often from genito-anal coinfections with various oncogene high-risk HPV subtypes. In recent years an increase of HPV-caused benign fig warts has been observed despite ART as well as intraepithelial neoplasms and carcinoma, both cervical and anal. In immunodeficient patients HPV-associated lesions have low rates of spontaneous remission and are very resistant to therapy (frequent relapses). The risk of developing anal cancer is 80 times higher in HIV+ MSM than in the general population. The incidence is 35/100,000 person-years (Chiao 2006, Silverberg 2012). Most HIV+ anal cancer patients have condylomata acuminata in their medical history (Hoffmann 2011). Screening and early treatment of genito-anal condylomata acuminata and intraepithelial neoplasia may reduce the incidence of anal cancer and recommended (DAIG 2013) in HIV+ persons.

Clinical course

Most HPV infections are asymptomatic or subclinical. Even symptomatic HPV infections may end with a spontaneous remission. The clinical manifestations of sexually transmitted HPV infections are genito-anal warts or Bowenoid papulosis as well as giant condyloma (Buschke-Lowenstein tumor), cervical or anal intraepithelial neoplasias (classified CIN or AIN I-III lesions including the erythroplasia of Queyrat) or at least carcinoma. In HIV-infected patients, the risk of persistent HPV infections is seven times higher and correlates inversely with the CD4 T cell count (Piketty 2003). In HIV+ patients, HPV infections are more often symptomatic and chronic. In addition, the risk of relapse is considerably higher, even after treatment. Malignant transformation is the most important complication involving the high-risk HPV subtypes. Condylomata acuminata are hyperkeratotic and verrucous papules of the anogenital region. Condylomata acuminata are usually caused by HPV 6 or HPV 11, so-called low-risk HPV types, which by themselves do not tend to induce malignant transformation.

Therefore, fig warts are not inevitably the beginning of genito-anal intraepithelial neoplasms and carcinoma but it is difficult to differentiate between them. Besides the preferred localization in genital as well as peri- and intra-anal regions, fig warts may also occur enorally and in the urethra. Condylomata are usually asymptomatic but can affect the sexual life of patients and may cause hygiene and psychogenic problems. Pruritus, burning or bleeding are rare and are generally caused by mechanical stress.

Diagnosis

Analogous to cervical intraepithelial neoplasia (CIN) and cervical cancer in women, regular screening (every 1 to 3 years) for condylomata acuminata, anal intraepithelial neoplasia (AIN) and anal carcinoma is advised for all HIV+ patients. Screening should include clinical inspection, palpation, colposcopy, proctoscopy, cytology (Pap smear) and, if necessary, a histopathological examination of biopsies. *Condylomata acuminata* is a clinical diagnosis. An exploratory biopsy is recommended before therapy starts to confirm it is not a malignancy. In case of therapy resistance, early relapse or a fast or infiltrating growth, an exploratory biopsy is imperative. Meanwhile, cytologic examination of microscopic preparations (smear tests) are done in order to differentiate from preliminary cervical or anal carcinoma. Cytological results of smears from the cervix are divided with the classification of Papanicolaou.
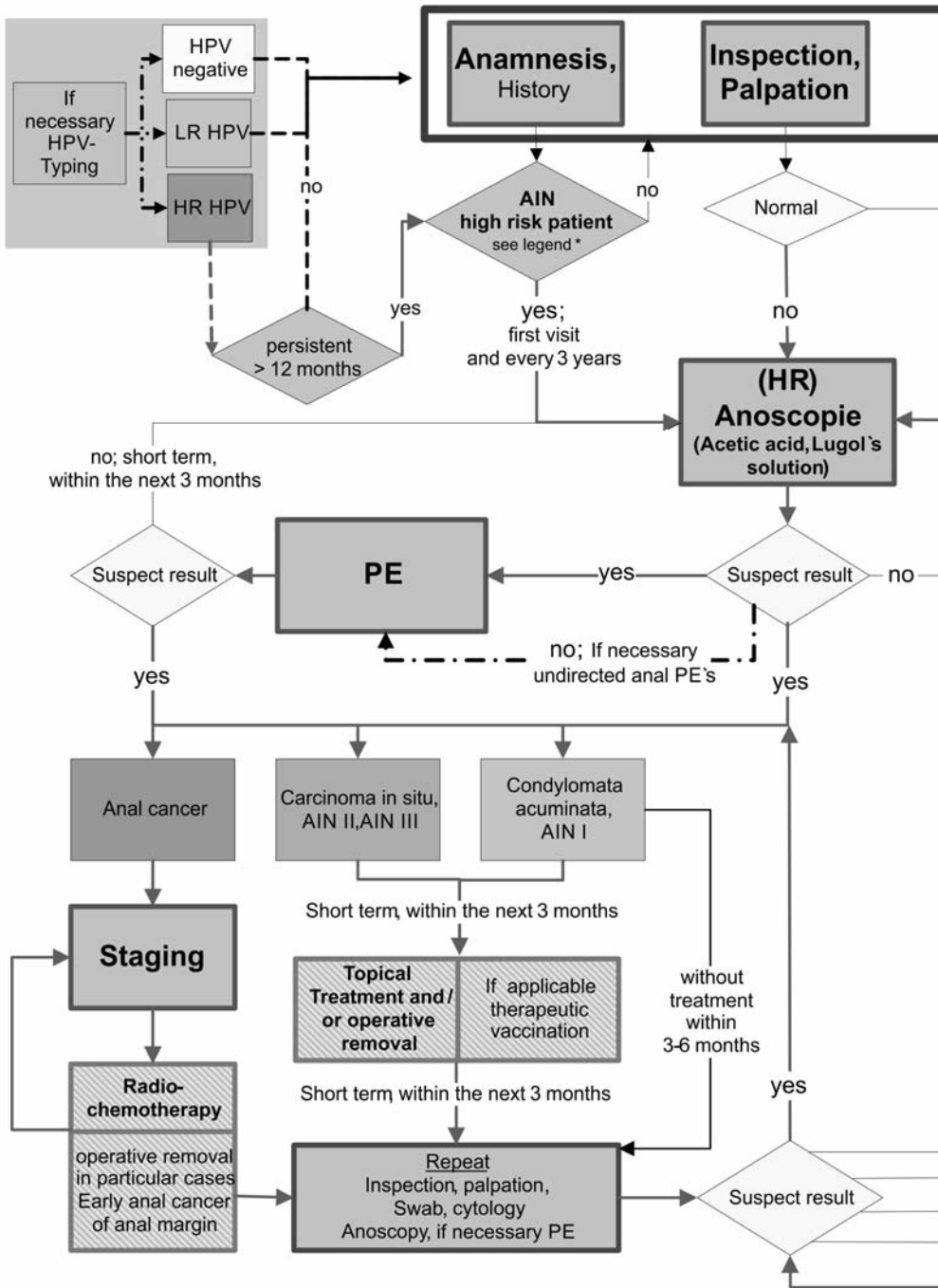
Algorithm: Anal cancer screening diagnosis and therapy of Condylomata acuminata, anal intraepithelial Dys-/Neoplasia and anal cancer in HIV-infected persons

Routine examinations once yearly for all HIV-infected persons → Swab, cytology → Normal

yes — yes — Normal

no

HSIL   LSIL / ASCH/ASCUS

short term, within the next 3 months → HSIL

within 3-6 months

Suspect cytology   yes: within 3-6 months → Repeat Swab, cytology

no

no — Normal

yes; within 3-6 months

Repeat Swab, cytology

no — Normal

Algorithm starts again   yes; 2x normal cytologic results

4x no; wthin 12 months

3x no; within 12 months
2x na within 3-6 months → Repeat Inspection, palpation, Swab, cytology Anoscopy, if necessary PE
no; within 3-6 months

Legend*:

AIN high risk patient, see legend*

Current or History of
- Condylomata acuminata
- HPV-associated intraepithelial Neoplasia (IN) independent of their localisation (oral, genital, anal)
- Anal cancer

For anal smears the Bethesda System is used: Normal results are differentiated from inflammation and atypical cells: atypical squamous cells (ASC: -US (undetermined significance), -H (cannot exclude HSIL), atypical glandular cells (ACG), low-grade or high-grade squamous intraepithelial lesion (LSIL or HSIL). However, the sensitivity and specificity of these tests are still not sufficient (Panther 2004, Jablonka 2011). A review of anal cytologic examinations has shown a prediction of biopsy results for anal dysplasia with a sensitivity of 69-93% and a specificity of 32–59% (Chiao 2006). Every suspicious cytologic finding should be monitored with a contemporary colposcopy or proctoscopy (Duerr 2006). Specialized centers offer the "High Resolution Anoscopy" as gold standard, which improves the test results of peri- and intra-anal inspections with regard to necessary exploratory biopsies, especially after the application of acetic acid (3 per cent mucosa, 5 per cent skin) and an additional staining with Lugol's solution. Suspicious lesions have to be biopsied. Histologically, examinations of intralesional biopsies differ in *Condylomata acuminata*, intraepithelial neoplasia divided in severity grades I-III (IN) and invasive cancer. The abbreviation of the anatomic location of the lesion is specified in front of the IN grade. The description AIN III is in accordance with an anal carcinoma *in situ*. The determination of the HPV subtype allows for differentiation between high- and low-risk types and is still not a routine diagnostic method, because of its subordinate role in therapy decisions (Ledger 2000). When high-risk HPV-types are detected, some experts recommend to shorten the period between control examinations of the affected region.

Just like women, HIV+ men, mainly those suffering from condyloma anamnesis, should have a proctological follow-up at least once a year (Chiao 2006, Scott 2008, Wexler 2008, Jamieson 2006, Esser 2011). To avoid fatal tumor growth and mutilating operations (rectum amputation, etc) it is recommended to do thorough genitoanal inspections and regular proctological exams by means of high resolution anoscopy with cytological smears and exploratory excision, which are timely and specific (Kreuter 2009, Pindea 2008). Rectal palpation and external inspection of the anogenital regions are not sufficient as a preventive medical checkup for HIV+ patients. Should an anal carcinoma be palpable, it has, in general, already progressed extensively. Until today, there are no good reports on how often intra-anal, HPV-associated lesions are isolated without involving the external genito-anal regions. Nowadays there are surveys trying to find out how often colposcopic and proctoscopic exams should be offered in addition to the routine genito-anal palpations and inspections, and exactly who should be examined.

Therapy

Until now there is no satisfying therapy for *Condylomata acuminata*. Relapses still occur frequently even after adequate treatment in immune competent HIV-negative patients (40-60%). However, therapy delays (watch & wait) should be avoided and all clinically striking findings should be removed at an early stage even at the risk of operating multiple times. Therapy includes the most complete operative removal possible with histological follow-up of the nature of the tumor and its invasive depth. Besides surgical excision, electrosurgery, the condyloma may be removed by means of laser surgery, infrared coagulation, caustica (trichloroacetic or podophyllotoxin) or cryotherapy with liquid nitrogen (high healing effect initially – high relapse risk). All the destructive treatments have disadvantages. Since virus-harboring keratinocytes can remain in the clinically normal surrounding tissue, relapses are as frequent as 50% in immunocompetent patients and in up to 70% in immunodeficient patients within 4 months. In clinical practice, attending physicians often try

to reduce the high relapse risk by an adjuvant local immunotherapy with imiquimod (Aldara®) cream or interferon beta. Both agents are expensive and a local therapy takes time (at least 3 months). Imiquimod is licensed for the topical treatment of HPV-associated lesions. As demonstrated in several controlled studies imiquimod treatment is safe and effective and has the lowest relapse rate of all treatments (6-13% in immunocompetent patients). Imiquimod is not approved for the treatment of anogenital warts in immunodeficient patients and intraepithelial neoplasias but results of successful treatments of genital warts (Cusini 2004), Bowenoid papulosis and Bowen's disease in HIV+ patients have been published (Kreuter 2008). In our own experience imiquimod can be successfully used as the sole therapy for flat, less hyperkeratotic condyloma. There are formulas for imiquimod-containing suppositories (off-label). However, the treatment period takes several weeks without surgical intervention, often complicated by compliance-reducing side effects such as inflammation, pruritus and burning. Condyloma may also be systemically treated with interferon (there are often problems with health insurance due to a low success rate of 31% in the intial stages, although there are reports of a significantly lower relapse rate in comparison with other invasive therapies). Herbal 10% *Camellia sinensis* ointment (Veregen®) is also approved for local therapy of genitoanal warts (Abramovits 2010). The only antiviral agent active against HPV is cidofovir but there is little experience in HIV+ patients (Snoeck 2001). In a prospective comparative trial, destruction of HPV-associated anogenital lesions with electrocautery was superior to local immunotherapy with imiquimod or topical chemotherapy in HIV+ MSM and had less adverse events (Richel 2013).

While various vaccines have been successfully used as prophylaxis for certain HPV-subtypes (HPV 6, 11, 16, 18), there is still no progress in the development of an effective therapeutic vaccination against symptomatic HPV infections. Primary results of the quadrivalent HPV vaccine in HIV+ men show that the vaccine is generally safe, well-tolerated and highly immunogenic. Efficacy studies are now warranted (Wilkin 2010). Case reports about less relapses after operative removal and vaccination have been published (Swedish 2012).

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Shigellosis

Shigella is a worldwide gram-negative bacteria related to the family of enteric bacteria which can be divided into different pathogenic serogroups (A-D) and serovars (group A: *S. dysenteriae*, B: *S. flexneri*, C: *S. boydii*, D: *S. sonnei*), depending on certain biochemical features and specific antigens. All shigella groups release an endotoxin which causes infection of the intestinal mucosa. *S. dysenteriae* type 1 also produces an exotoxin which often leads to severe symptoms involving insufficiency of the cardiovascular system and CNS disorders.

Humans are the only relevant hosts. Shigellosis is spread via fecal-oral transmission, most frequently through direct contact, i.e., by lack of proper hygiene and poor hand washing habits. Although shigella bacteria usually do not survive outside the human body, infection can be transmitted via contaminated water or food in warmer countries. As few as 100 perorally transmitted germs are enough to cause an infection. Shigella bacteria grow in the intestinal mucosa of humans and are shed in the feces. The incubation period is usually between 12-96 hours. Infections can be transmitted after a phase of acute illness and as long as the bacteria is excreted in stool, but usually no more than four weeks. Prevention of the spread of this highly contagious bacteria is complicated, as stool specimens can appear clinically normal.

With regard to sexual practices, sexually transmitted shigellosis appear rather frequently within certain risk groups such as MSM and cause regional outbreaks (RKI 2002+2005, Aragón 2007, Daskalakis 2007, Keay 2014). In a survey by the Robert Koch Institute, most infected patients stated that they had sexual contacts in bars,

parties or in saunas, where a direct or indirect contact of the mouth or anal regions by the fingers had taken place. In two outbreaks in Berlin in the years 2001 and 2004, the *S. sonnei* isolates in stool specimens showed similar isotopes and identical resistance patterns. Resistance was observed to TMP/SMX, tetracyclines, amoxicillin, ampicillin/sulbactam and mezlocillin (RKI 2002, Marcus 2004, RKI 2005). A more recent analysis investigated 52 cases of *S. sonnei* in MSM suffering with diarrhea in three major HIV clinics in Berlin and Hamburg. Results showed high rates of quinolone resistance especially high in HIV-infected MSM (for ciprofloxacin, 53% versus 21% in negative MSM). No resistance was found against carbapenem and newer cephalosporins, such as ceftriaxone, ceftazidime or cefepime (Hoffmann 2013). However, resistance to the commonly administered antibiotics is increasing worldwide (Niyogi 2007, Gaudreau 2010).

Clinical course

Many infections with shigella may present as a mild, self-limited illness. However, the clinical course of shigellosis varies widely, from asymptomatic disease, watery diarrhea, dysentery (bloody and mucoid stools) up to life-threatening septic courses. Mostly, shigellosis begins with watery diarrhea and can develop into an inflammatory colitis. Abdominal pain (colitis and tenesmus) is a typical sign. Frequent defecation (up to 50 times a day) can cause dehydration and loss of proteins. Usually, shigellosis resolves within 7 days. Fever, bloody, mucoid and ulcerous diarrhea are symptoms of severe cases. Focal ulcerations and necrosis appear most frequently in the distal colon that can develop into dilatation of the colon and colon perforation with following peritonitis and sepsis in extreme cases.

In rare cases (1-3%) shigellosis manifests outside the intestines: cytotoxin (Shiga toxin) produced by *S. dysenteria* serovar 1 is almost identical to Shiga toxin 1 (verotoxin 1) enterohemorrhagic *E. coli* (EHEC) that also causes a hemolytic uremic syndrome (HUS). Other possible sequels are infectious arthritis and Reiter's syndrome.

Diagnosis

Diagnosis is made by bacteriological investigation of freshly obtained stool or rectal smears. The stool sample is suspended in MacConkey agar to identify non-lactose fermenters such as shigella species. More selective media cultures and slide agglutination are then used to identify group and serotypes. The samples should be obtained before taking antibiotics and an antibiogram should be made. Results of resistance tests may be adjusted if therapy with antibiotics has already commenced. Identification of the infection sources and transmission paths help to define the serogroups and serovars involved.

Therapy

Since shigellosis is highly infectious, treatment with antibiotics is recommended. With antibiotics, the period of fecal shedding, diarrhea and illness is shortened (Christopher 2012). Quinolones, TMP/SMX, azithromycin, tetracyclines, doxycycline and ampicillin are suitable. Ampicillin is recommended for treatment of long-term carriers. In resource-limited areas, the drug of choice is ciprofloxacin (500 mg BID) or TMP/SMX (160 mg/800 mg BID) for five to seven days, respectively. However, in Western metropolitan areas and in cases of MSM infected with shigella, increasing resistance rates have to be considered (Niyogi 2007, Gaudreau 2010, Hoffmann 2013). In an analysis of 52 cases occurring in Hamburg and Berlin in 2010/2011, high resistance rates were found for doxycycline, tetracycline, aminoglycosides, all

cephalosporins of the first two generations tested, and TMP/SMX. In total, 30% of the cases were resistant to amoxicillin and ampicillin, while 48% were resistant to ciprofloxacin. Compared to HIV-negative cases, HIV+ patients had a significantly higher rate of quinolone resistance. For ciprofloxacin, resistance rates were 58% versus 20%, respectively. Most isolates were susceptible to newer cephalosporins such as cefixime and no resistance was found for carbapenems or newer cephalosporins such as ceftriaxone, ceftazidime or cefepime (Hoffmann 2013).

A symptomatic therapy with oral fluid replacement can suffice for patients in overall good and stable condition. In the case of comorbid, very young or older patients, loss of fluid and electrolytes should be replaced with parenteral nutrition. Motility inhibitors such as loperamide should be avoided.

Prevention

Basic measures to prevent shigellosis infection are clean and hygienic conditions (personal hygiene, clean water and food, hygiene in community facilities, prevention of fly contamination). As shigellosis is usually passed through direct contact from human to human, the most effective prevention is frequent and careful hand washing to avoid fecal and oral smear infections. Hands should be washed with soap or with an alcohol containing disinfectant. In countries with poor hygienic conditions one should follow the recommendation, "Peel it, boil it, cook it or forget it". As shigellosis is highly contagious and HIV+ patients possibly more vulnerable (Baer 1999), preventive measures against sexually transmitted shigellosis are more strict than with other STDs. Use of condoms for anal sex does not provide sufficient protection. Sexual contact should be avoided from the first days of diarrhea onwards until shigella bacteria are no longer detectable in the stool.

Early diagnosis and treatment prevents further infection. During the course of illness, measures should be taken to disinfect all objects and surfaces which may have come into contact with the patient's infectious excretions. Clothes, bed sheets and towels should be washed at least 60°C or be soaked in disinfectant for 12 hours before washing at normal washing temperature. Toilet seats and lids, as well as bed frames, sinks and bath tubs should be disinfected daily in health care facilities.

Owners of bars and darkrooms as well as organizers of sex parties should install soap dispensers in the washrooms. Sharing of used and inadequately disinfected dildos or tubes with lubrication gels should be avoided. Operators of saunas should chlorinate their whirlpools.

Other preventive measures for schools and other public facilities and food production companies, should follow preventive guidelines given by the authorities for disease control and prevention. People who are, or are suspected to be infected with shigellosis, are not allowed to work in facilities where food is produced or processed. This also applies to long-term carriers (asymptomatic shedders) of the infection. Admission to public facilities is possible after clinical recovery and three negative stool test results (stool samples after 1–2 days, respectively). The first sample should be taken after at least 24 hours after appearance of formed stool or 24 hours after ending a therapy with antibiotics. People in close contact with an infected patient should be tested after the incubation period and test negative. An exception may be made if typical symptoms do not show and otherwise hygienic measures are followed. Close personal contacts and a lack of hygiene, especially in community facilities encourage a spread of shigellosis. If a shigellosis outbreak is suspected, a quick identification of the source of the infection and transmission factors (i.e, food) can avoid further spread of the infection. In any case, the public health department should be informed as soon as possible.

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16. Vaccinations and HIV

THOMAS WEITZEL

HIV+ patients have an increased morbidity and mortality due to various infectious diseases that are vaccine preventable. On the other hand, vaccinations might cause a higher rate of adverse effects in HIV+ patients, who are also prone to a higher rate of failure in achieving a protective immune response. Indication and timing of vaccination should therefore reflect each patient's individual situation – the better the immune status, the higher the chances for an appropriate immune response. Thus, indications should be checked as soon as a patient is diagnosed with HIV (see chapter *Checklist: The new HIV patient*). In severely immunocompromised patients, vaccinations are usually not successful and might even be contraindicated. In such cases, the immunization status of close contacts should be checked and, if necessary, completed information about exposure and exposure prophylaxis should be provided. In certain situations, passive immunoprophylaxis might be indicated. When ART leads to a sustained rise in CD4 counts, vaccinations should be reconsidered and/or repeated. Recent studies demonstrate that many HIV+ patients do not receive the vaccinations that are internationally recommended (Molton 2010, Mohseni-Sadar 2010).

Benefits of vaccination

Depending on their immune status, a poorer response to previous vaccines and an accelerated decline of protective immunity over time must be expected. Until recently, the rule of thumb was that:
• the response to vaccination is reduced if CD4 T cells are <300/µl,
• no vaccination response is expected if CD4 T cells are <100/µl (Rosseau 1999).
Newer data question this concept since in patients with sufficient viral suppression some vaccines (e.g., influenza) exhibited an immune response that was independent from CD4 T cell counts (Hatakeyama 2011). Still, re-vaccinations should be reconsidered if CD4 T cells rise to >200/µl. To evaluate possible benefits of vaccinations, the anamnesis should include the following factors:

Current status of protection	Current risk of infection
• Prior infections • Prior vaccinations (problem: reduced effectivity in severely immunocompromised patients, consider antibody control)	• Sexual risks • Occupational risks • Contacts with infected individuals • Contacts with children • Traveling

Risks of vaccination

Some vaccinations might cause transient viral load increases. This effect reflects the stimulation of cellular immunity and does not occur in non-responders to the vaccine. The peak of this increased viral replication appears 1 to 3 weeks after the vaccination. Therefore, routine measurement of viral load should be avoided within four weeks after vaccinations. Numerous studies demonstrate that these transient elevations of the viral load are clinically and immunologically irrelevant. Still, genotyping before and after an influenza vaccine demonstrated in 2 out of 34 patients new mutations of the RT- or protease-gene (Kolber 2002). Furthermore, the elevated viral replication can (theoretically) increase the risk of materno-fetal transmission.

With inactivated vaccines, there is no higher rate of adverse events in HIV+ patients. In live vaccines, however, the risk of complications caused by an infection with the vaccine strain is increased. Severe and even fatal complications have been reported following vaccinations for smallpox, tuberculosis, yellow fever, and measles. Nevertheless, there is no general contraindication for live vaccines.

Vaccination of contacts

Whenever HIV+ patients are susceptible to vaccine-preventable infections, particular care should be taken to vaccinate close contacts (including yearly influenza vaccine). However, if contacts shed certain live vaccines, they might infect HIV+ patients. Therefore:
• Avoid oral polio and smallpox vaccinations of close contact persons
• Avoid stool contact (e.g. changing dipers) for 4 weeks after rotavirus vaccination (Rubin 2013)
• Avoid contact with skin lesions after varicella or zoster vaccinations; consider prophylactic use of acyclovir (German recommendation: STIKO 2005).
Other live vaccines of contacts bear no risk.

Vaccinations in HIV+ children

European Guidelines have recently been published (Menson 2012). With few exceptions, HIV+ children should be vaccinated according to national children vaccination schedules. BCG vaccination is generally not recommended. Children with severe immunodeficiency (relative CD4 T cell count <15%) should not receive live vaccines such as MMR and varicella vaccine. Above this level, children can be vaccinated with MMR and according to the latest US recommendations, also with varicella vaccine (Mofenson 2009). Due to lack of data, quadruple MMRV vaccine should be avoided. If one of those live vaccines cannot be applied, all family contacts (especially siblings) should be vaccinated. Antibody response might be controlled after vaccination, especially for measles and rubella (Menson 2012). HIV-infected children should receive a routine series of pneumococcal conjugate vaccine (PCV), starting in the second month of life. If and when to add polysaccharide vaccine (PPSV) is controversial (Menson 2012). Rotavirus live vaccine is now recommended for children in many countries. There are few data on the efficacy and safety. While American experts favor the use of this vaccine in HIV+ children (Rubin 2013), European guidelines are more restrictive (Menson 2012).

Post-exposure prophylaxis

With some infections, the risk of infection and/or disease severity can be reduced by post-exposure prophylaxis, including active and passive immunizations as well as chemoprophylaxis (Table 2). Usually, the time between exposure and the start of prophylactic measures is crucial and should be minimized.

Practical approach to vaccinations

Informed consent: The obligation to inform vaccines follows national recommendations (in Germany: see STIKO 2004). Patients should be informed about the risks and benefits of vaccines, with particular attention to HIV-related vaccination problems. In some countries, written informed consent is required. For vaccine information statements in different languages see www.immunize.org.

Timing: Vaccinations should be postponed when an acute infection is present; however, a mild afebrile infection is not relevant. Live vaccines such as MMR, varicella

or yellow fever must be given either simultaneously or at least four weeks apart from each other. After treatment with immunoglobulins, live vaccines should not be administered within the following three months (exception: yellow fever vaccine). When exact viral load measurements are crucial (e.g. decisions about ART), vaccinations should be postponed as they might influence viral replication.

Booster vs. complete series: As a rule of thumb, a complete series is necessary when no prior dose of the respective vaccination is reported or documented. That means that a past incomplete primary series can be completed independent of a time delay between the necessary shots (every shot counts!). This strategy does not take into account that vaccinations might be repeated if prior doses were given at a time when the patient was significantly immunosuppressed.

Route of application: Vaccination routes should follow the recommendations provided by the manufacturer. High immunogenicity and few complications make intramuscular injections the preferable application route (deltoid muscle, in infants also anterolateral thigh; gluteal applications are obsolete!). Many vaccines can also be administered subcutaneously (see product information). In hemophiliacs, subcutaneous vaccination followed by thorough compression (>2 minutes) usually allows vaccination without coadministration of clotting factors. Only a few vaccines have to be administered subcutaneously, including meningococcal polysaccharide, yellow fever, and some varicella vaccines. Intradermal rabies vaccination schedules, which are licensed in some countries, should not be administered to HIV+ patients due to reduced immunogenicity (Tantawichien 2001).

Combination vaccines: In general, it is recommendable to combine vaccines to minimize patient discomfort (and sometimes costs).

Documentation: Vaccinations should be documented in the patient's medical records as well as in a vaccination card kept by the patient. For the latter, a WHO recommended form can be ordered either through WHO or national providers. Documentation includes the brand, manufacturer, and lot number of the vaccine.

Details of selected vaccines in HIV+ patients

Tetanus/Diphtheria/Pertussis: Following a primary series during childhood, life-long protection against tetanus/diphtheria should be maintained by boosters every 10 years. According to a Danish study (Kurtzhals 1992), protection against diphtheria is often insufficient. Depending on their CD4 T cells, patients have a reduced response to boosters and an accelerated antibody waning (Moss 2003). Combination vaccines including polio and/or pertussis are available and suitable. Recently, most countries recommend a single booster of acellular pertussis vaccine for adults. Since adult pertussis vaccine is only available as a combination vaccine (e.g., Tdap), its use should be considered whenever tetanus and/or diphtheria vaccination is indicated.

Pneumococcal: Even under ART, there is an increased risk of invasive pneumococcal infections (Jordano 2004) which can be reduced with the 23-valent pneumococcal vaccine PPSV23 (Grau 2005, Rodriguez-Barradas 2008). The vaccine response in patients under ART with CD4 cell counts >200/µl is similar to healthy individuals (Falco 2006). However, a cohort study demonstrated that patients with VL >100,000/ml did not benefit independent of their immune status (Teshale 2008). Recent data indicate that pneumococcal conjugate vaccines (PCV) might induce a stronger and longer lasting protective effect (Nunes 2012). A placebo-controlled trial in Malawi showed an efficacy of 74% using PCV7 (French 2010). Therefore, most guidelines now recommend the combination of PCV and PPV. In the USA, unvaccinated HIV+ patients first receive PCV13 (independent of their CD4 counts); if they

have CD4 T cell counts ≥200/µl, they should then be vaccinated after ≥8 weeks with PPV23. If CD4 T cell counts are lower, this vaccine might be considered, if patients are under ART and have low VL, or be postponed until the immune status recovers. Patients already vaccinated with PPV23 receive additional PPV13 after a time span of at least 12 months. PPV23 is repeated once after 5 years, then again when the patient is 65 years old (CDC 2012, Panel on OI 2015). This strategy provides additional protection and is cost effective (Cho 2013).

Influenza: HIV+ patients have an increased risk of severe manifestations of influenza infection and a higher influenza-associated mortality (Lin 2001). Influenza vaccine is effective (Anema 2008). Since influenza remains a frequent cause of febrile respiratory infections even in vaccinated patients (Klein 2007), private and healthcare contacts should also annually be vaccinated. All patients older than six months should receive inactivated influenza vaccine at the beginning of each influenza season independent of their CD4 status (Geretti 2008). In children under 10 years of age, the first vaccination should include two doses at a 4-week interval. Intranasal live vaccines are not recommended by most experts.

Hepatitis B: According to international standards, every hepatitis B susceptible patient should be vaccinated; a recommendation that is not consistently followed in daily routine (Bailey 2008). The combination vaccine with hepatitis A should be preferred as it is more immunogenic (van der Wielen 2006). In HIV+ patients, the reduced immune response to hepatitis B vaccination is well known (van den Berg 2009). Depending on CD4 T cell counts and other factors such as viral load, gender, and age (Fisman 2002, Overton 2005), only 20-70% of patients will develop protective immunity (Laurence 2005). Success rates were higher in some studies using multiple and/or higher doses or more effective adjuvants (Whitaker 2012). Interestingly, patients taking ART have a better vaccine responses even if they have high CD4 counts (Landrum 2009). Although the optimal vaccination strategy is still under debate, there is a consensus to:
• Vaccinate early after diagnosis
• Control immune response 4 weeks after the last shot
• Revaccinate if the immune response is lacking or suboptimal (Germany: <100, USA: <10 IU/ml) and/or if there is substantial immunoreconstitution
It is recommended to start with a normal vaccination schedule (3 doses of 10–20 µg). If the initial schedule fails to generate sufficient response, revaccination using 3 or 4 dose schedules and normal or double dose vaccines (40 µg) is advisable (Panel OI 2015). The use of hepatitis A/B combination vaccine (Twinrix®) in double dose was also successful (Cardell 2008). British and US guidelines recommend annual controls of anti-HBs levels (Geretti 2008, Panel on OI 2015). The management of "isolated" anti-HBc is less clear (this constellation might be due to a false positive results, a loss of anti-HBs after infection or an occult HBV infection). Most experts recommend to consider these patients HBV susceptible and to vaccinate them as described above.

Hepatitis A: is common (Fonquernie 2001). The vaccine is indicated in patients with chronic liver disease or increased risk of exposure, e.g. MSM, hemophilia or traveling to high-prevalence areas. Routine pre-vaccination serology (HAV IgG) is not generally recommended, but can be considered in patients with possible prior exposure. Response is reduced especially if CD4 T cells counts are below 200/µl. Post-vaccination controls are recommendable. Non-responders should be revaccinated after CD4 counts rise using a normal 2-dose or a 3-dose schedule (Launay 2008). A combination vaccine with HBV is available and reduces costs (see above).

Measles: As measles causes severe disease in HIV+ patients (Kaplan 1992), patients without proven past infection or vaccination should be vaccinated (two doses sep-

arated by at least one month). The status of protection should be checked prior to trips to endemic areas (see chapter Traveling with HIV). It is possible to vaccinate patients with CD4 T cells >200/µl (different age-specific values in children) or >15%, who are mildly symptomatic or asymptomatic. Commonly, the MMR combination vaccine is used. For susceptible patients, immunoglobulin administration is indicated as post-exposure prophylaxis (in certain high-risk situations also pre-exposure prophylaxis).

Yellow fever (YF): Available data (<600 patients) indicate that asymptomatic patients with CD4 T cell counts above 200/µl can receive YF vaccine securely (Staples 2010). However, patients have reduced rates of seroconversion, depending on CD4 T cell status and viral load (Thomas 2012, Sidibe 2013, Barte 2014). One asymptomatic patient with a low CD4 count developed fatal encephalitis (Kengsakul 2002). Older individuals have a higher risk for severe adverse events (Khromava 2005). British guidelines disapprove YF vaccination in HIV+ patients >60 years of age (Geretti 2008). Due to reduced response rates, titre controls are often recommended. Another approach is the documentation of seroconversion in a paired serum sample (before and 2-3 weeks after vaccination. Patients who cannot receive the vaccine should not travel to YF endemic areas. Patients requiring a vaccination certificate only due to entry regulations (without a real risk of exposure) should receive a medical waiver stating that vaccination is not possible due to medical reasons. The new recommendations stating that most travelers do not need revaccinations every 10 years (WHO 2015, Staples 2015) does not apply to immunocompromised individuals.

Human papilloma virus (HPV): In many countries, HPV vaccination of juvenile girls is part of the routine vaccination schedule. In 2011, the US recommendations also included boys and young adults, especially MSM. The benefits in HIV+ patients are subject of ongoing studies. American guidelines favor catch-up vaccinations of all women, MSM, and HIV+ men up to the age of 26 years. Up to now, only the 4- and the newly introduced 9-valent vaccines are licensed for use in men; the 2-valent vaccine might have the advantage to be more immunogenic because of its adjuvants (Menson 2012, Toft 2013).

Varicella: Similar to measles, chickenpox is potentially life-threatening for HIV+ patients (Perronne 1990). Patients without a history of VZV infections (chickenpox or herpes zoster) or vaccination should be screened for antibodies. If susceptible and with CD4 T counts >200/µl, patients should be vaccinated (Geretti 2008, Kaplan 2009, Rubin 2014); although German recommendations are more restrictive (CD4 >25%, STIKO 2005). Vaccine complications should be treated with acyclovir. Zoster-like reactivations of the vaccine strain are possible but very rare. The zoster vaccine contains a higher dose of the Oka vaccine strain and was therefore initially contraindicated (Kimberlin 2007). Newer guides restrict this contraindication to those with CD4 T counts <200/µl (CDC 2011), but also do not generally recommend the vaccine to those with higher CD4 counts. It might be considered on an individual basis for patients >60 years with a good immune status.

Meningococcal infection: The risk of invasive meningococcal infections seem to be increased (Miller 2014). However, since the risk in general is very low, HIV infection alone is not considered an indication to vaccinate. In Germany, the 4-valent conjugate vaccine for people is recommended with immunodeficiency (without HIV being mentioned). Since clusters of severe meningococcal infections have recently been observed in MSM in several major cities, some experts suggested to vaccinate patients with a higher risk situation, e.g. attending mass events such as gay parades (Simon 2013). HIV+ patients, who have an indication for meningococcal vaccination, are vaccinated twice at an interval of 2-3 months (Cohn 2013).

The following tables summarize current recommendations.

Table 1: Vaccinations in HIV-infected individuals

Vaccine[1]	Type of vaccine	Indications[2]	HIV-specific recommendations[3] / Comments
Cholera	Inactivated + toxoid	Stay in endemic areas with increased risk of exposure	B Partly protects against some forms of travelers' diarrhea
Diphtheria	Toxoid	Generally recommended	B Age 6 years or older: reduced dosage
***Haemophilus influenzae* b (HiB)**	Polysaccharide	Children: generally recommended Asplenia	B Might be offered to unvaccinated HIV+ patients (Geretti 2008)
Hepatitis A	Inactivated	Chronic liver disease, hemophilia, increased risk (e.g., MSM, travel to endemic areas)	B British recommendations: booster every 5 years (Geretti 2008)
Hepatitis B	Recombinant antigen	Children: generally recommended Chronic diseases, increased risk (e.g., healthcare workers, sexual behavior, drug addiction, stay in endemic areas)	A Higher dose vaccines might be used (see text)
Human Papilloma Virus (HPV)	Recombinant	Girls aged 12–17: generally recommended USA: both genders	B
Influenza	I. Inactivated/fractionated antigen II. Live intra-nasal	Chronic diseases, age >60, and others (USA: all individuals >6 months)	I. A II. D Yearly different antigen combination
Japanese encephalitis	Inactivated[5]	Stay in endemic areas with risk of exposure	B Since 2009 new vaccine: Ixiaro®
Measles	Live	Children: generally recommended Susceptible individuals[4] especially work in healthcare, contact with kids or immunocompromised patients, travel to endemic areas	C Vaccinate susceptible HIV-patients if possible (see text) MMR combination vaccine
Meningococcal (groups A, C, W135, Y)	I. 2-/4-valent polysaccharide II. 1-/4-valent conjugate	Children: generally recommended in many countries Immunodeficiencies (e.g. complement deficiency, hypogammaglobulinemia, asplenia), travel to endemic areas	B GB: HIV+ age <25 y (Geretti 2008) USA: HIV+ juveniles (4-val. conjugate vacc. 2x) WHO: advanced HIV infection (WHO 2011)

Table 1 (continued)

Vaccine[1]	Type of vaccine	Indications[2]	HIV-specific recommendations[3] / Comments
Mumps	Live	Children: generally recommended Susceptible individuals[4] with contact with children	C MMR combination vaccine
Pertussis	Acellular antigens	Children: generally recommended Adults: 1 booster in many countries recommended	B For adults only available in combination with tetanus/diphtheria + polio
Pneumococcal	I. 23-valent polysaccharide II. 7- or 13-valent conjugate	I. Chronic diseases, immunodeficiencies, age >60 years (US: 65) II. In many countries recommended for all children, immunodeficiencies (US: age >65)	I. A II. A
Poliomyelitis	I. Inactivated (IPV) II. Live (OPV)	Children: generally recommended Booster if indicated (e.g. stay in endemic areas)	I. B II. D
Rabies	Inactivated	Risk of animal contact in endemic areas, travel to endemic areas with risk of exposure	B HIV: often poor response, serological testing, no intradermal schedules
Rota	Live	Children: generally recommended	Controversial: see text
Rubella	Live	Children: generally recommended Susceptible women[4] of child-bearing age, susceptible individuals[4] with frequent contact with children	C MMR combination vaccine
Tetanus	Toxoid	Generally recommended	B
Tick-borne encephalitis (German: FSME)	Inactivated	Risk of tick bite in endemic areas (usually April to November)	B German and European areas of risk: see www.rki.de
Tuberculosis	Live (BCG)	Depending on national guideline (Germany: not recommended)	D
Typhoid fever	I. Poly-saccharide II. Live	Stay in endemic areas with risk of exposure	I. B II. D

Table 1 (continued)

Vaccine[1]	Type of vaccine	Indications[2]	HIV-specific recommendations[3] / Comments
Varicella	Live	Children: generally recommended Susceptible women[4] of child-bearing age, susceptible individuals[4] with frequent contact to children or immuno-compromised patients, before immunosuppressive therapy	C Vaccinate susceptible HIV+ patients if possible (see text)
Yellow fever	Live	Stay in endemic areas, travel requirements in some countries	C Vaccination only by authorized institutions

[1] Use combination vaccines, if available (exception MMR+Varicella, see above)
[2] Indications mainly adapted to German standards. Strategies in other countries may vary.
[3] A, recommended; B, usable independent of immune status; C, usable dependent on immune status; D, contraindicated for HIV+ patients
[4] Susceptible: No documented history of disease or vaccination, no specific anti-bodies in serological testing
[5] Live vaccines (e.g. Imojev®) available in Australia and parts of Asia not recommended for HIV+ patients

Table 2: Post-exposure vaccines and chemoprophylaxis in HIV+ individuals.

Disease	Type of prophylaxis[1]	Indication	Comments
Diphtheria	VAC CH	Close contact (face-to-face) with diphtheria patient VAC: if last vacc. >5 y CH: independent of prior vaccinations	CH: oral macrolide x 7–10 d
***Haemophilus influenzae* b**	CH	Immunocompromised patients or their contacts after close contact with patient with invasive infection	Rifampicin 600 mg qd x 4 d (alternative: ciprofloxacin)
Hepatitis A	VAC IG	Exposure of susceptible individuals[2] Within 14 (maybe even 28) days after exposure	Immunocompromised patients: IG might be more effective than VAC (Victor 2007), HIV: IG recommended in USA (CDC 2007), VAC/IG simultaneously recomm. in GB (Geretti 2008)
Hepatitis B	VAC IG[3]	Depending on susceptibility and vaccination status	German recommendations: STIKO 2014

Table 2 (continued)

Disease	Type of prophylaxis[1]	Indication	Comments
Influenza	VAC CH	VAC: contact or outbreak with strain covered by vaccine CH: exposure of unvaccinated or insufficiently protected HIV patients	CH (Influenza A or B): Oseltamivir (Tamiflu®) 75 mg qd x 10 d Alternative: Zanamivir (Relenza®) 10 mg qd x 10 d
Measles	VAC IG	IG: exposure of HIV patient (independent of vaccine history or serology) VAC: Exposure of a susceptible individual[2] without immuno-suppression	IG: within 6 d after exposure (consider active vaccination 6 months later) VAC: within 72 h after start of exposure, if later: IG Never VAC + IG simultaneously!
Meningococcal	VAC CH	Following an index case: VAC: according to health authorities CH: all household members; persons in contact with oropharyngeal secretions; close contacts in child-care centers, dormitories etc.	CH: if possible, within 24 h, up to 14 d (index case contagious 7 d before onset of symptoms) Rifampicin 600 mg bid x 2 d or ciprofloxacin 500 mg once or ceftriaxone 250 mg i.m. once
Mumps	VAC	Exposure of susceptible individuals[2]	Within 3 (–5) d of exposure Consider contraindications
Pertussis	VAC CH	VAC: exposure and incomplete immunization CH: close contacts, e.g., household contacts	CH: within 7 d of exposure Oral macrolides, e.g., clarithromycin 500 mg bid x 7 d
Polio	VAC	Any exposure independent of immunization status	Avoid any delays!
Rabies	VAC IG[3] (simultaneous)	Depending on vaccination status, exposure, and national guidelines (German recommendations: STIKO 2014)	HIV patients: VAC: consider double dose on day 0 IG: if CD4 <400/µl use more liberally (even if vaccinated before exposure or minor exposure)
Rubella	VAC	Exposure of susceptible individuals[2]	Within 5 d of exposure Consider contraindications
Tetanus	VAC IG[2] (simultaneous)	Depending on vaccination status, wound, and national guidelines	German recommendations: STIKO 2014
Tuberculosis	CH	HIV patients after close contact with open TB case	Treat as latent TB (see chapter on TB)

Table 2 (continued)

Disease	Type of prophylaxis[1]	Indication	Comments
Varicella	VAC IG[3] CH	IG/CH: exposure[4] of susceptible immuno-compromised individual VAC: exposure[4] of susceptible individual[2] without immunosuppression	HIV: data lacking IG: availability limited CH: alternative to IG; in high risk situations also together with IG (e.g., acyclovir 800 mg qid x 5 d) VAC: within 3 (–5?) d after exposure, not together with IG or CH

h, hours; d, days; y, years; qd, once daily; bid, twice daily; qid, four times daily

[1] VAC, vaccination (active immunization); IG, immunoglobulin (passive immunization); CH, chemoprophylaxis

[2] Susceptible: No documented history of disease or vaccination, no specific anti-bodies in serological testing

[3] Specific hyperimmunoglobulin might be available in some countries

[4] Chickenpox exposure: >1 h in the same room, face-to-face contact, household contact; herpes zoster exposure: direct contact with skin lesions or their secretions, but indication for immunoprophylaxis under debate (data lacking)

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17. Traveling with HIV

THOMAS WEITZEL

About 10-15% of European and North American HIV+ patients travel abroad at least once yearly. Such travel activities frequently include visits to tropical and developing countries (Salit 2005). Many of those have a migration background (Sherrard 2009) and belong to the group of travellers visiting friends and relatives (VFRs), who have a high risk of travel-associated infections (recent reviews and guidelines: Igreja 2008, Franco-Paredes 2009, Nelson 2011, Smith 2012).

Travel preparations

Especially if CD4 T counts are below 200/µl, there is an increased risk of travel-associated infections and furthermore, the effectiveness of vaccinations is reduced. Trips should be planned at least 6 to 8 weeks in advance. In case of special health risks (e.g., tropical destinations), a travel medicine specialist should be consulted. For a general overview of travel recommendations, see the links below. Long-term travelers should clarify the treatment possibilities of HIV-related problems at their destination. A first-aid kit should contain local antihistamines, disinfectants, sun protection, analgesics, antipyretics, antiemetics, antidiarrheals and an antibiotic for the empirical treatment of traveler's diarrhea (see below).

Antiretroviral therapy (ART)

ART naïve patients with CD4 T cell counts <200/µl should postpone travel activities until their immune status has improved. An ongoing ART should be proven to be effective and well-tolerated. Depending on destination, planned activities and compliance problems (Salit 2005), a therapy interruption might be considered. If ART is continued during traveling, the following points are important:
• Pack sufficient amount of ARVs, preferably in the hand luggage
• Check availability of ART at destination beforehand. Consider carrying prescriptions and a medical letter in English.
• Pack ARVs in neutral packages if necessary (see entry regulations below)
• Check storage requirements for prescription drugs (e.g. refrigeration) in advance.
• Discuss unplanned therapy interruptions during travel in advance.

General precautions

HIV+ travelers should follow the five Golden Rules of travel medicine (cited by Dr. David Smith, Toronto):
• Don't get hit (accidents, crime)
• Don't get bit (mosquitos and other animals)
• Don't get lit (alcohol and other drugs)
• Don't do "it" (casual sex, tattoos, piercings, etc.)
• Don't eat shit (food and water hygiene)
Due to the particular risk of gastrointestinal infections (Hayes 2003), they should avoid the following foodstuff and drinks:
• Raw fruit or vegetables that are not peeled
• Raw or undercooked meat or fish dishes
• Tap water, ice cubes from tap water, unpasteurized milk or milk products
• Food distributed under insecure hygienic circumstances (e.g., street vendors)

Even brushing teeth or swimming carries the risk of swallowing small amounts of potentially contaminated water. High risk patients should use bottled water for brushing teeth. If no safe drinking water is available, tap water should be boiled. In areas up to 2000 meters above sea level, a boiling time of one minute kills all potential pathogens; at higher altitudes, the boiling time should be prolonged to three minutes. Chemical treatment and filtration methods are less reliable.

The prevention of vector-borne infections includes:
• Wearing long-sleeves and bright clothes if outdoors.
• Using repellents (e.g., DEET-based with concentrations of 30–50%) on uncovered skin areas (apply sun protection before repellent).
• Avoiding outdoor stays at dawn or night.
• Sleeping in mosquito-safe areas (mosquito nets or air conditioned rooms).
• Treating clothes and mosquito nets with permethrin for additional safety.

Since condoms and lubricants abroad are not always available, a sufficient amount of these products should be brought along to guarantee safe sex during the holiday. Because of possible *Strongyloides stercoralis* infection (see below), direct skin contact to fecally contaminated soil should be avoided. It is wise to wear closed shoes and place a towel underneath when lying on the ground.

Precautions against zoonotic infections such as salmonella or cryptosporidiosis include proper hand washing following animal contact.

Vaccinations

A travel medicine consultation is an opportunity to check and complete routinely recommended immunizations such as tetanus/diphtheria/pertussis, pneumococcal disease, influenza, and hepatitis B vaccinations (see chapter on HIV and Vaccinations). It has to be kept in mind that the southern hemisphere influenza season is from April to September, while in the tropics influenza can occur all year long.

Additional immunizations have to be considered according to destination, duration, and travel style. In general, most travel vaccines are more generously indicated for HIV+ travelers than in healthy travelers. This affects for example the parenteral typhoid fever vaccine (since *S. typhi* infections in HIV+ patients are more severe and relapse more often) or the pre-exposure rabies vaccination (Chadwick 2007). According to US American recommendations, immunocompromized travelers requiring hepatitis A vaccination shortly before departure (<14 days) should receive passive immunization (ACIP 2007). Other immunization questions usually require the consultation of a specialized travel medicine institution.

Malaria prophylaxis

Interactions between antiretroviral drugs and drugs for malaria prophylaxis such as chloroquine, mefloquine, doxycycline, and Malarone® (atovaquone/ proguanil) are not completely evaluated (Khoo 2005). A recent review (Skinner-Adams 2008) as well as internet-based databases (e.g., www.hiv-druginteractions.org) provide comprehensive information. Here a short summary:
• Chloroquine: Nowadays rarely used for prophylaxis. Potential interaction with ritonavir, dose adjustment, however, is not necessary.
• Mefloquine: Potential interactions with PIs, which might increase mefloquine levels and risk of side effects, although this was not confirmed in a study with ritonavir. Relevant interactions with other classes of ARVs are unlikely (although not studied). A recent study raised concerns discovering an increased viremia and mother-to-child transmission rates in mothers receiving mefloquine intermittent treatment together with different ART regimens (González 2014).

- Atovaquone/proguanil: Interactions with PIs and NNRTIs (efavirenz, nevirapin, etravirin) might reduce atovaquone levels. A reduction of the proguanil level induced by ritonavir, lopinavir or efavirenz is possible, although the clinical relevance is unclear. It is important to urge a proper intake of Malarone® (with a high-fat meal) and to be aware of the possibility of prophylaxis failures.
- Doxycycline: is not metabolized by the cytochrome P450 system. Relevant interactions are unlikely, which was confirmed by a current study (Abgrall 2013).

Available data and clinical experience indicate that chloroquine, Malarone®, and doxycycline can be safely and effectively used in patients taking antiretroviral therapy. Mefloquine with its potential of interactions (especially when taken with PIs) and its contraindication in patients with neurological comorbidity, might be considered as a second line option. The antiplasmodial effects of cotrimoxazole and PIs are not sufficient for malaria prophylaxis.

The German speaking countries (Germany, Switzerland, and Austria) recommend standby emergency treatment (SBET) for certain travelers to areas with low malaria risk (www.dtg.org). Drugs used for this indication depend on the resistance situation in the visited region and include chloroquine, Malarone®, and artemether/lumefantrine (Riamet®). The latter belongs to the ACT drugs (artemisinin combination therapy), which are considered first line treatment for uncomplicated malaria in many countries. Data on interactions of these drug combinations with ART are hardly known and conflicting (van Geertruyden 2014). Co-administration with PIs might lead to increased toxicity, but also showed a synergistic antimalarial effect, which might be a benefit for inhabitants of hyperendemic regions (Achan 2012). Studies involving efavirenz showed a significant reduction of lumefantrine leading to a recommended dose modification of Riamet® (Maganda 2015) and potential hepatotoxic effects if coadministrated with amodiaquine/artesunate, another ACT (German 2007). Therefore, if SBET is used in patients taking PIs or efavirenz, Malarone® might be preferred.

Entry regulations and travel insurance

Entry restrictions for HIV+ travelers violate internationally recognized basic human rights; furthermore, they are counterproductive as a measure of health policy and explicitly rejected by WHO and UNAIDS. In January 2010, the much criticized restrictions of HIV+ travelers to the US were finally lifted. However, many countries continue to refuse entry.

To avoid problems, information on entry regulations should be obtained beforehand. The brochure "Schnellfinder" (www.aidshilfe.de), which is available in various languages, provides a comprehensive overview on entry policies. In cooperation with the European AIDS Treatment Group, a regularly updated English version is available online at www.hivtravel.org. Travel insurance usually excludes existing illnesses and often refuses HIV+ individuals explicitly. Still, few travel insurances do not follow that policy (e.g., World First).

Special risks

Enteric infections

Reduced immunological defense and diminished gastric acid production increase the risk for gastrointestinal infections in HIV+ patients; in addition, MSM have a higher exposure risk to intestinal pathogens including parasites (Abdolrasouli 2009). Furthermore, bacterial enteropathogens such as *Salmonella*, *Shigella*, and *Campylobacter* bear a high risk of bacteremia and relapse (Angulo 1995). Infections by

intestinal coccidia (*Cryptosporidium, Cyclospora, Cystoisospora*) and microsporidia are dangerous due to their chronicity.

Prophylactic use of antibiotics, while it can reduce the prevalence of travel-associated diarrhea, is not generally recommended in HIV. In individual situations, e.g. HIV+ patients with advanced immunodeficiency traveling under high risk conditions for gastrointestinal infections, prophylaxis with ciprofloxacin (500 mg per day) should be considered. In regions with high rates of quinolone resistance such as Southeast Asia, azithromycin should be used.

Instead of prophylaxis, empirical self-treatment of acute episodes of traveler's diarrhea is usually preferred using ciprofloxacin (500 mg bid) or azithromycin (400 mg qd) for 3–5 days.

Malaria

The interactions between HIV and malaria are alarming, especially in endemic areas (Flateau 2011). In HIV+ patients, malaria episodes are more frequent and more severe (Laufer 2006, Cohen 2005). HIV infection and low CD4 T-cell counts are risk factors for malaria treatment failure (Shah 2006). Also, malaria leads to a long-term increase in HIV replication through proinflammatory cytokines (Kublin 2005) and might be associated with decreasing CD4 T cell counts (Mermin 2006). On the other hand, HIV-associated cotrimoxazole prophylaxis and ARVs might reduce the morbidity of malaria in some regions (van Geertruyden 2014).

Up to now, recommendations for malaria therapy are not influenced by a concomitant HIV infection with the exception of the proposed prolongation of therapy duration with atovaquone/proguanil if co-administered with efavirenz (Maganda 2015). Still, it has to be kept in mind that drug interactions of antimalarial and HIV drugs are insufficiently established. The treatment of complicated malaria is especially problematic since quinine, quinidine or artemisinin derivatives are metabolized by CYP3A4. The co-administration of these drugs with CYP3A4 inhibitors in patients with severe malaria requires intensive monitoring, drug level monitoring (if possible) or an interruption of ART.

Measles

In 2002, more than 200 million annual cases of measles with about 600,000 deaths were reported by WHO. In HIV+ patients, measles have a higher morbidity and mortality. The virus is shed for prolonged periods of time (Moss 2002) which is especially problematic in Africa (Moss 2006). American studies show a mortality rate of 40%, mostly due to giant-cell pneumonitis (Kaplan 1996). Non-immune HIV+ patients should receive active or passive immunization before traveling to areas with a high prevalence of measles (see chapter on *HIV and Vaccinations*).

Leishmaniasis

Visceral leishmaniasis (kala azar) is a life-threatening opportunistic infection with limited therapeutic options (see chapter on AIDS). In German travelers, most infections are acquired in Mediterranean countries. The infection is more frequent in HIV+ long-term travelers (Harms 2003, Weitzel 2005). Due to the infection's potentially extended latency period, symptoms can occur long after exposure in endemic areas. Diagnosis is challenging, requiring cooperation with a specialized center. Severely immunocompromised HIV+ patients must be informed of the risk of leishmaniasis even when traveling to Mediterranean countries. Preventive measures against mosquito bites should be followed (see above); because of the vector's small size, the use of impregnated mosquito nets of small mesh size is advisable. Cutaneous leishmaniasis does not seem to occur more frequently.

Tuberculosis

Globally, tuberculosis is the most prevalent HIV-associated opportunistic infection (see chapter on Tuberculosis). In most tropical and subtropical regions, the risk of tuberculosis is higher than in Europe. Before and after long-term travel to such areas, it is advisable to determine the TB reactivity by interferon-gamma release assay (IGRA) or PPD skin test (Rieder 2001). Patients with a positive reaction or with a known high-risk exposure and no further signs of active tuberculosis should receive a course of treatment for latent tuberculosis (see chapter on Tuberculosis). HIV+ travelers should avoid risk areas such as hospitals, prisons or homeless shelters or wear adequate facemasks.

Endemic mycoses

Endemic mycoses outside endemic areas are rare. Nevertheless, they are able to cause life-threatening opportunistic infections in HIV+ patients even years after a stay in an endemic area. Most agents of endemic mycoses are thought to enter the pulmonary tract after inhalation of infective spores. In areas endemic for *Penicillium marneffei* (South East Asia, Southern China) and *Coccidioides immitis* (south-west parts of the USA, parts of Central and South America), increased exposure to dust or soil should be avoided (e.g. construction sites, agriculture, garden work, excavations). *Histoplasma capsulatum* is prevalent worldwide in soil contaminated with bird and bat droppings. Exposure might happen during eco- or adventure-tourism and should be avoided. In certain cases, e.g., severely immunocompromised patients with a foreseeable contact with agents of endemic mycoses, primary prophylaxis can be considered. Depending on the expected pathogen, either fluconazole or itraconazole should be prescribed. Another fungus that can cause severe infections is *Sporothrix schenkii.* This pathogen, which occurs worldwide, enters the body through cutaneous lesions. Wearing gloves while working with plants, hay, or peat moss can reduce the sporotrichosis risk.

Sexually transmitted diseases

Traveling is associated with more frequent sexual encounters and less frequent use of condoms (Matteelli 2001). The risk of STDs is substantially increased (Richens 2006). Patients should be aware of this.

Other parasites

The following parasitic pathogens are relevant to HIV+ travelers:

- *Strongyloides stercoralis* is prevalent in most tropical and subtropical areas. The parasite is transmitted by cutaneous larval invasion after skin contact with contaminated soil. In HIV+ patients, there is the risk of a "hyperinfection syndrome" with a high fatality rate (Gompels 1991). Corticosteroids seems to be an important risk factor, as they may increase larval maturation triggering a cycle of massive autoinfection.
- *Trypanosoma cruzi* is endemic in large parts of Latin America. The protozoon that causes Chagas disease is transmitted by triatomine bugs but oral transmissions via contaminated fruit or sugarcane juice have also been reported. Chagas disease can persist asymptomatically for many years and reactivate in severely immunocompromised patients. In these cases, lesions radiologically resembling cerebral toxoplasmosis are found in the central nervous system (Rocha 1994).
- *Babesia sp*, a worldwide cause of zoonotic infections, is transmitted by ticks. Severe infections, clinically mimicking malaria or manifesting as fever of unknown origin, mainly occur in patients after splenectomy, but have also been reported in severely immunocompromised patients (Falagas 1996).

- Free-living amoeba (*Acanthamoeba sp.* and *Balamuthia mandrillaris*) are ubiquitous, living in soil and water. In immunocompromised patients, these organisms are capable of causing severe infections of the central nervous system (granulomatous encephalitis) as well as local infections of the skin and cornea (Sison 1995).
- *Schistosoma sp.* cause long-lasting and dangerous helminthic infections. In HIV+ patients, schistosomiasis treatment is less effective (Kallestrup 2006). The chronic stimulation of the immune system has a negative influence on HIV infection (Secor 2006). HIV+ travelers should avoid freshwater contact in endemic areas.

Medical problems after traveling

Every disease occurring during or after traveling should be checked in a timely manner. Because most tropical diseases are quite rare in temperate countries, diagnosis is often delayed. An analysis of imported visceral leishmaniasis in Germany revealed a median time span of 85 days until the diagnosis was established (Weitzel 2005). Furthermore, tropical diseases often manifest atypically (Karp 1999). In any event, differential diagnoses of diseases are very broad. After traveling abroad the clinical and diagnostic situation can become even more complex, calling for a close cooperation of HIV and Tropical Medicine specialists.

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Links

http://www.cdc.gov/travel/

http://www.who.int/ith/

http://www.tropenmedicus.de/

http://www.crm.de/

http://dtg.org/

18. HIV-2 Infection

DIRK BERZOW

HIV-2 infection is less prevalent than HIV-1. An estimated 1 to 2 million people worldwide are infected with HIV-2, most of them living in West Africa. As a consequence, data on clinical monitoring and treatment is limited.

Introduction

In 1986, Luc Montagnier and colleagues reported the isolation of a novel retrovirus, the human immune deficiency virus type 2 (HIV-2, previously LAV-2), from AIDS patients originating from West Africa (Guinea-Bissau, Cape Verde Islands). Hybridization experiments indicated substantial differences between the genomes of HIV-1 and HIV-2. Serological cross-reactivity was restricted to the major core protein, as the envelope glycoproteins of HIV-2 are not immunoprecipitated by HIV-1-positive sera (Clavel 1986).

HIV-2 bears all the hallmarks of a lentivirus but is more closely related to simian immunodeficiency viruses (SIV) than HIV-1, despite a similar biology (Clavel 1986, Kanki 1986). Whereas HIV-1 in humans resulted from at least four cross-species transmissions of SIVs from chimpanzees and gorillas in West Central Africa, HIV-2 resulted from at least nine independent transmissions of SIVs infecting sooty mangabeys in West Africa only There are at least nine different HIV-2 subtypes. The most prevalent HIV-2 subtype is A (Senegal, Gambia, Guinea-Bissau, Cape Verde Islands, Ghana, Ivory Coast), followed by B (Ghana, Ivory Coast). Others subtypes (C-I) are rarely seen and are seemingly dead-end transmissions (Peeters 2014).

A reduced rate of clinical progression indicates that HIV-2 has a reduced virulence compared to HIV-1 (Marlink 1994). However, there are several diagnostic, clinical and therapeutic challenges with HIV-2 infection to be discussed in this chapter.

Epidemiology

HIV-2 infection is endemic in West Africa. An estimated 1 to 2 million people in this region are infected with HIV-2. The proportion of HIV-2 among new HIV infections worldwide is estimated to be 0.3–1%. However, in recent years HIV-2 prevalence has declined markedly. The lower transmission rates of HIV-2 are probably due to its lower viremia in infected individuals. For example, in a rural area like Guinea-Bissau, a region with one of the highest numbers of HIV-2 infections worldwide, prevalence declined from 8.3% in 1990 to 4.7% in 2000. During the same period HIV-1 prevalence increased from 0.5% to 3.6% (van Tienen 2010). In Gambia, prevalence declined from 7.0% in 1988–1991 to 4.0% in 2001–2003 (Nyamweya 2013).

HIV-2 infection has also been reported in countries with historical and socio-economic ties to West Africa, among them mainly Portugal (2008: 1813 cumulative cases), but also France (2008: 572 cases in the ANRS cohort, 2% of all new infections), Spain (2013: 297 cases), Great Britain (2010: 137 cases), USA, India and Korea (Carvalho 2010, Drylewicz 2008, de Mendoza 2014, Gilleece 2010).

Diagnosis

Generally, Western Blot analysis leads to definite discrimination between an HIV-1 or HIV-2 infection (see Chapter *Test*). However, it may be difficult to distinguish between mono- and dual infection. Due to the close relationship cross-reactivity

leading to antibody reactions against both virus types can occur. In those cases, type specific PCR assays may help. There are no commercial PCR tests available, but some labs offer in-house tests. Viremia levels are usually lower than those seen with HIV-1 (see below), the detection limit is usually 100 copies/ml.

HIV-2 testing is strongly recommended in all patients (especially those from West African countries), showing HIV-associated or AIDS-defining illnesses (and/or low CD4 T cells) in the presence of low or undetectable HIV-1 viremia and/or an indeterminate or nonreactive HIV-1 Western Blot.

Natural Course

In general, there are no differences between HIV-2 and HIV-1 with regard to the clinical manifestation. If left untreated, HIV-2-infected patients with low CD4 T cells develop illnesses similar to those seen in HIV-1-infected patients. Almost all AIDS-defining infections and malignancies have been seen in HIV-2-infected patients. Given the endemic setting of HIV-2, some AIDS events such as TB, wasting syndrome and chronic diarrhea may be seen more frequently (Markovitz 1994, Ndour 2000, Matheron 2003). As HIV-1, HIV-2 is a neurotropic virus and can be isolated from cerebrospinal fluid in some patients (Arvidson 2004).

A reduced rate of clinical progression indicates that HIV-2 has a reduced virulence compared to HIV-1. The asymptomatic incubation period after infection with HIV-2 appears to be substantially longer. In a prospective clinical study on HIV+ woman from 1985 to 1993, HIV-1-infected women had a 67% probability of AIDS-free survival 5 years after seroconversion in contrast with 100% for HIV-2-infected women (Marlink 1994). So-called long-term non-progressors (LTNPs) and elite controllers (undetectable viral load in the absence of ARVs) are seen much more frequently than in HIV-1-infected patients (Marlink 1994, Hansmann 2005). Among 342 HIV-2-infected patients of a French HIV-2 cohort, the prevalence of LTNPs (i.e., asymptomatic for at least 8 years while maintaining CD4 T cell counts of at least 500 cells/µl) and of elite controllers (controlling HIV replication without ART for at least 10 years) were 6.1% (95% confidence interval 3.9-9.1) and 9.1% (95% CI 6.3–12.7), respectively. Most LTNPs (81%) were elite controllers, whereas only 55% of HIV controllers were LTNPs (Thiebault 2011).

HIV-2 viremia levels are much lower than those of HIV-1. In several studies, the median viral load was 30-100 fold lower, irrespective of the length of time infected or disease stage (Andersson 2000, Popper 2000, Hansmann 2005). A high viral load can already be considered when the HIV-2 RNA copy number is above 1,000/ml (Gilleece 2010). In total, mortality in patients with an undetectable viremia (<100 copies/ml) seems to be similar to that of the general population (van der Loeff 2010). However, there are also some patients showing clinical progression with low viremia or even with an absence of detectable viremia (Soares 2011, Hegedus 2014), implicating a dichotomy between amount of plasma virus and cell-associated viral burden. In general, CD4 T cells in HIV-2 infected patients are higher than in patients with HIV-1. Clinical progression mainly depends on plasma viremia and can be seen even at high CD4 stages (Sousa 2002, Hansmann 2005, van der Loeff 2010, Hegedus 2014). The main transmission routes of HIV-2 are sexual contacts, needle sharing, perinatal infections or blood products. The heterosexual spread of HIV-2 is significantly slower (3-9 fold) than that of HIV-1, which strongly suggests differences in the viruses' infectivity potential (Marlink 1994, Gilbert 2003). This may be due to the fact that HIV-2 levels are lower not only in plasma but also in the semen and in the female genital tract (Gottlieb 2006, Raugi 2013). In addition, the rates of mother-to-child transmission (MCT) of HIV-2 is lower compared to HIV-1. In a study from The Gambia

that enrolled 144 pregnant women positive for HIV-1 and 294 for HIV-2, the esti-
mated transmission rate of HIV-1 was 24.4% and that of HIV-2 was 4.0% (Ota 2000,
O'Donovan 2000). In a French cohort of 223 pregnant women, the mother-to-child
transmission rate for HIV-2 was only 0.6% although many women remained
untreated during pregnancy and delivered vaginally (Burgard 2010).

Pathogenesis

Is HIV-2 a model for a possible host control of HIV infection? If so, this would be
due to two main factors: 1. A better immune response of the host and/or 2. A lower
intrinsic pathogenetic potential of HIV-2. Current data suggest that both factors are
involved. Several differences between HIV-2 and HIV-1 infection are evident:
• Viral replication (plasma RNA, intracellular mRNA)
• Level of immune activation
• Response of the adaptive immune system (T cells and neutralizing antibodies),
• Activity of the *innate immunity*
• Activity and functions of viral proteins
In a French cohort of untreated patients (320 cases with HIV-1 and 160 with HIV-
2), CD4 T cell counts decreased less rapidly in HIV-2 than in HIV-1 patients. The
decline was -9 versus -49 cells/µl per year (Drylewicz 2008). The rate of decline cor-
relates with the level of immune activation (Sousa 2001, Soares 2011) and with the
level of plasma viremia (Gottlieb 2002).
The immune response to HIV-2 appears more protective against disease progression
suggesting that pivotal immune factors limit viral pathology (review: Nyamweya
2013).
Polyfunctional HIV-specific CD4 T cell responses are a hallmark of non-progressive
HIV-2 infection and may be related to good clinical outcome in this setting (Duvall
2006). HIV-2 viral control is also significantly associated with a greater CD8 T cell
receptor heterogeneity and functional flexibility (Lopes 2003) and strong CD8 gag
responses (Ledigdowicz 2010).
Immune activation is central to the pathogenesis of HIV. The lower decline of CD4
T cells in HIV-2-infected patients is associated with lower levels of immune activa-
tion, evaluated by HLA-DR expression on lymphocytes and sera concentrations of
IgG and beta2-microglobulin. *Ex vivo* apoptosis in all lymphocyte subsets, including
CD4 and CD8 T cells as well as B cells, is lower in HIV-2 than in HIV-1 infection
(Michel 2000, Jaffar 2005 Ledigdowicz 2010). HIV-2 non-progressors have minimal
immune activation; high viral load HIV-2 progressors have higher immune activa-
tion levels, similar to or exceeding those in HIV-1 infection (Hegedus 2014). In addi-
tion, programmed death (PD)-1/PD-L1 molecules, rather than markers of T cell
exhaustion, may act as modulators of T cell immune activation, contributing to the
slower course of HIV-2 infection (Tendeiro 2012).
In most studies, a considerable proportion (up to 40–50% of HIV-2 infection is
aviremic (which typically means a viral load of below 100 copies/ml). In many of
these patients, however, plasma RNA is detectable by sensitive assays such as quali-
tative RT PCR. HIV-2 plasma RNA is 30-fold lower than HIV-1. In contrast, the median
proviral HIV-2 DNA is 200 copies/10^5 PBMC and similar to those with HIV-1 (Popper
2000, Gottlieb 2002, Matheron 2003). Difference in the pathogenicity of HIV-1 and
HIV-2 may be explained by differences in viral replication, mainly at late stages after
integration and before late transcription (Soares 2012). Despite the lower levels of
plasma RNA, HIV-2 is able to establish a stable latent infection *in vivo* (MacNeil 2007).
Patients with higher viral load (>1000 copies/ml) show marked differences compared
to patients with low viral load (<100 copies/ml). The latter are mainly non-progres-

sors, with low CD4 and CD8 T cell turnover in memory cells, minimal immune activation and a minimal impairment of thymus function (Hegedus 2014). Highly viremic patients display no differences compared to HIV-1-infected patients.

Humoral responses in HIV-2 infection appear broader intratype neutralization responses. There is no cross-reactivity between HIV-1 and HIV-2 (Rodriguez 2007). HIV-2 isolates appear to have two mechanisms of immune evasion that are diminished in effectiveness relative to HIV-1: glycan shielding and conformational masking (Kong 2012). Potency and breadth of neutralizing antibodies decrease as the disease progresses. Resistance to antibody neutralization occurs in late stage disease and is usually associated with X4 viral tropism and major changes in V3 sequence and conformation (Marcelino 2012).

Natural killer cell function is well preserved in asymptomatic HIV-2 infection but similar to that of HIV-1 infection when CD4 T cell counts fall (Nuvor 2006). There are also differences with regard to restriction factors such as TRIM5, SAMHD-1 or the APOBEC3F/3G family of deaminase enzymes (Ylinen 2005, Nyamweya 2013, Bertine 2015).

Viral factors

Viral evolution occurs slowly in HIV-2 infection, which is consistent with the slow disease progression of HIV-2 and supports the notion that viral evolution may be a relevant correlate for disease progression. Longitudinal studies have shown a remarkable stability of *env*-C2V3 sequences over many years (MacNeil 2007). Accumulation of viral mRNA is attenuated in HIV-2 infection relative to that in HIV-1 infection. The differences in viral mRNA are consistent with the differences in plasma viral loads between HIV-1 and HIV-2 and suggest that lower plasma viral loads, and possibly the attenuated pathogenesis of HIV-2, can be explained by lower rates of viral replication *in vivo*. Changes in the genome of HIV-2 may have higher consequences on replicative fitness (MacNeil 2007).

The multifunctional accessory *Nef* protein may play an important role in the immunopathogenesis of HIV-2 infection. *Nef* proteins are able to downregulate the T cell receptor (TCR)-CD3 complex of the infected cell, thereby reducing the potential for deleterious activation. This *Nef*-mediated downmodulation is higher in HIV-2 infection and may help viremic HIV-2-infected individuals maintain normal CD4 T cell homeostasis by preventing T cell activation and by suppressing the induction of death receptors that may affect the functionality and survival of both virally infected and uninfected bystander cells (Khalid 2012).

Dual infection with HIV-1 and HIV-2

Dual infection with HIV-1 and HIV-2 was first confirmed in 1988, but difficulties in distinguishing between dual seropositivity and dual infection have hampered efforts to estimate the prevalence of this phenomenon. As a consequence, studies of clinical progression and outcomes are scarce (Raugi 2013). In a systematic review and meta-analysis, patients with dual infection had a similar mortality compared to HIV-1-infected patients which was higher than those of HIV-2-monoinfected persons. There was no evidence that HIV-2 delays progression to death in dually infected individuals (Prince 2014). In a cohort study from Senegal, after adjusting for CD4 T cell count, age and sex, HIV-1 RNA levels were significantly higher than HIV-2 levels in semen, cervicovaginal lavage, and oral fluids. Results suggest that with disease progression, HIV-1 outcompetes HIV-2 in dually infected individuals (Raugi 2013).

Antiretroviral therapy

Several therapeutic peculiarities have to be considered in patients with HIV-2 infection:
- some ARVs are intrinsically ineffective: all available NNRTIs, many PIs (nelfinavir, ritonavir, indinavir, fosamprenavir, atazanavir, tipranavir) as well as the fusion inhibitor T-20
- there are several polymorphisms in the genes of reverse transcriptase, protease and integrase, many at regions which are associated with resistance in HIV-1 infection
- resistance occurs more rapidly, even in the setting of undetectable viremia, and may show other resistance pathways (NRTIs, PIs)
- immune reconstitution with ART is slower compared to HIV-1 infection
- there is a lack of evidence for recommendations for initiation and modification of ART

Antiviral drugs effective against HIV-2 are all available NRTIs (and foscarnet), some PIs such as saquinavir, lopinavir, and darunavir. The three integrase inhibitors raltegravir, elvitegravir and dolutegravir are also effective, as is the CCR5-antagonist maraviroc in patients with R5 tropism. Data on antiretroviral therapy in HIV-2 infected patients is very limited (reviews: Camacho 2012, Ekouevi 2014, Menéndez-Arias 2014) and is based mainly on small and uncontrolled studies (Desbois 2008, Jallow 2009, Benard 2011, Charpentier 2011, Trevino 2011, Camacho 2012, Menéndez-Arias 2013). There are no randomized trials.

In ARCHIV2E, an observational study on 44 patients starting triple NRTI therapy (73% ABC+3TC+AZT) and 126 patients starting PI/r-based regimens (61% lopinavir/r), PI/r-containing regimens showed superior efficacy over triple NRTI regimens as first-line therapy. More patients achieved an undetectable viral load and immune reconstitution was better with PIs (Benard 2011).

The validity of HIV-2 plasma viral load as a control for treatment success is more limited compared to HIV-1. Viremia is lower and the decline is less impressive during ART (Drylewicz 2008, Camacho 2012). Viral replication, clinical progression and even resistance development may be seen in patients with low or even undetectable viremia (Popper 2000, Soares 2011). In addition, immune reconstitution is slower and less impressive with ART (Matheron 2006, Drylewicz 2008).

Resistance

Mutational pathways may differ from those seen in HIV-1 infection. No commercial resistance tests are available. Recently, some efforts have been made regarding standardized HIV-2 drug resistance interpretation (Charpentier 2015). Some authors recommend resistance testing prior to initiation of ART. Prevalence of transmitted resistance mutations have been reported to be between 3 and 6% (Charpentier 2013). According to EACS, resistance testing should be considered after treatment failure (Vandamme 2011). In the event of virologic, immunologic, or clinical failure, second-line treatment should be instituted in consultation with an expert in HIV-2 management.

NNRTIs: HIV-2 is intrinsically resistant to NNRTIs. This may be due to polymorphisms in the RT gene at the position 181 and 188. Resistance applies also to newer NNRTIs such as etravirine and rilpivirine which only showed minimal activity *in vitro*.

NRTIs: Several polymorphisms for HIV-2 have been described, among them T69N, V75I, V118I, L210N, T215S, K219E (Benard 2011, Camacho 2012, Menéndez-Arias 2014). In many cases, resistance is based on steric inhibition and Q151M (50%), K65R (13%) and M184I/V (25%) are the most frequently seen resistance-associated mutations. These RAMs occur more rapidly in HIV-2 infection and the genetic barrier seems to be lower. Thymidine-associated mutations (S215Y/F) are rarely selected and have been described only in a few patients (Jallow 2009, Ntemgwa 2009, Gilleece 2010, Trevino 2011, Menéndez-Arias 2014, Charpentier 2015).

PIs: Lopinavir, darunavir and saquinavir show *in vitro* similar efficacy in HIV-2 and HIV-1 infection (Desbois 2008). These agents should be preferably used in HIV-2 infection. However, as with NRTIs, resistance develops more rapidly (Ntemgwa 2007, Camacho 2012) mainly due to preexistent polymorphisms in HIV-2 protease (Raugi 2013, Menendez-Arias 2013). In contrast to HIV-1, even one or two RAMs seem to induce moderate or high resistance (Raugi 2013). This applies for V47A (lopinavir), I54M (lopinavir and darunavir) and L90M (saquinavir) (Menéndez-Arias 2013).

Common protease substitutions are V47A, I54M, I82F, L90M, L99F, V47A+L90M, V47A+L99F, I54M+L90M+L99F, I54M+I82F+L90M (Trevino 2011). The RAM combination I54M+I84V+L90M induces high resistance to lopinavir, darunavir und saquinavir. The HIV-2EU group associates G48V, I84V and L90M with resistance to saquinavir, V47A, I54M, I82F, I84V, L90M (and V62A+L99F) with resistance to lopinavir, and I50V, I54M, I84V and L90M with resistance to darunavir (Charpentier 2015). As a decrease of IC50 (hypersusceptibility) has been shown for saquinavir in the presence of V47A, some authors speculate on PI sequencing (Camacho 2012), i.e., first-line lopinavir, then second-line saquinavir.

INSTIs: the genetic pathways to integrase inhibitor resistance in HIV-2 and the extent of cross-resistance between different INSTIs seem to be similar to HIV-1 (Charpentier 2011). There is cross-resistance between raltegravir and elvitegravir. Both agents seem to have a low genetic resistance barrier. The main RAMs are N155H/R, Q148K/R, E92Q+T97A, Y143C/G/R+E92Q and Y143C/G/R+T97A for raltegravir, E92G/Q, Q148K/R, N155H and T97A+Y143C for elvitegravir. Dolutegravir was used in patients with resistance to first-line integrase inhibitors (Descamps 2015). Mutations associated with resistance to dolutegravir refer to Q148K, G140S+Q148R, E92Q+N155H, and T97A+N155H (Charpentier 2015).

CCR5 antagonists: HIV-2 tropism was assessed in 83 antiretroviral-experienced patients with virological failure. Tropism was predicted as X4 in 58% of patients and was associated with a CD4 cell count of less than 100 cells/µl, and with a higher number of drug resistance mutations. This high prevalence of X4 virus might compromise the use of CCR5 inhibitors (Visseaux 2012). There are some anecdotal reports that maraviroc is effective in HIV-2 with R5 tropism (Menéndez-Arias 2013, Descamps 2015).

Guidelines

In the current US DHHS guidelines (2015), a first-line therapy with a boosted PI/r (lopinavir/r, darunavir/r or saquinavir/r) plus 2 NRTIs is preferred. INSTI-based regimens are alternatives. Triple NRTIs, an option in the WHO Guidelines 2013, are recommended if lopinavir/r is not available. Monitoring of HIV-2 RNA levels, CD4 T cell counts, and clinical improvements can be used to assess treatment response, as is recommended for HIV-1 infection. Although the optimal CD4 T cell count threshold for initiating ART in HIV-2 infection is unknown, therapy should be started before there is clinical progression.

The British Guidelines (2010) recommend treatment initiation when HIV-2 plasma RNA is above 1,000 copies/ml. The preferred first-line regimen is TDF+FTC plus lopinavir/r, alternatives are AZT+3TC and darunavir/r (Gilleece 2010).

There is an uncertainty whether all patients with HIV-2 infection showed receive ART, regardless of their immune status. This applies in particular to elite controllers that are seen in considerable proportions in this setting.

Overall, clinical and immunologic and virologic outcomes in HIV-2 infected individuals treated with ART are suboptimal. There is a need for controlled trials to improve the management and outcomes (Ekouevi 2014).

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Women and Children

19. HIV and Gynecology

RAMONA PAULI

HIV+ women have a higher risk of cervical dysplasia and cervical cancer, genital ulcers, vaginal infections and genital condyloma than negative women. A gynecological examination including a Papanicolaou (Pap) smear and screening for sexually transmitted infections are part of the routine evaluation of female HIV+ patients at the time of first diagnosis as well as during the course of the disease.

Prophylaxis

Guidelines on Pap smear and breast cancer screening for the general population vary from country to country. In general, Pap smear screening starts at age 20 or 25 and continues until about age 50 or 60. Breast cancer screening starts in Germany at age 35. Regular gynecological checkups, including Pap smears, are especially important for HIV+ women because of their higher risk of cervical and anal dysplasia. In contrast, the risk of breast cancer in HIV+ women is not elevated, it seems to be lower than in negative women (Goedert 2006).

Physicians working with HIV+ women should stress the importance of gynecological evaluations. It cannot be taken for granted that all women will visit the gynecologist regularly even when it is covered by health insurance. In Germany for example only 50% of women take advantage of regular Pap smear and breast cancer screening. Therefore it is crucial to talk about the necessity and the reasons for gynecological screening. The frequency of screening depends on the clinical scenario. If the initial Pap smear after HIV diagnosis is normal, then a second screening should be done approximately 6 months later. If both results are normal, then an annual Pap smear is sufficient. Consider more frequent screening in women with a higher risk of cervical dysplasia, e.g., with abnormal Pap smear results, HPV infection, symptomatic HIV infection, CD4 T cell count <200/µl or after treatment for cervical dysplasia.

Table 1: Gynecological/Pap smear screening

Screening frequency	Clinical scenario
Every year	Routine control
6 months	First year of HIV diagnosis, then every year
<6 months	Abnormal Pap smear HPV infection After therapy for cervical dysplasia Symptomatic HIV infection CD4 T cells <200/µl

Basic gynecological evaluation

A full gynecological examination consists of inspection of the external genital and perianal region, bimanual examination of the inner genital area, rectal examination, colposcopy, microscopic examination of vaginal secretions and a Pap smear. In HIV+ women palpation of inguinal and axillary lymph nodes is important, since enlarged lymph nodes are often present and may need a rapid mammographic/ultrasound evaluation. At age 35 a basic mammogram should be performed. Since 2013, German-Austrian guidelines recommend an annual anal cytologic smear for all HIV+ men and women.

Menstrual cycle/menopause

Data on the influence of HIV on the menstrual cycle are conflicting. Older studies demonstrate a cycle prolongation (Shah 1994), whereas the WIHS study shows at most a slight increase of very short cycles (Harlow 2000). It is also unknown whether or not HIV accelerates the beginning of menopause. There is only limited data in small populations (Clark 2000, Greenblatt 2000). In contrast, it is clear that post-menopause as well as HIV infection and antiretroviral treatment have adverse effects on bone, lipid and glucose metabolism and may contribute to osteoporosis and cardiovascular disease.

Contraception

When choosing a contraceptive method be aware of the expectations of the woman. Condoms are the most common form of contraception (and they protect from STIs). Nevertheless their contraceptive effectiveness is comparatively limited. Condoms have a Pearl Index (number of pregnancies per 100 patient years) of 1–12 while contraceptive pills have a Pearl Index of 0.1–0.9. Other methods are contraceptive pills containing varying hormone combinations and dosages, depot and transdermal formulations as well as intrauterine devices (IUD). Hormone-containing contraception has no influence on the course of HIV infection, but this method may increase the risk of transmitting or acquiring HIV (Stringer 2009, Heffron 2012). Intrauterine devices made of copper as well as the levonorgestrel-containing device (Mirena®), which increases cervical mucus viscosity, have proved to be safe and effective in HIV+ women (Stringer 2007, Heikinheimo 2006).

In HIV+ patients on ART, interactions should be taken into account. Oral contraceptives interact with PIs and NNRTIs with almost unpredictable consequences. There are limited reliable studies of such interactions, and these interactions are agent-specific (El-Ibiary 2008, Heikinheimo 2008). The same is true of new parenteral oral contraceptives like the hormone-containing vaginal ring (NuvaRing®), the etonogestrel implant (Implanon®), transdermal hormone patches and emergency contraception and abortion pills. It is essential to inform patients about potential interactions when starting ART. Exceptions are unboosted atazanavir and indinavir, etravirine, maraviroc and raltegravir in combinations without ritonavir.

In an ACTG study depot formulations containing 150 mg medroxyprogesterone acetate (e.g., Depo-Provera®) proved equally safe and reliable with patients on efavirenz, nevirapine or nelfinavir (Watts 2008).

Infections

In the pre-ART era genital infections, especially genital herpes, vulvovaginal candidiasis and bacterial vaginosis, were more common in HIV+ women. The prevalence and severity of these infections correlate with the CD4 T cell count and HIV viral load. Today only vaginal candidiasis seems to be more common, which may be a consequence of a higher rate of antibiotic treatment (Watts 2006). Sexually transmitted infections (STI) are more common as well, though this depends on the sexual activity of the patient.

Bacterial Vaginosis

Bacterial vaginosis (BV) results from replacement of the normal lactobacillus-dominant vaginal flora by mixed flora, including anaerobic bacteria. This increases HIV expression in the genital tract and may promote HIV transmission (Olinger 1999, Cu-Uvin 2001). Persistence and severity of bacterial vaginosis increases with the

progression of immune deficiency and ART lowers the risk of vaginosis (Warren 2001). Most prevalent symptoms of bacterial vaginosis are a thin discharge and a "fishy" odour. In clinical practice BV is diagnosed when three of the following four criteria are present:
• thin, homogeneous discharge
• pH of vaginal fluid >4.5
• Fishy odour on adding Alkali-10% potassium hydroxide solution
• Clue cells on microscopy
Treatments of choice are metronidazole or clindamycin, and a topical application is preferred because of better compliance (DGGG 2008). Clindamycin is contraindicated in pregnant women. Oil-containing clindamycin vaginal cream may erode latex condoms.

Table 2: Therapy of bacterial vaginosis

	Agent	Dose
Therapy of choice	Metronidazole orally Metronidazole gel 0.75% Clindamycin cream* 2%	500 mg BID for 7 days 5 g vaginally QD for 5 days 5 g vaginally QD for 7 days
Alternatives	Clindamycin orally Clindamycin vaginal tablets	300 mg BID for 7 days 100 mg QD for 3 days

*may erode latex condoms, CDC 2015

Genital herpes

In most cases, genital herpes is caused by the human herpes virus type 2 (HSV-2). The virus remains latent in the body after the first infection. Genital herpes increases the risk of HIV infection and transmission (Heng 1994). ART lowers the frequency and severity of symptomatic episodes although there may be asymptomatic viral shedding (CDC 2015).
According to more recent studies reactivation of HSV-2 is associated with higher HIV replication (Rebbaprada 2007). Suppression of HSV-2 lowers HIV viral load in genital secretions and breast milk and has a positive impact on HIV progression (Nagot 2008, Drake 2011, Reynolds 2011). Whether this lowers the risk of HIV transmission is a matter that continues to be investigated. However, the suppressive treatment of HSV-2 does not influence the susceptibility to HIV infection in HSV-2-infected individuals (Celum 2008, Watson-Jones 2008).

Table 3: Therapy of genital herpes in persons infected with HIV (CDC 2015)

	Medication	Dose
Primary infection	Acyclovir Famcyclovir Valacyclovir	400–800 mg orally 2–3 times a day 500 mg orally BID 500 mg orally BID
Recurrent infection	Acyclovir Famcyclovir Valacyclovir	400 mg orally TID for 5–10 days 500 mg orally BID for 5–10 days 1.0 g orally BID for 5–10 days
Severe infection	Acyclovir	5–10 mg/kg IV every 8 hours
Acyclovir resistance	Foscarnet Cidofovir	40 mg/kg IV every 8 hours Topical Cidofovir gel 1%* over 5 days

* not available commercially, must be made up by pharmacist

HSV-2 infections are more common in HIV+ women and episodes are more severe and atypical. Typical lesions present as painful vesicles in groups on red skin, which ulcerate and heal without scarring. Primary infection may also be associated with signs of systemic viral infection like fever, headache, etc.

Diagnosis by clinical signs alone has low specificity and sensitivity (Sen 2007). Therefore, a clinical diagnosis should be confirmed by virologic and serologic tests, preferably by type-specific assays. Diagnosis should distinguish between syphilis and a 'chancroid' condition (*Haemophilus ducreyi*).

Vulvovaginal Candidiasis

Vulvovaginal candidiasis is more common and more persistent but not more severe in HIV+ women (Watts 2006). Low CD4 T cell count promotes the disease, though the more prevalent use of antibiotics and antifungals in immunodeficient patients may play an important role. Causative organisms are *Candida* strains in most cases, with *Candida albicans* being the most prevalent strain, but the incidence of non-albicans strains is rising. Typical clinical symptoms are pruritus, vulvar burning, vaginal soreness and thick white-yellow discharge. Dyspareunia and external dysuria may also be present.

Diagnosis can generally be made on the basis of physical examination and colposcopy. Thrush patches are usually found loosely adhering to the vulva and/or vagina. Bimanual examination is not painful. Budding yeast or pseudohyphae are documented on a wet mount or KOH preparation or gram stain of vaginal discharge. In case of recurrent disease yeast culture is mandatory. In case of dysuria a urine test is recommended.

The treatment of vulvovaginal candidiasis in is not different from negative women. Treatment of choice for uncomplicated acute vulvovaginal candidiasis is a short course of an -azole drug for 1–3 days. Alternatives are triazoles orally, e.g., 150 mg single dose or itraconazole 2 x 200 mg. In patients with advanced immunodeficiency topical treatment may be extended to 7 days. Treatment of the partner is only necessary in case of suspected sexual transmission.

Table 4: Therapy of acute uncomplicated vulvovaginal candidiasis (CDC 2006).

Agent	Dosage
Butoconazole 2% cream	5 g intravaginally QD for 3 days
Butoconazole 2% cream	5 g (Sustained Release) single application
Clotrimazole 1% cream	5 g intravaginally QD for 7–14 days
Clotrimazole 2% cream	5 g intravaginally QD for 3 days
Miconazole 2% cream	5 g intravaginally QD for 7 days
Miconazole 4% cream	5 g intravaginally QD for 3 days
Miconazole vaginal suppository	100 mg QD for 7 days
Miconazole vaginal suppository	200 mg QD for 3 days
Miconazole vaginal suppository	1200 mg single application
Nystatin vaginal tablet	100,000 units QD for 14 days
Tioconazole 6.5% ointment	5 g intravaginally single application
Terconazole 0.4% cream	5 g intravaginally QD for 7 days
Terconazole 0.8% cream	5 g intravaginally QD for 3 days
Terconazole vaginal tablet	80 mg QD for 3 days
Alternative	
Fluconazole orally	150 mg single application

Warning: oil-based intravaginal products may erode latex condoms

In recurrent vulvovaginal candidiasis prophylactic treatment with fluconazole 1 x 200 mg/week may be considered (Schuman 1997). Development of resistance against fluconazole is rare (Sobel 2001, Vazquez 2001). In contrast, resistance is more common in non-albicans strains. In this case itraconazole and ketoconazole are a good alternative.

HPV-associated diseases

Human papillomavirus (HPV) infections are very common. More than 50% of sexually active individuals get infected by one or more of the more than 100 HPV-subtypes. Normally the infection resolves within a few months (Evander 1995, Ho 1998). Chronic HPV infection may lead to condylomata acuminata as well as intraepithelial and invasive cancer in the lower female genital tract. Genital warts are caused mostly by the low-risk subtypes 6 and 11. The high-risk subtypes 16 and 18 play an important role in the development of cervical cancer.

HIV+ women have a higher prevalence and incidence of HPV (Ahdieh 2001, Branca 2003), a higher HPV viral load (Jamieson 2002), a longer persistence of HPV (Sun 1997, Ahdieh 2000) and more frequent infections involving multiple subtypes (Levi 2004) and oncogenic subtypes (Minkoff 1998, Uberti-Foppa 1998, McKenzie 2009). Prevalence and persistence of HPV correlate with HIV viral load and immune status (Palefsky 1999). In women with advanced HIV disease, oncogenic subtypes are more common (Luque 1999) and HPV reactivation is possible (Strickler 2005). HPV viral load correlates with persistence and is higher in patients with low CD4 T cell counts (Ahdieh 2001).

Testing for HPV is useful in patients over the age of 30 with a normal Pap smear since it allows detection of persistent high-risk subtypes for higher grade dysplasia (Ronco 2010). Specificity of the Hybrid Capture 2 (HC 2) assay is generally higher than that of the HPV PCR, while the sensitivity is comparable.

Condylomata acuminata

HPV-associated genital warts are more prevalent in HIV+ women, and the manifestation correlates with immune deficiency (Conley 2002, Silverberg 2002). Diagnosis is possible in most cases by inspection. A biopsy is only necessary if:
• diagnosis is uncertain
• warts do not respond to treatment
• warts progress in spite of therapy
• warts are pigmented, indurated, fixed, or ulcerated
For treatment of condylomata acuminata, see section on STIs.

Cervical dysplasia and cervical cancer

The risk of development of HPV-associated cancer is significantly higher in HIV+ women. Most common is cervical dysplasia, but other regions like the vulva and the perianal area are also affected (Maiman 1998, Massad 1999). In the pre-ART era 20% of HIV+ women developed cervical dysplasia within three years (Ellerbrock 2000). Manifestation and severity correlate with advanced immunodeficiency and high viral load (Davis 2001, Massad 2001, Schuman 2003). Reasons for the correlation are the higher prevalence of oncogenic subtypes and the higher HPV viral load (especially HPV-16) in patients with advanced HIV disease (Weissenborn 2003, Fontaine 2005, Harris 2005). HIV+ women have a nine times higher risk of invasive cervical carcinoma than negative women (Mbulaiteye 2003). Cervical cancer is an AIDS-defining illness. The incidence of cervical cancer in WIHS and HERS was 1.2 per 1000 person

years (Phelps 2001, Massad 2004). There seems to be no correlation with CD4 T cell count. Recent studies demonstrate no decline of cervical cancer as a result of ART (Dorrucci 2001, Moore 2002, Clifford 2005).

Anal dysplasia

Multifocal lesions of HPV infection are more common in HIV+ patients (Abercombie 1995). Therefore the risk of anal dysplasia in addition to cervical dysplasia is higher. The prevalence of dysplastic cells in cytological samples in the ART era reaches up to 16%, including women who do not partake in anal intercourse (Hessol 2009, Weis 2011). The risk for anal carcinoma is elevated by 2-28-fold in HIV+ women (Frisch 2000, Dal Maso 2003).

Diagnostic evaluation

Gynecological/cytological screening is indicated every six months in the first year after HIV diagnosis. In patients with no abnormalities, evaluations should be performed annually. A higher frequency of screening is indicated if:
• last Pap smear was abnormal
• HPV infection is present
• cervical dysplasia has been previously treated
• symptomatic HIV infection is present or CD4 T cell count is <200 cells/µl

Therapy

Treatment of cervical dysplasia (cervical intraepithelial neoplasia/CIN) and cervical cancer is the same in HIV+ and negative women. However, HIV+ women have a higher risk of recurrent disease and should be monitored closely (Fruchter 1996, Heard 2005). Surgical treatment of cervical dysplasia aims at complete removal of the transformational zone including all neoplastic lesions.
CIN I: If lesion is restricted to ectocervix (documented by colposcopy), repeat evaluation in 6 months. In persistent and ectocervical lesions perform CO_2 – laser vaporisation. In endocervical lesions, broad indication for conization.
CIN II: Repeat cytological and colposcopic evaluation in 6 months. Lesions persistent for more than 12 months should be treated like CIN III.

Table 5: Management of pre-invasive cervical lesions

Stage	Management	Surgical Method	Non-invasive/controls
CIN I	Colposcopic-cytological evaluation every 6 months	Loop conisation, laser (in case of persistence)	Up to 24 months watch and wait
CIN II	Colposcopic-cytological evaluation every 6 months	Loop conisation, laser	Up to 12 months watch and wait
CIN III	Therapy	Conisation (loop, laser, needle, knife)	Treat always, watch and wait only in pregnancy (conisation increases risk of premature delivery)
Lesion extends into deep endocervix	Colposcopic-cytological evaluation	Conisation (loop, laser or knife)	Possible in CIN I

Source: Interdisciplinary guidelines by German Cancer Association and German Gynecologic and Obstetric Association, 8/2008

CIN III: Surgical removal by loop excision or conization, ectocervical lesions where applicable by laser vaporisation, endocervical curettage. In case of R1 resection discuss further resection depending on individual situation (e.g., future pregnancy).

In CIN I documented by histology, perform regular screening only. This also applies to CIN II and III in pregnancy. With CIN II or III that is persistent more than 12 months in non-pregnant patients, surgery is indicated.

HPV vaccination: Public health officials in Australia, Canada, Europe and United States recommend prophylactic vaccination of young women against HPV to prevent cervical cancer and genital warts. Vaccination of HIV+ or HPV-infected women is not recommended. Studies in these populations are still ongoing.

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20. HIV and Pregnancy
Therapy for mothers and prophylaxis for neonates

MECHTHILD VOCKS-HAUCK

Perinatal (vertical) HIV infection has become rare since the introduction of anti-retrovirals as transmission prophylaxis and elective cesarean section. While vertical HIV transmission rates hovered around 15% in Europe at the beginning of the nineties, it is now at less than 1% (Connor 1994, European Collaborative Study 2005, Townsend 2014). Postpartum HIV infections are avoidable provided HIV-infected mothers do not breastfeed without prophylaxis. At the same time as transmission prophylaxis was introduced, the treatment of HIV infection changed. Nowadays, pregnancy is no longer a general contraindication for ART (Agangi 2005, CDC 2014). This chapter summarizes the German-Austrian guidelines for HIV therapy in pregnancy (DAIG 2014). Reference is made to the US (CDC 2014) and European (EACS 2014) Guidelines. Continuously updated recommendations can be found at www.europeanaidsclinicalsociety.org/guidelines.asp or www.AIDSinfo.nih.gov.

HIV therapy in pregnancy

Starting HIV therapy during pregnancy

It is important to distinguish between women with and without a therapy indication of their own. In the case of a maternal indication, treatment is generally begun in week 13+0 of pregnancy; if there is no maternal indication, i.e., solely transmission prophylaxis and low viral load, start at the latest at week 24+0 of pregnancy, and with a higher viral load (of more than 100,000 copies/ml) or high-risk pregnancy as of week 24+0 or earlier (DAIG 2014).

According to the US and/or European Guidelines transmission prophylaxis should be started at the beginning of the second trimester. The assessment of indications for therapy and drug selection is similar to that in non-pregnant patients (see chapter on *ART 2015*). Since the CD4 T cell count decreases physiologically by approximately 10–20% in pregnant patients, the threshold values should be adjusted upwards accordingly before treatment is started. Following the recommendations of the German-Austrian guidelines, antiretroviral therapy in symptom-free patients should begin when CD4 T cell count falls below 350–500/µl (15–20% relative). Before initiating therapy, a resistance test, and if necessary, subtyping, should be carried out (see chapter on *Resistance*).

When setting up a treatment plan, it is important that:
• AZT (Retrovir®) is part of the combination, but despite lack of approval in pregnancy, other NRTIs are also acceptable – if the result of the resistance test and the expected toxicity are favorable; and
• Efavirenz (Sustiva®, Stocrin®) is avoided because of possible teratogenic effects in the first trimester; and
• The combination of ddI+d4T is avoided and d4T should only be used when there are no appropriate alternatives, and for the shortest possible time.

A maximum suppression of viral activity (to <50 copies/ml) makes HIV transmission unlikely. In this case the intrapartum intravenous transmission prophylaxis with AZT can be waived (EACS 2014, see below).

Table 1. Special features of anti-HIV therapy in pregnancy

Explanation of risk: Only AZT is approved for perinatal transmission prophylaxis
HIV resistance testing, and if necessary HIV subtyping
No efavirenz (Sustiva®) in the first trimester before week 8 (teratogenicity)
No d4T+ddI (Zerit®+Videx®) because of mitochondriopathies, no d4T (whenever possible)
Nevirapine-related hepatotoxicity in women with CD4 T cell counts >250/µl
Raised toxicity with combination therapy, therefore monthly controls of lactate, hepatic
transaminase levels, viral load, CD4 T cell count
Therapeutic plasma drug level measurement (TDM) and possible dose ajustment

Continuation of ART during pregnancy

Most pregnant HIV+ women in the North are pretreated with antiretroviral agents. As a rule, if pregnancy is diagnosed after the first trimester, the current ART should be continued. Women in whom pregnancy is diagnosed during the first trimester should be informed about the benefits and risks of treatment in this period. In cases of reduced immune status in particular, ART could be continued in the first trimester under careful laboratory and ultrasonic controls. Embryonic toxicity seems to be low overall (Joao 2010, Watts 2011, Antiretroviral Pregnancy Registry 2015). However, agents with a toxic effect on the embryo should not be administered during early pregnancy (Table 1).

Interruption of treatment in the first trimester

Women who have to discontinue ART during pregnancy, e.g., because of hyper-emesis, should only restart ART when good drug tolerance is expected. In this case, as in all others, the rule is to withdraw all drugs (NRTIs and PIs) simultaneously and re-administer them simultaneously, with the exception of NNRTIs. Due to their long half-lives, NNRTIs should be withdrawn up to three weeks before NRTIs in order to prevent development of resistance. Alternatively, the NNRTI can be replaced by a boosted PI. In other cases – especially if pregnancy is diagnosed very early – the fear of possible embryotoxic effects may also lead to ART interruption until the end of the first trimester. Neural tube defects due to efavirenz can occur in the first 8 weeks of pregnancy.

However, there are reports that after interruption of treatment in pregnancy, return to complete viral suppression may be much more difficult (Liuzzi 2006) and the risk of transmission is higher (Galli 2009). As it is usually not possible to determine pregnancy duration exactly, restarting is mostly initiated at the gestational point of 13+0 weeks. A continuously updated summary of the current state of knowledge about antiretroviral drugs in pregnancy can be found at www.AIDSinfo.nih.gov.

Combination therapy for the duration of pregnancy

HIV therapy and/or perinatal prevention is recommended to be based on a boosted PI. The prolonged half-life of NNRTIs makes them less suitable for a short course of treatment for prevention only. The prevention of mother-to-child transmission starts from the second trimester (CDC 2014) onward or 24-28+0 weeks of gestation (DAIG 2014). Before starting therapy the risk of teratogenicity has to be weighed carefully against the risk of HIV transmission. The approach of an earlier start of HIV prevention is based on the assumption that any timely decrease in viral load translates into a lowering of the transmission risk (Tubiana 2010, Chibwesha 2011, Read 2012, Rachas 2013, Townsend 2014). With a viral load of less than 50 HIV RNA copies/ml, the advantage of cesarean section compared with vaginal delivery is no longer certain (Townsend 2014). For this reason, in most European countries vaginal delivery is

considered an option for women with undetectable HIV-RNA at the time of deliv-ery (under 50 copies/ml) and in whom no obstetric complications are expected. These cases are increasing in Western Europe, and the rates have now reached about 60% (Boer 2010, Brunet 2012).

Treatment monitoring

In addition to measuring the hemoglobin concentration to exclude an AZT-associ-ated anemia, transaminases for potential hepatic toxicity, especially in HIV and hepatitis virus coinfections, and lactate level to detect lactic acidosis early, the CD4 T cell count and viral load should be monitored at least bimonthly. If PIs are taken, it is of particular importance to monitor the blood glucose level closely (El Betuine 2006, Snijdewind 2011). Resistance and plasma level are determined at the beginning and, if appropriate, at the point of failure of treatment.

Special aspects of HIV prophylaxis/therapy in pregnancy

Because embryotoxicity cannot be excluded and hepatic metabolism is altered in pregnancy, and in some cases plasma levels are reduced, some basic rules must be taken into consideration (CDC 2014) (Table 1).

It is important to understand that a detectable plasma viral load always necessitates a resistance test. AZT resistance was verified in approximately 17% of women who received AZT monoprophylaxis between 1991 and 1997 (Palumbo 2001). In the year 2006, resistance mutations were diagnosed in up to 23% of perinatally HIV-infected children, mutations which limited future therapeutic options and thus potentially worsened their prognosis (Vignoles 2007).

HIV and hepatitis virus coinfections

In chronic hepatitis B (HBV) coinfection and pregnancy, tenofovir (TDF) and lamivu-dine (3TC) or emtricitabine (FTC) are recommended as NRTI backbone in HIV therapy (Shi 2010). The newborn of a mother with hepatitis B should receive hepatitis B vaccine and hepatitis B immunoglobulin (HBIG) within 12 hours of birth.

A hepatitis C coinfection should not be treated during pregnancy, because interferon is contraindicated during pregnancy and ribavirin is known to be embryo- and fetotoxic (pregnancy category X).

As hepatitis virus coinfections can enhance liver toxicity of ART (Snijdewind 2011), liver enzymes should be monitored monthly (CDC 2014, DAIG 2014).

Mode of delivery in HIV/hepatitis coinfection is managed following HIV criteria.

Antiretroviral agents in pregnancy

NRTIs

NRTIs cross the placenta and can cause toxic damage not only to the mother but also the child. According to experience to date, the main problems are anemia and, when using combination therapy, lactic acidosis.

On the basis of pregnancies observed to date, it can be maintained that frequently used NRTIs such as AZT, 3TC and d4T do not increase teratogenicity by more than two-fold (Antiretroviral Pregnancy Registry 2015). Most of our experience is with AZT. Follow-up of more than 20,000 children who received AZT prophylaxis did not show any serious side effects. An analysis of the causes of death of 223 children who died within the first five years of life ruled out drug-related causes (The Perinatal Safety Review Working Group 2011). In other studies no damage to mitochondrial DNA or neurological development dysfunction in HIV-exposed children after ART was detected (Alimenti 2006, Brogly 2010, Williams 2010).

Table 2: Antiretroviral agents in pregnancy

NRTIs	(full placental transfer)
AZT+3TC	AZT is metabolized in the placenta; mitochondriopathy risk: ddI>d4T>AZT>3TC>ABC>TDF
ABC+3TC	Alternative (with a negative HLA B*5701 Test)
TDF+FTC*	Renal clearance of TDF↑30%; lower trough levels, genotoxic signatures in neonatal blood cells
NNRTIs	**(placental transfer)**
Efavirenz	Neural tube impairment, earliest after 8 pregnancy wks if no alternative
Etravirine	PK studies
Nevirapine	General use in perinatal prophylaxis; hepatotoxicity especially in previously untreated mothers with >250 CD4 T cells; enzyme induction, rapid resistance
Rilpivirine	PK studies (IMPAACT P 1026): cord/maternal ratio 0.33–0.53
PIs	**(minimal placental transfer)**
Nelfinavir	Formerly frequent use; unboosted, less potent than boosted PIs
Indinavir	Hyperbilirubinemia, cord/maternal ratio 0.12
Ritonavir	Only as a booster
Lopinavir/r	Cord/maternal ratio 0.20
Saquinavir SGC	Some experience
Amprenavir	Low plasma levels, cord/maternal ratio 0.27
Fosamprenavir	Some experience, solution contraindicated, cord/maternal ratio 0.24
Atazanavir	Hyperbilirubinemia, also with neonates, cord/maternal 0.11–0.21
Tipranavir	Case reports: cord blood level > other PI
Darunavir	Twice daily; cord/maternal plasma 0.18–0.24
Entry inhibitors	**(no placental transfer)**
T-20	Some experience
Maraviroc	Case reports
INSTIs	
Raltegravir	Cord/maternal plasma ratio 1.03-1.48
Dolutegravir	Prolonged half-life in neonates
Elvitegravir	No data in pregnancy

In contrast to these findings, in a prospective study with 2,644 ART-exposed non-infected children, neurological symptoms with persistent mitochondrial dysfunction were reported in 0.26% (Barret 2003). Retardation of auditory evoked potentials (Poblano 2004), as well as nonspecific changes in cerebral MRTs in children perinatally exposed to AZT plus 3TC (Tardieu 2005) have been interpreted as a sign of neuro-toxicity. Even years after NRTI exposure, raised lactate values as well as impairment of hematopoiesis can still be demonstrated in children (ECS 2004, Vigano 2010, Brogly 2011). Severe mitochondriopathies have been observed during combination therapy of d4T+ddI. Tenofovir and FTC proved to cross the placenta easily (Bonora 2007, Hirt 2009a+b). Fetotoxicity has been demonstrated (Siberry 2014) but not all studies were performed on prenatally tenofovir-exposed children (Vigano 2011, Mora 2012).

NNRTIs

In perinatal prevention, nevirapine has been employed successfully, particularly in combination with AZT. Because of enhanced risk of liver toxicity during the first 18 weeks of treatment in previously untreated women with CD4 T cell counts of more

than 250/µl, treatment should be monitored closely and frequently, especially during the dose escalation period (Boehringer 2004). Nevirapine in pregnant women with over 250 CD4 T cells/µl is only recommended following very careful assessment of the benefit-risk ratio (CDC 2014). In a retrospective study of 197 pregnant women, toxic side effects were observed in 5.6%, leading to treatment discontinuation in 3.6% (Joao 2006). However, another study did not find higher liver toxicity with nevirapine during pregnancy than with other compounds (Quyang 2010). Perinatal single- and two-dose nevirapine prophylaxis resulted in the development of resistance mutations in more than 20% of cases (Flys 2005). This can be reduced by 50% or more by the additional administration of, for example, tenofovir and FTC (Chi 2007, Lehman 2009). The intrapartum single-dose administration of nevirapine to pregnant women receiving ART is NOT recommended by the CDC (2014) or EACS (2014). If a mother gives birth less than two hours following nevirapine administration, or has not received any prior nevirapine at all, the newborn should receive a dose of nevirapine immediately after birth and a further dose 48–72 hours later (Stringer 2003).

Efavirenz should not be used during the first trimester of pregnancy, due to embryonic toxicity in humans (neural tube impairment) and in rhesus monkeys (Bristol-Myers Squibb 2004). Efavirenz may be used used only after the second trimester in those who have no alternative treatment option, providing reliable contraception is practiced after delivery (Schwarz 2012, CDC 2014). The occurrence of isolated cases of neural tube defects caused the FDA to allocate efavirenz to category D. There are, unfortunately, only case experiences with etravirine and rilpivirine.

PIs

The use of PIs must be monitored carefully, especially in the later stages of pregnancy (monthly in the third trimester), due to possible diabetogenic effects (Beitune 2005) and hepatic toxicity. In other studies, however, no increased rate of gestational diabetes was seen (Hitti 2007, Azria 2009, Jao 2013). Hyperlipidemia occurred more frequently in another study (Floridia 2006).

Presently, most experience relates to nelfinavir (Timmermans 2005). Since nelfinavir is less potent than boosted PIs, it is seldom used today. Indinavir can lead to hyperbilirubinemia and nephrolithiasis; the plasma levels can be lowered (Kosel 2003, Cressey 2013). As with indinavir, saquinavir should also be boosted with ritonavir in pregnancy (Zorilla 2007). A twice daily dosage of saquinavir is highly effective (Brunet 2013), but a single dose is useful, too (Lopez-Cortez 2007). Lopinavir/r plasma levels are also lowered during pregnancy, especially in the third trimester (Manawi 2007, Aweeka 2010). With atazanavir/r, mild hyperbilirubinemia in neonates with low placental transfer of about 20% has been described (Ripamonti 2007, Ivanovic 2009, Mandelbrot 2011). Tipranavir reached higher concentration in umbilical cord blood compared to other PIs (Weizsäcker 2011). Darunavir does not cross the placenta (Ripamonti 2009). Fosamprenavir/r has been described as safe and effective (Martorelli 2010).

Monotherapy with lopinavir/r in pregnant women with an initial viral load under 30,000 copies/ml and CD4 T cell count over 350 cells/µl reduced the viral load in more than 88% to less than 200 copies/ml. Side effects with monotherapy were less than with triple ART (Tubiana 2011).

Previous speculation on increased rates of deformity when using PIs has been refuted, especially as PIs can barely cross the placenta due to their molecular size. An increase in premature births when using ART with a PI (EACS 2006, Cotter 2006, Grosch-Wörner 2008, Machado 2009, Townsend 2010, Powis 2011, Sibiude 2011) has also failed to be confirmed in other studies (Tuomala 2005, Kourtis 2007, Baroncelli 2009,

Carceller 2009, Patel 2010, Dola 2011, Lopez 2012). Alpha-fetoprotein levels are thought to be reduced on a PI regimen (Brossard 2006) although not the serum level of unconjugated estriol and human chorionic gonadotropin (Einstein 2004, Le Meaux 2008).

Despite data of increased preterm deliveries, especially in European studies, PIs are still recommended for treatment and transmission prevention in pregnancy (CDC 2014).

Entry, fusion and integrase inhibitors

Enfurvitide (T-20) was administered with some success to women with multiresistant viruses, also in combination with tipranavir (Wensing 2006). Therapy failures with perinatal HIV transmission have been described. In T-20 there is no placental transfer (Brennan-Benson 2006). Like T-20, maraviroc is assigned to FDA category B (see below), in macaques there is no placental transfer. The integrase inhibitor raltegravir (FDA category C) passes the placenta (Jaworsky 2010, McKeown 2010, Belissa 2015). Raltegravir and dolutegravir have a prolonged elimination half-life in (premature) neonates (Pain 2015).

FDA pregnancy classification for drugs

FDA has classified the potential toxicity during pregnancy into categories A-D. All HIV agents belong in categories B-D, since "harmlessness through studies on the human being" (category A) has not been shown for any HIV drug.

FDA category B is defined as "Animal studies have revealed no evidence of harm to the fetus; however, there are no adequate and well-controlled studies in pregnant women". FDA category B includes ddI, FTC, tenofovir (TDF), etravirine, nevirapine, rilpivirine, atazanavir, saquinavir, ritonavir, nelfinavir, T-20 and maraviroc, dolutegravir and elvitegravir/cobicistat/TDF/FTC.

FDA category C is defined as "Animal studies have shown an adverse effect and there are no adequate and well-controlled studies in pregnant women. Use in pregnancy should occur only after careful benefit/risk appraisal." All drugs not mentioned in category B fall into the FDA category C.

FDA category D is defined as "Adequate well-controlled or observational studies in pregnant women have demonstrated a risk for the fetus. Nevertheless, the benefits of therapy may outweigh the potential risk." For example, the drug may be acceptable if it is needed in a life-threatening situation or serious disease for which safer drugs cannot be used or are ineffective. Efavirenz falls into category D because of neural tube defects in humans after first trimester exposure.

FDA category X is defined as "Studies in animals or humans have demonstrated fetal abnormalities and/or there is a positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risk involved in use of the drug in pregnant women clearly outweigh potential benefits."

Prevention of perinatal HIV infection

In approximately 75% of cases, HIV is transmitted prior to, or during the last weeks prior to birth. About 10% of vertical infections occur before the third trimester, and 10–15% are caused by breastfeeding.

The probability of HIV transmission to a neonate correlates with the viral load (Warszawski 2008). This also applies to women receiving ART (Table 3). If the viral load is undetectable using currently available tests, the probability of transmission is extremely low (Tubiana 2011). Likewise, premature births and premature rupture of membranes are associated with an increased transmission risk, in particular when HIV suppression is insufficient.

For this reason, reduction of plasma viremia and improvement in the immune status of pregnant women are essential. If a mother is treated with antiretrovirals, these drugs should continue to be taken, if possible, during delivery at the usual scheduled intervals in order to achieve the maximum effect and to minimize the risk of resistance. About 20% of perinatal HIV transmissions (less than 2% total) are due to resistance (Parker 2003). For the general prevention of vertical transmission of HIV, pregnant women should be warned not to use intravenous drugs or to have unprotected sex (Birkhead 2010).

Table 3: Known risk factors for perinatal HIV transmission

High maternal viral load
Low CD4 T cell count, AIDS-defining illness of the mother
Vaginal delivery with viral load >50 copies without ART
Premature rupture of membranes of >4 h, preterm infants (<37 weeks of gestation)
Breastfeeding

In addition to the indicated or optional antiretroviral therapy of the mother, the following rules should be observed regarding chemoprophylaxis:
• Antiretroviral prophylaxis before and during delivery
• Elective cesarean section before onset of labor because vaginal delivery with a viral load of >50 HIV RNA copies/ml increases the transmission risk
• Post-natal chemoprophylaxis of the infants (post-exposure prophylaxis)
• No breastfeeding

Antiretroviral transmission prophylaxis

Combination prophylaxis

If the HIV+ pregnant woman is not already on treatment and if the viral load is far below 100,000 copies/ml, then combination therapy should be started latest at 24+0 weeks gestation (Table 4). In the case of high-risk pregnancies (e.g., multiples) prophylaxis is begun at week 13+0. A monoprophylaxis with AZT or the combination of AZT+3TC is not recommended because of the possible development of resistance (Mandelbrot 2001, CDC 2014). A triple combination with a boosted PI is increasingly being used as prophylaxis. Due to elevated hepatotoxicity with a CD4 T cell count above 250/µl, combinations containing nevirapine are only implemented after careful assessment of the benefit-risk ratio.

Table 4: Combination prophylaxis with combination therapy containing AZT in cases with a viral load >50,000 RNA copies/ml, but otherwise only standard risk

After resistance testing starting at the latest at 24 + 0 weeks gestation: Two NRTI + PI/r (alternative NNRTI)
During delivery (elective cesarean section earliest from 37+0 weeks gestation to week 37+6 or vaginal delivery at VL <50 copies/ml and ART): IV infusions of AZT as standard prophylaxis*   (if viral load >50 copies/ml):   2 mg/kg IV as a loading dose for 1 h to approximately 3 h preoperatively (prepartum)   1 mg/kg IV intraoperatively until delivery of the infant
In neonates AZT monoprophylaxis *within 6 hours postpartum:*   2 (4) mg/kg orally every 6 (12) hours for 2–4** weeks or   1.5 mg/kg IV every 6 hours for 10 days

* The benefit of intravenous AZT in a combination therapy and viral load <50 copies/ml is not certain (CDC 2014, EACS 2014, DAIG 2014) ** 4–6 weeks at VL of 1000–10,000 copies/ml

Prophylaxis in ART-pretreated pregnant women

More than half of all HIV+ pregnant women in the Global North are already treated with ART. In the case of efficient ART with fixed combinations, it is feasible to continue and not replace any of the NRTIs with AZT (Tariq 2011, CDC 2014).

Procedure in cases of additional pregnancy risks

Several risks (Table 5) require an intensified prophylaxis. Here too, the risk of transmission drops in the case of sufficient ART. In premature births, for example, perinatal HIV transmission only occurred when the mother had received no prophylaxis or only AZT monoprophylaxis (Aagaard-Tillery 2006).

Intrapartum prophylaxis without antepartum regimens

If the diagnosis of HIV infection is only established at the time of delivery, mother and newborn receive a dual or triple combination prophylaxis with AZT (plus 3TC and/or nevirapine) in cases of highly increased risk (high viral load and/or medical complications during delivery). Due to rapid resistance, nevirapine should only be administered in combination with other drugs.

Treatment during delivery

Elective cesarean section or vaginal delivery in cases of uncomplicated course of pregnancy

Cesarean section is carried out swiftly by experienced obstetricians prior to the onset of labor at the earliest from 37+0 up to 37+6 weeks of gestation using the Misgav-Ladach technique, which reduces bleeding. Blunt preparation and the delivery of the child within the intact amniotic sac are considered ideal (Schäfer 2001). In the case of undetectable viral load along with long-term ART, the advantage of elective cesarean over vaginal delivery is no longer recommended. For this reason, it is becoming more common in many countries to dispense with a C-section in favor of a later vaginal delivery (Townsend 2014).

High-risk pregnancy

Cesarean section in cases of multi-gravidity should be carried out using the same technique as for a cesarean section in a single pregnancy. In this context, the skill and experience of the operating surgeon are especially important. Cesarean sections in cases of premature infants are also important to avoid hypoxia in the neonate; the special aspects of chemoprophylaxis have been described above.

In cases with a premature rupture of membranes of less than four hours duration, a section is expedient for prophylactic reasons, providing that the clinical situation at that stage of delivery still allows this. If the rupture of membranes has lasted more than four hours, there is no longer an advantage of cesarean section compared to vaginal delivery. Nevertheless, vaginal delivery should occur as swiftly as possible, since the HIV transmission risk increases by about 2% per hour. The extension of the prophylactic scheme (Tables 5 and 6) is important if viral load in a high HIV transmission risk is not under 50 copies/ml at the time of delivery or 12 weeks before delivery has not yet been under 50 copies/ml. On the other hand in pregnancies with an increased transmission risk with a viral load of <50 copies/ml at least 12 weeks before delivery a mono-prophylaxis with AZT for 2–4 weeks is sufficient for the newborn.

Table 5: Prophylaxis in the case of increased transmission risk

Increased risk	Mother	Child
Multigravidity	Combination, e.g., AZT + 3TC + PI/r from 24+0 weeks gestation	Within 6 h postpartum AZT 4 x 2 (2 x 4)* mg orally for 4 weeks (if IV necessary, 10 days)
VL prepartum >1000 <10,000 copies/ml	Combination therapy, e.g., AZT + 3TC + PI/r	Within 6 h postpartum AZT 4 x 2 (2 x4)* mg orally for 4–6 weeks AZT dosage with premature birth <36 + 0 wks/gestation: 2 x 2 mg/kg orally or 2 x 1.5 mg/kg IV from day 15: 3 x 2 mg/kg orally (for premature <30 + 0 wks/gestation from day 29)
Premature infants >33+0 to 36+6 weeks of gestation*** VL>50 Premature infants <33 weeks and VL <50 less than 12 wks	Combination therapy, e.g., AZT+3TC+PI/r	AZT 4 x 2 (2 x 4)* mg/kg orally 6 weeks AZT dosage with premature birth as above

* For neonates >36+0 wks/gestation due to better adherence, also 2 x 4 mg/kg AZT
** See chapter on NNRTIs
*** For prematures, also triple prophylaxis (see below): 3TC, but cautiously with prematures

Unknown HIV status in cases of known risk

If, at the time of delivery, the HIV status is unknown and the existence of risk is known, an HIV test can be offered to the mother (Bulterys 2004). Although specificity is high, it is still considered inadequate. The combined use of two rapid tests from different manufacturers is ideal. If one of the two tests is negative, there is probably no infection.

Table 6: Prophylaxis when transmission risk is highly increased and VL is >50 copies/ml

Highly increased risk	Mother	Child
Premature infants <33+0 weeks of gestation and VL >50 copies/ml (or VL <50 for less than 12 weeks) Premature rupture of membranes >4 hours Amniotic infection syndrome Rise of viral load towards the end of pregnancy	In addition to AZT or combination therapy: nevirapine*	AZT (dosage, see above) over 4–6 weeks plus 3TC** 2 x 2 mg/kg over 4–6 weeks plus nevirapine* 2 mg/kg within 2–48 h + 2nd dose 48–72 h postpartum (if no NVP prepartum or <2h between ingestion and delivery) (If prepartum nevirapine, then only 1x after 48–72 h)***
Incision injury of the child Ingestion of hemorrhagic amniotic fluid HIV diagnosed only post partum		As above

* See chapter NNRTIs.** Premature babies: use 3TC cautiously.*** CDC 2014

Therapy of neonates

Standard postnatal prophylaxis

The postnatal transmission prophylaxis should begin, if possible, within the first 6 hours following birth with oral or – in the case of gastrointestinal symptoms – intravenous AZT prophylaxis. In Germany, the duration of the oral standard prophylaxis has been shortened from six to two (or four) weeks (Vocks-Hauck 2001, Neubert 2013).

Increased transmission risk (multiple neonates, premature infants)

In multiple-birth neonates without further risk, a two- to four-week AZT prophylaxis is recommended (without nevirapine). Premature infants (>33+0 weeks and VL >50 copies/ml and <33 weeks and VL <50 copies/ml) receive AZT monoprophylaxis for 6 weeks, if VL is <50 copies/ml at least 12 weeks before delivery.

Highly increased transmission risk

An additional transmission risk exists, e.g., in preterm babies <33 weeks and a viral load >50 copies/ml less than 12 weeks before delivery, a combination prophylaxis with AZT+3TC is recommended. A strongly increased risk exists, for example:
• after premature rupture of membranes
• in cases of amniotic infection syndrome
• when viral load >10,000 copies/ml prior to delivery
• when there has been no transmission prophylaxis
• if an incision injury of the child during cesarean section
• if the amniotic fluid sucked from the gastrointestinal or respiratory tract of the newborn is hemorrhagic
• if maternal viral load has been more than 50 copies/ml

In the case of children with additional transmission risks, a combination prophylaxis of AZT+3TC, as well as two doses of nevirapine are recommended. Nevirapine is given either once to the mother before delivery and once to the infant, or twice postnatally. If maternal nevirapine administration occurs less than an hour before delivery, then the newborn receives its first dose within the first 48 hours (Stringer 2003). If nevirapine was a part of the combination therapy for the mother, the dose is doubled to 4 mg/kg in newborns because of possible enzyme induction. In addition, newborns receive an AZT+3TC prophylaxis for six weeks (CDC 2014).

The pharmacokinetic data on ART in neonates are, however, extremely limited. According to the CDC guidelines (2014) the prenatal nevirapine dose to the mother is not applicable. Therefore the newborn receives nevirapine three times in the first week: immediately after birth and then after 48 and 96 hours. In addition, to lower toxicity only AZT is recommended as post-exposition prophylaxis for six weeks (two-drug regime, CDC 2014). In neonates whose mothers did not receive ART, prophylaxis with a two- or three drug regimen is superior to zidovudine alone (Nielsen Saines 2012). No differences were observed between single and combination neonatal drug prophylaxis in infants at high risk for MTCT in a European study (Chiappini 2013).

According to an FDA Safety communication (2011), lopinavir/r should not be administered to (premature) newborns during the first two weeks due to cardiotoxicity (McArthur 2009). Furthermore, transient adrenal insufficiency has been reported in newborns who have been exposed to lopinavir/r prenatally and for 30 days postnatally (Siman 2011). As such, lopinavir/r is no longer given to newborns in the first two weeks.

Raltegravir-based therapy induces rapid viral decay after short course treatment in late pregnancy (British Guidelines 2012), but may increase the risk of bilirubin neurotoxicity (Clarke 2013+2014).

Procedure in cases of no pre- or intranatal prophylaxis

Combination prophylaxis of AZT+3TC should start within the first 6 to 12 hours after delivery. In addition, a perinatal nevirapine prophylaxis is recommended. If HIV infection is discovered only after birth, a combination prophylaxis, begun within 48 hours, seems to be far more effective than a monoprophylaxis which is initiated only after 3 days (transmission rates 9.2% vs. 18.4%) (Wade 1998). However, even then, a certain positive effect of AZT prophylaxis as opposed to no prophylaxis can still be verified (18.4% vs. 26.6%) (Table 6). Even a late initiation of postnatal prophylaxis (>3 days) can still make sense.

Table 7: Studies on antiretroviral prophylaxis in neonates

Abbreviated name	Average daily dose	Most frequent side effects	Studies
AZT, Retrovir®	4 x 2 mg/kg, 2 x 2 mg/kg in PI* <35 GW, from 15th day: 3 x 2 mg/kg*, in PI <30 GW from 29th day	GI, anemia, neutropenia Mitochondriopathy in combination with 3TC	(P)ACTG 076, 316, 321, 353, 354, 358; HIVNET 012 III PACTG 331(PI)
3TC, Epivir®	2 x 2 mg/kg in neonates (<30 days)	GI, vomiting, mitochondriopathy in combination, incompatibility in premature infants	PACTG 358
FTC, Emtriva®	3 mg/kg in neonates to <3 months	Minimal toxicity, mitochondriopathy	ANRS 12109, Gilead PK study
ddI, Videx®	2 x 50 mg/m² from 14th day	Diarrhea, pancreatitis, mitochondriopathy in combination	PACTG 239, 249; HIV-NAT
d4T, Zerit®	2 x 0.5 mg/kg (0–13 days) 2 x 1 mg/kg ≥14 days	Mitochondriopathy, should be avoided	PACTG 332, 356; HIV-NAT
ABC, Ziagen®	1 x 2–4 mg/kg >1 month 2 x 8 mg/kg	HRS, mitochondriopathy, lactic acidosis	PACTG 321
TDF, Viread®	Mother 600 mg during labor, newborn 6 mg/kg daily for 7 days (not FDA approved)	Osteopenia, nephrotoxicity	NCT00120471, HPTN 057; ANRS 12109
NVP, Viramune®	1 x 2–4 mg/kg (or 1 x 120 mg/m²) for 14 days then 2 x 3.5–4 mg/kg (2 x 120 mg/m²)	Rash, hepatotoxicity, hyperbilirubinemia	PACTG 316, 356, HIVNET 012
NFV, Viracept®	2 x 40–60 mg/kg in infants <6 weeks (NFV powder no longer available, 250 mg tablets can be dispersed in water)	GI, particularly diarrhea	PACTG 353, 356, PENTA 7

Table 7: (continued)

Abbreviated name	Average daily dose	Most frequent side effects	Studies
RTV, Norvir®	Ritonavir 2 x 350–450 mg/m² in neonates <4 weeks	Hyperbilirubinemia, GI, especially nausea	PACTG 345, 354
Lopinavir/r, Kaletra®	2 x 300/75 mg/m² in infants >2 <6 weeks (avoid in newborns <2 weeks)	GI, especially diarrhea, cardiotoxicity, adrenal insufficiency in newborns	PACTG P 1030, IMPAACTG P1060
Raltegravir, Isentress®	PK in newborns; ≥4 wks 6 mg/kg twice daily	Hyperbilirubinemia	IMPAACT P1066 IMPAACT P1097

PI = premature infant; SD = single dose; (P)ACTG = (Pediatric) AIDS Clinical Trials Group; HIV-NAT = HIV-Netherlands Australia Thailand Research Collaboration; GI = Gastrointestinal side effects; GW = gestation week
Reference: Except for AZT in mature newborn infants, the dosage is from the studies. Antiretroviral agents that are not approved should not be used in neonates (except for clinical studies)

Further studies for HIV prevention in neonates

A survey of studies of the pharmacokinetics in pregnancy and neonates is listed in Table 7 (Ronkavilit 2001+2002, Blum 2006, Chadwick 2008, Hirt 2009a+b, Mirochnik 2005+2014). In order to continuously improve ART during pregnancy and the prophylaxis of perinatal HIV infection, a thorough documentation of clinical data is necessary. In the US, the Antiretroviral Pregnancy Registry is an extensive therapy register that helps to evaluate the potential teratogenicity of antiretrovirals on the basis of case reports of HIV-exposed neonates:
Antiretroviral Pregnancy Registry, Research Park, 1011 Ashes Drive, Wilmington NC 28405. Phone 1-800-258-4263, Fax 1-800-800-1052. For UK, Germany, France 0800-5913-1359, Fax 00800-5812-1658. Contact: www.apregistry.com/contact.htm

General references: US (CDC 2014) and European (EACS 2014) Guidelines. Detailed and continuously updated recommendations can be found on the internet at www.AIDSinfo.nih.gov or http://www.europeanaidsclinicalsociety.org/index.php?option=com_content&view=article&id=59&Itemid=41.

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21. HIV and Wanting to be a Parent

ULRIKE SONNENBERG-SCHWAN, MICHAEL WEIGEL

Introduction

For a growing number of men and women living with HIV/AIDS the perspective of parenthood is an important part in their planning of the future. Procreation without risk, or at very low risk of infection for the uninfected partner or prospective child, is achievable for couples in which one or both partners are HIV-infected. In an increasing number of countries reproductive counselling and/or support is provided to couples affected by HIV.

Procreative options for HIV-affected couples vary from unprotected intercourse to several techniques of assisted reproduction, donor insemination or adoption. In view of the strongly decreased risk of transmission with undetectable viral load, conception via intercourse without condoms has increasingly become an option under certain circumstances.

This has been greatly influenced by the "EKAF Statement" (Vernazza 2008, see also ART chapter) regarding the unlikeliness of HIV transmission while on effective ART. In January 2008, the EKAF (Swiss Commission on AIDS-related issues) stated that physicians could inform their patients about a negligible sexual transmission risk if three conditions are met:

• The HIV-infected patient is on a physician-monitored ART and is adherent
• Plasma viral load has consistently been undetectable for more than 6 months
• No sexually transmitted diseases are present in either of the partners

The statement also emphasized that only the HIV-negative partners can decide for themselves whether they want to stop using condoms with their seropositive partner. The background of the statement includes some longitudinal studies on serodiscordant couples. No infection occurred when the partners were on ART or the viral load in untreated partners was below 1,000 copies/ml (see chapter on Prevention). A retrospective Spanish study (Barreiro 2006) saw no infections in 74 HIV discordant couples (76 pregnancies) who conceived via timed intercourse. All HIV+ partners had a viral load below detection. However, data from couples who did not conceive were not available. This option was discussed prior to the Swiss Statement (Barreiro 2007). With a view to reproductive aspects the Swiss Statement puts on record that insemination with processed sperm is no longer indicated for prevention of HIV transmission if the viral load is below detection.

Studies on the association between viral load in sperm and blood show a high correlation, but data are limited (Kalichman 2008). Viral load in semen or genital secretions does not always correlate with plasma viral load (Pasquier 2009). HIV can sometimes be detected in semen or genital secretions even when viral load in blood plasma is undetectable. Since the publication of the HPTN 052 Study (Cohen 2011) showing a 96% reduction of HIV transmission with immediate use of ART, a growing number of studies has added support to the Swiss statement (Loutfy 2013).

The British fertility guidelines recommend to advise on the negligible risk of transmission to the female partner through unprotected sexual intercourse when the EKAF criteria are met for the HIV+ male partner (National Collaborating Centre for Women's and Children's Health 2012). The French guidelines consider natural conception as a reasonable option for serodiscordant couples with no detectable viral load, recommending self-insemination (when the woman is HIV+) or timed unprotected intercourse (when the man is HIV+) as the safest option (Mandelbrot 2012). The US guidelines (NIH 2014) recommend self-insemination or sperm preparation

techniques coupled with insemination or other reproductive procedures as the safest methods. HIV+ partners are advised to start ART before starting conception procedures. For couples with no access to reproductive services natural conception at ovulation is see as a choice, when the infected partner has no detectable viral load. Pre-exposure prophylaxis (PrEP) is seen as an additional option.

In view of the increasing worldwide access to ART, natural conception is increasingly discussed as a safe option for couples even in resource limited regions (Ong'ech 2012). For HIV+ women with an uninfected partner, self-insemination is a safe and affordable procedure also in these countries (Mmeje 2012).

Natural conception now has become an important issue for many HIV-discordant couples seeking reproductive counselling. The EKAF Statement and current data and the resulting reproductive options should be discussed. Usually, there are significant differences in individual risk estimation and the need for safety. In any case, couples who want to exclude even a minimal risk or who are facing fertility disorders are those who seek counselling. Furthermore it has to be considered that in some seropositive partners the viral load is not effectively suppressed or that they have not started treatment. In these cases insemination with processed sperm – in case of unimpaired fertility – can be the method of choice. The start of ART in patients with low viral load in order to open the option for natural conception also is an option.

The German-Austrian guidelines for diagnostics and treatment of HIV-affected couples (DAIG 2011) suggest the following options:

Fertile HIV-discordant couples, ART, viral load below detectability, no other STIs:
• Intercourse without condom during ovulation
• Intercourse without condom plus PrEP
• Self insemination in case of infection of the female partner
• Intrauterine insemination and sperm processing in case of infection of the male partner

HIV-discordant couples, fertility impaired, detectable viral load or no ART:
• Depending on medical indication, several methods of assisted reproduction. In case HIV+ male partner sperm processing and cryopreservation are advised.

Fertile HIV-concordant couples, undetectable viral load:
• Intercourse without condom
• HIV-concordant couples, fertility impaired, detectable viral load or no ART: Depending on medical indication, several methods of assisted reproduction

Donor insemination is an alternative safe option for a small number of couples, but due to legal restrictions it is only offered in a minority of centers. In the UK, for example, there are no restrictions on donor insemination, whereas in Germany access is limited. In addition, most couples wish for a child that is the biological offspring of both parents. Adoption often is only a theoretical option: HIV infection of a partner often renders this procedure very difficult or even impossible (e.g., in Germany). Egg cell donation might be an option for a small number of women facing severe fertility disorders, but is offered only in some countries (i.e., Spain).

Pre-conception counselling

The counseling of the couple should not only consider extensive information on all reproductive options, but also the psychosocial situation, the importance of a network of social support from family or friends, and planning and perspectives about the future as a family (Nakhuda 2005). A supporting, empathic and accepting mode of counselling is advisable, as couples can feel distressed if their motives for, or entitlement to, parenthood are questioned. The drastically reduced risk of transmission through unprotected intercourse if the viral load is undetectable should be

discussed as well as the individual risk perception and risk management strategies of the couple. In cases where professional psychosocial services are not integrated, cooperation with community organizations or self-help groups is advisable.

If reproductive assistance is planned, financial aspects and possible stress should be discussed as well as doubts or fears. Even with the very low risk of infection, anxieties regarding HIV transmission to the partner might occur (van Leeuwen 2008). The fear of results that might challenge their chance to become parents can also be a burden for couples. Sero-discordant couples need to know that the risk of HIV infection can be minimized, but not excluded completely. HIV+ women have to be informed about the risks of vertical transmission and the necessary steps to avoid it. In any case, couples should know that even using state-of-the-art reproductive techniques, achieving a pregnancy cannot be guaranteed. Table 1 shows the investigations as provided in the current German-Austrian guidelines (DAIG 2011).

Table 1: Pre-treatment investigations

General	Comprehensive medical and psycho-social history
Female exams	Gynecological examination, sonography, tubal patency test (hysterocontrast sonography, if necessary laparascopy) Endocrinological diagnostics (E2, LH, P, DHEAS, FSH, testosterone, SHBG, TSH, AMH) Cervical smear (PAP, chlamydia PCR) (UK: 2-5 FSH/LH and mid-luteal progesterone to evaluate female fertility) Serology (rubella, varicella, TPHA, CMV, HBV, HCV)
HIV-specific assessments	Blood glucose, creatinine, GOT, GPT, GGT, complete blood count Ultrasensitive HIV PCR, CD4/CD8 T cell counts and, if necessary, resistance testing HIV antibody test of the seronegative partner
Male exams	2 spermiograms, in case of pathologic results: semen culture, if necessary, sonography Serology (HBV, HCV; TPHA), urethral smear (GO), chlamydia PCR (urine)

Following the decision to conceive with reproductive assistance, the couple should undergo a thorough sexual health and infection screen, including information about the male partner's HIV status. The possibility of HIV infection in the female partner also has to be excluded. In some cases, it might be necessary to treat genital infections before starting reproductive treatment. Studies have indicated a frequent impairment of the sperm quality of HIV+ men (Duliost 2002, Pena 2003, Nicopoullos 2004, Bujan 2008). A prospective study revealed a significant impairment of sperm motility during ART, even with therapies that were not regarded as particularly mitochondriotoxic (van Leeuwen 2008). Data on the effect of these changes on fertility are limited (Prisant 2010).

After sperm washing and testing for HIV, spermatozoa can be utilized in three different reproductive techniques depending on whether the couples have any additional fertility issues: intra-uterine insemination (IUI), extracorporal fertilization by conventional *in vitro* fertilization (IVF) and intracytoplasmic sperm injection followed by embryonic transfer. According to the German recommendations, the choice of method depends on the results of gynecological and andrological investigations and the couple's preference. The success rate using IUI has been shown to be reduced if the sperm is washed and then cryopreserved before use. This is necessary in some centers where PCR testing of the washed sample for HIV cannot be done on the day of insemination. This, together with the possible impairment of semen quality results in a number of couples being advised to have IVF or ICSI.

Couples should be informed about further important aspects:
- Sperm washing and testing greatly reduce the risk of infection, but cannot exclude it completely. Following recent studies, this risk seems to be only theoretical.
- During treatment, consistent condom use is important, especially when the partner's viral load is not effectively suppressed. HIV infection of the woman in the early stages of pregnancy can increase the risk of transmission to the child.
- Most couples attending European centers have to pay for treatment costs themselves. These depend on the technique applied and range from about € 500 to € 5,000 per cycle. In some countries, couples have cost-free access to treatment.
- Even the most sophisticated techniques cannot guarantee successful treatment.

Following successful reproductive treatment, couples are usually monitored for HIV status for 6-12 months after childbirth, depending on the center.

The safety of sperm washing

The technique of processing sperm from HIV+ men prior to the insemination of their negative partners was first published by Semprini in 1992. The first inseminations with sperm washed free of HIV were carried out in Italy and Germany as early as 1989 and 1991, respectively. Up to mid-2003, more than 1,800 couples had been treated in about 4,500 cycles, applying various techniques of assisted reproduction. More than 500 children have been born with no seroconversion reported in the centers closely following the protocol of washing and testing the sperm prior to assisted reproductive techniques (Bujan 2007).

Native ejaculate mainly consists of three fractions: spermatozoa, seminal plasma and nuclear concomitant cells. The HIV progenome and virus have so far been detected in the seminal plasma, the concomitant cells, and occasionally in immobile spermatozoa. Several studies have indicated that viable, motile spermatozoa are not likely to be a target for HIV infection (Pena 2003, Gilling-Smith 2003).

Motile spermatozoa can be isolated by standardized preparation techniques. After separation of the spermatozoa from plasma fractions and NSC (non-spermatozoa cells), the spermatozoa are washed twice with culture medium and re-suspended in fresh culture medium. Incubation for 20–60 minutes allows motile sperm to "swim up" to the supernatant. To be more certain that it is not contaminated with viral particles, an aliquot of the sample should be tested for HIV nucleic acid using highly sensitive detection methods (Weigel 2001, Gilling-Smith 2003, Pasquier 2006).

Depending on the method, the limit of detection is 10 copies/ml. After having studied the effectiveness of several methods of sperm processing, Anderson (2005) concluded that the combination of gradient density centrifugation and swim-up allows a 10,000-fold decrease of HIV-1 concentration in sperm. Since HIV could theoretically remain undetected, sperm washing is currently regarded as a very effective risk reduction, although not risk-free.

Most of the European centers that offer assisted reproduction to HIV-discordant couples are part of the CREATHE network, which aims to optimize treatment and safety of the methods as well as to compile an extensive database. Compiled data from several centers hint on the safety and reliability of sperm washing (Bujan 2007).

Pre-Exposure Prophylaxis (PrEP)

Even before the FDA approval of Truvada® as the first antiretroviral agent for the prevention of HIV transmission through sexual intercourse, PrEP before periovulatory unprotected intercourse was an option for serodiscordant couples in some countries. Couples abstain from condom use only during the woman's fertile days.

Preconditions are an effectively suppressed viral load, the exclusion of sexually transmitted diseases, and unimpaired fertility status of both partners. Data from Switzerland and Germany shows high acceptance in couples. No case of HIV transmission has been reported in 53 couples, the pregnancy rate was 75% (Vernazza 2011). A growing number of studies shows the feasibility of this approach, especially in resource-limited settings (Adenji 2013, Whetham 2013). The fertility of HIV-negative men does not seem to be impaired by taking PrEP (Were 2014). Up to now, there is no evidence that PrEP further reduces the already negligible risk of infection when the viral load of the HIV+ partner is effectively suppressed. Nevertheless, some couples prefer this option because it increases their feeling of safety.

Female HIV infection

For many HIV+ women having a child now is an important part of planning for the future (Fiore 2008, Loutfy 2009). In France 32% of the HIV+ women of reproductive age want to become mothers (Heard 2007).

Treatment and care during pregnancy should be carried out according to the prevailing national or international guidelines (Fakoya 2008, DAIG 2011, Loutfy 2012). In some European countries reproductive options for women with unimpaired fertility include natural conception on the basis of the EKAF Statement as well as self-insemination, while self-insemination is still seen as the safest procedure.

Couples who decide for natural conception should undergo screening to exclude STDs. The transmission risk might be further reduced when the intercourse without condoms is limited to the time of ovulation. Women should be advised on the importance of adherence and regular checks of the viral load (Fakoya 2008).

If a woman is not taking ART, the viral load is not successfully suppressed, or concerns about the remaining risk are strong, self-insemination may be the method of choice. In some cases, ovarian stimulation may be advisable. This, however, requires highly qualified supervision to avoid multiple gestations.

It is important to time ovulation accurately (i.e., by use of computer-based ovulation kits or urine sticks). A simple inexpensive way of determining whether the cycles are ovulatory, helpful in women who have regular cycles, is a basal temperature chart beginning about three months before the first self-insemination.

At the time of ovulation, couples can either have protected intercourse with a spermicide-free condom and introduce the ejaculate into the vaginal cavity afterwards, or the ejaculate can be vaginally injected using a syringe or applied with a diaphragm or portio cap. Thus the conception remains within the private sphere of the couple.

After 6–12 months of unsuccessful self-insemination, the couple should have further fertility investigations with a view to assisted conception. Should the couple experience problems with self-insemination, intrauterine insemination (IUI) can be considered. HIV-specific and infective diagnostics are recommended. If no pregnancy has occurred over a period of 6–12 months (or earlier, if the couple so wishes) fertility diagnostics should be carried out (Table 1). If there are indicators of reduced fertility in one or both partners, fertility diagnostics might be carried out at an earlier stage in the counselling process.

Fertility disorders

In some cases, women will only be able to conceive by intercourse without condom or self insemination. Dependent on the fertility status of both partners, IVF and ICSI can be considered as methods of choice.

Fertility disorders in HIV+ women seem to have a higher prevalence than in an age-matched negative population (Ohl 2005, Gingelmaier 2010) and might lead to a

lower success rate of assisted reproduction (Coll 2006) although data show some conflicting results. Reasons might be infection of the upper genital tract (Sobel 2000), surgery due to cervical intraepithelial neoplasia (Gilles 2005) or a depletion of mitochondrial DNA in the oocytes (Garrabou 2006, Lopez 2008).

Data reported from a program in Strasbourg indicated infertility problems in most HIV+ women. IVF and ICSI were far more effective than IUI (Ohl 2005). In the Barcelona program, Coll (2006) observed a decreased pregnancy rate after IVF compared to age-matched HIV-negative controls and HIV+ women who received donated oocytes. Results indicated a decreased ovarian response to hyperstimulation. A slightly impaired ovarian response to stimulation during 66 ICSI cycles in 29 HIV+ women was also described by Terriou (2005). Martinet (2006) found no difference in ovarian response between HIV+ and HIV-negative women in Brussels.

Data concerning a possible association between ART and fertility disorders in women is limited (van Leeuwen 2006).

Although assisted reproduction for seropositive women with fertility disorders is offered in centers in various European countries as well as the US, access to assisted reproduction often is still more limited for women than for men.

HIV infection of both partners

A growing number of HIV-concordant couples are now seeking reproductive counseling. In some centers, these couples are also accepted for reproductive treatment in case of fertility disorders. If both partners are on effective ART and there are no fertility disorders present, timed unprotected intercourse can be the method of choice. The discussion pertaining to the transmission of mutated drug-resistant virus between partners is still ongoing. Following a recent review (Redd 2013), the transmission rate is higher than previously assumed, showing an incidence rate of up to 7.7%. Effective ART plays an important role here.

Couples should be offered the same range of fertility counseling and screening as HIV-discordant couples. The current health of each partner should be carefully evaluated with a full report from their HIV physician.

Psychosocial aspects

Experiences from more than a decade of counselling show the importance of offering professional psychosocial support to couples planning to conceive, especially if reproductive assistance is necessary. Accepting the desire to become parents and dealing with the underlying motives as well as the psychosocial situation in an empathic way enables couples to see obstacles as well as to develop alternative perspectives if this wish cannot be realized.

Frustration, strains and disappointment may accompany unsuccessful treatment cycles or premature termination of pregnancy. Psychiatric co-morbidities in one or both partners (i.e., substance abuse, psychoses) can be reasons to at least postpone treatment. Professional diagnosis and support is necessary in these cases.

Often, the central importance of the wish for parenthood of many migrant couples is overlooked in the medical and psychosocial counselling system. Language or communication difficulties on both sides, ignorance of different cultural backgrounds and lack of acceptance of other life-styles can lead to feelings of discrimination, isolation, helplessness or despair in couples.

Issues concerning the welfare of the child should be openly discussed during reproductive counselling. Many couples are concerned about a potential negative effect of antiretroviral drugs on their offspring. Severe impairment of the health of the prospective parents might lead to concerns for the future well-being of the child.

The future

Healthcare professionals are encountering a growing number of couples or individuals who are contemplating parenthood. Having a child is an expression of a fulfilled partnership and an important perspective in life. This is no less true in couples with HIV/AIDS. In the medical and psychosocial care of patients, it is important to create an environment where reproductive aspects and parenting can be discussed on an open and non-judgemental basis. Worldwide, there is a growing demand to establish reproductive health services, to support sexual rights of people living with HIV/AIDS and to provide reproductive counselling and assistance.

Recent data has encouraged a growing number of health care professionals in many countries to discuss natural conception as an option for HIV+ men and women with suppressed viral load. In most centers, sperm washing, self insemination and assisted reproduction are only recommended in the presence of fertility disorders, or detectable viral load. Future priorities include continued reporting of data pertaining to the applied methodologies as well as to the outcomes, reporting of adverse results and the follow-up of couples (Giles 2005). Steps towards optimizing semen-processing procedures, namely quality control of virus detection in processed sperm and laboratory safety, have already been taken (Pasquier 2006, Gilling-Smith 2005, Vitorino 2011).

Long-term outcomes in couples that receiving reproductive assistance, health outcomes among children, both in medical as well as in psychosocial terms, and consensus regarding best practice or surveillance of care provided by clinics have received little notice up till now. Many couples cannot afford the high costs of treatment, or travel long distances, sometimes even to other countries, to reach specialized units. There is an urgent need to develop strategies for the counseling and financial support of these couples in cases where natural conception is not possible or not advisable.

The use of donated oocytes in reproductive services for HIV+ women (Coll 2006) is limited in several countries due to legal and ethical considerations. It even enables treatment of women who have reached an age where reproductive assistance is not usually offered anymore due to the high risk of miscarriages and malformation and the low success rate of assisted reproduction techniques.

Medical and technical progress has opened a wider range of options, but aside from comparing higher or lower success rates, there is an urgent need to discuss psychological and psychosocial issues pertaining to the welfare of parents and child.

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22. Antiretroviral Therapy in Children

TIM NIEHUES

Characteristics of HIV infection in childhood

In 2014, the UNAIDS report estimated that worldwide 2.6 million children are living with HIV while another 150,000 died from it (http://www.unaids.org/).

Children are usually infected through perinatal transmission (vertical infection). In most cases (75–90%) HIV is transmitted peri- or intrapartum. Only a small proportion of children are infected *in utero* (10–25%). Transmission by breastfeeding is more common in resource-limited settings, but plays a minor role in developed countries, where breastfeeding by HIV+ mothers is strongly discouraged. The increasing knowledge about how HIV is vertically transmitted has led to highly effective interventions to prevent transmission and a significant reduction of the transmission rate to less than 2%. New infections in HIV-exposed children still occur

• if the HIV status of the mother is unknown

• if transmission prophylaxis is incomplete

• if the mother does not have access to transmission prophylaxis during pregnancy.

Without ART there is a rapid progression of the HIV infection with AIDS-defining symptoms and potentially lethal complications in a significant percentage of infants (10–25%). In the remaining children there is a much slower disease course with a mean duration of more than 8 years until AIDS-defining symptoms occur. The reason for this bimodal disease course is unclear, but the high mortality in infants (<1 year of age) has influenced the ART guidelines which strongly recommend to aggressively treat all HIV+ infants once they are diagnosed.

Viral dynamics in children are significantly different from the rapid increase and decrease of viral load seen in untreated adults within a few months of acute HIV infection. This reflects both the rapid somatic growth of the lymphatic system favoring viral spread and a less effective anti-HIV immunity in children as compared to adults (Figure 1).
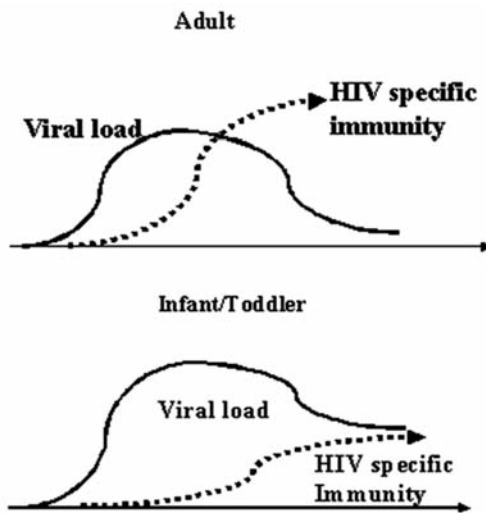

Figure 1: Differences in the natural course of HIV in the first months after infection/transmission of viral load and HIV immunity between adults and infants/toddlers

Table 1: 2007 WHO HIV Pediatric Classification System: Immune categories based on age-specific values. See also http://www.who.int/hiv/pub/guidelines

HIV-associated immunodeficiency	Age-related CD4 T-cell values, relative (%) or absolute (cells/µl)			
	<11 months	12–35 months	36–59 months	>5 years
None or not significant	>35%	>30%	>25%	>500/µl
Mild	30–35%	25–30%	20–25%	350–499/µl
Advanced	25–29%	20–24%	15–19%	200–349/µl
Severe	<25%	<20%	<15%	<200/µl or <15%

When assessing the immune system in infants and children, it is very important to compare the child's CD4 T cell count with the age-appropriate values (e.g., the mean CD4 T cell count for a 6-month-old baby is 3.0×10^9/l). Lymphocyte counts are very high in infancy and decline to adult levels after the age of 6 (Table 1).

In adults typical manifestations of the acute HIV seroconversion illness include fever, sore throat, lymphadenopathy and a mononucleosis-like disease. HIV seroconversion illness has not been described in perinatally-infected children. Symptomatic disease presenting in childhood has been classified according to severity of symptoms (Table 2) (http://www.who.int/hiv/pub/guidelines/art/en/).

If antiretroviral therapy in children is effective, opportunistic infections (OIs) become a rarity. However, in children who newly present with HIV (e.g., if HIV status in the mother is unknown and there was no transmission prophylaxis) opportunistic infections are observed and quickly put the infant at risk.

Diagnosis of HIV infection in children

A direct method of detecting HIV is necessary: the identification of HIV by RNA or DNA PCR is highly sensitive and specific. High titers of IgG are transferred transplacentally from mother to child. Maternal antibodies can be detected in children up to the age of 18 months or even longer. Thus, in infants, the detection of HIV antibodies does not prove infection.

Cord blood is not useful for diagnosis because it contains maternal cells which cause a false positive PCR test result. Within the first 48 hours after birth, 62% of all infected infants are still HIV PCR negative. Even 4 weeks after birth, 11% of the infections are still not detectable by PCR (Dunn 1995, Burgard 2012). PCR tests become reliable only after about 3 weeks after birth. Once a positive HIV PCR is found, a second independent blood sample should be taken as soon as possible. As diverse subtypes exist, it is advised to test paired samples from mother and infant by HIV PCR. If in doubt, expert advice should be sought but initiation of ART should not unduly be delayed. The disappearance of maternal IgG antibodies to HIV needs to be documented before HIV infection can be definitely excluded in the child. Tests with an increased sensitivity to detect HIV antibodies are not useful as they may detect maternal antibodies up to 28 months of age leading to anxiety and confusion in the affected families (Nastouli 2007). In the absence of breast-feeding two separate negative HIV PCRs (at least 2 weeks after cessation of post-exposure prophylaxis) are required to confirm that the child is not infected. Always keep in mind that babies can get infected after initially negative tests, if they are breast-fed (which the doctor may be unaware of). A negative HIV test in the mother early in pregnancy should not preclude testing the child, as the rate of mother to child transmission is high if the mother becomes infected later in pregnancy or during breastfeeding. In children older than 18 months, HIV infection is diagnosed in an analogous way to adults (see chapter on *HIV Testing*).

Table 2: WHO clinical staging for children with confirmed HIV infection,
http://www.who.int/hiv/pub/guidelines/

Clinical stage 1
• Asymptomatic
• Persistent generalized lymphadenopathy

Clinical stage 2
• Unexplained persistent hepatosplenomegaly
• Papular pruritic eruptions
• Fungal nail infection
• Angular cheilitis or lineal gingival erythema
• Extensive wart virus infection or molluscum contagiosum
• Recurrent oral ulcerations
• Unexplained persistent parotid enlargement
• Herpes zoster
• Recurrent or chronic upper resp. tract infections (otitis, otorrhea, sinusitis or tonsillitis)

Clinical stage 3
• Unexplained moderate malnutrition or wasting not responding to standard therapy
• Unexplained persistent diarrhea (14 days or more)
• Unexplained persistent fever (above 37.5°C intermittent or constant, >one month)
• Persistent oral candidiasis (after first 6–8 weeks of life)
• Oral hairy leukoplakia
• Acute necrotizing ulcerative gingivitis or periodontitis
• Lymph node or pulmonary tuberculosis
• Severe recurrent bacterial pneumonia
• Symptomatic lymphoid interstitial pneumonitis
• Chronic HIV-associated lung disease including bronchiectasis
• Unexplained anemia (<8 g/dl), neutropenia (<1000/µl), thrombocytopenia (<50,000/µl)

Clinical stage 4
• Unexplained severe wasting/malnutrition not responding to standard therapy
• Pneumocystis pneumonia
• Recurrent severe bacterial infections (such as empyema, pyomyositis, bone or joint infection or
  meningitis, excluding pneumonia)
• Chronic herpes simplex infection (orolabial or cutaneous of more than one month's duration or
  visceral at any site)
• Esophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
• Extrapulmonary tuberculosis
• Kaposi sarcoma
• CMV: retinitis or infection affecting another organ, with onset at age >one month
• Central nervous system toxoplasmosis (after one month of life)
• Extrapulmonary cryptococcosis (including meningitis)
• HIV encephalopathy or progressive multifocal leukoencephalopathy
• Disseminated endemic mycosis (coccidiomycosis or histoplasmosis)
• Disseminated non-tuberculous mycobacterial infection
• Chronic cryptosporidiosis (with diarrhea) or isosporiasis
• Cerebral or B-cell non-Hodgkin lymphoma
• Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

When to initiate ART

Children under 1 year of age

A randomized study in 377 infants under the age of 3 months in the South African CHER study (<u>C</u>hildren with <u>H</u>IV <u>E</u>arly Anti<u>R</u>etroviral therapy) examined whether to start directly after diagnosis (AZT+3TC plus lopinavir/r) or defer treatment until symptoms occur or CD4 T cells fall below 25% (Violari 2008). Deferred treatment is associated with a 4-fold higher mortality (16% versus 4%). These data are of fundamental importance as clinical practice and guidelines before this study did not advise to treat all infants.

Children over 1 year of age

Treatment is not an emergency. Many experts defer treatment in asymptomatic children (i.e., with a low viral load and without immunodeficiency). Commencing ART too early risks possible long-term side effects and early exhaustion of the limited supply of antiretroviral drugs that can be safely used in children. Commencing it too late may be associated with irreversible damage to the immune system and a larger viral reservoir throughout the body, complicating future curative treatment approaches (if they should become available).

Viral load and CD4 T cell counts are independent prognostic markers for AIDS or death. A computer program has been generated which can be used to give the risk of progression to AIDS or death within 6 or 12 months according to the age and either CD4 T cell count or viral load in the child (PENTA calculator, www.hppmcs.org). Some updated guidelines are listed here:
• European guidelines: http://penta-id.org
• US guidelines: https://aidsinfo.nih.gov/guidelines
• WHO guidelines for resource-poor settings: www.who.int/hiv/pub/guidelines/

A further simplification/harmonization of the pediatric guidelines can be expected as the results of the START trial in adults showed that initiation at 500 CD4 T cells/µl is superior to starting at 350 cells/µl (InsightStartStudyGroup 2015) and may provide further rationale to provide treatment to all HIV+ children/adults even beyond the first year of life, irrespective of CD4 T cells or viral load.

Table 3: Treatment indication, according to age and clinical, immunological and virological criteria (Bamford 2015)

Age, years		PENTA 2015 guidelines
<1	Start	All
1–3	Start	WHO stages 3,4 CD4 cells ≤1000/µl or <25% Consider all
3–5	Start	WHO stages 3,4 CD4 cells ≤750/µl or <25% Consider if HIV RNA >100,000 or additional indications*
≥5	Start	WHO stages 3,4 CD4 cells ≤350/µl Consider if CD4 ≤500 or HIV RNA >100,000 or additional indications *

*Coinfection with HCV or TB, autoimmune manifestations (e.g., thrombocytopenia), malignancy, growth or puberty delay, neurocognitive delay, prevention of transmission in sexually active adolescents, pregnancy, primary infection (e.g., after nosocomial or sexual transmission), child and family wish to start treatment (following full discussion of risks/benefits)

General considerations for treatment of HIV+ children

The treatment of children with antiretroviral drugs is complex. Successful treatment requires an interdisciplinary approach with the children and their families.

Good adherence is key to treatment success. Education of the child and the family regarding antiretroviral drugs is necessary. Sometimes a brief period of supervision in the hospital at the start of ART is useful to educate the child and family and gauge the tolerability of the regimen. In the prospective PACTG 377 study, adherence was defined as having not missed a single medication dose over the previous 3 days. According to this definition, only 70% of 125 children were found to be adherent within an observation period of 48 weeks (Van Dyke 2002). The modalities of the daily intake of medication need to be discussed in detail and adjusted to the daily and weekly routines of the family. Adherence is particularly problematic in adolescence, and successful reduction or control of viral load may only be achieved in a third of these patients (Ding 2009). In this age group, adherence often needs close follow-up including other health care professionals such as psychologists and social workers. In a meta-analysis, peer support and home-based nursing were shown to improve ART adherence (Bain-Brickley 2011). Sometimes (planned) periods off ART, despite the theoretical risk of clinical progression, have to be accepted in this group of patients. The BREATHER trial by the PENTA group in HIV+ adolescents (in whom virus was very well controlled) compared a 5-day short cycle to regular 7-day treatment. In this small study a short-cycle treatment was safe (www.ctu.mrc.ac.uk/our_research/research_areas/hiv/studies/breather/). Another promising approach to increasing adherence in children and adolescents is the use of once-daily regimens (e.g., the future PENTA 20 trial).

Underdosing by the doctor has been shown to be a problem in daily practice (Menson 2006). Dosing by weight instead of body surface area (given as an alternative in some older guidelines) may result in underdosing and ongoing growth may not be adjusted for. Particular genotypes are associated with hypermetabolism of NNRTIs and PIs.
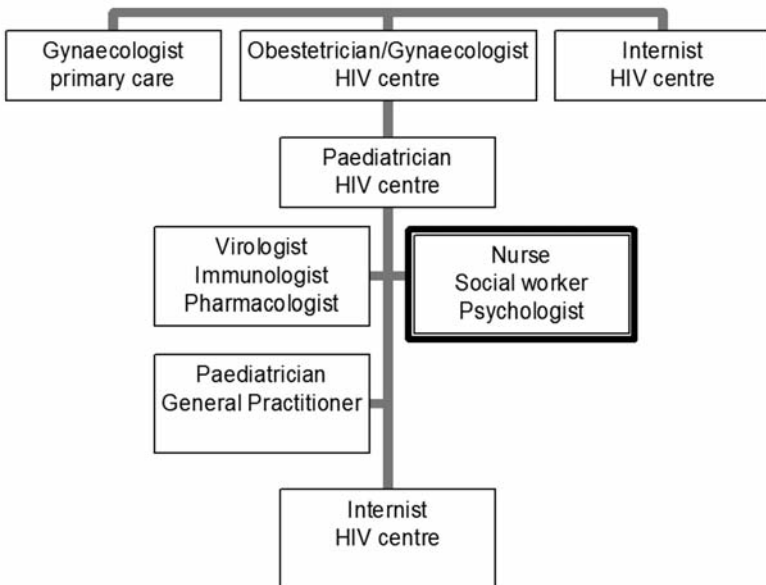

Figure 2: Interdisciplinary care for children and families who are affected by HIV

Plasma levels of NNRTIs and PIs can be measured (therapeutic drug monitoring, TDM) to detect inter-individual differences in drug metabolism and lack of adherence, to check on dosages that may be too low or to prevent toxicities from too high a dosage (Fletcher 2009).

Treatment strategy

At present, eradication of HIV cannot be achieved. In some children viral load remains below detection for years and subsequently there are no HIV-specific antibodies detectable but ultrasensitive assays still detect HIV.

The decision to start ART has fundamental consequences for the children and their families. From this point on it usually means that children will take the medication for life. A retrospective analysis of unplanned treatment interruptions in children demonstrated a significant decline of CD4 T cell percentages by 6.6% per year (Gibb 2004). In the randomized PENTA 11 trial of CD4 guided, planned treatment interruptions, there were no serious negative clinical outcomes. Younger children had better CD4 T cell recovery after treatment interruptions (PENTA 11). However, there are insufficient data on the long-term effects to recommend this strategy.

Table 4: Recommended first-line ART (without HBV or TB coinfection) (Bamford 2015)

	<1 year	1–3 years	3–6 years	6–12 years	>12 years
3rd Agent	LPV/r NVP	LPV/r NVP	LPV/r EFV	ATV/r EFV	ATV/r DRV/r EFV
Preferred Backbone	ABC[1]/3TC (+AZT if NVP)[3]	ABC[1]/3TC (+AZT if NVP and CNS involvement or high VL)[2]	ABC[1]/3TC	ABC[1]/3TC	TDF/FTC[4] ABC[1]/3TC (if VL<10[5])
3rd Agent	-	-	NVP DRV/r	NVP LPV/r DRV/r	NVP LPV/r RAL[6] DTG
Alternative Backbone	AZT[5]/3TC	AZT[5]/3TC	AZT[5]/3TC TDF/3TC(FTC)	AZT[5]/3TC TDF/3TC(FTC)	ABC[1]/3TC

[1] HLAB*5701 testing prior to abacavir. If positive, ABC should not be prescribed
[2] In children <3 years consider adding AZT to NVP-based regimen if very high VL or CNS involvement until VL suppressed for at least 3 months
[3] Four-drug induction for infants on NVP-based therapy may be considered until VL suppressed for at least 3 months, followed by 3-drug maintenance therapy
[4] TDF/FTC is preferred in older children with VL >100,000 copies/ml. Some clinicians would advocate deferring the use of TDF until after puberty
[5] AZT should be avoided if possible apart from the indications described above
[6] In rare instances (transmitted resistance, toxicity) RAL in children <12 years of age

Table 4 shows the current treatment concepts for choosing antiretroviral drug combinations. It appears useful to start with a combination that includes two classes (2 NRTIs plus a PI or an NNRTI) in order to spare one or two classes for future changes of ART and to minimize toxicity. In children >12 years integrase strand transfer inhibitor-based ART may be an alternative. As there are only small numbers of children and adolescents with HIV in Europe (after introducing successful transmission prophylaxis) it is highly recommended to include all children in multicenter clini-

cal trials (e.g., PENTA, http://www.pentatrials.org, Dr. Diana Gibb or Lynda Harper, Phone: + 44 20 7670 4825).

The randomized PENPACT 1 study with participation both of the PENTA and the PACTG groups has answered the question of whether initial therapy in children is more effective with 2 NRTIs and a PI or an NNRTI (n=263). There was no significant difference concerning viral load reduction over the observation period of five years (Penpact-1 Study Team 2011). The poor taste of boosted PIs precludes their use in young children. Transmitted viral resistance (from the mother) remains rare in children. Still, pretreatment resistance genotyping should be done.

Classes of antiretrovirals

Drugs of all antiretroviral classes can lead to nausea, vomiting, fever, headache, diarrhea, liver dysfunction, rash (sometimes severe) and anorexia. There is significant hyperlipidemia in a number of children and its long term consequences are unknown (Jacobson 2011). Some investigators found subclinical artherosclerosis and a high rate of coronary artery abnormalities in adolescents and young adults with long term ART exposure (Mikhail 2011). As with adults, dyslipidemia is associated with the use of PIs (Lainka 2002). This includes elevated total cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) and decreases in high density lipoprotein cholesterol (HDL-c). In lipodystrophy, there is a loss of subcutaneous fat (lipoatrophy) and/or a deposition of fat tissue subcutaneously or in visceral stores (lipohypertrophy). There are no clear diagnostic criteria. Studies from South Africa, Tanzania and Uganda estimate prevalence on ART as between 8–30% (Piloya 2012, Arpadi 2013, Kinabo 2013). Lipodystrophy and dyslipidemia coexist, and their interconnection is unclear. Other classes such as NRTIs (e.g., d4T) and NNRTIs (efavirenz, not nevirapine) also play a role in the pathogenesis of lipodystrophy.

Insulin resistance is another side effect that may present with or without fasting hyperglycemia, with new onset diabetes mellitus and exacerbations of pre-existing diabetes (Bitnun 2005). Moreover, PIs may influence bone mineral density and metabolism (Mora 2004). Taken together, the long-term consequences of PI-containing ART for growth and development of the child are currently not known. In the Swiss cohort, children exposed to PIs over a period of more than 10 years did not experience any major side effects (Rudin 2008).

NRTIs

The combination of 2 NRTIs as part of ART is effective and well-tolerated. For older children there are fixed dose combinations (see below). Severe side effects are rare but potentially life-threatening, such as lactic acidosis and hepatic steatosis. Neuromuscular dysfunction, cardiomyopathy, pancytopenia, pancreatitis and neuropathy are probably related to mitochondrial toxicity caused by NRTIs. On a laboratory level, mitochondrial toxicity is also seen in HIV+ pregnant women and their infants. Although it is uncertain whether this altered intrauterine metabolic programming poses a risk for their future health . Due to pharmacologic and antiviral antagonism as well as synergistic neurotoxicity, the following combinations are not recommended: AZT+d4T, ddI+TDF and FTC+3TC. The prevalence of lipoatrophy in children is unknown, as diagnostic criteria are not established. Whenever possible, d4T should be replaced by ABC or TDF.

Dosing of ART in children is done according to WHO weight bands (sometimes by age group) (http://www.who.int/hiv/paediatric/generictool/en/). Weight bands or age groups are shown in brackets.

Zidovudine (ZDV, AZT, Retrovir®) is available as syrup, capsules, tablets and concentrate for injection or intravenous infusion. Child dosing for liquid is: (4–9 kg): 12 mg/kg BID; (9-30 kg): 9 mg/kg BID; (≥30 kg): 300 mg BID; Child dosing for capsules is: (8–13 kg): 100 mg BID; (14–21 kg): 100 mg a.m. + 200 mg p.m.; (22–30 kg): 200 mg BID; (≥30 kg): 300 mg BID; Adult dosing is: 300 mg BID; for very sick children with gut failure, intravenous dosing of 120 mg/m² can be used. Maximum dosage is 300 mg every 12 hours.

Lamivudine (3TC, Epivir®) is available as oral solution and tablets. Child dosing (≥3 months) for liquid is: 4 mg/kg BID or 8mg/kg QD (max dose 300 mg per day). Well tolerated round up doses; Child dosing for tablet (150 mg) is : (≥3years): (14–21 kg): ½ tablet BID or 1 tablet QD; (>21–30 kg): ½ tablet a.m. + 1 tablet p.m. or 1½ tablet QD; (>30 kg): 1 tablet BID or 2 tablet QD; Adult dosing is: (≥12 years): 150 mg BID or 300 mg QD. In older children and adolescents (>35 kg body weight) fixed-dose combination with AZT (Combivir®) or abacavir (Kivexa®/Epzicom®) can be used. In adults, 3TC has antiviral activity against hepatitis B virus (HBV). In HIV-negative children with chronic hepatitis B early initiation of 3TC appears to achieve a high HBe and HBs conversion rate (Choe 2007). There are no data in HBV-coinfected children, and there is concern that using 3TC as the only drug active against HBV in dually infected children may select for 3TC-resistant HBV. In the PENTA 15 study the pharmacokinetics, feasibility and acceptability of dosing ABC or ABC+3TC QD in children aged 3 months to <36 months was studied. The AUC for QD dosing of both ABC and 3TC was bioequivalent to BID.

Didanosine (ddI, Videx®) is available as oral solution and tablets. It is not recommended for first-line therapy any more. Children on ddI should switch to a less toxic NRTI.

Abacavir (ABC, Ziagen®) is available as oral solution and tablets. Child dosing is: (≥3 months): 8 mg/kg BID or 16 mg/kg QD (max dose: 600 mg per day). Well tolerated round up doses; Child dosing for tablet (300 mg) is: (14–21 kg): ½ tablet BID or 1 tablet QD; (>21–30 kg): ½ tablet a.m. + 1 tablet p.m. or 1½ tablet QD; (>30 kg): 1 tablet BID or 2 tablets QD; Adult dosing is: (≥12 yrs): 300 mg BID or 600 mg QD in combination with 3TC (see PENTA 15 Trial above). In the PENTA 5 trial, the NRTI backbone of ABC+3TC showed better efficacy regarding viral load suppression than AZT+ABC and AZT+3TC. There is a potential risk of a hypersensitivity reaction (HSR). If ABC HSR occurs and the drug is stopped, it should never be restarted as, rarely, deaths have occurred in adults upon rechallenge. HLA B*5701 is associated with HSR, and should be tested before prescribing ABC. HLA B*5701 positive children should not receive abacavir.

Emtricitabine (FTC, Emtriva®) is available as capsules and oral solution. Child dosing for liquid is: (≥4 months): 6 mg/kg QD (max dose 240 mg OD); Child dosing for capsules is: (≥33 kg): 200 mg QD; Adult dosing is: capsule (≥33 kg): 200 mg QD; oral solution: 240 mg QD. The administration of capsules results in a 20% higher plasma level. Reduction in dosage is necessary in patients with renal impairment. There are no controlled trials regarding efficacy in children.

Tenofovir (TDF, Viread®) is currently available as 150/200/250 mg (tenofovir disoproxil fumarate 123/163/204 mg) (white) and 300 mg (245 mg) (blue) tablets and als granules TDF 40 mg/1g (33 mg/g TDF) (1g = 1 scoop). All doses are based on TDF. Child dosing for granule (1 scoop (scp) = 40 mg) is: (≥2 yrs) 8 mg/kg QD: (10–12 kg): 2 scp QD; (12–14 kg): 2.5 scp QD; (14–17 kg): 3 scp QD; (17–19 kg): 3.5 scp QD;

(19–22 kg): 4 scp QD; (22–24 kg): 4.5 scp QD; (24–27 kg): 5 scp QD; (27–29 kg): 5.5 scp QD; (29–32 kg): 6 scp QD; (32–34 kg): 6.5 scp QD; (34–35 kg): 7 scp QD; (≥35 kg): 7.5 scp QD; Child dosing for tablet (150, 200, 250, 300 mg) is: (≥2 yrs) (17–22 kg): 150 mg QD; (22–28 kg): 200 mg QD; (28–35 kg): 250 mg QD; (≥35 kg): 300 mg QD; Adult dosing is: (≥35 kg) 300 mg QD. It should be taken with meals. There are no controlled trials on the efficacy of tenofovir in children. Tenofovir has been shown to have metabolic, renal and bone side effects that may be significant for children and should be monitored closely. Tenofovir is also effective for treatment against HBV. In HBV-coinfected children who require treatment for HIV, a backbone of TDF+FTC (see fixed dose combinations below: Truvada®) should be considered as this will be effective against both viruses.

Stavudine (d4T, Zerit) is not recommended any more for first-line therapy as it has a high risk of causing lipoatrophy.

NNRTIs

NNRTIs have a low genetic resistance barrier. Suboptimal dosing or adherence can lead to cross-class resistance mutations within a few weeks, affecting all available NNRTIs. NNRTIs exist in palatable liquid preparations that are easier for children to tolerate than the liquid PI solutions. NNRTIs are contraindicated in severe hepatic impairment.

Efavirenz (EFV, Sustiva®, Stocrin®) is available as capsules, tablets and oral solution. Dosing is QD. Child dosing for liquid is: (≥3–5 years): (13–15 kg): 360 mg, (15–20 kg): 390 mg, (20–25 kg): 450 mg, (25–32 kg): 510 mg; (≥5 years): (13–15 kg): 270 mg, (15–20 kg): 300 mg; (20–25 kg): 360 mg; (25–32.5 kg): 450 mg, (32.5–40 kg); 510 mg, (≥40 kg): 720 mg.
Child dosing for capsules: (≥3 years): (13-15 kg): 200 mg, (15–20 kg): 250 mg, (20–25 kg): 300 mg, (25–32.5 kg): 350 mg, (32.5–40 kg): 400 mg, (≥40 kg): 600 mg; Adult: (≥40 kg): 600 mg (liquid:720 mg). It should be taken on an empty stomach before bedtime. High fat meals should be avoided. When using the solution, a 20% higher dosage than for capsules or tablets is necessary. Upon standard dosage EFV serum levels vary considerably in African children due to polymorphisms in the CYP2B6 drug metabolizing enzyme (Fillekes 2011). Central nervous system symptoms (somnolence, insomnia, abnormal dreams, confusion, abnormal thinking, lack of concentration, amnesia, agitation, depersonalization, hallucinations, euphoria) appear to be more common in adults than in children. Skin rash is observed in <10%. It is rarely severe and usually disappears within days despite continuation of efavirenz. Efavirenz may cause raised lipids in some patients.

Nevirapine (NVP, Viramune®) is available as immediate release tablets, as suspension and as extended release tablets. Child dosing for immediate release formulations (body surface area BSA) is: 150–200 mg/m² QD for 14 days (max 200 mg/day), then 150–200 mg/m² BID (max 400 mg/day) if no rash or LFTs abnormalities; Child dosing for immediate release formulations (bodyweight) is: 4 mg/kg QD for 14 days (max 200 mg/day), then (<8 years) 7 mg/kg BID or (≥8 years) 4 mg/kg BID (max 400 mg/day) if no rash or LFTs abnormalities; Child dosing for extended-release tablets (≥3 years) (body surface area BSA) is: (0.58–0.83 m²) 200 mg QD, (0.84–1.16 m²): 300 mg QD, (≥1.17 m²): 400 mg QD (all patients must initiate therapy with immediate-release formulations for 14 days); Adult dosing is: 200 mg QD for 14 days then increase to 200 mg BID or 400 mg QD if no rash or LFT abnormalities. The most common side effect is a skin rash. It occurs in up to 16% of children during the first weeks of treatment, may be quite severe (8%) and require hospitalization.

Life-threatening complications (Stevens-Johnson Syndrome, toxic epidermal necrolysis) are rare. Hepatotoxicity may also occur, and fatal cases have been reported in adults, but this appears to be less common in children.

Etravirine (ETV, Intelence®) is available as 200, 100 mg tablets and 25 mg tablets through compassionate use. The tablets are dispersed in water. Etravirine is taken with food. The AUC is decreased by 50% if it is taken on an empty stomach. Child dosing is: (≥6 years): (16–20 kg): 100 mg BID, (20–25 kg): 125 mg BID, (25–30 kg): 150 mg BID, (≥30 kg): 200 mg BID; Adult dosing is: (≥30 kg) 200 mg BID. Investigational adult dose: 400 mg QD. Side effects are pruritis and rash. The rash usually resolves in 1–2 weeks. Etravirine may be effective against HIV with some NNRTI resistance mutations, but is not used broadly due to the lack of a pediatric formulation, lack of pediatric pharmacokinetic data, lack of efficacy or safety data in children, and lack of data in antiretroviral-naïve patients.

Rilpivirine (RPV, Edurant®, also in **Complera®)** is not yet licensed in children.

PIs

All PIs can be used in combination with 2 NRTIs. PIs differ from each other in respect to their tolerability and side effects. All PIs should be boosted with ritonavir, which increases plasma concentrations of the therapeutic PI.

Lopinavir/r (LPV/r, Kaletra®) is a co-formulation of lopinavir and ritonavir, in which ritonavir acts as a pharmacokinetic enhancer (booster). It is available as 200/50 mg tablets (lopinavir/r), 100/25 mg tablets or 133.3/33.3 mg capsules in some countries. There is a liquid preparation with an unpleasant taste (5 ml = 400/100 mg). Liquid has to be kept in the fridge, contains 42% ethanol 153 mg/ml and proprylene glycol and is toxic to (premature) neonates. In ART-naive and -experienced children, the combination of LPV/r and NRTI or NNRTI shows a high efficacy (Saez-Llorens 2003, Fraaij 2004). Child dosing for liquid is: (without EFV/NVP): (≥14 days (PMA >42 weeks) -6 months) 16/4 mg/kg or 300/75 mg/m² BID, (≥6 months-18 years): 230/57.5 mg/m² BID or (<15 kg) 12/3 mg/kg BID, (≥15–40 kg): 10/2.5 mg/kg BID (max. 400/100mg BID); (with EFV/NVP): (≥6 months–18 years): 300/75mg/m² BID or (<15 kg) 13/3.25 mg/kg BID, (15–45 kg): 11/2.75 mg/kg BID (max 533/133 mg BID); Child dosing for tablet is: (without EFV/NVP): (15–25 kg or 0.5-0.9 m²): 200/50 mg BID, (25–35 kg or 0.9-1.4 m²): 300/75 mg BID, (>35kg or ≥1.4 m²): 400/100mg BID; (with EFV/NVP): (15–20 kg or 0.5–0.8 m²): 200/50 mg BID, (20–30 kg or 0.8–1.2 m²): 300/75 mg BID, (30–45 kg or 1.2–1.4 m²): 400/100 mg BID, (≥45 kg or ≥1.4 m²): 500/125 mg BID; Adult dosing is: 400/100 mg BID. It should be taken with meals. The dosage needs to be increased by up to 30% when combined with an NNRTI (TDM is useful). Cautious use is advised in patients with hepatic insufficiency.

Fosamprenavir (FPV, Telzir®) is available as 700 mg tablets and 50 mg/ml liquid. Liquid is given with or after food to aid palatability. Tablets are taken without food. Child dosing for liquid is: (≥6 years) (25–32 kg): 18 mg/kg BID+RTV 3 mg/kg BID, (33–38 kg): 18 mg/kg BID+RTV 100 mg BID, (≥39 kg) 700 mg BID+RTV 100 mg BID; Child dosing for tablet is: (≥39 kg) 700 mg BID+RTV 100 mg BID; Adult dosing is: (≥18 yrs and ≥39 kg): 700 mg BID+RTV 100 mg BID or (ARV-naïve) 1400 mg QD+RTV 100 mg QD. Alternatively, it can be given without RTV as booster at a dosage of 30 mg/kg BID (Fortuny 2014).

Ritonavir (RTV, Norvir®) is available as oral solution or capsules. It should be taken with meals. Ritonavir should be exclusively used as a booster for other PIs.

Atazanavir (ATV, Reyataz®) is available as capsules. It should be taken with meals. Omeprazole and other PPIs are contraindicated. Avoid indigestion remedies. ATV is interesting in children because of its once-daily application and somewhat lower incidence of dyslipidemia. Child dosing is: (≥6 years) (15–20 kg): 150 mg QD + RTV 100 mg QD, (20–40 kg): 200 mg QD + RTV 100 mg QD; (≥40 kg): 300 mg QD + RTV 100 mg QD; Adult dosing is: 300 mg QD + RTV 100 mg QD.

Darunavir (DRV, TMC114, Prezista®) is available as 75 mg, 300 mg, 400 mg and 600 mg given with or after food and a liquid formulation. It should be given with food. Child dosing for liquid is: (≥3 years, ≥10 kg): (10–11 kg): 200 mg BID+RTV 32 mg BID, (11–12 kg): 220 mg BID+RTV 32 mg BID, (12–13 kg): 240 mg BID+RTV 40 mg BID, (13–14 kg): 260 mg BID+RTV 40 mg BID, (14–15 kg): 280 mg BID+RTV 48 mg BID, (15–30 kg): 380 mg DRV BID+50 mg RTV BID, (30–40 kg): 460 mg BID+60 mg RTV BID, (≥40 kg): 600 mg BID+100 mg RTV BID; Child dosing for tablets is: (≥3 years): (15–30 kg): 375 mg BID+50 mg RTV BID, (30–40 kg): 450 mg BID+RTV 60 mg BID, (≥40 kg): 600 mg BID+100 mg RTV BID; Adult dosing is: (ART experienced): 600 mg BID + RTV 100 mg BID. No DRV-resistance mutations: 800 mg QD + RTV 100 mg QD.

Fusion and Entry Inhibitors

Enfuvirtide (T-20, Fuzeon®) The drug is injected subcutaneously. Child dosing is: (6–16 yrs): 2 mg/kg BID (max dose 90 mg BID), (11.0–15.5 kg): 27 mg BID, (15.6–20.0 kg): 36 mg BID, (20.1–24.5 kg): 45 mg BID, (24.6-29.0 kg): 54 mg BID, (29.1–33.5 kg): 63 mg BID, (33.6–38.0): 72 mg BID, (38.1–42.5 kg): 81 mg BID, (≥42.6 kg): 90 mg BID; Adult dosing is: (≥16 years): 90 mg BID.
After a two-year treatment duration only 6 of 14 children stayed on this therapy (Church 2004) . Reasons for treatment discontinuations were aversion to injections, local injection site reactions, inefficient viral load suppression, thrombocytopenia and edema. There are no controlled studies in children.

Maraviroc (MVC, Celsentri®) is available as 150 and 300 mg tablets. In adult patients, efficacy and safety have been proven. A tropism test is required prior to the use of CCR5 antagonists. There are no data on the use of maraviroc in children.

Integrase Strand Transfer Inhibitors (INSTIs)

This substance class allows for new treatment options in children. Insomnia, dizziness, headache, nausea and fatigue are reported in this class. As of yet, only raltegravir is licensed for children but studies with other INSTIs in children are underway. Dolutegravir and elvitegravir should be very attractive as they allow for once-daily dosing.

Dolutegravir (DTG, Tivicay®) is a promising drug for children as it will allow once-daily regimens. Child dosing: DTG is not approved for use in neonates/infants. Not recommended <12 years; in the US a clinical trial in treatment-experienced children aged <12 years is under way with an experimental dose of 50 mg in children weighing at least 40 kg. Dosing in children aged ≥12 years (>40 kg): 50 mg QD. If co-administered with efavirenz, fosamprenavir/r, tipranavir/r, or rifampin, dolutegravir should be given BID at 50 mg per dose.

Raltegravir (RAL, Isentress®) is available as 400 mg tablets. It is safe and effective in children (Perry 2014). Child dosing for chewable tablets is: (11–14 kg): 75 mg BID, (14–20 kg) 100 mg BID, (20–28 kg): 150 mg BID, (28–40 kg): 200 mg BID, (≥40 kg):

300 mg BID; Child dosing for film coated tablets is: (≥6 years and >25 kg or ≥12 years) 400 mg BID; Adult dosing (film coated tab) is: 400 mg BID.

Elvitegravir (ELV, Vitekta®) should only be used with a pharmacokinetic enhancer (boosting agent). Child dosing: Not recommended <18 years; Adult dosing is: (with atazanavir/r): 85 mg+RTV 100 mg QD (with lopinavir/r): 85 mg OD+RTV 100 mg BID, (with darunavir/r, fosamprenavir/r): 150 mg BID+RTV 100 mg BID. Preliminary data from an ongoing trial suggest the adult formulation in Stribild® may be appropriate for use in youth aged ≥12 years and body weight ≥35 kg.

Fixed Dose Combinations, FDC

In older children and adolescents (>35 kg) several fixed-dose combinations are available to reduce the daily burden of pills: Combivir® (300 mg AZT + 150 mg 3TC), Trizivir® (150 mg 3TC + 300 mg AZT + 300 mg ABC), Eviplera® (200 mg FTC + 300 mg TDF + 25 mg rilpivirine), Truvada® (300 mg TDF + 200 mg FTC), Atripla® (200 mg FTC + 300 mg TDF + 600 mg efavirenz), Kivexa® (300 mg 3TC + 600 mg ABC), Stribild® (elvitegravir 150 mg + cobicistat 150 mg + FTC 200 mg + TDF 300 mg), Triumeq® (3TC 300 mg + ABC 600 mg + dolutegravir 50 mg).

Drug interactions

There are many interactions that may complicate ART when it is co-administered with other drugs. Examples of dangerous interactions include commonly used drugs like oral contraceptives (Patni 2014), inhaled corticosteroids (Johnson 2006) and many others, e.g., tuberculosis and atypical mycobacterial treatment may interact with ART so very close monitoring and expert advice should be sought. Use http//www.hiv-druginteractions.org.

Monitoring efficacy and watching out for failure

There is no commonly used definition of treatment failure in children treated with antiretroviral drugs. In the PENPACT 1 study, children were randomized to change a failing treatment at either low or high viral rebound (>1000 or >30,000 copies/ml), outcome was not different in the two groups (PenpactStudyTeam 2011). Alternatively, therapy failure can be defined by a decrease in CD4 T cell counts, e.g., a decrease by at least a third of the absolute CD4 cell count in less than 6 months. In children with relatively low CD4 T cell percentage (of less than 15%), a decrease of more than 5% may be significant enough to consider therapy failure. The use of clinical criteria such as toxicity of the drugs, progression within the WHO classification, an increased susceptibility to infections, encephalopathy and failure to thrive may all indicate treatment failure.

Many children with multidisciplinary support and modern drug regimens now manage to maintain long-term (>5 years) viral suppression on first-line therapy, and the longer this can be maintained the better.

The most common cause of treatment failure is insufficient adherence, which is found in up to 25-30% of children. Assessment of adherence may be difficult as questionnaires may not be reliable. Determination of plasma levels and resistance tests (e.g., recurrence of wild type) are other options to assess adherence and monitor antiretroviral therapy more effectively.

Change of therapy

The suppression of viral load that can be reached on a second or third regimen depends on the preceding therapy, resistance status and ongoing adherence. In the NEVEREST-2 trial 195 children on lopinavir-based ART were randomized to switch to a nevirapine-based regimen (Arpadi 2013) or stay on lopinavir/r. The nevirapine group had somewhat favorable values for body fat and serum lipids. In adults, few randomized and prospective trials have shown that a change of antiretroviral therapy guided by resistance tests may lead to better treatment response. Usually, the initial treatment regimen contains a double NRTI backbone (e.g., AZT+3TC or ABC+3TC). When changing therapy, it appears useful to introduce a backbone with two new NRTIs plus a new class, e.g., INSTIs may be a good option for introducing a new class.

Supportive therapy and prophylaxis

OIs have become rare in perinatally infected children who experience immune reconstitution with ART. In most of these children respiratory and other infections are not much more common than in healthy children. The incidence of invasive pneumococcal disease among perinatally HIV+ children has decreased since the introduction of ART (Steenhoff 2008). Children who are treated with ART and who are clinically stable can even be given live varicella virus vaccine and show a specific response, which is an impressive sign of successful immune reconstitution (Taweesith 2011). In the vast majority of stable treated children treatment with intravenous immunoglobulins and PCP prophylaxis is no longer required (Nachman 2005). However, there are still life-threatening infections and deaths from HIV if perinatal HIV infection is unrecognized or ART has not led to immune reconstitution. A description of such infections in adults is given in other chapters of this book. An excellent and detailed guide for treatment of children with OIs can be found at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5811a1.htm.

Conclusion

In many aspects HIV infection in children is different from HIV infection in adults. The ongoing growth and development of children, their viral dynamics and immaturity of the immune system result in a different response to HIV compared to adults. This has important consequences for the diagnosis and treatment of HIV in children. The aim of therapy is to achieve maximum efficacy while avoiding long-term side effects. Sustained success in the treatment of children with HIV infection depends on:
• a multidisciplinary approach;
• standardized treatment protocols;
• participation in multicenter trials;
• appropriate formulations and treatment strategies for children & introduction of new classes of ART (e.g., INSTIs).

In developed countries the clinical picture of HIV infection in children has now changed from an often fatal to a treatable chronic infection, allowing children to lead a largely normal life. This picture is still different in developing countries but prevention of mother-to-child transmission and broader coverage with ART is getting better and the number of deaths from HIV infection in children estimated by UNAIDS is decreasing (2006: 380,000; 2014: 150,000).

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Interdisciplinary Medicine

23. HIV and Renal Function

ANSGAR RIEKE

Due to HIV+ patients' increasing age and comorbidities, kidney diseases will also be on the rise. Diabetes and arterial hypertension raise the risk of renal insufficiency by tenfold and account for 71% of dialysis cases in the US (Winston 2008). According to cohort studies, the prevalence of diabetes in male HIV+ patients is 12%, which is four times as common as in the age-based normal population (Winston 2008). The increase in renal insufficiency in the elderly is more pronounced (Goulet 2007). Renal insufficiency and the extent of proteinuria are also independent predictive factors for mortality in HIV+ patients, while half of all patients die of cardiovascular disease (USRDS 2010).

Increased creatinine is an indicator for kidney disease, the internationally valid classification of renal insufficiency follows the GFR (glomerular filtration rate) (given in ml/min/1.73 m^2):

Stage I   Kidney damage, normal or increased GFR   >90
Stage II   Kidney damage and slightly reduced GFR   60 – 89
Stage III   Moderate reduction in GFR   30 – 59
Stage IV   Severe restriction of GFR   15 – 29
Stage V   Kidney failure   <15

Nephroprotection

Acute renal failure is twice as common overall as in the non-infected, and the adjusted mortality rate is also significantly higher (Wyatt 2006). Despite the use of ART, the incidence of dialysis treatment in HIV+ patients remains unchanged. In the US, particularly Afro-Americans are affected, in whom the risk of kidney failure is tenfold higher than that of non-infected persons (Lucas 2007).

The following principles should be followed for nephroprotection – give up nicotine, keep blood pressure below 140/80 mm Hg (or <130/80 mmHg in the case of proteinuria) and provide for prevention or treatment of diabetes mellitus or other metabolic syndromes. The HIV-related changes in the glomerulum and the tubular system are a good reason to begin and continue ART. This has also been reflected in the international therapy guidelines in which kidney involvement is another reason for beginning ART (Choi 2009).

Clinical manifestation/diagnosis of nephropathy

The clinical picture of renal damage is often unspecific, with tiredness, poor concentration, loss of appetite, high blood pressure and possibly new edemas. Based on the cause of the kidney disease, one can differentiate between pre-renal, intra-renal (glomerular, tubular, interstitial) and postrenal. A sonography quickly supplies information about a post-renal drainage impediment (renal retention, prostate hypertrophy?) as well as about the size of the kidney (reduced with a narrow parenchyma in the case of chronic renal insufficiency). The anamnesis provides an indication for a pre-natal cause (NSAR, infections, sepsis, contrast agent?) The diagnosis is supplemented by a urine screening test or sediment and the determination of creatinine, electrolytes (K, Na, Ca) and phosphate. Several factors such the extent and severity of anemia, metabolic acidosis (blood-gas analysis), dysfunction of the calcium-phosphate, metabolism, possible venal thrombosis and newly diagnosed arterial hypertension are associated with the duration of the kidney disease. They can help to differentiate between acute and chronic renal failure.

Creatinine, Cystatin, GFR

Increased serum creatinine can be expected after occurrence of a more than 50% reduction of the glomerular filtration rate (GFR), and is dependent on muscle mass and gender, which means it is not a good sole marker for renal function. Creatinine is mainly subject to glomerular filtration, but is also secreted in the proximal tubulus via transporters which are blocked by dolutegravir and cobicistat (see below). The creatinine increase of 0.14 mg/ml thus indicates no deterioration of the true GFR. Cystatin C is constantly generated by all germ-bearing cells. It is a low molecular weight protein constantly generated by the organism, filtered freely and regardless of gender, muscle mass, or age, with a minor intra-individual variability (<5%). However, determination is by no means inexpensive. But its clinical value is nonetheless questionable when taking into account a chronic inflammation with HIV (Dhamidharka 2002, Jaroszewicz 2006). Clearance measurements can detect the "creatinine-blind" area of an early renal insufficiency faster and are particularly important when the kidney function is over-estimated due to lack of muscle mass. There are four procedures to determine GFR, of which the CKD-EPI formula has become established after scientific consideration, at least in mildly restricted renal insufficiency. In EuroSIDA, however, it was shown in more than 9,000 HIV+ patients and almost 125,000 measurements that both the Cockroft Gault and CKD-EPI formulas demonstrate renal insufficiency very well (Mocroft 2013). The four procedures are as follows:

1. Cockroft Gault formula:
   • (140 – age) x kg body weight) divided by serum creatinine mg/dl x 0.72).
   For women, the result is multiplied by 0.85.

2. MDRD formula (more precise, but requires additional laboratory data):
   • 170 x Krea $[mg/dl]^{-0.999}$ x age $^{-0.176}$ x (urea $[mg/dl]$ x 0.46$)^{-0.170}$ x albumin $[g/dl]^{-0.318}$ (for women: x 0.762), correlates well with HIV (Ravasi 2009).
   As an alternative[2] 186 x Krea $[mg/dl^{-1,154}$ x age^{-0203} x 1 (for women: x 0.762), (for people of color: x 1.212).

3. Cystatin C clearance:
   • 78 x 1/ CysC (mg/l) + 4 or 87 x 1/ CysC (mg/ ml) – 6.9

4. CKD-EPI formula (Levey 2009):
   • GFR = a x (serum creatinine /b)^C x (0.993)^{Age}
   The variable a conforms to race and gender (women of color = 166, caucasian women = 144, men = 141), the variable b to gender (women 0.7, men 0.9). The variable c adapts the formula to the serum creatinine value: women <0.7 mg/dl = -0.329; >0.7 mg/dl = -1.209 or -0.411 and -1.209 for men. The formula can be directly converted at www.nephromatic.com/egfr.php.

Proteinuria

The *extent of proteinuria* with loss of protein, the imbalance of serum protein fractions and residual kidney function with possible fluid retention all dictate edemas, loss of efficiency, susceptibility to infections, and hyperlipidemia. As with diabetes mellitus, microalbuminuria (Micral-Test in the urine) is an important indicator for the kidney and mortality due to cardiovascular events with HIV (Wyatt 2012). HIV+ patients with confirmed microalbuminemia are 25 times likelier to develop proteinuria, which, if it continues despite ART, is accompanied by a doubled risk of mortality (Wyatt 2012). HIV+ patients should be examined just as carefully for signs of kidney disease as diabetes patients.

Together with "*nephritic sediment*", proteinuria is a major symptom of glomerulonephritis (GN) and its extent should be quantified. Clinically, a difference is made between nephrotic syndrome (loss of protein), acute nephritic syndrome (acanthocytes as a sign of GN), rapid-progressive GN (loss of renal function within a few days), asymptomatic proteinuria or hematuria and chronic GN. These all need to be treated differently and require the collaboration of a nephrologist. HIV-associated nephropathy (HIV-AN) is a form of glomerulonephritis and is diagnosed in cases of nephrotic syndrome with edema, hypoalbuminemia, hyperlipidemia and proteinuria of more than 3.5 g/day. However, even a mild proteinuria is possible.

Urine sediment and sticks

Alongside salts and crystals (e.g., from HIV drugs such as indinavir), as well as epithelia, the existence of erythrocyturia together with the number and form of the erythrocytes is of significance for a differential diagnosis. The occurrence of proteinuria and erythrocyturia is pathognomonic for glomerulonephritis and, together with nephritic sediment, usually confirms the diagnosis. Under a polarizing microscope, a trained eye can easily identify the renal (glomerular) origin of the erythrocytes, on the basis of glomerularly deformed acanthocytes (erythrocytes). More than five acanthocytes per field of vision is a significant sign for GN. Extensive erythrocyturia (bleeding) below the renal pelvis (tumor of the urinary tract collection system) can be excluded by sonography and, if necessary, by cystoscopy. A *leukocyturia* must first be clarified microbiologically (Uricult®: midstream urine) in order to administer antibiotics according to the resistance situation, whereby a bacterial interstitial nephritis may also exist. In the case of a sterile leukocyturia, the possibility of urogenital tuberculosis should also be considered. However, it can also be the expression of an interstitial kidney disease (e.g., when taking indinavir).
Glucosuria (with a normal blood sugar level/drop in the normal glucose level of the kidney) or *phosphaturia* are signs of a tubular disorder, such as can occur with medication (e.g., with TDF).

Routine tests for renal impairment

The routine checkup of an HIV+ person should include tests for sodium, potassium, calcium, phosphate (every three months) and serum creatinine (creatinine clearance). The urine should be tested for glucosuria, proteinuria, erythrocyturia and leukocyturia every 3 months.
If there is a significant elevation in proteinuria or serum creatinine, or a drop in GFR to below 60, a nephrologist should be consulted (renal biopsy if necessary). There is no time to waste in the case of a rapid increase of creatinine (to look for rapid-progressive glomerulonephritis), an increase of LDH connected with hyperbilirubinemia and thrombocytopenia (hemolytic uremia syndrome, HUS), or severe electrolyte imbalance (especially hyperkalemia), or acidosis that cannot be controlled, which can also occur on therapy as lactic acidosis.
An asymptomatic, mild proteinuria with no rise in creatinine can be observed in up to one third of untreated patients and should be monitored quarterly. The extent of the proteinuria can be assessed based on the urine protein / creatinine ratio of spontaneous urine, which when normal is <1 (e.g., urine protein 120 mg/dl and urine creatinine 30: proteinuria of 4 g/day).
A decrease in renal function could be interpreted as symptomatic HIV infection, and ART may be considered. When employing imaging techniques, the use of a contrast
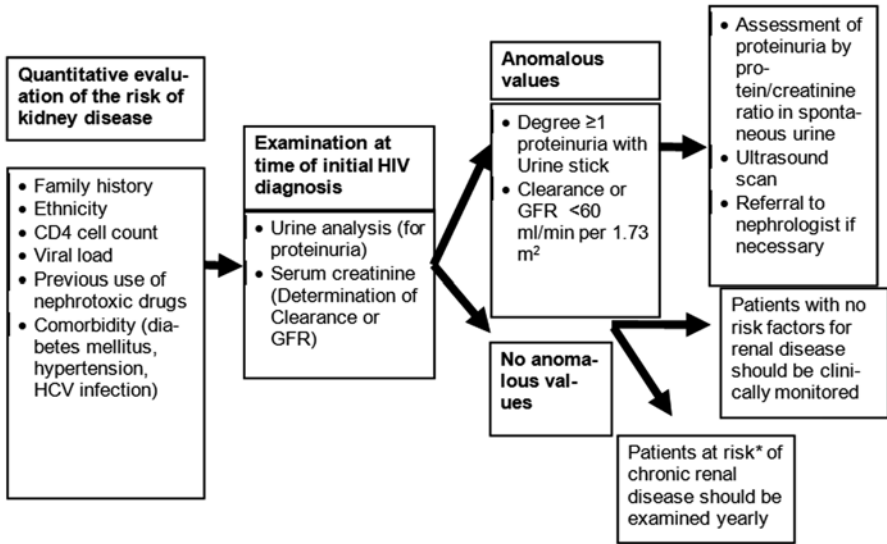
Figure 1: Screening Algorithm for HIV-associated disorders
Modified in accordance with the recommendations of the Infectious Diseases Society of America (IDSA)
*Patients at risk of chronic renal disease: Africans/African-Americans; diabetics, patients with hypertension or HCV infection; CD4 T cell counts <200 cells/µl; HIV RNA >4,000 copies/ml. According to Gupta 2005

medium (CM) for the urinary tract should be avoided, especially in cases of renal insufficiency, proteinuria and all forms of low intravasal volume (including cirrhosis of the liver), in order to avoid causing CM-induced renal failure.

HIV-associated nephropathy (HIV-AN)

HIV-AN is characterized by rapid loss of renal function, especially in African-Americans, and was first described in 1984. In the US, HIV-AN is the third most common reason for dialysis in African-Americans aged 20-64 years (Winston 2008). The genetic predisposition probably results from the reciprocity of the human gene MYH9 (nonmuscle myosin heavy chain 9) with HIV and a neighboring apolipoprotein L1 gene as promoter of HIV-AN. As mutations of the ApoL-1 gene led to an evolutionary advantage in coping with sleeping sickness, HIV-AN is found almost exclusively in people of black African origin (Soleiman 2011, Kopp 2008, Kao 2008). Despite hemodialysis, the one-year survival rate arrives at approximately 50%; ART has reduced the dialysis risk through HIV-AN by 40%. In addition, the one-year survival rate on dialysis has increased from 25 to 75% thanks to ART (Winston 2008). Most patients have a poor immune status with <100 CD4 T cells/µl (only 20% are in the normal range). Individual cases of sudden renal insufficiency within acute HIV syndrome have been reported. But there seems to be no correlation with HIV viral load or duration of infection.

Nephrotic proteinuria usually presents clinically as more than 3.5 g/day, but a minor proteinuria is also possible. Progression is fast and can lead to end-stage renal disease (dialysis) in only a few months (Szczech 2001). Blood pressure is normal or slightly increased; the kidneys are within the normal size range when examined by ultrasound scan. The histological findings in biopsies correspond mostly (70%) to a focal

segmental sclerosing glomerulonephritis (FSGN), as well as cystic tubular changes and degenerations. However, other causes of a glomerulonephritis, such as an amyloid kidney are also possible with HIV (Daugas 2005). Single case descriptions with the histological course of disease have confirmed the direct infection of the glomerular basal membrane with HIV, and have documented an impressive positive effect of ART on histological changes in the kidney (Bruggemann 1997, Winston 2001).

Experience with other FSGN forms has shown that only early intervention with ART – i.e., before scarring of the glomeruli due to the underlying disease – has a chance of success. This calls for a rapid reaction: HIV-AN must be treated quickly and independently from the immune status and viral load, and if this is initiated early, the prognosis for the kidney is also improved (Lucas 2004)! There is no specific recommendation for the selection of therapy. However, the different means of renal elimination (adjustment of dose from <60 ml GFR with NRTIs) should be taken into consideration. ACE inhibitors or angiotensin receptor blockers should be added (see Table 2). The use of steroids is controversial (1 mg/kg/day for 2 to 11 weeks), but is favored in the US alongside starting ART, particularly in cases with a course similar to lupus (Haas 2005, Gupta 2005, Choi 2009).

The question of whether a case of HIV-AN needs to be confirmed by means of a kidney biopsy is the subject of discussion. Should the ethnicity suggest such a diagnosis, ART should be started immediately. Over an observation period of 3 months, viral load should be completely suppressed, the blood pressure well adjusted, if necessary diabetes treated and the therapy supplemented with a lipid therapy (e.g., pravastatin along the lines of cardiological recommendations following infarction) (Szczech 2009). It is often the case that renal function improves and proteinuria lowers with this therapy. The decision to perform a biopsy should be placed in the hands of a nephrologist, depending on the extent of proteinuria and restriction of GFR (<60 ml/min/1.73 m^2). Thanks to triple diagnostics made up of light and electron microscopy together with immunohistochemistry, kidney puncture can clarify the many causes of kidney damage and their prognosis.

Other cases of post-infectious glomerulonephritis

In caucasian patients, IgA nephropathy, membranous and membranoproliferative GN must all be regarded as typical results of HIV infection. At 6–32%, non-black African HIV+ patients show a considerably higher prevalence of proteinuria than non-infected persons (Soleiman 2011). Furthermore, many pathogens are able to trigger or support a post-infectious or other chronic GN. Viral infections such as CMV, EBV, HZV, influenza, adenovirus, hantavirus or parvovirus B19 do this as well as HIV. After malaria, syphilis and infections with staphylococci, pneumococci, legionella, salmonella and other infectious agents, an acute post-infectious glomerulonephritis can also occur. In addition, there is a risk of circulatory renal failure in the case of profuse diarrhea in the context of an infectious bowel disorder.

In the case of *membranous glomerulonephritis,* malignant tumors and hepatitis (B and C) must be ruled out as a classical "secondary GN". Chronic hepatitis C can lead to a membranoproliferative GN, or through cryoglobulinemia cause vasculitis with renal involvement.

The most common form of renal disease in Germany is IgA nephropathy, which can also be triggered by HIV, respiratory infections or infection with hepatitis. With post-infectious GN, the underlying infection is treated first, and is then monitored so that the necessity of a possible additional immunosuppression can be deliberated between nephrologist and HIV specialist. Irrespective of the liver histology, HCV-associated

GN can also be a reason for therapy, especially in cases of cryoglobulinemia-associated vasculitis (observe dosing interval adjustments). However, only a greatly reduced dose or no ribavirin at all should be used if the creatinine clearance is less than 50 ml/min/1.73 m² because of the danger of prolonged anemia. For example, an alternating daily dose of 200 and 400 mg should be administered.

In untreated HIV+ patients, hemolytic-uremic syndrome (HUS) or thrombotic-thrombocytopenic microangiopathy syndrome (TTP) can occur, characterized by the combination of creatinine increase, signs of hemolysis (increased LDH, thrombopenia) and neurological symptoms with kidney failure. Pathophysiologically, the induction of procoagulatory effects of gp120 (HIV) on endothelial cells can probably be assumed (Mikulak 2010). In these cases, plasma separation or a therapy with immunoabsorption is necessary, in order to improve the otherwise bad prognosis and to prevent dialysis.

Principles of therapy of glomerulonephritis

The underlying cause of all forms of post-infectious glomerulonephritis should be treated first, including HBV, HCV and HIV infection. Kidney failure caused by hantavirus (transmitted through mouse or rat droppings) has a positive prognosis in Europe and thus its spontaneous course is to be expected.

Particular attention should be paid to the adjustment of blood pressure. Target values are <140/80 mmHg or, in the presence of proteinuria, <130/80 mmHg. ACE inhibitors as well as AT-II receptor antagonists are used to control blood pressure, usually in combination with diuretics.

Proteinuria should be treated with an ACE inhibitor, at high doses if necessary, irrespective of the blood pressure measurement, and should be combined additionally with AT-II receptor antagonists if the proteinuria is more than 0.5 to 1 g/day. The protein intake is reduced to 0.6–0.8 g/kg/day (the Mediterranean diet may be helpful).

High proteinuria (>3.5 g/24 h) calls for anticoagulation if the serum albumin concentration drops to levels below 25 g/l, as the renal loss of coagulation factors (AT-III and many others) results in hypercoagulability and otherwise deep vein thrombosis can be expected (phenprocoumon at INR 2-3 or NMH, if necessary, dosed according to factor Xa determination in case of renal insufficiency).

Fluids should be restricted to 1.5 to 2 l/day and adapted according to body weight and amount of edema. Not smoking is of vital importance because nicotine causes an increase in the risk of progression of glomerulonephritis.

Hyperlipidemia should be treated after dietary arrangements have been exhausted. HMG-CoA reductase inhibitors are ideal, provided that they are combined with anti-retroviral therapy (see chapter on *Drug Interactions*). Fibrates or fibrates plus statins may only be used carefully when renal function is reduced. Analgesics should be avoided as much as possible, especially the "small" analgesics such as ASA and paracetamol. If creatinine clearance reaches a value of less than 50 ml/min/1.73 m², treatment should be managed by a nephrologist, especially if renal puncture and immunosuppressant therapy are indicated.

Practical treatment of hypertension in HIV

Antihypertensive drugs offer an array of side effects, including hyperkalemia with ACE inhibitors. At a creatinine level of higher than 1.4 mg/dl, potassium-saving diuretics should be avoided; above >1.8 mg/dl only loop diuretics such as furosemide or torasemide should be used.

Table 1: Blood pressure adjustments

Category	Drug	Dosage (examples)
ACE inhibitors	Lisinopril, benazepril-HCL, fosinopril sodium, enalapril, etc	Fosinopril 5 mg QD, increase slowly to 20 mg/day
Beta blockers	Metoprolol, bisoprolol	Metoprolol 50 mg 1x1
AT-II receptor antagonists	Valsartan, candesartan, telmisartan, etc	Candesartan first 2-4 mg/day, increase carefully to 16 mg/day
Diuretics	Hydrochlorothiazide + triamterene	Dytide H® or Dyazide® 1x1
Ca antagonists	Amlodipine	Norvasc® 5 mg QD, after >1 week increase to BID if necessary

Nephrotoxicity

The spectrum of allergic or autoimmune reaction in the kidney is no different from that in the skin or other internal organs. Reactions can be humoral or T cell-mediated and can lead to renal insufficiency. Even the one-off use of an analgesic (e.g., ibuprofen) can lead to renal failure. This is also possible with ARVs. Any change of treatment should always be followed by a check of renal function after 14 days in case of any noticeable renal changes, then every 4-6 weeks in the first year, especially with TDF.

Acute renal failure or acute tubular necrosis can also occur during treatment with acyclovir, gancyclovir, adefovir, aminoglycosides or pentamidine. Tubular dysfunction may also occur with ddI, d4T or 3TC. An acute allergic interstitial nephritis can arise in connection with a hypersensitivity reaction when taking abacavir. In patients taking atazanavir and T-20, membranoproliferative GN has been observed. Ritonavir and efavirenz can also cause kidney damage (Winston 2008). A number of specific renal damage toxicities should be addressed individually:

Renal side effects of antiretroviral therapy
Crystal-associated nephropathy

Renal problems due to crystalluria and nephrolithiasis that have seen mainly with indinavir have become rarer with newer PIs. Many medications can cause crystalluria and it is often a combination of agents that leads to nephrolithiasis. These agents include ampicillin, acyclovir, aspirin, ciprofloxacin, methotrexate, vitamin C, sulfonamide and other drugs that lead to an increase in uric acid. Forced fluid intake, Buscopan® and analgesics often lead to resolving acute renal colic without the need for hospitalization. Should it become necessary to consult a urologist, the risks involved in using contrast medium must be clarified.

Elevation of creatinine with long-term indinavir therapy was often seen in the late 1990s (Fellay 2001, Boubaker 2001). Typical signs of indinavir nephropathy include sterile leukocyturia with loss of renal function (Gagnon 2000, Dielemann 2003) and an echogenic transformation of the renal parenchyma in otherwise normal kidneys. Discontinuing indinavir leads to normal function in most cases. Tuberculosis in the urinary tract with sterile leukocyturia should be considered.

Hypophosphatemia, tubulotoxic damage, Fanconi's syndrome

Alongside glomerular filtration of substances such as creatinine, an alternative form of secretion via transporters in the tubulus is possible. In the proximal tubulus, the transporter OCT2 leads to an intake of creatinine from the the blood in the tubular cell, which in turn is secreted into the urine via MATE 1. The integrase inhibitor dolutegravir inhibits OCT2, whereas the pharmacoenhancer cobicistat inhibits MATE 1. Thus, both substances block the alternative secretion route via the tubular cells, which leads to a slight increase in creatinine (0.14 mg/dl) or a drop in estimated eGFR of around 15 ml. Of course, this does not change the true GFR and therefore has no effect on the glomerulus or the renal function. Tivicay® can thus also be used in patients with limited kidney function. However, care must be taken regarding the combination with TDF, as it may be difficult to identify a true impairment of renal function, and TDF must be reduced in cases of a GFR of less than 60 ml. The fixed-dose combination Stribild® should thus only be used in patients with an eGFR >90 ml and should be avoided below 70 ml.

Tenofovir itself is absorbed into the tubular cell not only via glomerular filtration but also via the transporter OAT 1 and is then secreted actively (dependent on ATP) via MRP 2 and 4 into the urine of the proximal tubulus. Should true filtration in the glomerulus decline (for example, in cases of acute kidney failure), attempts are made to eliminate more TDF via the tubular cell (increased activity of OAT 1). This results in an increased concentration of TDF in the tubular cell, which in turn leads via a disruption of the mitochondrial polymerase gamma to a decline in energy-dependent MRP 2 and 4 transport capacity and can thus end in tubular damage (Perazella 2010). Indeed, concentration-dependent damage caused by TDF to the tubular cell can be demonstrated: patients with low body weight are more vulnerable (Nishijirna 2012).

When the agents filtered from the glomerulus in primary urine exceed the transport capacity of the reabsorbing tubular cells they are excreted with the urine. The most prominent example is the glucose threshold of the kidneys (180 mg/dl). However, a transport dysfunction in the tubular system can also be caused by drugs such as cidofovir, tenofovir and adefovir. This is known as secondary (drug-induced) Fanconi's syndrome and is distinguished by a malfunction of the tubular system without there necessarily being any impairment of the GFR. There is an increased amount of phosphate, amino acids and glucose in the urine, whereas phosphate in the blood is reduced. The loss of amino acids, phosphate, glucose, bicarbonate and other organic and inorganic substances, as well as water, can become clinically manifest in the form of increased urination, thirst, tiredness, bone pain or weakness, and lead to secondary changes in the bone metabolism.

Not every **hypophosphatemia** (<0.8 mmol/l) is Fanconi's syndrome. Special caution is required when dealing with fixed-dose combinations (FDCs) such as Stribild®. At a serum phosphate level <0.48 mmol/l (1.5 mg/dl) or a creatinine clearance which has dropped to <70 ml/min with Stribild®, the renal function should be checked again within a week. This also includes determination of blood sugar, potassium in the blood and glucose concentration in the urine. In patients whose creatinine clearance is confirmed to drop to <50 ml/min or whose serum phosphate level drops to 1.0 mg/ml, treatment with Stribild® should be discontinued, as dose adjustment with FDCs is not possible.

Hypophosphatemia also occurs under the influence of alcohol, with diabetes, cachexia, diarrhea or a disorder of vitamin D metabolism or hyperparathyroidism. About 10% of cases are found in untreated HIV+ patients, 23% in people on ART and up to 31% in those taking tenofovir. The reasons are many and varied, includ-

ing a low phosphate absorption (normal: 1200 mg/day). Unusual levels (<0.8 or 0.6 mmol/l) should be monitored and the patient examined for other symptoms of Fanconi's syndrome. The determination of intact parathormone, vitamin D or an anamnesis with diuretics, vomiting or a tumor may indicate other causes of hypophosphatemia. The secretion ratio of phosphate to creatinine can be a sign of tubular damage if, despite hypophosphatemia, more that 10% of the filtrated phosphate is secreted (Jamison 1982).

Secretion ratio: <u>(urine phosphate, mg/dl) x (serum creatinine, mg/dl)</u>
(serum phosphate, mg/dl) x (urine creatinine, mg/dl)

In case reports, renal failure has above all been described in patients with other reasons for renal insufficiency, mostly in ART combinations that include boosted PI regimens and tenofovir as well as secondary disorders and cirrhosis of the liver or hepatitis. Nephrologists advise caution in selecting antiretroviral therapy for patients with proteinuria, nephritic syndrome, cirrhosis of the liver, and/or dyslipoproteinemia. Potentially nephrotoxic agents such as cidofovir, adefovir, tenofovir or fixed-dose combinations should be avoided in these patients. In principle, it is possible to administer NRTIs (e.g., ABC or ABC+3TC at a GFR >50 ml).

Renal insufficiency and ART

In advanced cases (with appropriate resistance testing), NRTI-sparing combinations of a PI/r plus raltegravir, two boosted PIs, a combination of an NNRTI plus a PI or combinations of dolutegravir or maraviroc can be considered as kidney-neutral solutions. The application of NRTIs is often not possible. Careful monitoring of serum creatinine, proteinuria, erythrocyturia and serum phosphate is recommended.

Tenofovir and the kidney

In view of the broad use of tenofovir, more attention must be devoted to long-term renal toxicity in the future. The increased renal risk observed in early cohort studies was less explicit in more recent analyses – possibly because TDF is now being used more carefully by the treating physicians. Studies verified an increased tubular risk with TDF (Dauchy 2011) higher than that with ABC+3TC (Moyle 2010). Although a meta-analysis of 17 studies showed only a slight reduction of GFR on TDF (-3.92 ml/min) and a slightly increased risk of renal failure (+0.7%), TDF should not be used uncritically or without regular monitoring of renal function (Cooper 2010). In the large D:A:D cohort (n=22,603), a decline in GFR of more that 20 ml to less than 70 ml/min correlated with the use of TDF, boosted atazanavir and lopinavir (Derek 2013). This was also seen in the EuroSIDA cohort, in which renal failure incidence amounted to 1.05 per 100 patient years. Again, there was a correlation between the use of TDF, atazanavir/r and lopinavir/r. In contrast to the D:A:D group, patients with renal insufficiency were not excluded in EuroSIDA study (Derek 2013). The incidence of renal events in the manufacturer's database since drug approval amounts to 29.2 per 100,000 patient-years (Nelson 2006). In two prospective studies (GS903E and GS934) on patients with good renal health, a creatinine increase to >1.5 mg/ml was observed in less than 1% of patients during an observation period of 144 weeks, and proteinuria of more than 100 mg/dl in less than 5%. However, patients suffering from renal insufficiency were excluded from these studies (Gallant 2008).

The leading renal event during TDF treatment is Fanconi's syndrome (22.4/100,000 patient-years, a combination of **hypophosphatemia**, glucosuria (renal diabetes mellitus with normal blood sugar) and a mild proteinuria. It occurs 7-10 months after starting therapy and disappears 4-6 weeks after discontinuation (Izzedine 2004).

An isolated hypophosphatemia without glucosuria in HIV+ patients can also be due to malnutrition, vitamin D deficiency, diuretics or alcohol, and doesn't necessarily require TDF discontinuation.

The risk of kidney damage with TDF is increased through coadministration with nephrotoxic agents, kidney disease or prior renal insufficiency, sepsis, dehydration, or severe hypertension (Nelson 2006). Other risk factors are CD4 T cells <50/µl, age >45 years, diabetes mellitus and long-term ART exposure (Moore 2007).

Like other NRTIs, tenofovir is eliminated renally, requiring dose-adjustment with renal insufficiency. Although ritonavir increases the Cmax and the AUC of tenofovir by about 30%, combination with boosted PIs is possible. This is also confirmed by *in vitro* studies (Izzedine 2005, Ray 2005). In cohort studies, a reduction of GFR of 7-10 ml/min was observed, whereby the total GFR remained in the normal range, but there was an apparently faster drop in GFR when tenofovir is combined with PIs instead of NNRTIs (Goicoechea 2008, Winston 2008). The use of TDF during pregnancy does not seem to be associated with damage to renal function in neonates (Linde 2010, personal correspondence).

During the first year of TDF treatment, even patients with healthy kidneys should be monitored monthly, followed by quarterly monitoring. The determination of creatinine, calculated GFR, phosphate and glucose in serum and urinstix with a check for proteinuria, glucosuria and erythrocyturia are sufficient. Patients with kidney dysfunction should be monitored more often. In the case of additional nephrotoxic agents or drugs which are also excreted via the renal transporter (aminoglycosides, amphotericin B, famcyclovir, gancyclovir, pentamidine, vancomycin, cidofovir, IL-2), renal function is monitored weekly. Special care must be taken with the TDF-containing FDC Stribild® which should only be considered for patients with a GFR >90 ml (see above).

Caution is required with TDF-containing regimens in the following cases:
• Limited renal function at onset of therapy (GFR <80 ml/min)
• Proteinuria of >1g/24 h, regardless of cause
• Combination with PI/r (especially when renal function is already reduced at onset)
• Low body weight or BMI <19
• Poorly regulated arterial hypertension or diabetes mellitus
• Nephrotoxic concurrent medication, especially NSAR and others
• Active drug use (IVDU) (cocaine and heroin, among others)
• Extra-hepatic manifestation of hepatitis in the kidney (except hepatitis B)

Tenofovir alafenamide (TAF) is a novel NRTI that has demonstrated high antiviral efficacy at a dose less than one-tenth that of TDF, as well as improved renal and bone laboratory parameters in clinical trials. Approval is expected in 2016 (see chapter 6.3)

Atazanavir and the kidney

In cohort analyses atazanavir also was associated with renal changes. In EuroSIDA, for example, a 21% higher (reversible) risk of renal insufficiency was observed (Mocroft 2010, Dauchy 2011). The prevalence of nephrolithiasis is also contentious and goes from "rare" (Calza 2012) to an FDA case analysis of 30 cases who fully recovered kidney function after discontinuation (Chan-Tack 2007). For patients with known nephrolithiasis, renal colic or hematuria in their medical history, atazanavir is clearly not a preferred agent (EACS 2013).

Dosage of antiretrovirals in cases of renal insufficiency

In all cases, the prescribing information of the individual agents should be considered. Because NNRTIs, PIs, INSTIs (including dolutegravir) and maraviroc are almost exclusively hepatically eliminated, a dose rate adjustment is normally only necessary for NRTIs, unless hepatic insufficiency is also present. In the case of limited renal function and combination with a CYP3A4 inhibitor, maraviroc must be dosed according to agent and GFR (see chapter on *ART*). In fixed-dose combinations (FDCs), the most strongly accumulating substance is always decisive. Thus, FDCs should be avoided in patients with a GFR <50 ml.

Table 2: Dosage of antiretroviral medications in presence of renal insufficiency (in each case diurnal dosages, if not otherwise stated). HD=Hemodialysis

Drug	Standard dose	CrCl (ml/ min)	Dose in renal insufficiency
AZT (Retrovir®)	2 x 250 mg	>10 <10	2 x 250 mg 300–400 mg
3TC (Epivir®)	1 x 300 mg or 2 x 150 mg	>50 30–49 <30 <5	Standard dose 1 x 150 mg 150 mg (15 ml) on day 1; 100 mg (10 ml)/day thereafter 50 mg (5 ml) on day 1; 25 mg (2.5 ml)/day thereafter
AZT+3TC (Combivir®)	2 x 1 tab.	>50 <50	Standard dose Not recommended
ABC (Ziagen®)	2 x 300 mg	>50 <50	Standard dose Contraindicated
AZT+3TC+ABC (Trizivir®)	2 x 1 tab.	>50 <50	Standard dose Not recommended
ddI (Videx®)	1 x 400 mg (>60 kg) 1 x 250 mg (<60 kg) (combined with TDF never exceed 1 x 250 mg)	>60 30–59 10–29 <10	Standard dose Half standard dose 1 x 150 or 100 mg 1 x 100 or 75 mg
TDF (Viread®)	1 x 300 mg (TDF disoxoproxil fumarate)	>50 30–49 10–29 HD patients	Standard dose 300 mg every 2 days 300 mg every 72–96 hrs 300 mg every 7 days past HD
FTC (Emtriva®)	1 x 200 mg	>50 30–49 15–29 <15 (incl. HD)	Standard dose 200 mg every 2 days 200 mg every 72 h 200 mg every 96 h
ABC+3TC (Kivexa®)	1 x 1 tab.	50	Standard dose Not recommended
TDF+FTC (Truvada®)	1 x 1 tab.	>50 30–49 <30 and HD	Standard dose every 24 hrs 1 tablet every 48 hrs Not recommended

Table 2: (continued)

Drug	Standard dose	CrCl (ml/ min)	Dose in renal insufficiency
TDF+FTC+EFV or RPV (Atripla®, Eviplera®)	1 x 1 tab.	>50 <50	Standard dose every 24 hrs Not recommended
TDF+FTC+ELV/c (Stribild®)	1 x 1 tab.	<90 <70	Standard dose Not recommended
MVC (Celsentri®)	2 x 300 mg	50–80 <50–30 <30	Reduction of the standard dose Only in combination with CYP 3A4-Inhibitors: see literature
Dolutegravir (Tivicay®)	1 x 50 mg	>15 <15 (incl. HD)	Standard dose, monitoring! No data

Note: d4T is no longer listed (should be avoided)

In the case of hepatitis C therapy, DAAs are possible, but ribavirin should be omitted in patients with renal insufficiency (prolonged anemia) if the creatinine clearance is less than 50 ml/min/1.73 m². T-20 (Fuzeon®) can be used up to an endogenous creatinine clearance of 30 ml/min/1.73 m² without dose reduction; no data is available for more severe renal insufficiency.

OIs and renal insufficiency

The following tables show the treatment of the most significant OIs.

Table 3: PCP treatment in renal insufficiency

	GFR normal	GFR >50 ml/min	GFR 10–50 ml/min	GFR <10 ml/min	Dose adjustment for HD/CAPD/cont. NET
*Cotrimoxazole	160/800mg 3 x TID (total of 120 mg/kg daily)	(100% every 12 h)	(100% every 12–24 h)	(50% every 24 h)	HD: + half dose after dialysis CAPD: no adaptation CAVH: GFR 10–50 CVVHD: GFR <10
Dapsone	100 mg every 24 h	50–100%	50%	Avoid	Avoid
Atovaquone	750 mg every 12 h	100%**	100%**	100%**	HD: no adaptation CAPD: no adaptation* CAVH: (GFR <10)**
Pentamidine	4 mg/kg every 24 h	100%	100% every 24–36 h	100% every 48 h see text	HD: (GFR <10)** CAPD: (GFR <10)** CAVH: (GFR <10)**

* no studies available, normal dosage recommended ** no studies available, dosage as for GFR <10ml/min recommended (cont. NET = continuous dialysis, HD = intermittent hemodialysis, CAPD = continuous ambulant peritoneal dialysis; CAVH = continuous arteriovenous hemofiltration, CVVHD = continuous veno-veno hemodiafiltration)

Table 4: Treatment of cerebral toxoplasmosis with renal insufficiency

	GFR normal	GFR >50 ml/min	GFR 10–50 ml/min	GFR <10 ml/min	Dose adjustment for HD/CAPD/cont. NET
Pyrimethamine	50–75 mg every 24 h	100%	100%	100%	HD: no adjustment CAPD: no adjustment CAVH: no adjustment
Clindamycin	150–300 mg every 6 h	100%	100%	100%	HD: no adjustment CAPD: (GFR <10)* CAVH: (GFR <10)* CVVHD: GFR normal
Sulfadiazine	2 g every 6 h	Avoid	Avoid	Avoid	Avoid

* no studies available, dosage as for GFR <10 ml/min recommended (cont. NET = continuous dialysis, HD = intermittent hemodialysis, CAPD = continuous ambulant peritoneal dialysis; CAVH = continuous arteriovenous hemofiltration, CVVHD = continuous veno-veno hemodiafiltration)

Table 5: Treatment of viral infections such as CMV, HSV, HZV in renal insufficiency

Drug	GFR normal	GFR >50 ml/min	GFR 10–50 ml/min	GFR <10 ml/min	Dose adaptation for HD/CAPD/cont. NET
Acyclovir	5–10 mg/kg every 8 h	5 mg/kg every 8–12 h	5 mg/kg every 12–24 h	2.5 mg/kg every 24 h	HD: Dose after dialysis CAPD: GFR <10 CAVH: 3.5 mg/kg every 24 h CVVHD: 6.5-15 mg/kg every 24 h
Gancyclovir	5 mg/kg every 12 h	3 mg/kg every 12 h if GFR 25–50 ml	3 mg/kg every 24 h if GFR 10–25 ml	15 mg/kg every 24 h	HD: Dose after dialysis CAPD: GFR <10 CAVH: 3.5 mg/kg every 24 h CVVHD: 2.5 mg/kg every 24 h
Valgancyclovir	900 mg every 12 h	GFR 40–59 ml/min 450 mg every 12 h GFR 25–39 ml/min 450 mg every 24 h GFR 10–24 ml/min 450 mg every 48 h for induction		Unknown	Unknown
Foscavir	90 mg/kg every 12 h	50–100%	10–50%	Avoid	HD: Dose after dialysis CAPD: 60 mg/kg every 48–72 h CAVH: GFR 10–50
Cidofovir	5 mg/kg every 7 days	100%	0.5–2 mg/kg every 7 days	Avoid	HD: GFR 10–50 CAPD: GFR 10–50 CAVH: avoid
Famcyclovir	250 mg every 8 h p.o.	every 12 h	every 48 h	50% every 48 h	HD: Dose after dialysis CAPD: ? CAVH: GFR 10–50

cont. NET = continuous dialysis, HD = intermittent hemodialysis, CAPD = continuous ambulant peritoneal dialysis; CAVH = continuous arterio-venous hemofiltration, CVVHD = continuous veno-veno hemodiafiltration

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24. HIV and Cardiac Diseases

ACHIM BARMEYER, MARKUS UNNEWEHR

With growing age and duration of the disease, the prevalence of cardiovascular diseases is increasing in HIV+ patients. The increase of cardiovascular morbidity results from an elevated cardiovascular risk profile as well as being a direct consequence of HIV infection itself. Knowledge of the diagnosis and therapy of HIV-associated cardiovascular disease is becoming more and more important (Neumann 2002a, Dakin 2006).

Coronary artery disease (CAD)

HIV+ patients show a higher prevalence of CAD (Currier 2003) and a higher incidence of acute coronary syndromes (ACS) (Klein 2002, Triant 2007), especially acute myocardial infarctions (MI), compared to HIV-negative individuals. It also appears that cardiovascular events occur earlier. The higher cardiovascular morbidity might be attributable to three possible major causes: negative effects of ART, a direct impact of HIV infection and a higher cardiovascular risk profile.

The effect of ART on cardiovascular morbidity was investigated in a number of studies. ART was associated with a higher incidence of CAD (Currier 2003), the development of atherosclerosis (Jericó 2006, de Saint Martin 2006) and incidence of coronary vascular events (Iloeje 2005). In the D:A:D study, including more than 23,000 patients, a 26% increase in MI incidence was found with each year of ART exposure (Friis-Moller 2003, Law 2006). However, the event rate was low, with 3.5 MIs per 1000 patient-years. Antiretroviral therapy was an independent risk factor for CAD along with the classical cardiovascular risk factors like age, gender and particularly smoking (Law 2006).

Of interest is the effect of NRTIs on the occurrence of myocardial infarction. The D:A:D study reported an increased rate of MI for abacavir and ddI (Sabin 2008). An elevated incidence of cardiovascular events was also found in a retrospective analysis of the SMART study as well as in another retrospective analysis of a Danish workgroup (Obel 2010). Inflammation may be the cause of this increased cardiovascular event rate (Lundgren 2008b). On the contrary, a recent FDA meta-analysis plotting data of almost 10,000 patients could not find a statistically significant difference of MI events between subjects receiving abacavir-containing ART and other ART regimens (Ding 2011). When using PIs, the increased event rate was associated with classical risk factors such as diabetes and hyperlipidemia which may explain some of the events. Patients undergoing therapy with abacavir also were likely to be male and had an increased rate of risk factors like increased age, diabetes and pre-existing cardiovascular disease. Further investigation is needed to clarify how much classical risk factors contribute to the MI event rate. When looking at NNRTIs and some PIs (nelfinavir, saquinavir) and NRTIs (AZT, d4T, 3TC, tenofovir) there was no hint of an increased cardiovascular event rate (Worm 2010). To what extent these results have an effect on medical care of HIV-infected patients remains unclear.

However, the SMART study did show an increase in the cardiovascular event rate in patients in whom ART was discontinued intermittently compared to patients who received ART continuously (El-Sadr 2006). It was suggested that increased inflammation during treatment interruption is responsible for this (Kuller 2008).

Summing up, the evidence for negative effects of ART on CAD is not strong enough to influence the decision of when to start or switch ART regimens in terms of the cardiovascular risks.

Besides ART there is some evidence that HIV itself may promote atherosclerosis by chronic inflammation. HIV+ men exhibited slightly more positive arterial remodeling on coronary CT than negative controls (Miller 2015). Also, the prevalence of coronary calcium is higher (Chow 2015). However, in a sonographic controlled study of the development of carotid plaques HIV+ patients with high baseline CD4 T cells did not have an increased risk compared to normal controls. It was concluded that the degree of immunodeficiency might play a role in the progression of atherosclerosis. Studies focusing on subclinical atherosclerosis predict an increasing prevalence of CAD in the near future as the population continues to age (Triant 2009, Lo 2010). It has been shown that HIV+ subjects exhibit a marked cardiovascular risk profile (Neumann 2003+2004a+b). Most notably, cigarette consumption is two- to three-fold higher than in non-infected populations. In addition, uncontrolled blood pressure is frequent in HIV+ patients (Nuernberg 2015) and a recent publication revealed that treatment of risk factors is frequently insufficient (Reinsch 2012). Especially patients with known CAD and diabetes exhibit a high risk for subsequent cardiovascular events (CAD: 7.5-fold; diabetes: 2.4-fold) (Worm 2009).

Prevention and treatment of CAD

Prevention and early diagnosis of CAD in patients older than 45 years and with an elevated cardiovascular risk profile should, therefore, be routine in current therapeutic management of HIV infection. Primary and secondary prevention should aim at modifying known risk factors (Lundgren 2008a). Prevention of CAD is based on the most recent general guidelines (Smith 2006, Perk 2012) (Table 1) and EACS guidelines (Lundgren 2008a). A number of different scores for calculation of the cardiovascular risk profile is proposed. However, all of them take into account the classical risk factors hyperlipidemia, diabetes, hypertension and smoking. Primary prevention of CAD aims at the control of these risk factors to lower the future risk of cardiovascular events.

Primary prevention of CAD starts by modifying lifestyle and comprises cessation of smoking and a balanced diet with a low content of saturated fatty acids and trans-unsaturated fatty acids, a reduced salt intake as well as a high amount of fibers, fruits and vegetables. Moderate intensity physical activity with a cumulative duration of 2.5–5 hours per week is recommended. Weight reduction which aims at a BMI of 20–25 is beneficial for the control of blood pressure and metabolic imbalance.

If control of blood pressure cannot be achieved with lifestyle modifications only, drug therapy should be initiated. Drug therapy can comprise any of the standard antihypertensives (diuretics, ß blockers, calcium channel blockers, ACE inhibitors angiotensin receptor blockers and renin antagonists) or a combination (caveat: do not combine ACE inhibitors, angiotensin receptor blockers and renin antagonists). When using calcium channel blockers, interactions with ART (boosted PIs!) should be considered. When multiple metabolic risk factors are present, diuretics and ß blockers are not recommended. The aim of blood pressure control should be a systolic blood pressure <140 mmHg and a diastolic blood pressure <90 mmHg.

Hyperlipidemia can be approached with lipid lowering drugs. Statins are the therapy of first choice but might interact with ARVs. In particular, several PIs act as substrates for isoenzyme 3A4, a subgroup of the cytochrome p450 system. Inhibition of the isoenzyme 3A4 can increase the blood concentration of statins and induce side effects. In contrast to most other statins, pravastatin and fluvastatin are not modulated by isoenzyme 3A4. Therefore, these two drugs are preferred in HIV+ patients. Simvastatin is contraindicated in patients receiving ritonavir-boosted PI-based ART. The goal of statin therapy is lowering the LDL-cholesterol level to targets shown in

Table 1. In case of insufficient control of LDL cholesterol levels the cholesterol uptake inhibitor ezetrol can be added.

Diabetes in HIV+ patients should be treated according to the general guidelines. From the cardiovascular point of view, an HbA1c of <7mg/dl should be aimed for.

Table 1: Prevention of coronary heart disease

- Stop smoking
- Balanced diet
- Moderate intensity exercise training (2.5–5 h per week)
- Normalize weight (BMI of 20–25 kg/m²)
- Reduce alcohol consumption (<15 g/d)
- Optimize blood pressure (systolic: <140 mmHg, diastolic <90 mmHg)
- Modulation of LDL cholesterol (ESC/EAS Guidelines for the Management of Dyslipidemias – DYSLIPguidelines-Dyslipidemias-FT.pdf n.d.)
  - Moderate risk (2 or more risk factors):   <115 mg/dl (3.0 mmol/l)
  - High risk (i.e., diabetes mellitus):   <100 mg/dl (2.5 mmol/l)
  - Very high risk (i.e., known CAD):   <70 mg/dl (1.8 mmol/l)
- Optimize blood glucose value (HbA1c <7%)

Secondary prevention of CAD aims at inhibition of platelet aggregation in unstable coronary lesions and at control of risk factors to prevent recurrent cardiovascular events. The control of risk factors should be a goal in patients with a very high risk of cardiovascular events. In randomized clinical trials, low dose aspirin (100 mg/d), ß blockers, ACE inhibitors and statins decrease the risk of mortality and re-infarction in patients with CAD. If aspirin is not tolerated it may be substituted by drugs that blocks the ADP receptor on platelets such as clopidogrel 75 mg/d. A calcium antagonist, nitrates, ranolazine and/or procoralan can be supplemented for symptomatic treatment. In patients with acute coronary syndrome a dual antiplatelet therapy should be maintained for at least 12 months.

The indication for invasive vascular diagnostic and intervention depends on current guidelines (http://www.escardio.org/knowledge/guidelines). Clear indications for coronary angiography are a documented exercise-induced ischemia, typical clinical symptoms together with ST alterations in the ECG, increases in cardiac enzymes and/or a marked cardiovascular risk profile. It is worth emphasizing that HIV infection is not an exclusion criteria for invasive procedures. Successful coronary interventions have been performed on HIV+ patients, including catheter procedures with implantation of drug-eluting stents (DES) (Saporito 2005, Glazier 2006, Neumann 2010) and coronary artery bypass operations (Filsoufi 2006). However, there are some reports that show an increased rate of re-stenosis after DES and increased rate of "major adverse cardiac events" following surgical revascularization in HIV+ patients (Boccara 2008, Ren 2009).

Recommendations for follow-up

HIV+ patients with cardiovascular risk factors should undergo an annual cardiac check-up, including a resting ECG and estimation of the cardiovascular disease risk based on the available risk scores. Symptomatic patients need further cardiovascular examinations (exercise ECG, stress echocardiography, laboratory work-up and, in some cases, myocardial scintigraphy or coronary angiography).

Congestive heart failure

Congestive heart failure includes a variety of myocardial alterations. In HIV+ patients, HIV-associated dilated cardiomyopathy is of major interest. It corresponds to a reduced systolic function with a dilated and less contractile left ventricle (Dakin 2006, Butt 2012). Myocarditis is still the most thoroughly studied cause of congestive heart failure in HIV disease. Until now, a variety of pathogens has been found in the myocardial tissue of HIV+ patients (Patel 1996, Wu 1992). Furthermore, HIV itself appears to infect myocardial cells in a patchy distribution. Myocardial damage by gp120 and cytokine-mediated apoptosis is presumed (Fiala 2004). Especially, HIV-1 is known to cause cardiomyopathy (Lopes de Campos 2014). In addition to a direct impact of HIV or other pathogens, dilated cardiomyopathy was reported in association with an autoimmune reaction. Cardiac-specific autoantibodies (anti-α-myosin antibodies) have been reported in up to 30% of HIV+ patients with cardiomyopathy. However, several studies indicate that dilated cardiomyopathy is associated with cardiotoxic agents (e.g., pentamidine, interleukin-2, doxorubicin) or caused by malnutrition (Nosanchuk 2002). Furthermore, it is under discussion whether antiretroviral drugs may induce cardiac dysfunction due to mitochondrial toxicity (Lewis 2006, Purevjav 2007). A retrospective study of a large cohort showed an association of tenofovir intake and incident heart failure (Choi 2011).

The prevalence of congestive heart failure in the pre-ART era was between 9% and 52% (Ntsehke 2005) and 29% in patients with AIDS (Levy 1989). Since the introduction of ART the prevalence of dilated cardiomyopathy seems to have decreased. In an Italian cohort a prevalence of 1.8% at the end of the 1990s was reported (Pugliese 2000). In a recent register study the rate of death due to cardiomyopathy was higher than in negative controls. However, the rate of death due to heart failure was lower (Whiteside 2015).

In recent years, growing evidence has been found that not only systolic function can be impaired in HIV+ patients but also diastolic function. (Schuster 2008, Hsue 2010, Reinsch 2011, Blaylock 2012). However, impairment of diastolic function was often asymptomatic. There is uncertainty about the causes for diastolic impairment, although there is evidence that it seems to be mainly related to HIV infection itself rather than to ART (Fontes-Carvalho 2015).

Chronic heart failure is associated with a reduced life expectancy. In cases of NYHA III-IV, the annual mortality rate rises to 25%. While in some cases a total recovery has been described (Fingerhood 2001, Tayal 2001), the majority of patients with HIV-associated dilated cardiomyopathy have a progression of left ventricular dysfunction and a poor prognosis (Felker 2000). It is unclear whether ART has an influence on the recovery of ventricular function. Potentially helpful for the assessment of prognosis in HIV cardiomyopathy is the evaluation of contractile reserve by stress echocardiography (Wever-Pinzon 2011). Early diagnosis and conventional therapy seem to be the most promising ways to reduce disease progression.

Unfortunately, heart failure is often not recognized. In a prospective study of 416 HIV+ patients with unknown heart disease the frequency of cardiac dysfunction was 17.7% (Twagirumukza 2007). Diastolic dysfunction was found in up to 48% of subjects in the HIV-HEART study (Reinsch 2010). Besides left ventricular dysfunction, cardiomyopathy often includes dilatation and reduced contraction of the right ventricle. However, a Danish study that enrolled 90 HIV+ patients did not find an increased rate of right ventricular dysfunction (Kjaer 2006).

The diagnosis of chronic heart failure is based on clinical findings and symptoms. In addition to exercise intolerance, patients often exhibit dyspnea and edema. Nocturia, night cough (cardiac asthma), peripheral cyanosis and weight increase may

also occur. In these cases, ECG, x-ray and echocardiography may lead to the diagnosis of heart failure.

Of proven value for diagnosis and follow-up is the serum parameter b-type natri- uretic peptide (BNP or NT-proBNP). The diagnostic value of BNP has been confirmed in the setting of HIV infection and heart failure (Neumann 2009). Exercise intoler- ance can be determined by a 6-minute walk test, exercise ECG or spiroergometry. In some cases, MRI or CT reveal scar tissue or coronary artery calcification (Breuckmann 2007). Invasive diagnosis including myocardial biopsies is often recommended in unexplained cases of chronic heart failure. Stable chronic heart failure patients in an early stage should be monitored annually. In advanced stages the monitoring should include ECG, echocardiography and occasional BNP measurements every 3 to 6 months.

Treatment of congestive heart failure

Since no randomized trials exist to investigate treatment of heart failure in HIV+ patients, recommendations are based on consensus and relate to the current guide- lines (www.escardio.org/).

Lifestyle modifications comprise moderate and regular exercise in combination with a healthy diet, including a reduced fluid and salt intake. Therapeutic options that could eliminate the causes of heart failure (such as revascularization, operative replacement in the case of a valvular heart disease or intensive antibiotic therapy for bacterial myocarditis) have priority. In these cases, cooperation with a special- ized center is recommended.

Contemporary treatment of congestive heart failure includes medication with a beta blocker, an ACE inhibitor and an aldosterone antagonist for neurohumoral block- age as a fundamental treatment that should at least be considered for every patient suffering from heart failure. Diuretics are often added for symptomatic relief.

In the setting of sinus rhythm, a heart rate <70/min, ejection fraction <35% and symptomatic heart failure, additional ivabradine can reduce hospitalization rate and increase LV function and quality of life. If the ejection fraction remains below 35% despite optimal medical treatment for 3 months, primary prevention with an implantable cardioverter defibrillator (ICD) to reduce the risk of sudden death is indi- cated. Cardiac resynchronization therapy has to be considered in symptomatic patients in cooperation with a cardiologist.

Comorbidities such as anemia, diabetes, COPD, gout, depression and disordered breathing while sleeping are associated with worse prognosis. Case reports also describe heart transplantation and treatment with an assist device in HIV patients (Sims 2011). For these cases cooperation with a specialized center is mandatory.

Non-steroidal antirheumatics (NSAR), class I antiarrhythmics, dronedarone, calcium channel blockers (verapamil, diltiazem and short acting dihydropyridine deriva- tives), glitazones for the treatment of diabetes and addition of angiotensin receptor antagonist or renin inhibitor to established therapy with an ACE inhibitor and an aldosterone antagonist should be avoided.

Recommendations for follow-up

HIV+ patients should be questioned and examined on an annual basis for clinical signs and symptoms of heart failure. If positive, the patient should undergo further evaluation comprising measurement of BNP, an ECG and a transthoracic echocar- diography. In case of abnormalities invasive coronary angiography should be taken into account to rule out CAD. In case of further deterioration of cardiac function despite optimal medical therapy additional examinations like cardiac MRI and

myocardial biopsy must be performed to rule out differential diagnosis like amyloidosis or different types of myocarditis that demand specific therapy (e.g., giant cell myocarditis). Once the diagnosis of cardiomyopathy is confirmed and therapy is initiated, patients must be seen regularly depending on their clinical presentation. For asymptomatic patients a follow-up interval of six months appears reasonable. However, for patients who continue to be symptomatic under optimal therapy a more frequent schedule is necessary.

Pericardial effusion

While pericardial effusion in the context of HIV infection is still common in African cohorts (Sliwa 2011, Chillo 2012) the prevalence in a German cohort studied in the HIV-HEART study was below 1% (Lind 2011).

However, the majority of HIV-associated pericardial manifestations are described as asymptomatic. The spectrum ranges from acute or chronic pericarditis to an acute pericardial tamponade (Silva-Cardoso 1999, Park 2010). Causes may include HIV itself, opportunistic pathogens, immune reconstitution syndrome or neoplasms (Stotka 1989). In HIV+ patients, infectious pericarditis by rare pathogens such as rhodococcus equi (Gundelly 2014) or mycobacterium avium complex (Babu 2014) can be found. Yet, in African cohorts by far the most frequent cause of pericardial effusion is tuberculous pericarditis (Reuter 2005). However, non-HIV-associated causes of pericardial effusion, such as uremia, trauma, irradiation, and drugs have to be considered. In some cases of lipodystrophy an increase in the cardiac lipid tissue could simulate an extensive pericardial effusion (Neumann 2002b). Chronic pericardial effusion can lead to constrictive pericarditis, which is characterized by impairment of diastolic filling due to a rigid and non-compliant pericardium.

Echocardiography is referred to as the standard method for diagnosis and follow-up of pericardial disease. Nevertheless, further diagnosis should be performed by computer tomography and/or magnetic resonance tomography if neoplasm or an increase in the cardiac lipid tissue is suspected. Diagnostic pericardial puncture can be performed to confirm the cause.

Treament of pericardial effusion

If possible, a causative therapy should be applied. Additional treatment comprises 10–14 days NSAID plus 3 months colchicine (2 × 0.5 mg; 1 × 0.5 mg in patients <70 kg). Pericardial puncture and pericardial tamponade can be performed in symptomatic patients. Pericardiotomy might be an option in chronic pericardial effusion. In cases of constrictive pericarditis, pericardiectomy must be considered.

Cardiac arrhythmias

HIV infection appears to lead to alterations of the autonomic nervous system and of cardiovagal autonomic function with a reduction in heart rate variability (Chow 2011). Cardiac arrhythmias can depend on medication. Antiretroviral drugs, e.g., efavirenz, foscarnet, pentamidine, or therapy with methadone, are expected to prolong the QT interval, an alteration in ECG that may cause Torsade de pointes tachycardia (Castillo 2002). Further drug combinations such as macrolides and chinolones may have the same effect on the QT interval. Results of the HIV-Heart study showed that prolongation of the QT interval is frequently found (20%). However, a correlation with antiretroviral drugs was not established (Reinsch 2009). Another prospective study also showed no correlation between QT prolongation and therapy with PIs (Charbit 2009).

Initiation or change of medication that might influence the QT interval should be controlled regularly by ECG. In case of arrhythmias, electrolyte and glucose concentrations have to be determined and corrected if necessary. Magnesium may be used for termination of Torsades de pointes tachycardia. Furthermore, heart rhythm disorders may occur together with cardiomyopathy. Dilatation of the ventricles carries an increased risk of life-threatening arrhythmias and sudden cardiac death (Lanjewar 2006). Ventricular arrhythmias were observed in the context of immune reconstitution syndrome (Rogers 2008). Conduction abnormality, bundle branch block and sinus arrest have been reported to occur with lopinavir/r and in combination with atazanavir (Chaubey 2009, Rathbun 2009).

The new anti-arrhythmic substance dronedarone is contraindicated with ritonavir because of metabolism by the CYP3A4.

Valvular heart disease/endocarditis

Valvular heart disease of HIV+ patients often occurs as bacterial or mycotic endocarditis. The hypothesis that HIV infection alone makes someone more susceptible to infective endocarditis has not been validated. However, intravenous drug users have a ten- to twelve-fold increased risk for infective endocarditis than non-intravenous drug users. Also, in intravenous drug users infection of the tricuspid valve is more frequent. The most frequent germ is *Staphylococcus aureus*, detected in more than 40% of HIV+ patients with bacterial endocarditis. Further pathogens include *Streptococcus pneumoniae* and *Hemophilus influenzae* (Currie 1995). Mycotic forms of endocarditis, which may also occur in patients who are not intravenous drug users, mostly belong to *Aspergillus fumigatus*, Candida species or *Cryptococcus neoformans* and are associated with a worse outcome.

A retrospective study showed no difference in the clinical outcome of *Staphylococcus aureus* endocarditis comparing HIV+ and -negative patients (Fernandez 2009). Signs of infective endocarditis include fever (90%), fatigue and lack of appetite. An additional heart murmur may also be present (30%). In these cases, repeated blood cultures should be taken and transesophageal echocardiography is mandatory (Bayer 1998). Due to the fact that detection of the infectious agent is often difficult, antibiotic therapy should be started early when endocarditis is presumed (Duke criteria), even without the microbiology results.

Antibiotic prophylaxis of endocarditis is not generally recommended. According to current guidelines for infectious endocarditis, antibiotic prophylaxis is only recommended for a very small patient population. For detailed information go to http://www.escardio.org/.

HIV-associated pulmonary arterial hypertension

One complication of HIV infection is the development of pulmonary arterial hypertension that clinically and histologically resembles idiopathic pulmonary arterial hypertension (PAH). HIV infection was included as one cause of PAH in the classification of pulmonary hypertension (Classification of Nice 2013, Galie 2014). Pulmonary hypertension is defined as mean pulmonary artery pressure >25 mmHg at rest (Badesch 2009).

Recent data show a 10% prevalence of PAH in an unselected HIV+ cohort (Quezada 2012). In another echocardiographic study a substantial proportion showed signs of increased pulmonary pressure on echocardiography (Schwarze-Zander 2015). In a study that compared HIV+ patients with normal controls, a high proportion had elevated pulmonary arterial pressure, even after elimination of other factors that could

have contributed to the elevation. Therefore, HIV seems to be an important cause of PAH. Compared to other forms, HIV-associated PAH has a worse prognosis.

The etiology of HIV-associated PAH is a combination of vasoconstriction and vascular remodeling with endothelial dysfunction, proliferation of endothelial and smooth muscle cells and finally vessel obliteration. As the majority of HIV+ subjects does not develop pulmonary hypertension, a complex genesis including genetic predisposition is likely. At the beginning there is inflammation which is fueled by HIV proteins tat, PG120 and in particular nef (Hassoun 2009). Pulmonary hypertension leads to an increased afterload of the right ventricle with hypertrophy, right heart dilatation and finally heart failure.

In a prospective study, women were more likely to be affected (1:1.4) by HIV-associated PAH, and intravenous drug abuse was the most common route of infection (53%) (Krings 2007, Reinsch 2008).

Fortunately, only a small proportion of patients with PAH become symptomatic. Dyspnea on exertion is the most common symptom of pulmonary hypertension. At the time of diagnosis 2/3 of patients can be classified in advanced stages of heart failure (NYHA III/IV). Further symptoms are lower limb edema, (dry) cough, syncope, angina, fatigue and weakness.

On physical examination one should pay attention to signs of right heart failure, such as edema, tachycardia, jugular vein distention and hepatomegaly. On auscultation one may find right parasternal systolic murmur indicating tricuspid insufficiency and a split second heart sound.

Based on clinical suspicion (dyspnea, syncope, edema, cough) further diagnostic work-up should consider pulmonary hypertension as a possible diagnosis. ECG and chest x-ray show indirect signs. With the help of transthoracic echocardiography one can estimate systolic pulmonary artery pressure (sPAP) and assess right ventricular form and function. In the case of elevated sPAP and signs of right ventricular strain pulmonary hypertension is probable. For definite diagnosis and acute vasodilator testing right heart catheterization is mandatory. To rule out other possible reasons for pulmonary hypertension such as chronic obstructive pulmonary disease or pulmonary thromboembolism, a CT scan and lung function test are often needed.

Treatment of PAH

Patients with HIV-associated pulmonary hypertension should be diagnosed and followed in a specialized center. There are several therapeutic options to reduce pulmonary artery pressure (Montani 2013). There is conflicting evidence if ART can reverse PAH. In a small retrospective study, decrease of pulmonary artery pressure was observed under ART (Zuber 2004). However, in another study, no improvement of hemodynamic parameters was observed under ART (Degano 2010).

The world consensus conferences in Dana Point and in Nice acknowledged the endothelin receptor antagonists bosentan and ambrisentan as well as sildenafil as class A for PAH WHO functional class II. Metabolism of sildenafil interferes with PIs such as ritonavir so that increased plasma level concentrations can be expected. Therefore, careful dosing is required (Chinello 2012). Further therapeutic substances in more advanced stages of PAH include derivates of prostacyclin intravenously, subcutaneously and by inhalation (Barst 2009).

HIV-associated PAH has only been studied in uncontrolled clinical trials. Bosentan and long-term infusion of epoprostenol seem to improve hemodynamics and exercise tolerance (Sitbon 2004). General measures include diuretics, oral anticoagulation, oxygen and if appropriate digoxin and rehabilitation. Physical stress and pregnancy should be avoided. Every effort should be made to prevent pneumonia.

Before initiating vasodilative therapy acute vasodilator testing is mandatory. However, a long-term response to calcium channel blockers after positive testing was rarely observed in patients with HIV-associated PAH (Montani 2010).

Further cardiac manifestations

Cardiac neoplasms are rarely found in HIV+ patients. These manifestations occur predominantly in advanced stages of the disease. On autopsy, the rates of cardiac-localized Kaposi sarcoma and lymphoma are less than one percent. Some cardiac infections in HIV+ subjects may not only result in myocarditis but in abscesses. Several opportunistic pathogens including *Toxoplasma gondii* and trypanosomes have been reported to cause cardiac abscesses. These manifestations are believed to decrease with ART. As well as neoplasms and abscesses, vascular alterations including vasculitis and perivasculitis have been described as further cardiovascular manifestations in HIV+ patients.

Table 2: Cardiac diseases in HIV-infected patients

Pericardial diseases
• Pericardial effusion
• Pericarditis (viral, bacterial, mycotic)
• Neoplasm (Kaposi sarcoma, lymphoma)

Myocardial diseases
• HIV-associated dilated cardiomyopathy
• Myocarditis (acute or chronic)
• Neoplasm (Kaposi sarcoma, lymphoma)
• Drug side effects (especially antiretroviral therapy)

Endocardial diseases
• Infective endocarditis (bacterial, mycotic)
• Nonbacterial thrombotic endocarditis

Vascular diseases
• Atherosclerosis
• Vasculitis, perivasculitis
• Pulmonary arterial hypertension

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25. HIV and Respiratory Diseases

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The spectrum of lung diseases in encompasses typical HIV-related complications such as TB and PCP, bacterial pneumonia, lymphomas and HIV-associated pulmonary hypertension, but also includes usual respiratory problems like acute bronchitis and asthma (see Table 1). Due to the better management of HIV+ people, comorbidities of the older patient become more important, such as COPD, bronchial carcinomas and lung fibrosis (Staitieh 2014, Feldman 2014). With ART, PCP and TB have become less frequent and pulmonary mortality has decreased (Grubb 2006, Morris 2011). HIV influences toll-like receptors and other factors of immune function that increase the risk of pneumonia (Morris 2011). Particularly in patients with respiratory problems and advanced immune deficiency, it is essential to take all differential diagnoses into consideration, of which this chapter presents an outline. PCP, mycobacterial infections and pulmonary hypertension are covered in detail in other chapters.

Table 1: Pulmonary complications in HIV+ patients

Infections	Neoplasia	Other
Pneumocystis jiroveci	Kaposi sarcoma (KS)	Lymphocytic interstitial pneumonia (LIP)
	Non-Hodgkin lymphoma	Non-specific interstitial pneumonia (NSIP)
Bacterial pneumonia	Hodgkin lymphoma	Cryptogenic organizing pneumonia (COP)
S. pneumoniae	Bronchial carcinoma	Pulmonary hypertension
S. aureus	Multicentric Castleman's	COPD
H. influenzae	disease (e.g., mediastinal	Bronchial hyperreactivity
B. catarrhalis	lymph nodes)	
P. aeruginosa		**Side effects of ART:**
Rhodococcus equi		Dyspnea + cough in hypersensitivity
Nocardia asteroides		reaction to abacavir
		Dyspnea + tachypnea in lactic acidosis
Mycobacteria		Pneumonia with T-20 therapy
M. tuberculosis		Pulmonary infiltration, lymph nodes and
Atypical mycobacteria		fever in IRIS
Other		
Cytomegalovirus (CMV)		
Aspergillus spp.		
Cryptococcus neoformans		
Histoplasma capsulatum		
Toxoplasma gondii		

Talking with the patient

The most important question: What is the immune status? The number of CD4 T cells is an excellent marker of the patient's individual risk of opportunistic infections. More important than the trough level (nadir) is the current CD4 T cell count. Above 200 cells/µl, typical opportunistic infections are unlikely. In these patients, generally "usual" problems such as acute bronchitis and bacterial pneumonia can be expected. However, TB should always be considered. Although the risk increases with immunodeficiency, more than half of HIV+ TB patients have more than 200 cells/µl (Wood 2000, Lange 2004).

In patients with less than 200 CD4 T cells/µl the most common pulmonary disease is bacterial pneumonia, and PCP is also typical. Pulmonary Kaposi sarcoma and pulmonary *Toxoplasma gondii* infection tend to appear at less than 100 cells/µl but are rarely seen. Below 50 cells/µl, pulmonary infections with CMV (mostly in combination with PCP), invasive pulmonary aspergillosis (IPA), endemic fungi *(Histoplasma capsulatum, Coccidioides immitis)* and infections with atypical mycobacteria occur. Especially in patients with advanced immunodeficiency, pulmonary disease might be an indicator of a systemic infection (e.g., aspergillosis). Rapid invasive diagnostics are advisable in patients with low CD4 T cells.

What are the signs and symptoms? PCP patients typically have dyspnea and a non-productive cough. A large quantity of discoloured sputum is more likely to indicate a bacterial cause or a combination of infections. Usually, bacterial pneumonia is of acute onset. These patients usually see a doctor after 3-5 days of discomfort (Cilloniz 2014).

What is the medical history of the patient? Someone who has had PCP previously is at higher risk of having it again. A patient with COPD might have just an exacerbation of his pulmonary disease.

What medication should the patient be on? Under 200 CD4, PCP is unlikely with cotrimoxazole prophylaxis and the risk of bacterial pneumonia may be reduced (Beck 2001). When pentamidine inhalation is used for PCP prophylaxis, however, atypical PCP can present in the upper lobes.

Has the patient recently started ART? Respiratory symptoms after starting ART might result from immune reconstitution and inflammatory syndrome (IRIS). IRIS may have infectious and non-infectious causes (Grubb 2006). Low CD4 T cell count and high viral load are risk factors. In a retrospective analysis, IRIS was seen in 30% of patients with TB, atypical mycobacteriosis and cryptococcosis (Shelburn 2005).
Because of HLA testing, hypersensitivity to abacavir is rarely seen today. Dyspnea (13%), cough (27%) and pharyngitis (13%) are common symptoms of hypersensitivity (Keiser 2003). Some patients even develop pulmonary infiltrates. T-20 seems to increase the risk of bacterial pneumonia, at least among smokers. Dyspnea and tachypnea are also seen as symptoms of lactic acidosis secondary to NRTI therapy.

Does the patient smoke? Smoking is more harmful to HIV+ than to negative persons, and is more common (Crothers 2011). Smoking promotes local immunodeficiency in the lung. It reduces the number of alveolar CD4 T cells and the production of important pro-inflammatory cytokines (Wewers 1998) and suppresses the phagocytic capacity of alveolar macrophages (Elssner 2004). Both HIV-associated and -independent pulmonary diseases are more common in smokers. This applies to bacterial pneumonia and PCP, but also to asthma, COPD and pulmonary carcinoma (Hirschtick 1996, Crothers 2011).
Motivating the HIV+ patient to quit smoking is important. Promising strategies that are supported by scientific evidence are brief verbal interventions, participation in motivational groups, nicotine substitutes and bupropion medications. For bupropion, interactions with boosted ARVs should be taken into consideration. The Smoking Cessation Handbook of the US Department of Veterans Affairs (http://www.va.gov/vhapublications/ViewPublication.asp?pub_ID=2826) provides practical assistance (Veterans Health Administration 2012).

What is the patient's geographical background? An important question is the travel history and/or background of the patient. Histoplasmosis, for example, is more widespread in certain parts of the US and in Puerto Rico than PCP, yet is rare in Europe. Coccidioidomycosis can occur endemically. TB is more frequent in immigrants from high-prevalence countries.

How did the patient become infected with HIV? Intravenous drug users suffer more often from bacterial pneumonia or tuberculosis (Hirschtick 1995). Pulmonary KS is almost exclusively found in MSM.

What does the chest X-ray look like?

Table 2: What does the chest X-ray look like?

Chest X-ray	Typical differential diagnosis
Without pathological findings	Pneumocystis pneumonia (PCP), asthma, KS of the trachea
Localized infiltrates	(Myco)-bacterial, fungal infections, lymphoma, lung cancer
Multifocal infiltrates	Bacterial pneumonia, mycobacteriosis, PCP, KS
Diffuse infiltrates	PCP (ground glass, predominantly central), CMV, KS, LIP, cardiac insufficiency, fungal infections
Miliary pattern	Mycobacterial, fungal infections
Pneumothorax	PCP
Cavernous lesions	Mycobacteriosis (CD4 >200), bacterial abscess (Staph., pseudomonas), lung cancer
Cystic lesions	PCP, fungal infections
Pleural effusion	Bacterial pneumonia, mycobacteriosis, KS, lymphoma, cardiac insufficiency
Bihilar lymphadenopathy	Mycobacteriosis, KS, sarcoidosis, lymphoma, multicentric Castleman's disease

KS = Kaposi sarcoma, LIP = Lymphoid interstitial pneumonia

Pulmonary complications and comorbidities

COPD, lung cancer, pulmonary hypertension, lung fibrosis und pulmonary infections are more common in HIV+ patients (Crothers 2011). In patients on ART, pulmonary infections are less frequent while non-infectious pulmonary diseases are more frequent (Crothers 2011, Morris 2011).

Bacterial pneumonia

Bacterial pneumonia occurs more often in HIV+ patients (Crothers 2011) and like PCP, leaves scars in the lung. This often results in a persistent restrictive lung function impairment (Alison 2000) and significantly worsens the long-term prognosis of the patient (Osmond 1999). The risk increases with higher immunosupression and age. Thus, acquiring bacterial pneumonia more than once a year is regarded as AIDS-defining. The introduction of ART resulted in a significant decrease of bacterial pneumonia (Jeffrey 2000, Grau 2005, Madeddu 2009, Crothers 2011).

However, HIV+ patients more often present with fewer symptoms and a normal leucocyte count (Feldman 1999). Below 200 CD4 T cells/µl, multifocal and interstitial manifestations are more common in pneumococcus infections (Rizzi 2008). The CD4 T cell count is important for the risk stratification (Lim 2003), in addition to the usual criteria of the CRB-65 score (confusion, respiratory rate, blood pressure, age >65 years). The mortality of patients with less than 100 CD4 T cells/µl is increased by more than six-fold. To have a low threshold to admit severely immunocompromised patients to hospital is probably more reasonable than to rely on risk scores

validated for immunocompetent patients (Cordero 2000). For details of microbiologic pathogens and treatment see the chapter *AIDS*.

In a recent analysis, patients with bacterial pneumonias more often report less days of symptoms and have a higher CRP level compared with PCP patients, whereas PCP patients have a lower white blood cell count, a higher LDH level and more multilobular infiltrations (Cilloniz 2014).

Pneumococcus vaccination is recommended (Nunes 2012). However, at a CD4 T cell count lower than 200/µl there is no proof of benefit. Due to the frequency of secondary bacterial infections, an annual influenza vaccination is advisable.

COPD and Emphysema

COPD is, along with pneumonia, the most common pulmonary complication in HIV+ patients (Crothers 2011). Probability of developing lung emphysema is higher (Crothers 2006) and quality of life is reduced (Drummond 2010). Each patient should be asked about COPD symptoms such as cough, dyspnea and sputum, and a spirometry test should be offered. A pathogenetic synergy from smoking and pulmonary infiltration with cytotoxic T cells due to HIV infection is possible (Diaz 2000, Yearsley 2005, Caner 2009). ART was shown as an independent factor for developing bronchial obstruction and COPD if this occurs in IRIS (George 2009). Smoking cocaine (crack) increases the risk of pulmonary emphysema even more. In this case, it seems that superficial epithelial and mucosal structures are destroyed (Fliegil 1997). Furthermore, crack can sometimes cause pneumothorax or alveolar infiltrates.

Bronchial Asthma

Besides COPD, bronchial asthma is the most common pulmonary comorbidity in HIV+ patients and is more frequent than in negative individuals (Drummond 2014). In case of cough, dyspnea or recurrent bronchitis, a hyperreactive bronchial system as a sign of asthma should be considered. It is not clear whether the immunosuppression of HIV protects patients from exaggerated immune reactions like allergies and asthma. In the pre-ART era the incidence of asthma seemed to be not influenced by the presence of HIV (Wallace 1997). A recent US study shows a lower incidence of asthma with ART and low viral load (Crothers 2011).

Inhaled corticosteroids should not be combined with boosted ARVs because of the risk of hypercortisolism (Cushing syndrome). Other inhaled medications, however, do not seem to be influenced by ART. Since steroids are the treatment of first choice for asthma, an ART change may be reasonable. Integrase inhibitors do not affect the cortisol levels. In COPD patients, corticosteriods should be considered with caution (Drummond 2014). Of note, HIV+ children have more asthma (Siberry 2012). Immunoreconstitution with ART is associated with an increase of asthma incidence in children (Foster 2008).

Lung Fibrosis and Lymphoid Interstitial Pneumonia (LIP)

Lung fibrosis is a rare disease, but more frequent in HIV+ patients (Crothers 2011). Manifestations like COP, NSIP, UIP and alveolar proteinosis have been described (Crisan 2009). LIP is a form of pneumonia with a chronic or subacute course and is extremely rare in adults. Its reticulonodular X-ray pattern is similar to PCP. LIP occurs paraneoplastic, rarely idiopathic, and can be caused by infections such as HIV and EBV. In contrast to PCP, patients with LIP usually have a CD4 T cell count of more than 200/µl and normal LDH values. A CD8-dominated lymphocytic alveolitis with

no pathogen detection is characteristic. Definite diagnosis often calls for an open pulmonary biopsy. LIP is considered sensitive to steroids. LIP can occur in the context of the Diffuse Infiltrative Lymphocytic Syndrome (DILS), at which DC8-lymphocytic infiltrates manifest in the parotid glands and other organs (Ghrenassia 2015). The role of ART is unclear, especially as LIP has occasionally been observed in the context of immune reconstitution during ART. Other interstitial pneumonias, like cryptogen organizing pneumonia (COP with BOOP as histology) or nonspecific interstitial pneumonitis (NSIP) are also seen in association with HIV (Khater 2004).

Bronchial Carcinoma (BC)

Multiple studies, including one meta-analysis, show a two- to eight-fold increased incidence of bronchial carcinoma (Hessol 2006, Shiels 2009, Polesel 2010, Crothers 2011, Hoffmann 2013). In the ART era more patients die from BC than from most AIDS-defining malignancies (Engels 2008). See chapter on *Non-AIDS-defining Malignancies*.

Less common opportunistic infections

In HIV+ children, CMV pneumonia is more often seen than PCP (Zampoli 2011), while in adults it is less frequent. The significance of the pathogen in the later stages may be underestimated, since histological examination of autopsy material showed pulmonary CMV infections in up to 17% (Waxman 1997, Afessa 1998, Tang 2005). However, in respiratory insufficiency due to PCP, CMV pneumonia should be considered and perhaps treated, because a coinfection has a higher mortality (Boonsarngsuk 2009). The detection of CMV in BAL repeatedly gives rise to discussion regarding clinical relevance. At over 90%, seroprevalence is high, and colonization of the respiratory tract is common. Transbronchial biopsy may prove CMV infection, blood markers (CMV PCR or pp65 antigen) may be helpful. Regarding invasive pulmonary aspergillosis (IPA), which only occurs in the late stages and usually in conjunction with additional risk factors such as neutropenia or steroid therapy (Mylonakis 1998), refer to the chapter AIDS.

Diagnostic strategy for pulmonary infiltrates

The intensity of the diagnostic workup in a patient with pulmonary infiltrates is based on the stage of HIV and the expected spectrum of pathogens. With a CD4 T cell count of more than 200/µl, non-invasive basic diagnostics and a calculated antibiotic therapy are justified. At 25–60%, the rate of bacteremia is higher than in immunocompetent patients (Miller 1994), so two pairs of blood cultures and a microscopic and cultural sputum examination including mycobacteria should be done in inpatient settings.

In advanced stages (below 200 CD4 T cells/µl), and if rapid diagnostic management is possible and does not delay treatment, bronchoscopy is recommended (Dalhoff 2002). The diagnostic success rate in HIV+ patients with pulmonary infiltrates is 55–70% and reaches 89–90% when all techniques including transbronchial lung biopsy are combined (Cadranel 1995). The sensitivity of a bronchoalveolar lavage (BAL) is 60–70% in bacterial pneumonia in patients without previous antibiotic treatment, and 85–100% in PCP (Baughman RP 1994). Due to the high sensitivity of the BAL, transbronchial biopsy with possible complications is only recommended in the diagnosis of PCP with a negative initial diagnostic workup and in patients taking chemoprophylaxis (Dalhoff 2002).

In individual cases the possibility of antigen detection in the urine should be considered (e.g., pneumococcus, legionella, cryptococcus, histoplasma). The determination of the cryptococcus antigen in serum has a high predictive value for the detection of invasive cyptococcosis (Saag 2000).

A chest CT is helpful in the diagnostic workup (high resolution CT or multi-slice CT). PCP, for example, might be depicted in CT, but might be missed in a conventional chest X-ray. Surgical open biopsies and CT-guided transthoracic pulmonary biopsies are rarely necessary.

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26. HIV-related Thrombocytopenia

HEINZ-AUGUST HORST

Thrombocytopenia is one of the most frequently observed hematological complications of HIV infection. The incidence increases among patients not receiving adequate antiretroviral treatment and does not appear to vary according to the mode of acquisition of HIV (Heyward 1988, Finazzi 1990, Sloand 1992). A 10-year cumulative incidence of up to 45% has been reported (Eyster 1993). In patients with previously well controlled HIV infection a discontinuation of ART can lead to the rapid occurrence of thrombocytopenia (Bouldouyre 2009). Thrombocytopenia is mostly mild and asymptomatic. Platelet counts of <30,000/µl have only been seen in less than 10% of the cases with HIV-related thrombocytopenia (Mientjes 1992, Vannappagari 2011). HIV-related thrombocytopenia has been generally attributed to two different mechanisms: First, an immunologically driven destruction of the platelets and second, an insufficient platelet production by the megakaryocytes. While in early HIV infection increased platelet destruction appears to be predominant, production failure is often the main cause of thrombocytopenia in late-stage patients (Najean 1994).

Table 1: Differential diagnoses of thrombocytopenia, except HIV

- Pseudo-thrombocytopenia
- Toxic bone marrow suppression: drugs, e.g., TMP-SMX, rifampicin, ethambutol, radiation
- Infection: HCV, *H. pylori*, CMV, MAC
- Malignant lymphoproliferative B cell disorders: e.g., chronic lymphocytic leukaemia, diffuse large B cell lymphoma
- Immunologic: Systemic lupus erythematodes, immune thyroiditis, Evans syndrome, heparin
- Other causes: HUS, TTP, PNH, hypersplenism, liver cirrhosis

Clinical manifestations

The clinical course is often asymptomatic. However, a spectrum of bleeding problems including petechiae, epistaxis, ecchymosis, menorrhagia, hemorrhage of the gingivae may occur. Severe bleeding of the gastrointestinal tract or the CNS are rarely observed and are most likely at platelet counts <30,000/µl. In contrast to patients with immune thrombocytopenic purpura (ITP) patients often present with splenomegaly and lymph node enlargement. Spontaneous remissions of HIV-related thrombocytopenia have been observed in 10–20% of the cases, mostly with mild thrombocytopenias (Walsh 1985, Abrams 1986).

Recently, the evaluation of the EuroSIDA data showed a possible association between thrombocytopenia and non-AIDS-related cancer (Borges 2014).

Diagnosis

HIV-related thrombocytopenia is a repeatedly confirmed isolated decrease of the platelet count <100,000/µl. In the peripheral blood the platelets often show an increased variability in size. In the bone marrow the number of megakaryocytes is normal or increased.

HIV-related thrombocytopenia has to be distinguished from cases of EDTA-induced pseudo-thrombocytopenia and from other causes of "true" secondary thrombocytopenias, which include myelotoxic drugs, hepatitis C virus (HCV), cytomegalovirus (CMV) and *Mycobacterium avium complex* (MAC) infections. The risk of heparin-induced thrombocytopenia is probably increased in HIV+ patients (Thompson 2007).

In rare cases thrombocytopenias induced by ART have been observed (Lebensztejn 2002, Camino 2003). Furthermore, the distinction from thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome is of great importance. These diseases show a peripheral platelet destruction not related to an immune mechanism, occur in higher frequency with HIV infection, and are life threatening. Important causes of thrombocytopenia are summarised in Table 1.

Therapy

The therapy is based on two principles: antiretroviral therapy, and in severe cases an additional treatment with agents used in non-HIV immune thrombocytopenia, i.e., glucocorticoids, intravenous immunoglobulins, or anti-(Rh)D. In refractory cases splenectomy is also a treatment option (George 1996, Godeau 2007). The treatment besides ART is based on the recent international consensus report and the guidelines of the American Society of Hematology (Provan 2010, Neunert 2011). Treatment options are summarized in Table 2.

Table 2: Therapy of HIV-related thrombocytopenia

Clinical Situation	Therapy
Asymptomatic and thrombocytes >30,000/μl	ART
Thrombocytes <30,000/μl or thrombocytes <50,000/μl and significant mucous membrane bleeding	ART plus **First-line therapy:** glucocorticoids **Subsequent therapies*:** intravenous immuno-globulins, anti-(Rh)D, rituximab, splenectomy
Severe bleeding	Platelet transfusions, high-dose glucocorticoids, intravenous immunoglobulins, either alone or in combination

* Subsequent therapies after failure of glucocorticoids should be given according to the experience of the treating physician since only a few prospective randomised studies are available (Vesely 2004)

ART: leads to a significant recovery of the platelet count within three months of treatment in most patients (Arranz Caso 1999, Servais 2001). This effect is independent of the antiretrovirals utilised and the platelet count at the start of therapy (Arranz Caso 1999). Importantly, during treatment interruptions often thrombocytopenias develop, particularly in patients with a history of HIV-related thrombocytopenia (Ananworanich 2003, Bouldouyre 2009). A therapy in addition to ART is indicated for patients with a platelet count <30,000/μl or a platelet count of <50,000/μl with a significant concomitant mucous membrane bleeding or risk factors for bleeding, such as peptic ulcers or hypertension (George 1996).

Glucocorticoids: are currently the standard first-line therapy of HIV-related thrombocytopenia. A dose of 0.5-1.0 mg/kg body weight prednisolone or prednisone results in a significant increase in platelet counts in 60–90% of the patients (Gottlieb 1983, Abrams 1986). After a response, which can be expected within a few days, the initial dose should be continued for 3-6 weeks. Then, depending on the platelet count, which should be kept >60,000/μl, the glucocorticoid dose should be tapered within weeks and discontinued if possible. In the case of a life-threatening bleed we recommend higher dosages (i.e., 1 g methylprednisolone/day for three days with subsequent dose reduction). In order to avoid a long-lasting therapy with prednisolone or prednisone and possible side effects, a short-term protocol with high-dose dexamethasone may be used. After treatment with 40 mg of dexamethasone

for four consecutive days in patients with non-HIV immune thrombocytopenia a response can be seen in 85% of patients. A relapse does occur in 50% of the responding patients within six months. These patients require a prolonged therapy with glucocorticoids or a different treatment (Cheng 2003). After four cycles of dexamethasone given for four days every 14 days in 74% of the patients a long-term response (median time of 8 months) can be seen (Mazzucconi 2007). Using steroids it has to be kept in mind that particularly prolonged treatment is associated with a high risk of even fatal infectious complications (Portielje 2001, Zimmer 2004).

Intravenous immunoglobulins: are costly and often given after failure of glucocorticoids, in the case of contraindications against glucocorticoids or in a situation with life-threatening bleeding. The standard dose is 1 g/kg body weight for 1–2 days. The response rate is approximately 60%. Without maintenance therapy the platelet count will decrease in most patients and it drops to the pre-treatment levels after about a month (Godeau 2007).

Anti-(Rh)D: The intravenous anti-(Rh)D application is an interesting treatment option. The mechanism of action is assumed to be mediated through the destruction of antibody-coated (Rh)D positive red blood cells (RBC). The preferential clearance of antibody-coated RBC by macrophages particularly in the spleen leads to an Fc receptor blockade sparing the destruction of autoantibody-coated platelets (Scaradavou 1997). The response rate in HIV-related thrombocytopenia was 64% (Scaradavou 1997). The peak platelet count was significantly higher and the duration of response significantly longer in HIV+ patients treated with anti-(Rh)D compared to intravenous immunoglobulins (Scaradavou 2007). WhinRho◊ SDF (Cangene Corporation) is the first anti-D immunoglobulin approved for use in HIV-related thrombocytopenia. The recommended initial dose for adults is 50 µg/kg body weight administered i.v. by a 3–5 minute infusion. In patients with a hemoglobin level less than 10 g/dl a reduced dose is recommended. It has to be kept in mind that anti-(Rh)D is only suitable for (Rh)D positive patients who are not splenectomized. An important adverse event is a decrease of the hemoglobin level by hemolysis. In a large study of 272 patients the mean hemoglobin decrease was 0.8 g/dl (Scaradavou 1997). Patients with pre-existing hemolysis (Evans syndrome) should not be treated with anti-(Rh)D.

Splenectomy: is effective even after failure of treatment with glucocorticoids and intravenous immunoglobulins. Most studies in HIV+ patients showed a high response rate of more than two-thirds of patients with a normalization of the platelet count in most responders. Although relapses occur, most of the patients show a sustained increase of their platelet count (Oksenhendler 1993). Worsening of the immunodeficiency by splenectomy leading to an acceleration of the HIV infection was a major concern about this procedure which, however, was not seen at long term follow up (Oksenhendler 1993). Independent of HIV status patients undergoing splenectomy are at increased risk of life-threatening bacterial infections. For prophylaxis polyvalent pneumococcal vaccine, *Hemophilus influenzae* type b, and meningococcal vaccine should be given at least two weeks prior to splenectomy. A response in HIV+ patients with CD4 T cells of less than 400/µl is uncertain (Greub 1996). Considering the other treatment options splenectomy should only be performed in individuals presenting with therapy-resistant severe HIV-related thrombocytopenia. Particularly because of morbidity splenectomy should be postponed for at least 6 months after diagnosis since late partial or complete responses can occur subsequent to efficient HIV suppression and additional therapy of the thrombocytopenia.

Rituximab: received increasing attention as a promising drug for the treatment of refractory non-HIV immune thrombocytopenia (Godeau 2007). Successful treatment was also reported in HIV-related thrombocytopenia (Ahmad 2004). However, particularly in patients with low CD4 T cells (<100/µl), rituximab should only be used after thoroughly considering the possibly increased risk of infections caused by B cell depletion through the anti-CD20 antibody. Several HIV-negative cases of progressive multifocal leukoencephalopathy with fatal outcome have been observed after rituximab therapy (Carson 2009). A systematic review of the literature on the efficacy of rituximab in adults (age >15 years) with non-HIV immune thrombocytopenia revealed a response rate (thrombocytes >50,000/µl) of 62%. A response was usually seen 3–8 weeks after the first infusion of rituximab and lasted from 2–48 months (Arnold 2007). In patients with relapsed/refractory immune thrompocytopenia a replacement of splenectomy by rituximab is being discussed (Godeau 2007).

Interferon-α: significantly increased platelet counts in a small randomized, placebo-controlled study on patients with HIV-related thrombocytopenia. At a dose of 3 million units three times weekly for four weeks an increase of >60,000 platelets/µl was observed within three weeks of treatment. Subsequent to therapy interruption the platelet counts slowly returned to pre-treatment values (Marroni 1994). They can be increased again on reinstitution of interferon-α therapy. Treatment of refractory HIV-related thrombocytopenia may be particularly promising in patients coinfected with HCV. Adverse events of interferon-α are flu-like symptoms, depression and, less frequently, cytopenias.

Thrombopoietin receptor agonists: are a new treatment option in non-HIV immune thrombocytopenia. In a phase III study a platelet response occurred in 79% of the splenectomized and in 88% of the non-splenectomized patients with non-HIV immune thrombocytopenia after s.c. treatment with the peptide romiplostim. These responses were durable (platelet count >50,000/µl for >6 weeks) in 38% of the splenectomized and in 61% of the non-splenectomized patients (Kuter 2008). The recommended starting dose for romiplostim is 1 µg/kg given s.c. once weekly. It is than adjusted to 1–10 µg/kg weekly according to the platelet count. An increment can be expected after 7–10 days.
A response rate of more than 80% in non-HIV thrombocytopenia was also reported for the small molecule eltrombopag, which can be administered orally (Bussel 2006+2007). The recommended starting dose is 50 mg once daily. It has to be adjusted to 25–75 mg daily according to the platelet count. A platelet response can be expected after 7–10 days. For patients of East Asian ancestry or patients with moderate or severe hepatic insufficiency, eltrombopag was approved at a starting dose of 25 mg once daily. Furthermore, a positive effect of eltrombopag on the platelet count was shown in HIV- and in HCV-associated thrombocytopenia (McHutchison 2007, Quach 2012). It might be necessary to adjust the dose of eltrombopag when given with ART. The co-administration of the PI lopinavir/r lopinavir/ritonavir with eltrombopag decreased the plasma concentration of eltrombopag by 17% (Wire 2012). Romiplostim and eltrombopag received FDA and EMA approval. A systematic evaluation regarding the role of thrombopoietin receptor agonists in HIV related thrombocytopenia and data on the long-term safety, however, are still missing.

Platelet transfusion: Since the increased platelet destruction is an important mechanism in HIV-related thrombocytopenia, platelet transfusions are only useful in the rare situation with life-threatening bleeding. In this situation platelet transfusions are combined with high dose glucocorticoids (e.g., methylprednisolone 15 mg/kg

for 3 days) and intravenous immunoglobulins (1 g/kg for 2 days) (Godeau 2007). Platelet transfusions are also recommended before splenectomy if the platelet count is <10,000/μl, despite adequate therapy.

Additional treatment options: Promising results for many other drugs have been reported including cytotoxic and immunosuppressive agents, i.e., azathioprine and cyclosporin A. However, in most studies the numbers of patients are few and long-term safety data are missing (Vesely 2004). This is particularly true for the treatment of HIV-related thrombocytopenia.

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27. HIV-associated Skin and Mucocutaneous Diseases

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Introduction

In comparison to the general population HIV+ patients develop skin diseases more often (Rothengatter 2009). Skin and mucocutaneous diseases are important at the first diagnosis of HIV infection and in determining the clinical stage. In 10% of cases, the diagnosis of HIV infection is based on diseases of the skin and the mucous membranes (Itin 2008). High HIV prevalence was detected in patients with sexually transmitted diseases (STDs) and skin diseases like seborrheic dermatitis in the HIV Indicator Diseases across Europe Study (HIDES I, Sullivan 2011).

The spectrum of HIV-associated dermatoses has dramatically changed in recent years with ART. KS (Friedman-Kien 1981), OIs of the skin (e.g., the rodent ulcer herpes simplex infections) and the mucous membranes (e.g., *Candida* infections) have been observed as marker diseases of acquired immunodeficiency. Almost any HIV-associated or AIDS-defining disease can be manifested on the skin and mucous membranes before other symptoms appear. The broad spectrum comprises infections from viruses, fungi and bacteria as well as protozoa and parasites (Gottlieb 1981, Siegal 1981, Schöfer 1991+1999). KS and OIs have been reduced by the increasing use of ART in industrial countries, while side effects and incompatibilities with drugs, virus- and UV-associated epithelial tumors as well as other STDs are on the rise (Costner 1998, Sepkowitz 1998, Kreuter 2002, Calista 2002). HIV+ patients should, have regular dermatologic screening tests and treatment.

Skin and the mucous membranes are "independent organs" of the immune system. An immunodeficiency allows even harmless saprophytes on the body surface and follicle openings to penetrate into deeper tissue layers, and thus develop life-threatening infections. An increase in wound infections and pyoderma, as well as in clinically relevant methicillin-resistant infections have been seen in (Burkey 2008). In addition to common dermatoses (e.g., oral candidiasis, herpes zoster, seborrheic dermatitis) other diseases that have rarely or never been reported have been diagnosed in progressive immunodeficiency (cutaneous cryptococcosis, bacterial angiomatosis, oral hairy leukoplakia, *Penicillium marneffei* infections).

In tropical and subtropical regions, STIs like herpes genitalis, chancroid and other ulcerating diseases occurring on the genitals play a decisive role in spreading HIV. Syphilis and lymphogranuloma venereum (LGV) are experiencing a renaissance in Europe. Homosexual men are the main carriers in this new epidemic. Since 2000, the number of male syphilis patients in big cities has grown tremendously. In Germany, in 45% of newly registered syphilis infections, HIV infection is diagnosed at the same time (RKI 2008). Circumcisions decrease the risk of sexual transmission of HIV infection (Warner 2009, Giuliano 2009) for men but the best protection is the use of condoms.

The immune system protects the skin and the mucous membranes against the development of various malignant tumors (Schöfer 1998). Oncogenic viruses plus immunodeficiency increase the ratio of many neoplasias in. Some of these are KS (HHV-8), NHL (EBV, HHV-8) as well as cervical and anal carcinoma (HPV, especially HPV-16 and -18) (Esser 1998). In HIV+ patients, younger people appear more at risk from cancer (Mitsuyasu 2008). The longer a cellular immunodeficiency exists, the more likely epithelial tumors will develop that affect the skin and the mucous membrane (basal cell carcinomas, cutaneous and mucocutaneous squamous cell carcinomas).

This is also true for malignant melanomas. Despite ART, HPV-associated diseases are increasing. Due to a rising incidence of anal carcinomas, regular proctological examinations are recommended in addition to the current colposcopic monitoring, especially for HIV+ MSM with known *condylomata acuminata* (Kreuter 2003, DAIG 2013). Avoiding risk factors and regular checkups may help to prevent cancer. The skin and the mucous membranes are easily accessible and suspicious lesions can be removed at an early stage.

Knowledge of diagnosis and therapy of HIV-associated dermatoses is interdisciplinary and indispensable for an efficient treatment.

Dermatological examination and therapy in HIV+ patients

Inspection of the whole skin surface, the mucous membranes of the mouth, the genitals, the anal region as well as palpation of the lymph nodes can be done without any special effort or expense. But even for an experienced physician, diagnostic and therapeutic problems may arise when examining HIV+ patients – the clinical picture may differ from textbook knowledge. Skin and mucocutaneous diseases often show an unusual, more serious, faster and therapy-refractory clinical course (Ameen 2010). The spectrum of causes of an infection may differ considerably from HIV-negative patients (Imaz 2010). The coexistence of several infections means a serious immunodeficiency. Therefore, it is important to examine lesions correctly before starting therapy. In case of inconclusive test results or in patients who are in advanced HIV stages punch biopsies should be done to obtain histological reports.

Standard treatment of the skin and the mucous membranes might fail in HIV+ patients. The main reasons for this are an advanced immunodeficiency as well as resistance. In such cases, a higher dose over a longer period of time should be given, keeping in mind possible toxic side effects (Osborne 2003). Interactions, e.g., with azole antimycotics or aromatic retinoids, should be considered regarding patients on ART. Immunosuppressive therapies should be used cautiously, and only for a short period of time. UV treatment (e.g., PUVA therapy for psoriasis) should be considered carefully and closely supervised when used as viral infections may potentially be provoked, malignant tumors induced and the HIV viral load increased (Popivanova 2010). On the other hand, good therapy results of the UVB 311nm phototherapy in therapy-refractory itching papular dermatoses has been observed without showing deterioration of the immunological situation in individual cases. Diagnostics and therapy can require the whole repertoire of a clinical center primarily specialized in infectious diseases as well as the interdisciplinary cooperation of different expert groups.

ART: Influence on skin and mucocutaneous diseases

In the context of life-long treatment, ART-associated side effects are of decisive importance for prognosis, in particular regarding the skin and the mucosa. Regarding exanthemas, the differentiation of a drug reaction from other causes, e.g., an immune reconstitution syndrome, syphilis or viral exanthema presents a big challenge. The identification of the agent as the cause of exanthemas is often difficult in patients on multiple treatments.

The typical side effects of some drugs (nevirapine, abacavir) are exanthemas. Pharmacogenomic HLA-B*5701 tests help to avoid hypersensitivity reaction against abacavir (Mallal 2008). ART may cause lipodystrophy. Lipoatrophy can probably develop with some NRTIs, whereas lipohypertrophies are seen with some PIs (Carr 1998, Carr 2000). These disorders of adipose tissue are often stigmatizing. But the incidence of the lipodystrophy syndrome has decreased since new antiviral substances and classes with better tolerability have become available (Potthoff 2010).

Appendix: Frequent especially HIV-associated skin diseases

Acute HIV exanthema: after HIV transmission, 40-90% of patients develop an acute, febrile, mononucleosis-like disease with constitutional symptoms and exanthema (see chapter on *Acute HIV-1 Infection*). This nonspecific eruption starts 1 to 3 weeks after transmission, and weeks before HIV seroconversion. The macular exanthema favors the upper trunk and is characterized as fairly non-pruritic with erythematous macules from 0.5 to 1 cm in diameter. Morbilliform or rubella-like eruptions and palmoplantar hyperkeratotic eczema occur less frequently. Histopathology reveals a non-specific perivascular and interstitial infiltrate in the upper- and mid-dermis (Barnadas 1997). Oral aphthous ulcers frequently in combination with shallow genital ulcers (bipolar aphthosis) are another important clinical symptom (Hulsebosch 1990, Porras-Luque 1998). Differential diagnosis includes viral infections (EBV, CMV), Mediterranean spotted fever (Segura 2002), secondary syphilis, drug eruptions (Hecht 2002, Daar 2001) and Behcet's disease.

Anal cancer: See chapters on *STDs* (*Condylomata acuminata*) and *Cervical and Anal Cancer.*

Aphthous ulcers: At least three different kinds of aphthous ulcers can occur in the oral cavity of HIV+ patients. The most frequent diagnosis is recurrent aphthous stomatitis (canker sores) (1) with single or few painful lesions usually localized in the vestibule of the mouth. The ulcers occur at sites of mechanical injuries, are 3 to 10 mm in diameter and heal spontaneously after a few days. Single or multiple large aphthae (2) which are >1 cm in diameter and usually persist for several weeks are less common. Both variants are of unknown origin (Rogers 1997). In a few cases, especially when multiple small lesions occur, herpes simplex viruses can be involved. Large ulcers in combination with severe immunodeficiency can be caused by cytomegalovirus, usually part of a generalized CMV infection. Bipolar aphthosis (3) involving the oral and genital mucosal membranes is an important clinical symptom of acute HIV infection or Behcet's disease. In addition to these clinical variants of aphthous ulcers several authors have discussed the direct role of HIV in aphthous stomatitis (Kerr 2003). The treatment of recurrent aphthosis is based on topical anesthetics and corticosteroids. Large persistent aphthae can require intralesional corticosteroids or systemic prednisone. Immunomodulators such as thalidomide are suggested for use as prophylaxis in patients with frequent and painful recurrences.

Folliculitis: pustular, papular or edematous-papular follicular lesions, involving the proximal limbs and the upper trunk. Possible causes include *Staphylococcus, Malassezia furfur, Demodex folliculorum* and drugs like indinavir. Treatment depends on the etiologic agent detected by bacterial swabs and histopathology if needed. Antimicrobials against staphylococcus and malassezia or changing the antiretroviral regimen may be required. DADPS, a 10% crotamiton or polidocanol ointment or low-dose UVB 311 nm radiation are effective against severe pruritus in these patients (Holmes 2001, Simpson-Dent 1999). Today, it is well-established that ART-naïve patients with pruritic eosinophilic folliculitis significantly improve with ART.

Genital warts (*condylomata acuminata*): See chapters on *STDs* (*Condylomata acuminata*) and *Cervical and Anal Cancer.*

Herpes simplex virus / Herpes zoster infections: see chapter on *AIDS*.

Immune reconstitution inflammatory syndrome (IRIS)-related skin reactions: ART supports the TH-1 immune response and the tuberculin test reactivity recovers (Girardi 2002). In association with this immune reconstitution clinical manifesta-

tions of herpes zoster, mucocutaneous herpes simplex infections, mycobacterial infections, eosinophilic folliculitis, foreign body granulomas and cutaneous sarcoidosis have been reported (Handa 2001, Hirsch 2004, Beatty 2010). These infectious, as well as some non-infectious inflammatory skin diseases occur within a few days to 3 months after the initiation of ART. The therapy depends on the severity of clinical manifestations and consists of specific antibiotics, steroidal and non-steroidal anti-inflammatory drugs (see chapter on *IRIS*).

Kaposi sarcoma: the most frequent malignant tumor of the skin and mucosal membranes associated with HIV infection (see chapter on *Kaposi's sarcoma*).

Lipodystrophy: See chapter on *Lipodystrophy syndrome*.

Malignant cutaneous lymphomas: Malignant B and T cell lymphomas are rare in HIV-infected patients (Beylot-Barry 1999, Biggar 2001). Cutaneous B cell lymphomas usually grow as red to violaceous nodules and are easily mistaken for Kaposi's sarcoma. They can also look like persistent hematoma or non-specific asymptomatic papules. A biopsy should be performed on any clinically unclear tumor of the skin. Cutaneous T cell lymphomas are rare malignancies in HIV+ patients. The prevalence among 2,149 HIV-infected patients in Frankfurt was 0.06%. The clinical course starts with non-specific eczematous patches (Stage I), which are usually not diagnosed as cutaneous lymphoma even after several biopsies because of the paucity of findings such as cellular atypia. These lesions are usually diagnosed as eczematous dermatitis. A linear pattern of patchy or slightly infiltrated lesions in the relaxed skin tension lines can be an early clinical indication of cutaneous T cell lymphoma known as parapsoriasis (Munoz-Peres 1999). Histopathology becomes more evident during the plaque stage (Stage II), and is striking when in Stage III multiple tumors of the mycosis fungoides present. Biggar (2001) calculated a relative risk for cutaneous T cell lymphomas in HIV+ patients of 15.0 in comparison to the general population. The leukemic phase (Sézary syndrome) is characterized by erythroderma involving the palms and soles. In patients with erythroderma who have darker skin types and lack the histopathological signs of cutaneous T cell lymphoma the so-called pseudo-Sézary syndrome has to be considered in the differential diagnosis (Picard-Dahan 1996). Therapy with potent topical steroids (e.g., clobetasol) is effective in the patch and plaque stages. Solitary tumors can be controlled by radiotherapy (20–24 Gy) or photodynamic therapy (Paech 2002). Widespread, multiple tumors and Sézary syndrome are treated with a combination of retinoids and interferons or chemotherapy. Recently, remission of a CD8-positive pseudolymphoma treated solely with ART was reported (Schartz 2003).

Molluscum contagiosum: A benign viral infection of the skin usually seen in children and often in association with atopic dermatitis. The pox virus causes multiple papular skin-colored lesions with a typical central umbilication. The diagnosis is usually made on clinical grounds. After several weeks or months, an inflammatory reaction indicates the onset of spontaneous healing. In adults, mollusca are detected in the anogenital area and regarded as a sexually transmitted disease (Agromajor 2002). In HIV+ patients, the clinical manifestations can differ significantly from those seen in the normal host. Spontaneous healing is rare; most patients have high numbers of lesions, typically occurring in the face and neck region, which otherwise is a rare location. The presence of multiple mollusca on the face is a typical disease marker indicating advanced cell-mediated immunodeficiency (CD4 T cell count <100/µl) (Schöfer 1991, Schwartz 1992). The growth of mollusca in the immunocompromised host is not always exophytic, sometimes endophytic lesions occur. Multiple mollusca have to be differentiated from hematogenous dissemina-

tion of cryptococcosis, histoplasmosis and coccidioidomycosis, which are usually associated with fever, headache and sometimes pulmonary infiltrates. In such cases, skin biopsies (and tissue culture) and chest x-rays are indicated. Single molluscum can exceed 1 cm in diameter and grow exophytically, which can cause confusion with keratoacanthoma, squamous cell carcinoma, basal cell carcinoma or common warts. Mollusca are treated surgically with a special type of forceps, electrocautery, curettage or with liquid nitrogen. Recently, photodynamic therapy with 5-Aminolevulinic acid (Moiin 2003) and imiquimod 5% cream have also shown to be effective (Hengge 2000, Calista 1999, Calista 2000, Liota 2000, Smith 2002). Imiquimod is applied by the patient 3x/week (off-label). An inflammatory reaction (erythema) occurring after 3 to 4 weeks of topical treatment indicates the beginning of the immune reaction, which leads to complete resolution of the mollusca after 6-8 weeks.

Oral hairy leukoplakia (OHL): is a clinical manifestation of Epstein-Barr virus infection, almost exclusively found in patients with untreated advanced HIV disease. Non-cytolytic viral replication in the glossal epithelium, especially in the lateral parts of the tongue, leads to asymptomatic white verrucous plaques that do not rub off. OHL is clinically diagnosed; initially parallel white or grayish hyperkeratotic rows arranged vertically on the lateral aspects of the tongue are characteristic. Unilateral lesions are possible, but bilateral occurrence of several plaques is more typical. Important differential diagnoses include other leukoplakias, lichen planus mucosae and oral candidiasis (Patton 2002, Cherry-Peppers 2003). If the diagnosis is in doubt, a biopsy or cytology can confirm the diagnosis. As the lesions will respond to antiviral drugs such as acyclovir, gancyclovir, or foscarnet (Walling 2003) but not antifungals, treatment can be used as a diagnostic tool to distinguish OHL from candidiasis. Both diseases respond well to ART, which has led to a significant decrease (Triantos 1997, Ramirez-Amador 2003).

Prurigo nodularis: The stimulus for the development of Prurigo nodularis is Pruritus. Psyche-pruritus-scratch cycles support lesional proliferation of skin nerves for years. Nodules (0.5-3cm) develop at a local site in which persistent picking and scratching occur. Lesions appear as dome-shaped nodules, which often have an eroded surface with scale and crusts. Multiple lesions may be distributed throughout the extremities. The intervening skin shows scales, excoriations, lichenification, post-inflammatory pigmentary changes and scars, which can remain even after the healing process. Manifold underlying disorders were described along with HIV infection (Liautaud 1989). Psychiatric disorders and emotional tension are often associated with Prurigo nodularis. There is an affinity to lichen simplex chronicus. Complications of the important dermatoses like atopic dermatitis or insect bites. Therapy: Local: Potent topical glucocorticoids (under occlusion) or intralesional injection. Polidocanol, calcipotriol, capsaicin; phototherapy (UVB, UVA1) or PUVA therapy. Some patients have been successfully treated with cryotherapy, laser, electrosurgery and even with excisions. Systemic therapies with sedating antihistamines (interactions may occur), psychopharmacy (neuroleptics, antidepressives), corticosteroids and retinoids have been used. Good results have been shown with oral thalidomide up to 400 mg/day – be aware of possible neurotoxicity, teratogenicity (Matthews 1998, Maurer 2004). Occlusive bandaging can protect against mechanical irritations.

Pruritus: Chronic, often unremitting pruritus is one of the most frequent clinical symptoms of HIV infection. One in three patients is affected. In most cases, etiology remains unclear and only symptomatic treatment can be offered which may be unsatisfying (Moses 2003, Singh 2003). Pruritus can be a complication of infectious dis-

eases, such as viral, bacterial, fungal infections (e.g., *Malassezia furfur* folliculitis) or scabies. Also, dry eczematous skin (xerosis), papulosquamous skin diseases, systemic lymphomas, renal insufficiency and hepatic disease are causative conditions. Finally, many antiretrovirals and other drugs can cause pruritus (with or without rash).

To diagnose idiopathic pruritus it is necessary to exclude all skin and systemic diseases mentioned above. In patients on ART it can be useful to change the treatment regimen. Systemic antihistamines and topical corticosteroids are symptomatic treatment standards. If they are ineffective, or a prolonged systemic treatment is necessary, phototherapy (UVA-1, UVB 311nm) or photochemotherapy (PUVA) is an alternative or adjuvant therapy (Smith 1997, Gelfand 2001, Zirwas 2001, Singh 2003). Concerning the immunosuppressive effects of ultraviolet light, it seems that patients on ART are at less risk.

Papular dermatoses: Patients can present either with monomorphic skin colored to red papules (size 2–5 mm) or with combined eruptions consisting of papules and pustules (sterile eosinophilic pustulosis, Ofuji's disease). There is no special predilection for any site. The etiology of papular eruptions is heterogeneous. According to the clinical presentation and laboratory findings (elevation of IgE, eosinophilia in peripheral blood and affected skin) they resemble the prurigo of atopic dermatitis found in adults. Autoimmune reactions against follicular antigens have also been discussed , such as eosinophilic folliculitis (Fearfield 1999). These papules can be due to a hypersensitivity reaction to drugs, microbiological agents (viruses, bacteria, fungi), parasites or saprophytes (*Sarcoptes scabiei*, *Demodex folliculorum*, *Pityrosporum ovale* and others). A thorough history of drugs, microbiological and histological examinations (including special stains such as PAS) are required for a correct diagnosis. If possible, specific infectious agents are treated. In case of sterile eosinophilic pustulosis (Ojufi's disease) or papular dermatosis of unknown origin, therapy is symptomatic. Depending on the clinical situation, antihistamines, itraconazole (200 mg/d for 2 weeks), isotretinoin, dapsone, mild PUVA or UVB (311nm narrowband UVB is the most effective therapy) or 5% permethrin cream can be tried (Ellis 2004). Topical tacrolimus (0.1%) has also been shown to be effective (Kawaguchi 2004).

Paronychia and ingrown nails: Ingrown toenails and inflammatory reactions of the proximal nailfold are a well known complication in diabetics, but also in patients on beta-blockers or retinoid therapy. A few cases might be due to local pressure (wrong shoes) or occur spontaneously. Patients on ART are the latest group of patients to regularly develop ingrown nails. These are ascribed to retinoid-like side effects of several antiretrovirals, especially indinavir, but also 3TC. Usually, the large toenails are involved, but all other toenails and fingernails can be affected. Complete remission is often seen when indinavir or 3TC are replaced by other antiretrovirals. Surgical measures such as Emmert-plasty or its modification after Hanneke, should only be performed when changing ART has not led to remission after 3 to 6 months (Tosti 1999, Alam 1999, Garcia-Silva 2002).

Psoriasis vulgaris: Today, psoriasis is regarded as a polygenic dispositional, chronic systemic autoimmune disease determined by multifactorial inheritance with variable penetrance and affects approximately 2% of the general population. Characteristic cutaneous lesions result from inflammatory reactions with increased proliferation and inhibited differentiation of keratinocytes. Psoriatic arthritis has a prevalence rate of 7% to 26% of the patients with psoriasis. Psoriasis is increasingly recognized as a systemic inflammatory process. Physical stimuli such as friction and less UV light or endogenous factors such as infections, drugs, and stress trigger the course psoriatic flares. Psoriasis may appear for the first time or can be aggravated after exposition

to such factors. The incidence of psoriasis in HIV+ persons has been reported to be between 2.5% (Braun-Falco 1988) and 4.9% (Schöfer 1990). The use of antiretrovirals reduces inflammation and improves psoriasis.

Typical psoriatic plaques can be eruptive, guttate or chronic and stationary. Atypical findings include inverse localization on the palms or soles and in the genital region and axillae, exudative, pustular or erythrodermic manifestations. In general, the severity of psoriasis parallels the impairment of the immune system. Besides infection, drugs have to be considered as possible triggers. In the final stages of HIV infection, psoriasis can be generalized and extremely resistant to therapy. Alternatively, the disease may disappear completely.

The typical psoriatic plaque is a sharply demarcated, erythematous plaque covered with silvery scales. Clinically and histologically, it may be difficult to differentiate it from seborrheic dermatitis.

Triggering factors should be eliminated if possible. Treatment is more difficult if the immune system is impaired. Antiretroviral therapy should be initiated or optimized. Localized lesions can be treated topically with corticosteroids, anthralins, dithranol, calcium-agonists (calcipotriol or tacalcitol), vitamin D3 or the topical retinoid tazarotene. The scalp and nails can be treated topically with corticosteroids. Phototherapy or photochemotherapy have no detrimental effect for HIV patients compared with other psoriasis patients and that they are justifiable (Akarapathanth 1999, Schoppelrey 1999). These treatments are as effective as in patients without HIV infection. UVB 311 (narrowband UVB) is well tolerated and effective. Broadband UVB is an alternative. In case of treatment failure, photochemotherapy can be instituted (local = bath or cream PUVA, or systemic PUVA). Systemic therapy is used additionally in patients with severe psoriasis or topical treatment refractory clinical course. Also generalized or exudative eruptions are usually treated systemically. Methotrexate, cyclosporine, fumaric acid esters and retinoids are systemic treatment options (DDG 2011). Interactions with ART as well as adverse events and immune suppressive effects of the systemic psoriasis therapy have to be considered. Fumaric acid esters reduce the CD4 and CD8 T cell counts and long term therapy in HIV-negative psoriasis patients was associated with higher incidences of Kaposi sarcomas (Philipp 2013). Biologicals can modulate the inflammation cascade by reducing the secretion and the effects of pro-inflammatory cytokines like TNF-alpha. Adalimumab, etanercept, infliximab and ustekinumab are highly effective additional or alternative treatment options for patients with severe and therapy refractory psoriasis (DDG 2011). Before TNF-alpha blocker are initiated tuberculosis, hepatitis B infection and other clinically relevant opportunistic infections have to be diagnostically excluded. Etanercept and infliximab do not increase the viral load in HIV+ patients (Bartke 2004, Ting 2006, Sellam 2007, Morar 2010). Although the total number of cases is rare a higher incidence of progressive multifocal leukoencephalopathy has been observed in HIV+ patients during treatment with biologicals (Bharat 2012). Interactions of the mentioned antipsoriatics with antiretroviral agents are unknown.

Reiter's syndrome: Reiter's syndrome is regarded as a variant of psoriasis in patients who carry HLA-B*27. This rare chronic-relapsing disease mainly affects young men, the incidence being higher than in the general population (0.6% to 6%) (Kaye 1989).

The classical triad consists of urethritis (sterile yellow urethral discharge), conjunctivitis (serous or purulent) and arthritis (mainly knee-, foot- or sacroiliac joints, causing pain and leading to immobility). The triad can be found in about 30% of patients. Furthermore, constitutional symptoms (attacks of fever, malaise, leukocytosis, elevated ESR) and skin lesions can be found. The skin lesions are characterized

by erythema with sterile pustules on the palms and soles and later, hyperkeratotic, scaling, exudative lesions known as keratoderma blenorrhagicum. Psoriatic plaques can be seen as well as the typical circinate balanitis presenting as crusting, dessicated plaques in circumcised men and shallow, moist, serpiginous, painless ulcers with slightly raised borders in uncircumcised men.

The diagnosis depends on the typical pattern of arthritis plus one or more of the mentioned clinical symptoms. Gonorrhea or *Chlamydia urethritis* have to be excluded by microbiological methods. Psoriatic arthritis should have other clinical signs of psoriasis (nail changes) and lacks fever.

Initially symptomatic therapy with non-steroidal anti-inflammatory agents, or possibly corticosteroids (short-term, high-dose pulse therapy) should be given. Acitretin (25–75mg/d) in combination with topical fluorinated corticosteroids have also been shown to be effective. Alternatively, sulfasalazine has been used successfully. Arthritis is also treated with oral gold. There is one report on the successful use of infliximab in a patient with Reiter's syndrome without negative effects on the viral load (Gaylis 2003).

Scabies: Scabies can be found worldwide; prevalence varies from <1% to 30% depending on the socio-economic circumstances. Scabies is characterized by extreme pruritus, especially at night. In general, the clinical presentation does not differ from that seen in HIV-negative persons. In the interdigital areas (volar sides) of the joints of the hands, breasts, axillae, periumbilical region, or penile shaft, fine red burrows (S-shaped or straight lines) may be found. There may be a small papule or vesicle at one end. Excoriations and/or secondary infections make the identification of burrows difficult. Generalized eczematous eruption may be seen. Typically in the groin or on the genitals red-brown pruritic nodules can be found. These scabies granulomas can persist for months even after successful therapy.

In the case of severe cellular immunodeficiency crusted scabies or Norwegian scabies can occur. Besides HIV+ patients, persons with general physical or mental debilitation are affected. Over weeks or months, eczematous lesions covered with asbestos-like crusts extend over large areas and the plaques can be mistaken for psoriasis. Crusted scabies is extremely infectious and carries many more mites than regular scabies – up to 10,000 mites/g scales. The history of unremitting and intractable itching is suggestive of scabies. The diagnosis is made by the clinical picture and proven by the demonstration of the mites, their ova, or fecal droppings in the scales. On histology, the female mite can be seen in the stratum corneum.

A single application of permethrin 5% cream is performed (whole body application from chin to toes, usually excluding the face; leave on skin for 8 hours, then shower off). In cases of crusted scabies, the scales have to be removed over several days (salicylic ointments) and therapy has to be repeated over 3–4 days. Alternative therapies are hexachlorocyclohexane (lindane), benzoylbenzoate, pyrethrum extracts or allethrin/piperonyl butoxide, all applied for 3 days. It is important to treat all contact persons at the same time.

Linens and bed clothes have to be changed daily. Depending on the clinical presentation another treatment one week later is sometimes recommended (as a safety). In cases of severe immunodeficiency the scalp has to be treated too. If more than 50% of the skin is affected or several recurrences have occurred a combination of keratolytic/topical therapy against scabies and systemic treatment with ivermectin is recommended. Hygienic measures to prevent contact infections are extremely critical. A single therapy with 2 tablets (6 mg each; or 200 µg/kg) is generally sufficient. Ivermectin is not licensed for this indication. There are no reports on complications after this therapy in HIV-infected patients (Dourmishev 1998).

Seborrheic dermatitis: The incidence in the general population is estimated to be 3–5%. The lipophilic yeast *Malassezia furfur* (formerly *Pityrosporum ovale*) is believed to be of pathogenetic relevance. Here the specific subtype appears to be more important than the density of colonization. In HIV infection 20–80% of untreated people are affected depending on the immune status (Chatzikokkinou 2008). Seborrheic dermatitis appearing *de novo* or exacerbation could indicate conversion of HIV infection from a latent state to a symptomatic state (Ippolito 2000).

Areas rich in sebaceous glands, such as the scalp, forehead, eyebrows, nasolabial folds, over the sternum, between the shoulder blades, external ear canal and retroauricular area, develop yellowish oily scales and crusts on mildly erythematous to very red plaques. The lesions may be pruritic.

The clinical picture is typical in most cases. Differentiation from psoriasis may be difficult both clinically and histologically. Initially other forms of eczema such as allergic contact dermatitis and atopic dermatitis may have similar presentations.

Due to the pathogenic role of *Pityrosporum ovale*, topical antifungals such as ketoconazole cream, other topical imidazoles or triazoles, or alternatively selena disulfide, metronidazole, and low-dose dithranol or lithium succinate- and zinc-sulfate-creams are used. For the scalp antimycotic shampoos, zinc pyrithione or tar-containing products are used. In severe cases systemic antimycotics are given like ketoconazole (200 mg QD), itraconazole (100 mg QD) or terbinafine (250 mg QD).

Syphilis: see chapter on *HIV and Sexually Transmitted Diseases*.

Tinea (dermatophytosis, ringworm infections): Infections of the skin, hair or nails with dermatophytes (in Western Europe predominantly *Trichophyton, Microsporum* and *Epidermophyton* species). Tinea has a high prevalence in the general population. There is no significant difference between HIV-negative and HIV+ adults. The prevalence depends upon climate, profession, clothing, and participation in team sports. Typical clinical findings are superficial, scaling, round or oval erythematous plaques that expand centrifugally with an inflammatory edge and central clearance. Deep infections with tissue destruction and abscess formation are rare in Europe and North America but common in tropical regions. According to Torssander (1988) onychomycosis due to dermatophytes is frequent in ART-naïve patients and difficult to treat. Nails are discolored (white, yellow, green, black), thickened and show growth disturbances (onychodystrophy). Subungual hyperkeratosis and onycholysis are common.

Psoriasis, yeast infections and trauma can imitate onychomycosis so it is necessary to identify the causative organisms on KOH and fungal culture. Direct microscopic examination with the addition of 10-15% KOH solution shows translucent, septated hyphae (mycelium) and arthrospores. Calcofluor or Blankphor microscope slides can be used for diagnostic immunofluorescence microscopy. Culture on Sabouraud's or Kimmig's medium identifies different fungi by their growth characteristics.

Treatment of superficial fungal infections of the skin is best achieved with topical broad spectrum antifungals such as ciclopirox or -azoles applied twice daily. In severe inflammatory disease it is helpful to start with combination therapy including topical corticosteroids for 3 or 4 days to achieve quick relief. Deep infections and infections involving terminal hairs (tinea capitis, tinea barbae) require systemic treatment with griseofulvin 500–1000 mg/day, terbinafine 250 mg/day, fluconazole 50 mg/day, or itraconazole 100–400 mg/day (Elewski 2001, Millikan 2001). There are different regimens to treat onychomycosis. Itraconazole and terbinafine are typically used for two months for fingernails and three months for toenails. Griseofulvin may be used for up to 9 months or longer, until the infection clears (Aly 1996, Myskowski 1997, Torssander 1988). If only the distal part of the nail plate is infected topical treatment

with nail varnish containing antifungals, which are able to penetrate the nail plate, are advised to avoid drug interactions between systemic antifungals and antiretroviral medications (see chapter on *Drug Profiles*). If systemic therapy is necessary, fluconazole has fewer drug interactions than other antifungals.

Xerosis/Dry skin: Dry skin is a very frequent complication of any kind of immunodeficiency. In the pre-ART era, we diagnosed dry skin in one in three HIV+ patients (Table 1). The patients complain of dry, itchy skin, which is exacerbated by any stimulus. Overall, these skin problems are very much like atopic dermatitis (Rudikoff 2002) and can culminate in acquired ichthyosis. The prevalence of dry skin decreases after the introduction of ART but can sometimes be seen in patients on indinavir (Garcia-Silva 2000). Some years ago, we found that the lipid film of the skin surface has a different composition in HIV+ patients although not diminished in quantity (Semrau, unpublished data).

Dry itchy skin is treated with the application of emollients that contain 5 to 10% urea, or 3 to 4% lactic acid, and dexpanthenol. Patients should be advised to take maximum one shower every (other) day. 1 to 2 oil baths per week should be recommended. In cases with severe inflammation and fissures (eczema craquele) topical Class 3 or 4 corticosteroids are very helpful in reducing symptoms. They should not be used for longer than 3 to 5 days.

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28. HIV-1-associated Neurocognitive Disorder (HAND) and Myelopathy

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HIV-1-associated neurocognitive disorder (HAND)
Terminology, etiology and epidemiology

In 2007 an international panel (Antinori 2007) devised three categories of HAND in order of descending severity: HIV-1-associated dementia (HAD), HIV-1-associated mild neurocognitive disorder (MND), and HIV-associated asymptomatic neurocognitive impairment (ANI) ("Frascati criteria"). This replaces the older terms HIV encephalopathy, AIDS dementia complex, and HIV-associated cognitive motor complex.

Table 1: The classification system of HAND ("Frascati criteria") (Antinori 2007)

HIV-associated asymptomatic neurocognitive impairment (ANI)	Neuropsychological testing with impairment (≥1 standard deviation) in cognitive function in ≥2 functional domains*. The impairment does not interfere with everyday functioning.
HIV-1-associated mild neurocognitive disorder (MND)	Cognitive results as in ANI. At least mild interference in daily functioning (at least one of the following): a) Self-report of reduced mental acuity, inefficiency in work, homemaking, or social functioning. b) Observation by knowledgeable others that the individual has undergone at least mild decline in mental acuity with resultant inefficiency in work, homemaking, or social functioning.
HIV-1-associated dementia (HAD)	Marked acquired impairment in cognitive functioning. Cognitive results as in ANI, but typically multiple domains affected, and impairment ≥2 standard deviations. Marked interference with day-to-day functioning (work, home life, social activities).

For all categories, delirium must be excluded, and there must be no alternative plausible cause.
*considering age- and education-adjusted norms. Cognitive domains are: verbal/language; attention/working memory; abstraction/executive; memory (learning; recall); speed of information processing; sensory-perceptual, motor skills

The primary cause of HIV-1-associated neurocognitive disorder (HAND) is an infection of the CNS caused by HIV. If untreated, there is a high level of replication of HIV in the macrophages and microglial cells of the brain. Neural cells have not consistently been shown to be infected. However, different immunopathological mechanisms lead to structural damage of these cells with subsequent neurocognitive impairment (NCI). With respect to viral replication and viral quasispecies, the CNS is partially independent from the hematolymphatic compartment (Eggers 2003, Eggers 2013), and this may lead to "viral excape" with direct clinical consequences (Canestri 2010, Peluso 2012). A recent autopsy study on patients with advanced HIV infection found aspects of Alzheimer's pathology and unspecific histological changes as equally associated with HAND as the classical HIV pathology (Everall 2009).
As the life expectancy of HIV+ individuals in the developed world now comes close to that of the general population (May 2014), the prevalence of HAND has risen to

20-50% (Sacktor 2002, Heaton 2010). With ART, the prevalence of severe cases has decreased while that of the minor variants has increased (Heaton 2011). Among individuals in the WHO/CDC clinical stage A, however, a slight to moderate impairment is more frequent than in the pre-HAART-era. Longitudinal cohort observations have shown that many patients with asymptomatic neurocognitive impairment (ANI), even with suppressed plasma viral load, will eventually develop symptomatic NCI (Cole 2007, Grant 2014). Patients who were diagnosed and treated early after infection had a low prevalence of NCI (Crum-Cianflone 2013). A study of treated subjects with initially low but increasing CD4 T cell counts showed some improvement of cognitive function, but this remained worse than that of an HIV-negative control group (Mc Cutchan 2007). Frequent subjective complaints of reduced cognitive performance with and without objective correlates on formal neuropsychological testing have been found in many patients with longstanding suppression of plasma viral load (Simioni 2010). HAND is associated with a shortened survival (Sevigny 2007) and with poor medication adherence (Albert 1999).

It is generally accepted that HAND, in untreated patients, at least in its more severe stages, is a treatable condition. However, the extent and sustainability of the effects of ART on cerebral function are still unclear. Progressive, clinically relevant, and, at times, fluctuating neurocognitive impairment may occur in patients on suppressive ART (Brew 2004, Antinori 2007, Canestri 2010, Peluso 2012).

HIV-1-associated dementia (HAD) as the most severe HAND manifestation is now rare on ART (Price 2008). However, more subtle but, with regard to working performance, significant dysfunction may be seen in everyday clinical practice, and it now occurs at earlier stages of HIV-induced immunosuppression (Sacktor 2001, Dore 2003).

In the pre-HAART era, the time course of the CSF and plasma viral load and the current CD4 T cell count were predictors of HAND, but this has now changed. Longitudinal studies in ART-treated subjects without dementia show low nadir CD4 T cell counts, previous AIDS, longer duration of HIV infection, low educational status, older age, plasma levels of TNF-alpha and MCP-1, illicit drug use, and comorbidity in general to be predictors for the development of HAND (Robertson 2007, Sevigny 2007, Tozzi 2007, Bhaskaran 2008, Heaton 2010, Mind Exchange Group 2013). The occurrence and/or persistence of HAND, despite effective suppression of plasma viral replication, might be associated with chronic immune activation within the CNS, as suggested by persistently elevated levels of neopterin and anti-MOG antibodies in the CSF (Eden 2007, Lackner 2010), and by microglial activation on brain positron emission tomography (PET) (Garvey 2013). This observation might suggest some "uncoupling" of mechanisms within the CNS from those in the hematolymphatic compartments. Contrary to earlier estimates, HCV coinfection seems not to confer NCI (Clifford 2015).

Cases of severe HAD with high levels of CSF viral load were observed in patients with well-suppressed plasma viral load ("viral escape") on ART (Venkataramana 2006, Canestri 2010, Peluso 2012). In an autopsied patient, numerous CD8-positive lymphocytes were found in the perivascular spaces and the parenchyma, partly in close spatial association with neurons. This condition may be interpreted as an immune reconstitution phenomenon directed against HIV itself (Venkataramana 2006).

Clinical manifestation

HAND is considered to be a subcortical dementia. With the introduction of ART, signs of cortical involvement and memory impairment have become more prominent, while motor signs have become less important (Heaton 2011).

HAND emerges over the course of weeks and months. Acutely developing symptoms point to another etiology. Fever, exhaustion, the effects of tranquilizers, reduced physical condition and even major depression may all mimic dementia. In these cases, diagnosis of HAND can only be made after repeated examinations when the condition mimicking dementia has improved.

Expressing complaints about neurocognitive dysfunction is not equivalent to actually being impaired. Patients in whom cognitive testing actually demonstrates NCI tend to underestimate the degree of their dysfunction, while the opposite is true for patients with depression (Thames 2011). This is why a history given by informants close to the patient is important. Typical complaints are slowing of reasoning, forgetfulness, difficulties concentrating, lack of energy, mild depressive symptoms and emotional blunting (Tables 2 and 3). In terms of clinical findings, impairment of alertness, neck stiffness, focal or lateralizing neurological signs (e.g., hemiparesis, aphasia), and focal and generalized epileptic seizures are not typical for HAND. Psychotic symptoms without cognitive or motor disturbance do not warrant a diagnosis of HAND. The coincidence of psychosis with HAND is rare. Non-lateralizing and mostly subtle signs of pyramidal, extrapyramidal, oculomotor and autonomous dysfunction may be present in advanced stages. The severity of HAND may be functionally categorized according to the Memorial Sloan Kettering scale (Table 4) (Price 1988).

Table 2: Symptoms of HAND including history given by close relatives or companions

Cognition	Forgetfulness, difficulties concentrating, mental slowing (apprehension, processing)
Emotional	Loss of drive and initiative, withdrawal from social activities, failure to manage the financial and administrative aspects of one's life. Depressive mood, emotional blunting
Motor	Slowing and impairment of fine movements (e.g., typing, buttoning up) and disturbance of gait
Autonomous	Impaired micturition (urgency), loss of sexual libido, erectile dysfunction

Table 3: Signs of HAND

Psychological findings	Early stages: emotional blunting, disappearance of strong personality traits, distractability, loss of initiative Later: problems with recalling events in the correct time order, disorientation to time, space and situation. Finally mutism
Neuro-psychological findings	Slowing of psychomotor speed (e.g., naming the months in reverse), impairment of short term memory (recall of verbally presented items, digit span), and mental flexibility (spelling simple words backwards)
Neurological findings	Early stages: impaired gait, slowing of rapidly alternating movements, hypomimia, occasionally tremor and short-stepped gait Later: brisk tendon reflexes, positive Babinski sign, slowing of gaze saccades, sphincter impairment including incontinence. Palmomental, grasp and glabellar reflexes. Occasionally accompanying polyneuropathy In the terminal stages: spastic tetraplegia and dual incontinence

Table 4: Severity of HAND (Memorial Sloan-Kettering (MSK) Scale) (Price 1988)

Stage 0	(normal) normal mental and motor function
Stage 0.5	(equivocal/subclinical) no impairment of work or capacity to perform activities of daily living (ADL); normal gait; slowing of ocular movements and movements of extremities may be present
Stage 1	(mild) able to perform all but the more demanding aspects of work or ADL, but with unequivocal signs or symptoms of functional, intellectual or motor impairment; can walk without assistance
Stage 2	(moderate) able to perform basic activities of self-care, but cannot work or maintain the more demanding aspects of daily life; able to walk, but may require a single prop
Stage 3	(severe) major intellectual incapacity (cannot follow news or personal events, cannot sustain complex conversation, considerable psychomotor slowing); motor disability (cannot walk without assistance, usually manual slowing and clumsiness)
Stage 4	(end stage) almost mutistic. Intellectual and social comprehension and output are at a rudimentary level; almost or completely mute; paraparetic or paraplegic with urinary and fecal incontinence

Diagnostic workup

Making the diagnosis of HAND requires a synopsis of clinical information and laboratory tests. No laboratory test result on its own can warrant a diagnosis of HAND. Rather, the diagnosis requires the exclusion of other conditions (Table 5).

Clinically, cognitive deficits prevail. Psychological and behavioral as well as motor signs and symptoms may be subtle in the early stages. Motor signs are often encountered in the later stages (Tables 2 and 3). Formal neuropsychological cognitive testing, the gold standard, should be done. This should encompass the domains verbal/language, attention/working memory, abstraction/executive function, learning/recall, speed of information processing, and motor skills (Mind Exchange Group 2013). Where a trained neuropsychologist is not available, the HIV dementia scale as an easy-to-use bedside instrument may be used, but its sensitivity and specificity are limited (Morgan 2008).

Laboratory tests are mainly employed to exclude differential diagnoses. MRI is preferred to CT. The MRI may show patchy, diffuse, and relatively symmetrical hyperintense lesions in the white matter. These changes indicate leukoencephalopathy. In addition, atrophy with enlargement of the ventricles and the extraventricular CSF spaces may be seen. However, none of these findings are specific for HAND, and the disease may evolve with a normal MRI. Unlike in PML, the white matter lesions do not affect the cortical U-fibers, i.e., they do not reach the cortical ribbon. Edema and space occupying lesions are not compatible with HAND and should raise suspicion of other conditions.

CSF analysis mostly shows a normal white cell count, and with severe immunosuppression this may even be decreased. In patients with an at least partially effective ART, CSF pleocytosis may be seen, suggesting an immunological response to HIV in the context of immune reconstitution. Total protein and albumin concentrations may be slightly elevated (blood-brain barrier disruption). Oligoclonal bands and increased IgG index indicate autochthonous immunoglobulin production within the CNS. However, these findings are non-specific, and they are frequently present even in the asymptomatic stages of HIV infection.

In untreated patients there is a weak but statistically significant correlation of (higher) CSF viral load with HAND. However, this association is no longer true for individuals on ART (Mc Arthur 2004, Heaton 2011). The electroencephalogram (EEG) shows no or only mild signs of generalized slowing. Moderate or severe slowing or focal arrhythmic delta activity are atypical for HAND.

With a large part of the HIV+ population growing older, other types of dementia such as Alzheimer's disease, vascular dementia, Lewy body dementia, etc. need to be differentiated by appropriate diagnostic steps.

Screening and Treatment

Screening for HAND is recommended in all HIV+ patients, regardless of treatment status (Mind Exchange Group 2013). This should ideally be carried out before initiation of ART in order to generate baseline data. Screening should be done every 6 to 24 months, according to the risk profile of the patient. The HIV dementia scale, the MoCA test, and the NEU screen have been validated in HIV+ patients as screening instruments (Morgan 2008, Munoz-Moreno 2013, Brouillette 2015). When results are abnormal, further neurological and neuropsychological work-up should be done. According to the pathogenesis of HAND, **treatment** should aim at suppressing viral replication in the CNS. Although the CNS is a separate compartment of viral replication, the initiation of ART in a treatment-naïve patient leads to a rapid decline of the CSF viral load in most patients (Eggers 1999+2003). In this situation of no prior ART the clinical improvement may be dramatic in cases of severe HAND (HAD) while the effect size is lower in less severe cases (MNC). HAD patients may regain working ability and independence from caregivers, and this takes some 3 to 9 months to evolve (Cysique 2009). During the first months of treatment and despite clinical improvement, the radiological signs of leukoencephalopathy may become more prominent, but eventually will regress.

The indication for ART in treatment-naïve patients with symptomatic HAND (MNC or HAD) is undisputed. In the situation of a patient with established ART it is obviously important to make sure that plasma virus replication is suppressed. Beyond this step, it is largely unknown what antiretroviral compounds and in what combination these are best suited for the treatment of HAND. The extent of penetration into the CSF and the brain parenchyma is generally assumed to be essential, and this view is supported by early findings of little suppression of the CSF viral load by regimens containing only PIs (Gutmann 2010). A CNS penetration efficacy score (CPE) was devised by Letendre et al. (2011). It is composed of the relative values of the CNS penetration of the substances and comprises four categories, where lower scores indicate lower CNS penetration (Table 6). Most studies showed a higher CPE score to be associated with lower CSF viral loads e.g. (Letendre 2008, Cysique 2011, Cusini 2013). However, whether a higher CPE score is associated with better clinical, i.e., neurocognitive performance is less clear. The majority of studies, though, did show a modest but significant positive effect (Cysique 2011, Vassallo 2014). Two small randomized trials that prospectively examined ART regimens with higher vs. lower CPE scores were published. One showed slightly better cognitive results with higher CPE scores (Winston 2010), while in the other such an effect was significant only in the subgroup with suppressed plasma viremia (Ellis 2014). These diverging results are likely due to the differing methodology and the uncertainty of how to measure and define CNS penetration as well as to other factors with impact on HAND. The notion of the importance of suppressing the CNS viral replication is, however, supported by case series of patients with long-standing suppression of the plasma viremia but detectable viral replication in the CSF (viral escape). These subjects had

clinically overt neurological disease, and on optimization of their ART according to the CPE score and resistance testing, all improved clinically and in terms of CSF viral load (Canestri 2010, Peluso 2012). A further argument for achieving high enough levels of antiviral compounds in the CNS is its role as a viral reservoir and the finding of resistant viral strains in the CSF (Smit 2004, Canestri 2010). It is therefore recommended that antiviral regimens contain CNS-penetrating compounds, and this is even more important with symptomatic CNS involvement of HIV (Mind Exchange Group 2013).

As mounting evidence suggests a major pathogenic role of monocytes, a monocyte efficacy score has been published that is closely associated with cognitive functioning (Shikuma 2012).

Several non-antiviral substances have been tried as an adjunctive treatment for HAND (minocycline, memantine, selegiline, lithium, valproate, lexipafant, CPI-1189, peptide T, nimodipine, psychostimulants, rivastigmine). Although all proved to be safe, none exhibited meaningful clinical effects (Sacktor 2011, Simioni 2013).

Non-pharmacological interventions include the treatment of concomitant conditions such as liver disease, major depressive disorders, the management of cardiovascular and metabolic risk factors, as well as the improvement of drug adherence (Mind Exchange Group 2013).

Neurotoxicity of antiretroviral substances may be considered in patients developing or maintaining neurocognitive and psychiatric dysfunction. Neuropsychiatric side effects are best documented for efavirenz, but these are mostly transient. Some authors reported on cognitive dysfunction with suppressive ART that resolved with withdrawal of ART, but these results have been questioned by others (Munoz-Moreno 2010, Grund 2013). In view of its systemic effects, however, treatment interruptions are not recommended. If neurotoxicity is suspected, the ART regimen might be altered (Mind Exchange Group 2013).

While there used to be a discussion about the optimal time point to start antiviral treatment in relation to cognitive impairment, in some guidelines ART is now recommended in all HIV+ subjects irrespective of the stage of the disease. With the results of the START study (see *ART* chapter), almost all patients will begin ART. The current EACS guidelines (November 2014) recommend to screen for and, when appropriate, to perform the diagnostic steps for HAND. In case of established diagnosis, CNS-active drugs should be considered. The US DHHS guidelines (April 2015) recommend to initiate ART at any stage of HIV infection, but does not recommend specific antiretroviral drugs in HAND-affected patients.

Some evidence suggests an early initiation of ART for the prevention of HAND (Ellis 2011), but the value of CNS-penetrating compounds is unclear.

Depression is frequent in HIV infection (Pence 2012). Depressed people tend to over-report cognitive symptoms (Thames 2011), while on formal testing, a skilled neuropsychologist/neurologist will find normal or near-normal cognition. Patients complaining of cognitive symptoms should therefore be examined for depression as the depression may actually be the cause of the cognitive complaints (so called "pseudodementia").

Table 5: Differential diagnoses of HIV encephalopathy and diagnostic workup

Condition	Adequate diagnostic step (commentary)
Neurosyphilis	Antibody testing and CSF analysis (pleocytosis >15/µl) (serological findings may be atypical for active neurosyphilis)
CMV encephalitis	CSF (pleocytosis, potentially granulocytic; decreased glucose elevated total protein) PCR for CMV in CSF, CMV antigen (pp65) in blood antibody testing in blood and CSF (IgG and antibody index may be increased) MRI (potentially subependymal hyperintensity and contrast enhancement) Occurs mostly in association with manifestation of other organs (retinitis, colitis, pneumonitis, esophagitis)
Toxoplasmosis	CT / MRI (single or multiple lesions found most frequently in basal ganglia or thalamus, space occupying effect, edema, frequently with contrast enhancement [patchy or ring-shaped]) Presence of toxoplasma specific IgG in blood and CSF (rarely total seronegativity). PCR for Toxo DNA in CSF has low sensitivity (Disease may rarely run as diffuse microglial nodule encephalitis)
Primary CNS lymphoma	CT / MRI (single or multiple lesions most frequently adjacent to ventricles, space occupying effect, edema, contrast enhancement) CSF cytology EBV PCR in CSF (HIV-associated CNS lymphomas EBV-induced) PET or SPECT (tracer enhancement in lesion)
VZV encephalitis	CSF (marked inflammatory signs) VZV specific IgG in blood and CSF (IgM may be absent) VZV PCR in CSF Mostly antecedent or accompanying cutaneous zoster lesions
Cryptococcal meningitis	CSF (opening pressure frequently elevated, cell count and protein may be normal), India ink stain Cryptococcal antigen in blood and CSF, fungal culture
Tuberculous meningitis and other bacterial infections	CSF, culture, PCR for mycobacteria appropriate tests
Progressive multifocal leukoencephalopathy (PML), classical form	MRI (single or multiple lesions of white matter, no space occupying effect, no edema, no contrast enhancement) CSF (no signs of inflammation but PCR for JC virus positive)
PML in the context of immune reconstitution inflammatory syndrome	MRI (white matter lesions with contrast enhancement, space occupying effect) CSF (variable signs of inflammation, JCV DNA PCR positive)
Intoxication	Determination of drug levels / screening for illicit drugs
Metabolic encephalopathy and impaired general physical condition	Determination of electrolytes, renal and hepatic markers, hormones (thyroid, cortisol), blood count, vitamin B12 deficiency Hypoxemia? (blood gas analysis) Reduced physical state? (bed ridden, wasting, pyrexia)
Depression with "pseudodementia"	Psychiatric examination
Other forms of dementia	Alzheimer's disease, Lewy body dementia, normal pressure hydrocephalus, Parkinsonian syndromes, subcortical arteriosclerotic encephalopathy, other neurodegenerative conditions

Table 6: CNS penetration effectiveness score (CPE) (Letendre 2011+2014)

CPE rank	4	3	2	1
NRTIs	AZT	Abacavir FTC	ddI 3TC d4T	Tenofovir
NNRTIs	Nevirapine	Etravirine Efavirenz	Rilpivirine	
PIs	Indinavir/r	Darunavir/r Fosamprenavir/r Indinavir Lopinavir/r	Atazanavir Atazanavir/r Fosamprenavir	Nelfinavir Ritonavir Saquinavir/r Tipranavir/r
Entry Inhibitors		Maraviroc		T-20
INSTIs	Dolutegravir	Raltegravir	Elvitegravir/c	

HIV-associated myelopathy

Clinical characteristics

HIV+ patients may rarely develop HIV-associated myelopathy (HIVM), without the neuropsychological signs and symptoms of HAND. The histopathological hallmarks are prominent vacuoles in the cervical and thoracic parts of the spinal cord and lipid-laden macrophages, hence the term "vacuolar myelopathy" (Petito 1985). These changes are reminiscent of severe combined degeneration (i.e., vitamin B12 deficiency).

As HIV viral products have only inconsistently been shown to be part of the lesions, the role of HIV is uncertain. A disturbance of cobalamin-dependent transmethylation has been discussed. Like HAND, HIVM occurs mainly with advanced immunosuppression. Not all patients with autopsy findings of vacuolar myelopathy had shown clinically apparent myelopathy during life (dal Pan 1994).

A patient may be suspected of having HIVM if he has a spastic-atactic gait, hyperreflexia with positive Babinski sign, disturbance of sphincter control, erectile dysfunction, and slight signs of sensory dysfunction in a glove and stocking distribution. The diagnosis of an independent HIVM should only be made when the concomitant cognitive impairment is significantly less prominent than the myelopathy. Increased latencies of somatosensory-evoked potentials (SEP) and motor-evoked potentials on transcranial magnetic stimulation (MEP) are compatible with the diagnosis. CSF, microbiological and spinal imaging studies are inconspicuous or non-specific, and they have their importance in the exclusion of other diagnoses, as listed in Table 7. Spinal imaging should include MRI of the cervical cord and possibly the thoracic cord.

Treatment

Early observations of significant improvement with AZT monotherapy (Oksenhendler 1990) were later confirmed with ART. Any patient with HIVM should be offered ART. A controlled trial showed L-methionine to bring about improvement on electrophysiological but not clinical parameters.

Table 7: Differential diagnoses of HIV myelopathy and diagnostic workup

Condition	Adequate diagnostic step (commentary)
Mechanic compression of the myelon (cervical myelopathy, disk herniation)	Degenerative changes of the cervical spine MRI shows reduced CSF spaces around the spinal cord with hyperintense lesions of the myelon
Neurosyphilis	Antibody testing and CSF analysis (pleocytosis >45/3) (serological findings may be atypical)
CMV myelopathy	CSF (signs of inflammation), PCR for CMV in CSF Antibody testing in blood and CSF (IgG and antibody index may be increased)
Toxoplasmosis	Contrast enhancing cord lesion on MRI
VZV myelitis	CSF (marked inflammatory signs) VZV specific IgG in blood and CSF (IgM may be absent) VZV PCR in CSF Mostly antecedent or accompanying cutaneous zoster
HSV myelitis	CSF (inflammatory signs may be absent), HSV PCR in CSF
HTLV-1 (tropical spastic paraparesis)	Travel to the Caribbean, West Africa or East Asia Slow evolution of symptoms, bladder dysfunction charac-teristic, CSF inflammation, HTLV-1 specific antibodies
Severe combined degeneration	Vitamin B12 levels, increased MCV, homocysteine, holo-transcobalamin
Heredodegenerative diseases (hereditary spastic paraparesis, adrenoleukodystrophy, Friedreich ataxia, etc.)	Appropriate tests

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29. Neuromuscular Diseases

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Polyneuropathy and polyradiculopathy

Peripheral neuropathy is the most common neurologic complication of HIV infection. Even in the era of modern antiretroviral therapy neuropathic signs and symptoms are found in about 30% of patients (Evans 2011). Neuropathies can be classified as primarily HIV-associated or as secondary diseases caused by neurotoxic agents or opportunistic infections. Distal symmetrical sensory polyneuropathies (DSSP) related to HIV infection have been on the decline since the introduction of ART, but there has been an increase in the prevalence of medication-related toxic neuropathies (Gonzalez-Duarte 2008). Even though the incidence rate of the other types of neuropathies is low, they require a precise and rapid diagnosis because many of them do benefit from specific therapies.

Clinical features

Acute, inflammatory, demyelinating polyneuropathy (AIDP), Guillain-Barré syndrome (GBS)

AIDP usually occurs at seroconversion or during the asymptomatic stages of HIV infection. It seems to be rarely associated with immune reconstitution. Typical clinical signs are areflexia, symmetrically ascending weakness and relative sparing of sensory nerve fibers. Involvement of cranial nerves and cervical and thoracic spinal nerves leads to respiratory insufficiency, dysarthria and dysphagia. Parasympathetic and sympathetic nerve involvement may cause life threatening cardiac arrhythmias and severe arterial hypo- or hypertension. CSF typically shows a raised concentration of protein caused by the dysfunction of the blood-brain barrier. In contrast to HIV-negative patients with AIDP, a moderate pleocytosis of up to 50 leucocytes/µl CSF is found in most HIV-infected patients. The progressive stage is followed by a few days or weeks of stable disease until recovery begins. If secondary axonal damage has occurred, recovery can last up to two years. A persistent disability of varying degrees develops in about 30%.

Chronic, inflammatory, demyelinating polyneuropathy (CIDP)

Whereas AIDP is a monophasic, self-limiting disease, the course of CIDP is chronic progressive or relapsing-remitting. Weakness and sensory disturbances commonly develop over several months. In some cases relapses, incomplete remissions and periods of stable disease alternate with each other. In CIDP, as in AIDP, the CSF is abnormal with an elevated protein level. A moderate pleocytosis is often found instead of the classical acellularity. CIDP is a rare complication of seroconversion or the early stages of HIV infection.

Vasculitic neuropathy

Necrotizing vasculitis with involvement of the peripheral nerves is a rare cause of neuropathy in HIV infection. Most patients develop a mononeuritis multiplex characterized by acute relapsing dysfunction of individual peripheral nerves. Prognosis of the disease is determined by involvement of other organs such as heart, kidneys or muscles in the vasculitic process. An immune complex attack associated with hepatitis C virus infection or cryoglobulins appears to play an essential role in the pathological mechanism.

Table 1: Polyneuropathies and polyradiculopathies in HIV infection

Type	HIV infection	Clinical features	Findings
Primary HIV-associated polyneuropathies			
Acute, inflammatory, demyelinating poly-neuropathy (Guillain-Barré syndrome, GBS)	Seroconversion, asymptomatic, no or early immuno-suppression	Symmetrical weakness > sensory loss, areflexia	ENG with demyelinating features, elevated CSF protein and moderate CSF-pleocytosis (<50 c/μl)
Chronic demyelinating inflammatory poly-neuropathy (CIDP)	Asymptomatic early immunosuppression, rarely AIDS	Distal and proximal weakness > sensory loss, areflexia	ENG with demyelinating features, elevated CSF protein and moderate CSF-pleocytosis (<50 c/μl)
Vasculitic neuropathy	Asymptomatic no or early immunosuppression, rarely AIDS	Mostly asymmetric, acute loss of function of single nerves, rarely distal symmetrical sensory and motor disturbances	Elevation of ANA, cryoglobulinemia, HCV coinfection; vasculitis in nerve biopsy but also in muscle, kidney and other organs
Neuropathy in diffuse, infiltrative leukocytosis syndrome (DILS)	Early immuno-suppression	Mostly asymmetrical weakness and sensory loss, rarely distal symmetrical	Disease resembling Sjögren's syndrome; CD 8 T cells >1200/μl
Distal symmetrical sensory polyneuro-pathy (DSSP)	AIDS or advanced immuno-suppression	Distal symmetrical sensory loss, paresthesia and pain of the legs	ENG with axonal features predominantly involving sensory nerves of the legs
Secondary polyneuropathies			
Medication-related toxic neuropathy	Early or advanced immunosuppression	Distal symmetrical sensory loss, paresthesia and pain in the lower legs	Treatment with ddI, ddC, d4T, vincristine, dapsone
Acute neuromuscular weakness syndrome	Early or advanced immunosuppression	Acute progressive tetraparesis	Lactic acidosis (NRTIs) axonal nerve damage, additional myopathy
Mononeuritis multiplex in CMV-infection or non-Hodgkin lymphoma	AIDS	Asymmetric, acute loss of function of single nerves	CMV infection of other organs, CMV DNA detection in plasma; non-Hodgkin lymphoma
Polyradiculitis in CMV or *M. tuberculosis* infection or due to meningeal lymphoma	AIDS	Flaccid paraparesis, sensory loss, bladder dysfunction	CMV or mycobacterial infection at other sites, detection of CMV DNA or mycobacteria in CSF, malignant cells in CSF

Diffuse infiltrative lymphocytosis syndrome (DILS)

DILS is a rare cause of distal symmetrical, often painful neuropathy. It resembles Sjögren's syndrome, but has multivisceral infiltration characterized by CD8 hyperlymphocytosis (CD8 T cell count >1000/µl). Sicca syndrome with parotidomegaly, lymphadenopathy, splenomegaly, pneumonitis and renal dysfunction may occur in association with axonal neuropathy (Gulbus 2012).

Distal symmetrical sensory polyneuropathy (DSSP)

DSSP is still the most common neuropathy in HIV-positive patients and becomes symptomatic in the later stages of infection. Risk factors are older age, diabetes mellitus, HTLV-1 coinfection, hypertriglyceridemia and the use of statins (Banerjee 2011, Evans 2011, Robinson-Papp 2012, Silva 2012). The clinical course is predominated by slowly progressive sensory symptoms such as numbness, dys- and paresthesia in the feet and lower legs (Table 2). Approximately 30-50% of patients complain of burning, lacerating or stabbing pain. It mainly involves toes and soles of the feet and sometimes makes walking difficult. The most important clinical findings are depressed or absent ankle reflexes, an elevated vibration threshold at toes and ankles and a decreased sensitivity to pain and temperature in a stocking distribution, whereas proprioception is usually normal. Weakness and atrophy of intrinsic foot muscles are mild and are not features of the disease. The fingers and hands are rarely involved.

Involvement of the upper legs and trunk, significant weakness of leg muscles or decreasing proprioception are not typical for DSSP and should raise suspicion of other disorders, for instance a conjoined myelopathy. Loss and dysfunction of small sympathetic and parasympathetic nerve fibers are essential features of the disease and may cause postural hypotension, erectile dysfunction, gastroparesis and alterations of skin or nails in many DSSP patients.

Table 2: Clinical features of distal symmetrical sensory polyneuropathy

Numbness, pain, dysesthesia and paresthesia in the feet and lower legs
Decreased or absent deep ankle tendon reflexes
Decreased or absent vibratory senses of the toes and ankles
No or only minimal motor dysfunction
No or only minimal involvement of the hands and arms
Slowly progressive course
Electrodiagnostic studies with features of axonal nerve damage
Autonomic dysfunction: orthostatic hypotension, erectile dysfunction

Medication-related toxic neuropathy

A distal symmetrical sensory peripheral neuropathy occurs in about 10–30% of patients treated with ddI, d4T (and formerly, ddC). It is indistinguishable from HIV-induced DSSP on clinical examination or in electrodiagnostic studies. The only difference is in the exposure to neurotoxic nucleoside antiretroviral medication. Brew (2003) found an elevation of serum lactate in over 90% of patients with d4T-related neuropathies.

NRTI neuropathy develops after a mean of 12–24 weeks of treatment. After withdrawal, there can be a temporary worsening for 2–4 weeks and improvement usually begins after 6-12 weeks. In several cases the restitution remains incomplete. In these cases there may have been an additional pre-existent damage to the peripheral nerves

due to HIV infection itself. Subclinical disturbance of peripheral nerve function confirmed by pathological findings in electrodiagnostic studies elevates the risk of developing NRTI-related neuropathy.

PIs seem to have a very low additional neurotoxicity. In combination with d4T, ddI or ddC they seem to be an additional risk factor for neuropathy (Ellis 2008, Evans 2001). The instruction leaflets of many PIs list peripheral neuropathy as a possible side effect, because neuropathic symptoms were slightly more often reported in the PI arms of clinical trials. In combination with ddI, d4T and ddC, PIs seem to be a risk factor for neuropathy on their own (Ellis 2008, Evans 2011). But there are no reports of cases of neuropathy that developed while on PI treatment that resolved after withdrawal. In clinical experience, the risk of PI-induced neuropathy is very low. A few cases of neuropathy due to darunavir have been reported, but it remains unclear if the PI is really the cause of neuropathy in these cases (Lorber 2013).

Table 3: Neurotoxic drugs frequently used in HIV medicine

NRTI	ddI, d4T (ddC, no longer manufactured)
Antibiotic	dapsone, metronidazole, isoniazid
Cytotoxic	vincristine, etoposide

Acute neuromuscular weakness syndrome

In the course of an NRTI-induced lactic acidosis a life threatening tetraparesis resembling AIDP may occur. In most cases axonal peripheral nerve damage is found, but in a few patients demyelination is also detected. In addition, muscle biopsy reveals myositis or mitochondrial myopathy in some cases (Simpson 2004).

Polyneuropathy and polyradiculopathy due to other diseases

In patients with advanced HIV disease, mononeuritis multiplex may be caused by CMV infection or lymphoma. Acute or subacute polyradiculopathies of the cauda equina with rapidly progressive flaccid paraparesis of the legs, bowel dysfunction and sensory disturbances occur in the course of opportunistic infections (CMV, *M. tuberculosis*) or meningeal non-Hodgkin lymphoma. Other important causes of polyneuropathy are alcohol abuse, diabetes mellitus, malnutrition in patients with long-lasting gastrointestinal diseases, neoplastic diseases or cachexia.

Diagnosis

A diagnosis of neuropathy can usually be made based on medical history and clinical examination. Electrodiagnostic studies may be performed for confirmation and for differentiation from other diseases such as myelopathy. Cerebrospinal fluid analysis may be necessary if there is a suspicion of infection with, for example, CMV or syphilis. Sural nerve and muscle biopsy may be necessary only in atypical cases – for instance, painful DSSP with a high CD4 cell count and low viral load and without neurotoxic medication or other risk factors. Table 4 gives some recommendations for clinical practice.

Occasionally, patients report complaints of burning feet, aches, pain and tingling but clinical examination and nerve conduction studies are unremarkable. In these cases symptoms might be due to an isolated small fiber neuropathy exclusively affecting the small unmyelinated vegetative nerve fibers. Diagnosis requires a punch skin biopsy with histological assessment of intraepidermal nerve fiber density or pain-related evoked potential conduction testing (Obermann 2007).

Table 4: Diagnostic work-up

Procedure	Findings	Condition
Basic examinations (recommended for all cases)		
Medical history	Drugs	Medication-related toxic PNP
	Opportunistic diseases	Neuropathy associated with CMV infection or lymphoma
	Alcohol abuse	Alcoholic PNP
Neurological examination	Clinical type of PNP (distal symmetrical, mononeuritis multiplex, etc.)	Symptoms not due to myelopathy or myopathy
Electromyography Electroneurography	Confirmation of neuropathy	Symptoms not due to myelopathy or myopathy
	Demyelinating features	AIDP, CIDP
	Axonal features	DSSP, Multiplex Neuropathy, DILS
Blood tests	HbA1c, glucose	Diabetic polyneuropathy
	Vit B12, B1, B6, Fe, ferritin	PNP due to malnutrition or malassimilation
	ANA, cryoglobulins, HCV-serology, circulating immune complexes, ANCA	Vasculitic neuropathy
	TPHA	Neurosyphilis
	CD8 cells >1200/µl	Neuropathy associated with DILS
	lactate	NRTI-induced toxic neuropathy
		Mononeuritis multiplex due to
	CMV DNA	CMV-infection
	(if CD4 cells <100/µl)	
Additional tests (necessary only in particular cases)		
CSF	Elevated total protein	AIDP, CIDP
	Pleocytosis (granulocytes), CMV DNA	Polyradiculitis due to CMV infection
	Lymphoma cells, EBV DNA	Lymphomatous meningitis
	Elevated IgA, acid fast bacilli, mycobacterial DNA	Tuberculous polyradiculitis
Autonomic tests (sympathetic skin reaction, heart rate variability)	Involvement of sympathetic or parasympathetic nerves	Additional autonomic neuropathy
MRI (lumbar spine)	Compression of the cauda equina	Spinal lymphoma Spinal toxoplasmosis
Nerve and muscle biopsy	Necrotizing vasculitis	Vasculitic neuropathy
	Perivascular CD8 infiltration without necrosis	DILS-associated neuropathy

Treatment

Causative treatment options only exist for some of the rare neuropathies or polyradiculopathies. Intravenous immunoglobulins and plasmapheresis have proven effective in the therapy of AIDP. Corticosteroids are also effective in CIPD. In clinical trials on the treatment of CIDP, no difference in the efficacy of immunoglobulins, plasmapheresis or corticosteroids has been shown. However, an individual patient may only respond to one of the three options. In patients who only respond to higher dosages of corticosteroids, other immunosuppressive agents such as azathioprine, low dose weekly methotrexate or cyclosporine may replace long-term steroid therapy. We have seen CIDP patients who were in partial remission after temporary steroid therapy and who have remained stable for years with ART alone.

In medication-related neuropathy the offending agent needs to be withdrawn. The intake of 2 g L-acetylcarnitine significantly reduced pain in HIV patients with neurotoxic neuropathy (Youle 2007).

A causative treatment for DSSP does not exist. ART might improve the function of sensory nerves in a few cases, and therefore starting ART or optimizing a current ART should be considered in newly diagnosed DSSP. In most cases the neuropathic symptoms still persist.

Symptomatic treatment is directed at irritative symptoms such as pain and paresthesia. It is not effective against deficits of nerve function including sensory loss or weakness. The agents listed in Table 6 are recommended because they have proven useful in daily practice and because they interfere only slightly and in a predictable way with ART. A controlled study showed that lamotrigine was effective in reducing the symptoms of neurotoxic neuropathy (Simpson 2003). The drug is well tolerated if one adheres to the slow dose escalation regimen and stops treatment or reduces the dose when a skin reaction occurs. In a small study, gabapentin was shown to be effective in reducing DSSP-induced pain (Hahn 2004). The advantages of this agent are good tolerability and lack of interference with ART. Pregabalin, an anticonvulsant drug similar to gabapentin, effectively relieves pain in studies of patients with painful diabetic peripheral neuropathy (Rosenstock 2004). Like gabapentin, it does not interfere with ART and is well tolerated. It is commonly used in DSSP, although a recent trial in HIV patients did not show efficacy (Simpson 2010).

The tricyclic antidepressants amitriptyline and nortriptyline both have significant anticholinergic side effects. The dose necessary for reducing neuropathic pain is in the same range as for treating depression and many patients can not tolerate these dosages. However, lower dosages have proved ineffective in DSSP. Nortriptyline has no sedative side effects. We use this agent with good success rates, although clinical trials for its use in HIV-associated neuropathy are lacking. The antidepressant duloxetine, a serotonin-norepinephrine reuptake inhibitor, has been approved for the treatment of painful diabetic neuropathy. In our experience it is also useful in reducing pain in DSSP and toxic neuropathy in HIV+ patients. The anticonvulsant carbamazepine is widely used for the treatment of neuropathic pain. However, it induces some enzymes of the CYP450 system and interferes significantly with ART. Thus, its use in HIV medicine is very limited.

A high-concentration capsaicin patch has recently been shown to be effective in the treatment of pain in DSSP patients (Mou 2013). The patch is now available in Europe and in US, where it is OTC. The responsiveness varies considerably from patient to patient, but the somewhat laborious application is worth a try.

In two trials smoked cannabis has proven effective against neuropathic pain in DSSP (Abrams 2007, Ellis 2009). However, the effect was rather short-lived. Oral cannabinoids have not been tested yet in painful HIV neuropathy.

Table 5: Causative treatment of polyneuropathies and polyradiculopathies

Condition	Treatment
AIDP	Intravenous immunoglobulins 0.4 g/kg daily for 5 days or: plasmapheresis (5 x in 7–10 days)
CIDP	Intravenous immunoglobulins 0.4 g/kg daily for 5 days or: plasmapheresis (5 x in 7–10 days) or: prednisone 1–1.5 mg/kg daily for 3–4 weeks with subsequent tapering for 12–16 weeks
Vasculitic neuropathy	Prednisone 1–1.5 mg/kg daily for 3–4 weeks with subsequent tapering for 12–16 weeks
Neuropathy due to DILS	Starting or adjusting ART **plus** prednisone 1–1.5 mg/kg daily for 3–4 weeks with subsequent tapering for 12–16 weeks
Distal symmetrical sensory polyneuropathy	A causative treatment is not known, ART may improve nerve function, for symptomatic treatment. See Table 6
Medication-related toxic neuropathy	Withdrawal of the neurotoxic agents, if possible.
Mononeuritis multiplex or polyradiculitis due to CMV-infection	Intravenous foscarnet 2 x 90 mg/kg daily **plus** intravenous ganciclovir 2 x 5 mg/kg daily.
Lymphomatous meningitis	Starting or adjusting ART **plus** intrathecal methotrexate (intraventricular shunt or lumbar puncture) 12–15 mg 2 x/weekly until CSF is free of malignant cells, subsequently 1 x/week for 4 weeks and subsequently 1 x/month **plus** 15 mg oral folinate after each injection **plus** systemic treatment of lymphoma (see chapter on Malignant Lymphoma)
Polyradiculitis due to infection with *M. tuberculosis*	Treat tuberculosis (see chapter on OIs)

Table 6: Symptomatic treatment of painful neuropathy

	Treatment	Adverse effects
Step 1	Physical therapy, supporting measures (wide shoes, etc.), 5% lidocaine patch	Rarely allergy
Step 2	Temporary trial of 3–4 x 1000 mg paracetamol or 2–3 x 50 mg diclofenac or 4 x 40 drops novaminsulfone for 10–14 days or 8% capsaicin patch	Nausea, vomiting, allergy (rarely) Transient skin irritation
Step 3	Gabapentin 300 mg at night, dose escalation of 300 mg a day every third day up to a maximum of 1200 mg TID *or* Pregabalin 2 x 75 mg for 1 week, dose escalation to 2 x 150 in the 2nd week, possible escalation up to 2x300 mg *or*	Sedation, nausea, dizziness, rarely pancreatitis Nausea, vomiting, diarrhea, allergic drug rash

Table 6: (continued)

	Treatment	Adverse effects
	Lamotrigine 25 mg at night, dose escalation of 25 mg every 5 days up to 300 mg *or*	Allergy, sedation, cephalgia, nausea
	Amitriptyline 25 mg at night, dose escalation of 10–25 mg every 2–3 days up to 3 x 50 mg *or*	Sedation, orthostatic hypotension, constipation, dizziness, dry mouth, dysrhythmia, retention of urine, except for: glaucoma
	Nortriptyline 25 mg in the mornings, dose escalation of 25 mg every 2–3 days up to 2–3 x 50 mg *or*	Orthostatic hypotension, constipation, dizziness, dry mouth, dysrhythmia, retention of urine, except for: glaucoma
	Duloxetin 1 x 60–120 mg	Nausea, diarrhea, agitation
Step 4	Flupirtine 3 x 100, dose escalation up to 3 x 600 mg *or*	Sedation, constipation, nausea
	Retarded morphine 2 x 10 mg gradual escalation up to 2 x 200 mg	Sedation, constipation, nausea
General practice	Proceed to the next step if symptoms persist. Agents in step 3 may be combined (for instance an anticonvulsant and an antidepressant), agents in step 3 and step 4 may also be combined (for instance flupirtine and an anticonvulsant). If a rapid relief of symptoms is necessary, treatment should be started with step 4 agents and a low dose step 3 drug should simultaneously be started with slow escalation. The slower the escalation the greater the possibility of reaching an effective dosage.	

Potent opioids may be used to manage moderate or severe pain if a slow dose escalation of an antidepressant or anticonvulsant is not possible and an immediate analgesic effect is desired. Even in cases of substituted or non-substituted drug abuse, opioids can be used (Breitbart 1997). Sometimes, the dosage of methadone only needs to be moderately increased for a sufficient analgesic effect.

Myopathy

Myopathies occur in 1–2% of all HIV+ patients. They may appear at any stage of disease.

Table 7: Most important myopathies in HIV infection

Primary HIV-associated	Secondary
Polymyositis	AZT myopathy
Nemaline (rod body) myopathy	Vasculitic myopathy
Vacuolar myopathy	Lymphomatous muscle infiltration
Inclusion body myositis	Infectious myositis
	Medication-related toxic rhabdomyolysis

Polymyositis mediated by cytotoxic T cells is the most common HIV-associated myopathy. AZT-induced myopathy occurs very infrequently with the dosages used today (500–600 mg/day). Other agents such as ddI, cotrimoxazole, pentamidine, sulfadiazine, lipid-lowering drugs and the integrase inhibitor raltegravir (Zembower 2008) may rarely cause acute rhabdomyolysis with tetraparesis and marked elevation of serum CK levels. Notably, PIs raise the serum concentration of statins, increasing the risk of myopathy and rhabdomyolysis (Hare 2002). Raltegravir causes rarely a chronic limb girdle myopathy with paresis and myalgia (Lee 2012). An elevated serum CK activity is frequently observed during treatment with TDF, especially in patients with HBV/HCV coinfection. This is due to a type 2 macroenzyme creatine kinase (macro CK) and must not lead to suspicion of ischemic heart or of muscular disease. The accumulation of this liver-derived isoenzyme seems to be the result of an insufficient macro CK-2 clearance capacity mediated by TDF (Schmid 2005).

Clinical features

Myopathy in HIV infection usually presents with exercise-induced myalgia of proximal muscles followed by slowly progressive, symmetrical weakness and atrophy of proximal muscles. Limb girdle muscles are most commonly involved, but distal muscles and muscles of the trunk, neck, face or throat may also be affected.

Diagnosis

Myalgia, fatigue and elevated serum CK levels are frequently found in HIV infection. Some of the antiretroviral substances, mainly AZT, nevirapine and maraviroc, may cause myalgia. But these unspecific symptoms and signs on their own do not warrant the diagnosis of myopathy. The diagnosis of probable myopathy requires weakness, muscle atrophy or myopathic features demonstrated by electromyography. A muscle biopsy confirms the diagnosis and may give some additional clues to the classification and pathogenesis of the muscle disease.

Treatment

Moderate myalgia may respond to non-steroidal anti-inflammatory drugs. Prednisone (100 mg daily for 3–4 weeks, subsequent tapering) or intravenous immunoglobulin (0.4 g/kg for 5 days) have been shown to be effective in treatment of polymyositis (Johnson 2003, Viard 1992). The treatment of AZT myopathy is cessation of the drug. Myalgia usually resolves within 1–2 weeks. If symptoms persist beyond 4-6 weeks, prednisone as described above may be effective.

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30. The new HIV+ Patient

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The initial interview

Should be spread over several appointments at short intervals.

What the patient should know after the first consultation

- In general terms, how the virus causes illness.
- The difference between being HIV-infected and having AIDS.
- The importance of CD4 T cells and viral load.
- How others can become infected and how this can be avoided.
- How HIV therapy works and how helpful it is.
- Good prognosis: Nowadays, the vast majority of HIV patients under treatment live a normal life.
- Additional sexually transmitted diseases (STDs) and viral hepatitis should be avoided, as these can worsen the course of HIV infection. If there are symptoms of STDs, the patient should be able to calmly talk about them.
- It is theoretically possible to become infected with another more pathogenic or resistant strain of HIV (reinfection, super-infection).
- A balanced diet and regular physical exercise can help to improve the prognosis.
- Smoking increases the risk of countless complications.
- Where to find further medical and social information.
- The support groups (NGOs, community-based organizations) available in the area for the support of HIV+ patients.
- Planned tests and their usefulness for future treatment.

What the doctor should know after the consultation

Infection and risk

- When, where and why was the HIV test performed? Was there a negative test prior to this? What risks did the patient take in the meantime? In the case of no recognizable risk, the test result may be held until confirmation (see below).
- Knowing sexual behavior helps for detecting STDs and supports prevention counseling.
- Family history of diabetes, coronary heart disease, cancer, tuberculosis or other infectious diseases.
- Has the patient traveled recently and what is his geographical background? (prevalence of infections may vary by region)
- What recreational drugs does the patient consume regularly and how (IV, inhaled, etc.)?
- What about smoking? Cumulative amount (pack-years)?
- Tuberculosis among contacts of the patient.

Concomitant illnesses

- Previous illnesses, concomitant illnesses?
- Previous infections, tuberculosis, STDs including syphilis and hepatitis B/C?
- What medications are they on?
- Is there a history of allergic reactions?
- Vaccination status (record?). Did the patient take part in disease screening programs?

Social

- If they have a partner, has the partner been tested for HIV and STDs? What about children or plans for pregnancy?
- What is the social background of the patient? What is his/her profession / occupation? Work schedule? What duties does (s)he have to fulfill? Is it possible to pay for some medical aspects due to local medical care insurance?
- What about migration? Residential status, legal status, insurance?
- What about religious background? Are there any restrictions on taking ART or other risk factors and sexual orientation?
- Who knows about the infection? Who will help if the patient becomes ill? Who does (s)he talk to about problems? Does (s)he have friends who are infected? Is (s)he interested in getting in touch with social workers or support groups (NGOs)?
- Is psychotherapeutic support necessary?
- Is there a legal guardian?

Laboratory tests

- The HIV test is double-checked; Reactive Quick and Elisa antibody tests will be double-checked by Western Blot (WB) or Immunoblot test (see Test Chapter)
- Complete blood count: 30–40% of all HIV+ patients suffer from anemia, neutropenia or thrombocytopenia
- CD4 T cell count and CD4/CD8 ratio. Allow for variations (percentage with less fluctuation; HTLV-1 coinfection leads to higher counts despite existing immunodeficiency)
- Plasma HIV RNA (viral load) and HIV resistance test (genotype)
- HLA-B*5701 testing is mandatory before starting abacavir, tropism test before maraviroc
- Electrolytes, creatinine, calculated creatinine clearance, urine status (proteinuria is often a sign of HIV-associated nephropathy), AST (GOT), ALT (GPT), γGT, AP, LDH, lipase, total protein, protein electrophoresis
- Fasting blood glucose and lipid profile
- Hepatitis serology: A, B, C, D (vaccination? B also in order to choose an ART that might also be useful for hepatitis B); consider PCR testing in cases of acute infections
- TPHA test and cardiolipin, if TPHA positive
- If appropriate, STD screening of chlamydia, gonorrhea with tissue swabs (oral, urethral, anal if necessary) and PCR testing
- If clinical suspicion and / or low CD4 count: toxoplasmosis serology IgG. If negative: important for differential diagnosis, if CD4 T cells <200/µl – prevention of infection (such as no raw meat). If positive: medication for prophylaxis if necessary
- If clinical suspicion and / or low CD4 count: CMV serology (IgG). If negative: important for differential diagnosis, inform well about prevention (i.e., safe sex). In cases of severe anemia, transfusion of CMV-negative blood only. If positive and CD4 <100: PCR or pp65 antigen for CMV viral load; eye examination for retinitis
- If clinical suspicion and / or low CD4 count: varicella, measles, rubella serology. If negative: active vaccination with attenuated pathogens is contraindicated, but at >400 CD4 T cells/µl refer to the vaccination guideline
- If clinical suspicion: folic acid, vitamin B12 and D (often under normal range)
- Blood culture in acute diseases

Examinations

- Physical examination, including an exploratory neurological examination (vibration sensitivity and mini-mental status exam if appropriate)
- Neurological impairment should prompt CT or MRT scan of the brain to screen for cerebral infections or malignancies
- If CD4 T cells are above 400/μl, a T cell interferon gamma release tests (TIGRA, e.g., ELISpot® or Quantiferon®) should be carried out. The tuberculin skin test (TST, PPD) is less specific and sensitive than TIGRA. A negative test does not exclude active or latent tuberculosis. Chest X-ray only in case of positive TST or TIGRA or clinical suspicion of disease of the thoracic organs
- Sonographic scan of the abdomen in case of suspicion or elevated risk. A harmless, informative examination as a baseline finding (for liver, spleen, kidney, lymphoma)
- In case of previous or suspected cardiac / pulmonary diseases: ECG and pulmonary function test. Simple tests to assess cardiovascular and pulmonary status; n-BNP and / or echocardiography in cardiac diseases; risk scores for CHD; check QTc interval for drug toxicity
- For women, a PAP smear upon initial diagnosis, after 6 months and then, if negative, once a year for CIN screening
- For those who practice passive anal sexual intercourse, an anal PAP smear for AIN screening, proctologic investigation should be offered
- Fundoscopy, especially in case of visual disturbances and at low CD4 T cells (<100/μl) to rule out active CMV retinitis or scars
- Nutritional advice and/or treatment of malnutrition
- Check for osteoporosis risk
- Verifying vaccinations (see chapter on Vaccinations)
- Checking for necessity of OI prophylaxis
- Checking the indication for antiretroviral therapy

31. Post-Exposure Prophylaxis (PEP)

THORE LORENZEN

Transmission routes and risks

Transmission of HIV may occur if someone comes into contact and incorporates the blood, semen or vaginal fluids of an HIV+ source person. Exposure of intact skin to HIV-contaminated material (e.g., blood) is not sufficient. Besides vertical transmission, HIV transfer is possible if HIV-containing material enters the body by:
• needlestick injury or incision by surgical instruments
• exposure of damaged skin or mucosal membranes
• unprotected sexual intercourse with an infected person
• IDU needle or equipment sharing
• transfusion of HIV-contaminated blood or blood products

Overall, HIV is a low contagious pathogen. The transmission rate ranges between 1:100 and 1:1000. The transmission rate for hepatitis C and B are approximately 10 and 100 times higher, respectively. Factors associated with transmission risk include the amount of source-incorporated virus transmitted and the length of exposure time. Contact with body fluids of a patient with a high viral load probably holds a greater risk than a similar contact with body fluids of a patient on ART with a viral load below level of detection. Additionally, rapid removal of infectious material, e.g., from damaged skin or mucosal membrane by washing or disinfection, presumably decreases the risk of transmission. For percutaneous contact with HIV-containing blood, a transmission rate of 0.3% on average is assumed. Using retrospective data, rates have been calculated more precisely (Table 1).

Table 1: Calculations to assess estimated individual transmission risk after HIV exposure*

Type of Exposure	Relative Risk
Deep needle-stick injury or cut	16:1
Fresh blood on the penetrating instrument	5:1
Penetrating needle previously placed in blood vessel	5:1
Source person with high viral load	6:1
Exposition of mucosal membrane	1:10
Exposition of inflammatory damaged skin	1:10

* Source: German-Austrian recommendations for PEP against HIV infection 2013

For information about assumed transmission risk of other types of exposure, please refer to first chapter of this book (*Introduction*). Simian models show that in mucosal membranes, HIV primarily infects the local immunocompetent cells such as Langerhans cells. These cells and/or their sibling cells migrate to regional lymph nodes; detection of HIV in the blood occurs days later. The process of local infection and migration of the cells to the lymph nodes takes approximately 24–48 hours (Spira 1996, Otten 2000). Theoretically, immediate treatment may avert a systemic infection.

Effectiveness and limitations of PEP

Early reports on the use of AZT after occupational needle-stick injuries date from 1989. An analysis of retrospective case-control studies shows that even prophylaxis with a single antiretroviral agent after exposure reduces the probability of an infection by approximately 80% (Tokars 1993). In theory, the combination of multiple drugs seems to be even more potent.

Transmission of HIV cannot always be prevented and there have been reports of HIV infections despite the use of PEP, mostly with AZT mono-PEP but also with ART combinations (Cordes 2004, Roland 2005). Furthermore, transmission from patients on ART may lead to transfer of resistant virus strains. The rate of primary resistances in naïve patients varies by region and country, but over the years it has stabilized at approximately 10 to 15% for at least one agent or drug class. How to deal with this issue concerning PEP initiation still remains unclear since resistance testing takes some days or more. Results would arrive too late to avoid the spread of HIV using the appropriate antiretrovirals.

When is PEP indicated?

The decision to provide PEP should be made by a physician experienced in HIV treatment. It is important to ascertain whether the source person has a supposed or confirmed HIV infection. A rapid test may be helpful in such a situation, but confirmation of the result should be performed by established laboratory-based methods. However, the sooner the PEP is initiated, the better the chances to avoid transmission. In unclear cases, it makes sense to start PEP and to stop it in case of a negative result. If the source person is HIV-infected, current viral load, stage of disease, treatment history and resistance tests may be considered (Puro 2003).

The exposed person should also be asked about any first aid procedures that have already been performed.

Table 2: Overview of recommendations for usage of PEP

Occupational Exposure	
Percutaneous needlestick injury with hollow needle (body fluids with high viral load: blood, liquor, material from biopsies, cultured virus)	Recommended
Deep injury (e.g., cuts), apparently blood-stained	Recommended
Intravenous injection with a previously used needle	Recommended
Superficial injury (e.g., with surgical needle)	Considered
- if source person has AIDS or high viral load	Recommended
Contact of mucosal membrane or damaged skin with fluids with high viral load	Considered
Percutaneous contact with body fluids other than blood (e.g., urine, saliva)	Not Recommended
Contact of intact skin with blood (including high viral load)	Not Recommended
Contact of skin or mucosal membranes with body fluids such as urine or saliva	Not Recommended
Non-occupational Exposure	
Transfusion of HIV-containing blood products (or if HIV contamination is highly probable)	Recommended
Unprotected insertive or receptive sex with an HIV-infected person	Considered or Recommended*
Sharing contaminated needle or equipment with IDUs	Recommended*
Unprotected receptive oral sex with ejaculation with an HIV-infected person	Considered
Kissing and other sexual contacts without semen-/blood-mucosal membrane contact	Not Recommended
Needlestick from a discarded needle in the community	Not Recommended

Sources: CDC Guidelines for the management of occupational exposure to HIV 2005; UK guidelines for the use of post-exposure prophylaxis for HIV following sexual exposure 2011; Updated US Public Health Service Guidelines for the Management of Occupational Exposures to Human Immunodeficiency Virus and Recommendations for Post-exposure Prophylaxis 2013
*Only recommended if source person has detectable viral load. Consider if serostatus of source is unknown and person belongs to or comes from high prevalence group/area

Following clarification of these queries, the exposed person has to be informed about the possible risks of PEP. It should also be emphasized that no antiretroviral agent is approved for use in this setting (although Truvada® is approved for PrEP in the US, it is not licensed for PEP!). Besides the legal responsibility of the prescribing physician, these facts are also important with regard to the coverage of cost, especially after sexual exposure. For example, in Germany, although some regional and national plans will cover a limited program of PEP, the medication cannot be prescribed at the expense of health insurance companies; however, PEP for occupational exposure is usually covered by statutory accident insurance.

Table 2 gives an overview of situations in which PEP is recommended. Of note, risk assessment has changed in the last years: following the Swiss statement (EKAF 2008, see chapter on *ART and Prevention*), the newest British PEP guidelines have modified their recommendations: in case of an HIV+ source person without detectable viral load, PEP should only be provided after receptive anal intercourse. In cases of detectable viral load, PEP is recommended in direct homo- or heterosexual intercourse. In case of unknown serostatus of the source person, the use of PEP is very restrained (Benn 2011). Similar opinions are found in current French and German guidelines. The overview of recommendations for PEP usage should serve as an orientation, although alterations can occur in individual cases.

Potential risks of PEP

Adverse effects of the antiretroviral drugs have to be taken into account, most frequently gastrointestinal symptoms such as nausea, vomiting or diarrhea. Changes in hematology, liver enzymes, and/or creatinine are less frequent. Additionally, there have been reports of elevated triglycerides and cholesterol levels, and insulin resistance even with short-term use of PIs (Parkin 2000).

It is unknown whether the temporary use of ARVs may lead to long-term side effects. However, all this is secondary since the main emphasis is to prevent a chronic and potentially life-threatening disease. For pregnant women, particular caution is required since data concerning teratogenicity are lacking.

Initial interventions

Different procedures are recommended following exposure to HIV, depending on the type of exposure. Following needlestick or cut injuries with HIV-contaminated instruments, fluid should be expressed by squeezing the tissue surrounding the wound and striking out proximal blood vessels towards the wound. Very intense massage or contusions should be avoided. The wound should be flushed with an alcoholic virucidal antiseptic for a minimum of 10 minutes. For skin that has been in contact with blood or body fluids removal of the infectious material and subsequent extensive disinfection with a skin antiseptic appears sufficient. After contamination of an eye, immediate flushing with water or antiseptic solutions is recommended. The oral cavity should be rinsed several times (10-15 seconds each) with an aqueous solution or alcohol after contact with potentially infectious material.

Persons who, through sexual exposure, have had contact with anal or genital mucosae from infectious material, should wash the penis with soap and water; genital mucosae should be flushed with water after urination in order to wash contaminated material from the urethra. Intense deep washing of the vagina or rectal enemas are not recommended due to an elevated risk of injuries. Following these initial interventions, an expert in HIV treatment should be consulted for the decision on whether pharmaceutical PEP needs to be initiated.
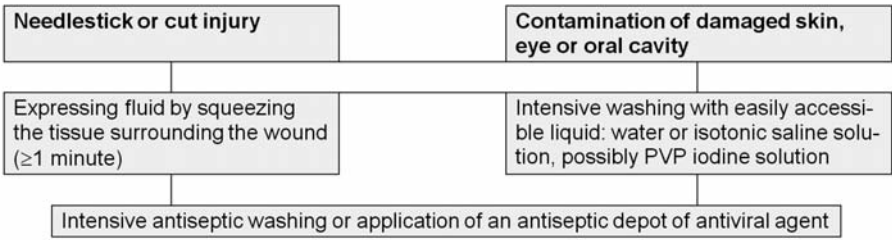
Figure 1: Recommended initial interventions after HIV exposure (Source: German-Austrian recommendations for Post-Exposure Prophylaxis against HIV infection 2013)

Comprehensive evaluation and accurate documentation of the course of the accident is very important, especially for occupational exposure. The process of informing the patient about the risks of PEP needs to be documented carefully and the patient should sign an informed consent.

Initiation of PEP

Timing is the most crucial factor as the best chance to prevent transmission is within the first 24 hours of exposure, preferably within 2 hours after exposure. A deferred initiation increases the risk of systemic spread of the virus. Initiating PEP after more than 72 hours following exposure does not seem reasonable. In this short time frame, if consultation with a physician experienced in HIV treatment is not possible, it might be advantageous to just initiate PEP. Interrupting a regimen that is not indicated is always an option.

For a long time most recommendations have favored a regimen with a combination of antiretroviral agents given for 4 weeks, preferably consisting of two NRTIs and one PI. In current updates the integrase inhibitor raltegravir is most preferred due to its excellent tolerability. NNRTIs, especially nevirapine, should not be used for PEP because of the risk of severe adverse effects such as severe hepatotoxicity (CDC 2001). For efavirenz, CNS effects limit its use for PEP.

When starting PEP, existing viral resistance mutations should be taken into account as far as possible; in many cases, however, this information will not be available. Recommended combinations are shown in Table 4.

For entry inhibitors such as T-20 (Fuzeon®) and maraviroc (Selzenty® or Celsentri®) data on PEP is limited. These agents, however, may be useful in this setting due to their mode of action.

Table 3: Recommended antiretroviral combinations for HIV post-exposure prophylaxis*

NRTIs	plus	Third agent
TDF + FTC (Truvada®) or TDF + 3TC or AZT + 3TC (Combivir®)		raltegravir (Isentress®) or lopinavir/r (Kaletra®) or atazanavir/r (Reyataz® plus Norvir®) or darunavir/r (Prezista® plus Norvir®) or efavirenz (Sustiva® or Stocrin®)

* Source: Updated US Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis 2013; UK guidelines for the use of post-exposure prophylaxis for HIV following sexual exposure 2011; German-Austrian Recommendations for PEP against HIV infection 2013
Note: Efavirenz often causes CNS side effects and is contraindicated in pregnancy

During pregnancy, PEP should be used only after careful consideration of the benefits, since there are only limited data on the teratogenic effects. In any case, advice of a physician experienced in HIV treatment and pregnancy should be obtained.

After contact with potentially infectious material, not only HIV, but other diseases might be transmitted. Hepatitis B/C testing should also be performed. Persons exposed to HBV should receive hepatitis B immunoglobulin and a vaccine series simultaneously if they do not have sufficient vaccination status.

Unprotected sexual contacts should highlight the possibility of transmissions of other STDs such as syphilis or gonorrhea. STD testing is recommended between 2–4 weeks after exposure.

Management of PEP

After initiation of PEP, the patient should not be discharged without a follow-up consultation. Persons exposed to HIV may be under high psychological pressure. It should be emphasized that there is generally a low risk of transmission. Adverse effects often include gastrointestinal symptoms. Changes in hematology, liver enzymes, and/or creatinine are less frequent. However, tests for these side effects should be conducted after 14 days and at the end of the course of PEP. Despite close monitoring, different studies report discontinuation rates of 15-30% (Lancombe 2006, Sonder 2005+2007).

At the end of a completed course or discontinued PEP, HIV testing should be performed after 6 weeks and 3 months. An HIV PCR which may indicate an early infection before seroconversion would only be helpful if there is reasonable suspicion of transmission of HIV infection.

In any case, the patient should be counseled to remember to practice safer sex.

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SECTION 7

Drugs

32. Drug-Drug Interactions

JAN THODEN

With an increasing number of antiretroviral drugs there is an growing risk of adverse drug-drug interactions. These might interfere with therapeutic success. Moreover, antiretroviral drugs are used by an aging patient population with a variety of co-morbidities requiring additional medications.

By inducing or inhibiting enzyme production, the elimination of a drug and hence its plasma levels are influenced. Especially metabolization by cytochrome-P450 plays a crucial role. As many drugs, PIs and NNRTIs are mainly metabolized by CYP3A4 in liver and the gastrointestinal tract. Another pathway is the glucuronidation by glucuronosyltransferases, though this usually does not cause clinically relevant interactions. Moreover, there are major interindividual differences in enzyme activity and drug metabolization rates. Other factors that need to be considered include genetic polymorphisms, ethnicity, age, sex, and co-morbidities.

The tables provide a brief overview of drug combinations deemed safe (+) as well as those that should be avoided (☹). However, for many combinations the interactions are uncertain, unknown or can only be assumed based on theoretical calculations (☺). In these cases, use might still be safe and should be controlled by TDM.

The first part is focused on ART/ART interactions, the second part on those between ART and concomitant medications.

Among the INSTIs, elvitegravir is listed as the fixdose tablet Stribild® (STB). Cobicistat stand-alone (Tybost®) which is approved in combinations with atazanavir and darunavir, is not listed, as well as irrelevant drugs such as d4T, ddI indinavir, nelfinavir. All PIs are assumed to be given boosted with ritonavir or cobicistat. T-20 is only mentioned in the first part as there are no known relevant interactions.

This chapter is intended as a tool to support rapid decision making in the daily practice, but should not replace a literature search. On rare occasions, drug combinations with known adverse effects might be unavoidable due to a lack of alternatives. In these cases, close monitoring (including TDM) is necessary.

Individual questions regarding interactions can be answered by experts (e.g. www.ifi-interaktions-hotline.de). Several APPs serve this purpose, too (e.g. HIV iChart of the University of Liverpool).

Abbreviations:

+	Combination of these drugs possible
☺	Potential interactions or unknown, combination of these drugs is often possible, therapeutic drug monitoring suggested
☹	Combination of these drugs should be avoided or is contraindicated
↑	up to 50% increased drug levels, ↑↑ up to 100%, ↑↑↑ >100%
↓	up to 50% decreased drug levels, ↓↓ up to 100%, ↓↓↓ >100%
BID	Twice daily (TID = Three times daily. QD = Once daily)
TDM	Therapeutic drug monitoring

Part 1: ART + ART

NRTIs + NRTIs

	3TC	ABC	FTC	TDF	AZT
3TC		+	☹[1]	+	+
ABC	+		+	☺	+
FTC	☹[1]	+		+	+
TDF	+	☺	+		☺
AZT	+	+	+	☺	

[1] Antagonism

NRTIs + NNRTIs

	3TC	ABC	FTC	TDF	AZT
EFV	+	+	+	+	+
ETV	+	+	+	+	+
NVP	+	+	+	+	+
RPV	+	+	+	+	+

NRTIs + PIs

	3TC	ABC	FTC	TDF	AZT
ATV	+	+	+	☺[1]	+
DRV	+	+	+	+[2]	+
FPV	+	+	+	+	+
IDV	+	+	+	+	+
LPV	+	☺[3]	+	+[2]	+
NFV	+	+	+	+	+
RTV	+	+	+	☺	+
SQV	+	+	+	+	+
TPV	+	☺[3]	+	+	☺[3]

[1] ATV ↓, TDF ↑, ATV always boosted   [2] TDF ↑, caveat: combination with nephrotoxic drugs, increased nephrotoxicity possible   [3] NRTI ↓ (unknown relevance)

NRTIs + EIs/INSTIs

	3TC	ABC	FTC	TDF	AZT
T-20	+	+	+	+	+
DTG	+	+	+	+	+
MVC	+	+	+	+	+
RAL	+	+	+	+	+

STB as a single tablet regimen should not be coadministered with other ARTs

NNRTIs + EIs/INSTIs, EIs/INSTIs + EIs/INSTIs

	EFV	ETV	NVP	RPV	T-20	DTG	MVC	RAL
EFV	☹, NNRTIs				+	☺[4]	☺[1]	☺[2]
ETV	should not				+	☹[5]	☺[1]	+
NVP	be combined				+	☹	+	+
RPV	with each other				+	+	+	+
T-20	+	+	+	+		+	+	+
DTG	☹[4]	☹[5]	☹[4]	+	+		+	☹
MVC	☺[1]	☺[1]	+	+	+			+[3]
RAL	☺[2]	+	+	+	+	☹	+[3]	

[1] MVC ↓↓, increase MVC to 2 x 600 mg/d, if not combined with PI or potent CYP3A4 inhibitor
[2] RAL ↓, relevance unclear   [3] RAL ↓, MVC ↓, probably without clinical rele-vance   [4] DTG↓ increase DTG to 50mg BID   [5] DTG↓ no combination with EFV without co-administration of ATV, LPV or DRV

STB as a single tablet regimen should not be coadministered with other ARTs

NNRTIs + PIs, EIs/INSTIs + PIs

	EFV	ETV	NVP	RPV	T-20[7]	DTG	MVC	RAL
ATV	☺[1]	☹[1]	☺	☺	+	+	☺[2]	☺
DRV	☺	+	+	+	+	+	☺[2]	+
FPV	😐	☹[3]	☺	☺	+	☺[8]	+	+
LPV	☺[4]	+	☺[4]	+	+	+	☺[2]	+
RTV	☺	☺	+	☺	+	☺	☺[2]	+
SQV	☺	+[5]	☺	☺	+	☺	☺[2]	+
TPV	+	☹[6]	+	☺	☺	☺[8]	+	☺

[1] ATV ↓↓, ATV always boosted   [2] MVC ↑↑↑, reduce MVC to 2 x 150 mg/d
[3] FPV ↑↑, relevance unclear, monitor FPV levels   [4] LPV ↓, increase LPV to 2 x 3 tablets (controversial in combination with NVP, use TDM)   [5] SQV ↓↓, always boosted   [6] ETV ↓↓, TPV↑, combination therefore not recommended   [7] T-20 can be increased by PIs, PIs by T-20, too, no clinical relevance; TDM if problems   [8] DTG↓, increase DTG to 50 mg BID

PIs + PIs

	ATV	DRV	FPV	LPV	RTV	SQV	TPV
ATV		+	☺	☺	+	+[1]	☹
DRV	+		☺	☹	+	☹	☹
FPV	☺	☺		☺	+	+[2]	☹
LPV	☺	☹	☺		+	+	☹
RTV	+	+	+	+		+	+
SQV	+[1]	☹	+[2]	+	+		☹
TPV	☹	☹	☹	☹	+	☹	

[1] ATV ↑, SQV ↑, combination well tolerated   [2] FPV with 200 mg RTV, combination possible
Comment: The combination of two PIs is probably not more effective compared to second generation PIs (DRV and TPV)

Part 2: ART + concomitant medications

Gastrointestinal agents

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Cimetidine	+	+	+	☺	+	+	+	+	☺[2]
Famotidine	+	+	+	+	+	+	+	+	☺[2]
Loperamide	+	+	+	+	+	+	+	+	+
MCP	+	+	+	+	+	+	+	+	☺
Mesalazine	☺	+	☺	☺	+	+	+	+	+
Ondansetrone	+	+	+	+	+	+[1]	+[1]	+[1]	☺
Ranitidine	+	+	+	+	+	+	+	+	☺[2]
PPIs	+	+	+	+	+	+	+	+	☹[3]

[1] NNRTIs are strong enzyme inductors, ondansetrone levels can be decreased   [2] RPV should not be coadministered with H2-blocking agents, alternatively H2-blocker >12h before or 4h after RPV.
[3] No combination of RPV and PPIs, RPV-levels strongly decreased
MCP = metoclopramide, PPIs = proton pump inhibitors

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Antacids	☺[1]	+	+	+	+	☺[1]	+	☺[6]	☺	☺
Cimetidine	☺	+	☺	+	+[2]	☺	+	+	+	☺
Famotidine	☺	+	☺	+	☺	☺	+	+	+	☺
Loperamide	☺	☺	+	☺	☺	☺	☺	☺[7]	+	+
MCP	+	+	+	+	☺	+	☺	+	+	☺
Mesalazine	+	+	+	+	+	+	+	+		+
Ondansetrone	+	+	+	+	+	☺	☺	+	+	☺
PPIs	☹[3]	+	+	+	☺	☺	☺[4]	+	+	☺[5]
Ranitidine	☺	+	☺	+	+	☺	+	+	+	☺

[1] PIs ↓, take antacids at least 2 hours apart   [2] Cimetidine ↑, SQV ↑↑↑   [3] ATV boosted, TDM recommended, avoid this combination   [4] Potential interactions with esomeprazole, other PPIs probably without relevant interactions   [5] RAL ↑↑, relevance unclear   [6] Potential interactions, TDM (e.g. take antacids containing aluminum or magnesium >2 h after STB; Elvitegravir ↓)
[7] Potential interactions, TDM. MCP = metoclopramide, PPIs = proton pump inhibitors

Antiarrhythmic drugs

Most PIs increase the drug levels of antiarrhythmic drugs. In combination with NNRTIs the levels might be fluctuating. Antiarrhythmic drugs should be introduced with the lowest possible dosage. Calcium channel inhibitors will be discussed separately. Maraviroc: TDM in combination with amiodarone. No interactions with altegravir expected.

PIs/NNRTIs

	ATV	DRV	FPV	LPV	SQV	TPV	EFV	ETV	NVP	RPV
Amiodarone	☺	☹	☹	☹	☹	☹	☺	☺	☺	☺
Chinidine	☹	☹	☹	☺	☹	☹	☺	☺	☺	☺
Flecainide	☹	☺	☹	☹	☹	☹	☺	☺	☺	+
Lidocaine	☺	☹	☺	☺	☺	☺	☺	☺	☺	+
Propafenone	☹	☺	☹	☺	☹	☹	☺	☺	☺	+

Antibiotics
NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Amoxicillin	+	+	+	+	+	+	+	+	+
Azithromycin	+	+	+	+	+	+	☺	+	+
Ciprofloxacin	+	+	+	+	+	+	+	+	+
Clarithromycin	+	+	+	+	☺[1]	☺[2]	☺[2]	☺[2]	☺
Clindamycin[5]	+	+	+	☺[3]	+	+	+	+	+
Cotrimoxazole	☺	+	☺	☺	☺[4]	+	+	+	+
Dapsone	+	+	+	+	☺[4]	☺	☺	☺	+
Ertapenem	+	+	+	+	+	+	+	+	+
Erythromycin[5]	+	+	+	+	+	+	☺[7]	☺[7]	☺
Ethambutol	+	+	+	+	+	+	+	+	+
Gentamycin	+	+	+	☺[3]	+	+	+	+	+
Isoniazid	+	+	+	+	+	+	+	+	+
Meropenem	+	+	+	☺	+	+	+	+	+
Metronidazole[5]	+	+	+	+	+	+	+	+	+
Moxifloxacin	+	+	+	+	+	+	+	+	☺
Ofloxacin	☺	+	☺	+	+	+	+	+	+
Pentamidine[5]	+	+	+	☺[3]	+	☺	+	+	☺
Pyrazinamide[5]	+	+	+	☺[3]	+	+	+	+	+
Pyrimethamine	+	+	+	☺[3]	☺[4]	+	+	+	+
Rifabutin	+	+	+	+	+	☺[8]	☹[9]	☺	☹
Rifampicin	+	☺	+	+	☺	☺[10]	☹[11]	☹	☹
Rifapentin	+	+	+	+	+	☺	☹	☹	☹
Streptomycin Tetracyclines	+	+	+	☺[3]	+	+	+	+	+
Vancomycin	+	+	+	☺	☺	+	+	+	+

[1] AZT ↓, take 1–2 h apart   [2] active metabolite ↑, consider alternatives e.g. azithromycin.
[3] Caveat: renal function   [4] Caveat: hematotoxicity   [5] Theoretical data on interactions with NRTIs
[6] NNRTI ↑, consider alternatives (azithromycin)   [7] Rifabutin ↓, increase dosage to 450–600 mg/d
[8] ETV ↓, rifabutin ↓, avoid this combination   [9] EFV ↓, increase EFV to 800 mg/d   [10] ETV approved in combination with PI/r – rifampin contraindicated

Comment: No relevant interactions with azithromycin, ciprofloxacin and tetracyclines

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Amoxicillin	+	+	+	+	+	+	+	+	+	+
Azithromycin	+	+	+	+	+	+	+	+	+	+
Ciprofloxacin	+	☺	+	☺	☺	☺	+	+	+	+
Clarithromycin	☺[1]	☺	+	☺	+	☹[2]	+[3]	☺[8,9]	+	+
Clindamycin	+	+	+	+	+	+	+	+	+	+
Cotrimoxazole	+	+	+	+	+	☺	+	+	+	+
Dapsone	+	+	☺	+	☺	☺	+	+	+	+
Ertapenem	+	+	+	+	+	+	+	+	+	+
Erythromycin[4]	+	+	+	+	☺	☺	☺	☺[8]	+	+
Ethambutol	+	+	+	+	+	+	+	+	+	+
Gentamycin	+	+	+	+	+	+	+	☺[8]	+	+
Isoniazid	☺	☺	+	☺	+	☺	+	☺[8]	+	+
Meropenem	+	+	+	+	+	+	+	+	+	+
Metronidazole[5]	☺	☺	+	☺	+	☺	☺	+	+	+
Moxifloxacin	☺	+	+	☺	+	+	+	+	+	+
Ofloxacin	☺	+	+	☺	+	+	+	+	+	+
Pentamidine[5]	+	+	+	☺	+	+	☺	+	+	+
Pyrazinamide[5]	+	+	+	+	+	+	+	+	+	+
Pyrimethamine[5]	+	+	+	+	+	+	+	+	☺	+
Rifabutin[6]	☺	☺	☺	☺	☺	☺	☺	☺[8]	+	+
Rifampicin	☹	☹	☹	☹	☹	☹	+[7]	☹	☺[10]	☺
Rifapentin	☺	☺	☺	☺	☺	☺	☺	☺[8]	☺	+
Streptomycin[5]	+	+	+	+	+	+	+	☺[8]	+	+
Tetracyclines	+	+	+	+	+	+	+	+	+	+
Vancomycin	+	+	+	+	+	+	+	☺[8]	+	+

[1] QT-prolongation possible, clarithromycin ↑ by 50%, reduce dose   [2] TPV ↑↑   [3] MVC ↑↑, reduce MVC to 2 x 150 mg/d   [4] PIs ↑, erythromycin ↑, consider azithromycin   [5] Little data, probably no relevant interactions   [6] Rifabutin ↑↑, reduce to 150 mg every other day   [7] Increase MVC to 2 x 600 mg/d, if not combined with PI or potent CYP3A4 inhibitor   [8] Potential interactions, consider TDM.
[9] If creatinine clearance <50–60 ml/min, consider reduction of Clarithromycin by 50%
[10] DTG ↓, increase DTG to 2 x 50 mg or consider alternative therapy

Comment: (Probably) no relevant interactions with ciprofloxacin, clindamycin and streptomycin

Antidepressants

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Amitriptyline	☺	☺	☺	☺	☺	+[1]	+	+	+
Bupropion	☺	☺	☺	☺	☺	☺[1]	☺	☺	+
Citalopram	☺	☺	☺	☺	☺	☺[1]	☺	☺	+
Desipramine	☺	☺	☺	☺	☺	+[1]	+	+	+
Doxepin	☺	☺	☺	☺	☺	+[1]	+	+	+
Fluoxetine	☺	☺	☺	☺	☺	+[1]	+	+	+
St. John's w.	☺	☺	☺	☺	☺	☹	☹	☹	☹
Lithium	+	+	+	+	+	+	+	+	+
Mirtazapine	☺	☺	☺	☺	☺	☺[1]	☺	☺	+
Nortriptyline	☺	☺	☺	☺	☺	+	+	+	+
Paroxetine	☺	☺	☺	☺	☺	+[1]	+	+	+
Sertraline	☺	☺	☺	☺	☺	☺[1]	+	+	+
Trazodone	+	+	+	+	+	☺[1]	☺	☺	+
Venlafaxine	+	+	+	+	+	☺[1]	☺	☺	+

[1] CNS-effects of EFV can be increased

Comment: No data exists for most antidepressants and their interactions with NRTIs.

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Amitriptyline[1]	☹	☺	☹	☺	☺	☺	☺	☺[7]	+	☺
Bupropion	☺	☺	☺	☺[1]	☺	☺	☺	☺[7]	+	☺
Citalopram[4]	☺	☺	☺	☺	☺	☺	☺	☺[7]	+	☺
Desipramine[1]	☺	☺	☺	+	☺	☺	☺	☺[7]	+	☺
Doxepin[4]	☺	☺	☺	☺	☺	☺	☺	☺[7]	+	☺
Fluoxetin[4]	☺	☺	☺	☺	☺	☺	☺	☺[7]	+	☺
St. John's w.	☹	☹	☹	☹	☹	☹	☹	☹	+	+
Lithium	☺	+	+	+	+	+	+	+	+	+
Mirtazapine	☺	☺	☺	☺	☺	☺	☺	☺[7]	+	☺
Nortriptyline[1]	☺	☺	☺	☺	☺	☺	☺	☺[7]	+	☺
Paroxetine	☺[2]	☺[2]	☺[2]	☹[4]	☺[4]	☺[4]	☺	☺[7]	+	☺
Sertaline	☺	☺[3]	☺	☹	☺	☺[4]	☺	☺[7]	+	☺
Trazodone	☹	☹[4]	☹	☺	☹	☹	+	☺[7]	+	+
Venlafaxine[5]	☺	☺	☺	☺	☺	☺	☺	☺[7]	+	+

[1] Tricyclic antidepressants and boosted PIs: PI ↑, antidepressant ↑   [2] Paroxetine ↓−↓↓, adjust if applicable   [3] Sertraline ↓, adjust if applicable   [4] Antidepressant↑, titrate dose!   [5] Boosted PIs ↑ and venlafaxine ↑, TDM of PIs, careful titration!   [6] Tricyclic antidepressants and boosted PIs: PI ↑, antidepressants ↑   [7] STB increases drug levels of SSRI and tricyclic antidepressants; titrate carefully if combination is necessary

Antidiabetics (oral)

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Exenatide	+	+	+	+	+	+	+	+	☺
Glibenclamide	+	+	+	+	+	+	+	+	+
Glimepirid	+	+	+	+	+	☺	+	+	+
Metformin	+	+	+	+	+	+	+	+	+
Pioglitazone	+	+	+	+	+	☺	☺	☺	+
Repaglinide	+	+	+	+	+	☺[1]	☺[1]	☺[1]	+
Rosiglitazone	+	+	+	+	+	+	+	+	+
Sitagliptin	+	+	+	+	+	+	+	+	☺

[1] TDM of NNRTIs recommended

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Exenatide	+	+	+	+	+	+	+	+	+	+
Glibenclamid	+	+	+	+	+	+	+	☺	+	+
Glimepirid	+	+	+	+	+	+	+	☺	+	+
Metformin	+	+	+	+	+	+	+	☺	+	+
Pioglitazone	☺	☺	☺	☺	☺	☺	+	☺	+	+
Repaglinide	☺	☺	☺	☺	☺	+	+	☺	+	+
Rosiglitazone	☺	+	+	☺	+	+	+	+	+	+
Sitagliptin	+	☺	+	☺	☺	☺	+	☺	+	+

Antihistamines

No relevant interactions with NRTIs, MVC, DTG and RAL to be expected.
STB should **not be combined** with Astemizole and Terfenadine.
Potential interactions with other antihistamines, consider Cetirizine.

PIs/NNRTIs

	ATV	DRV	FPV	LPV	SQV	TPV	EFV	ETV	NVP	RPV
Astemizole[1]	☹	☹	☹	☹	☹	☹	☹	☹	☺	☺
Cetirizine	+	+	+	+	+	+	+[2]	+	+	+
Fexofenadine	☺	☺	☺	☺	☺	☺	☺[2]	☺	☺	+
Loratadine	☺	☺	☺	☺	☺	☺	☺	☺	+	+
Terfenadine[1]	☹	☹	☹	☹	☹	☹	☹	☹	☺	☺

[1] Cave at: Arrhythmia   [2] CNS-effects of EFV can be increased

Comment: No relevant interactions with NRTIs

Antihypertensive therapy

Calcium-channel blockers (CCB) can be increased (separate chapter), especially in combination with PIs or STB. They should be introduced carefully. In combination with NNRTIs variations in drug levels are possible. In general, alternatives should be considered.

The combination of **beta blockers** and Atazanavir can lead to QT-prolongation. There are interactions between STB and beta blockers, their levels may increase. Atenolol considered to be relatively safe.

Anticonvulsants

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Carbamazepine	☺	☺	☺	☺	☺	☺[1,2]	☹	☺[1]	☹
Gabapentine	+	+	+	+	+	+[2]	+	+	+
Lamotrigine	+	+	+	+	+	+[2]	+	+	+
Levetiracetam	+	+	+	+	+	+	+	+	☹
Oxcarbazepine	+	+	+	+	+	+	☺	☺	☹
Phenobarbital	+	☺	+	+	☺	☺	☹	☺	☹
Phenytoin	+	☺	+	+	☺	☺	☹	☺	+
Pregabaline	☺	+	☺	☺	+	+	+	+	+
Valproic acid	+	☺	+	+	☺[3]	+	+	☺	+

[1] EFV ↓, NVP ↓, avoid combination or monitor closely (TDM)   [2] CNS-effects of EFV can be increased
[3] AZT ↑↑, monitor for side effects

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Carbamazepine	☺[1]	☺[1]	☺[1]	☺[1]	☺[1]	☺[1]	☺	☹	☺[3]	☺
Gabapentine	+	+	+	+	+	+	+	+	+	+
Lamotrigine	☺	+	+	+[2]	+	+	+	+	+	+
Levetiracetam	+	+	+	+	+	+	+	+	+	+
Oxcarbazepine	☺	☺	☺	☺	☺	☺	+	☺	☺[3]	+
Phenobarbital	☺	☹	☺	☺	☺	☺	☺	☹	☺[3]	☺
Phenytoin	☺	☹	☹	☺	☺	☺	☺	☹	☺[3]	☺
Pregabalin	+	+	+	+	+	+	+	+	+	+
Valproic acid	☺	+	+	☺	+	☺	☺	☺	+	☺

[1] PIs ↓, Carbamazepine ↑, avoid if possible or monitor closely (TDM)   [2] Lamotrigine ↓, increase if necessary   [3] Avoid this combination, DTG ↓

Anthelmintic agents

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Albendazole	+	+	+	+	☺	☺	☺	☺	+
Diethylcarbamazine	+	+	+	+	+	+	+	+	+
Ivermectin	+	+	+	+	+	☺	☺	☺	+
Levamisole (Ergamisol)	+	+	+	+	+	+	+	+	+
Mebendazole	+	+	+	+	☺	☺	☺	☺	+
Niclosamide	+	+	+	+	+	+	+	+	+
Oxamniquine	+	+	+	+	+	+	+	+	+
Praziquantel	+	+	+	+	+	☺	☺	☺	+
Pyrantel	+	+	+	+	+	+	+	+	+
Suramin sodium	+	+	+	+	☺	+	+	+	+
Triclabendazole	+	+	+	+	+	☺	☺	☺	☺

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Albendazole	☺	☺	☺	☺	☺	☺	+	☺	+	+
Diethylcarbamazine	+	+	+	+	+	+	+	+	+	+
Ivermectin	☺	☺	☺	☺	☺	☺	+	☺	+	+
Levamisole (Ergamisol)	+	+	+	+	+	+	+	+	+	+
Mebendazole	☺	☺	☺	☺	☺	☺	+	☺	+	+
Niclosamide	+	+	+	+	+	+	+	+	+	+
Oxamniquine	☺	☺	☺	+	☺	☺	+	☺	+	+
Praziquantel	☺	☺	☺	☺	☺	☺	+	☺	+	+
Pyrantel	+	+	+	+	+	+	+	+	+	+
Suramin sodium	+	+	+	+	+	+	+	+	+	+
Triclabendazole	☺	☺	☺	☺	☺	☺	☺	☺	+	+

Antimycotic agents
NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Amphoter. B	☺	+	☺	☹[1]	☺[1,2]	+	+	+	+
Caspofungin	☺	☺	☺	☺	☺[1,2]	☺[8]	☺[8]	☺[8]	+
Fluconazole	+	+	+	+	☺[1,2,3]	+	+	☺[4]	☺
Flucytosin	☺	+	☺	☺	☹	+	+	+	+
Itraconazole	+	+	+	+	+	☺[6]	☺[5]	☹[4]	☺
Ketoconazole	+	+	+	+	+	☺[6]	☺[5]	☹[7]	☺
Posaconazole	+	☺	+	+	☺	☹[10]	☺[5]	☺[4]	☺
Terbinafine	☺	☺	☺	☺	☹	+	+	+	+
Voriconazole	☺	☺	☺	☺	☺	☺[6,9]	☺[5]	☺[4]	☺

[1] Caveat: additive nephrotoxicity possible   [2] Increased hematotoxicity   [3] AZT ↑↑, Fluconazole ↓
[4] NVP ↑↑, monitor liver function tests; in combination with azoles Fluconazole still preferred
[5] azoles increase ETR levels, relevance unclear   [6] NNRTIs ↑, azoles ↓   [7] NVP ↑, Ketoconazole ↓↓
[8] Caspofungin ↓, dose 70mg/d recommended.   [9] Efavirenz ↑ (reduce or TDM), Voriconazole ↓↓,
dose 400mg BID recommended.   [10] Posaconazole ↓↓

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Amphoter. B	+	+	+	+	+	+	☺	☺	+	☺
Caspofungin	☺	☺	☺	☺	+	☺	☺	+	+	☺
Fluconazole	+	+	+	+	+	☺[1]	+	☺	+	+
Flucytosine	+	+	+	+	+	+	☺	+	+	☺
Itraconazole[2]	☺	☺	☺	☺[3]	+	☺	☺[4]	☺	+	+
Ketoconazole[2]	☺	☺	+	☺	+	☺	☺[4]	☺	+	+
Posaconazole	☹[7]	☺	☺	☺	☺	☺	☺[6]	☺	+	+
Terbinafine	+	+	+	+	+	+	☺	☺	+	☺
Voriconazole[5]	☺	☹	☺	☹	☺	☺	☺[6]	☺	+	+

[1] Fluconazole ↑↑, do not excess 200 mg/d   [2] PIs ↑, Itra-/Ketoconazole ↑, avoid doses >200 mg/d
[3] LPV ↑, Itraconazole ↑; avoid doses >200 mg Itrac./d   [4] Keto-/Itraconazole: MVC 150 mg BID
[5] Voriconazole ↓ by RTV, avoid boosted PIs if possible   [6] TDM recommended, if necessary decrease.
MVC to 150 mg BID   [7] ATV-Clearance ↓, TDM!

Calcium channel antagonists (CCB)

The serum levels of CCB can be increased, especially if combined with PIs. Lercarnidipine is contraindicated in combination with boosted PIs.

In combination with NNRTIs serum levels might be fluctuating.

STB increases drug levels of CCB, too. Start CCB at low dose and titrate to full effect, monitor BP or discuss alternatives.

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Amlodipine	+	+	+	+	+	☺	☺	☺	+
Diltiazem	+	+	+	+	+	☺	☺	☺	☺
Felodipine	+	+	+	+	+	☺	☺	☺	+
Lercarnidipine	+	+	+	+	+	☺	☺	☺	+
Nifedipine	+	+	+	+	+	☺	☺	☺	+
Verapamil	+	+	+	+	+	☺	☺	☺	☺

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Amlodipine	☺	☹	☺	☺	☺	☺	☺	☺	+	☺
Diltiazem	☺	☹	☺	☺	☺	☺	☺	☺	+	☺
Felodipine	☺	☹	☺	☺	☺	☺	+	☺	+	+
Lercarnidipine	☹	☹	☹	☹	☹	☹	+	☹	+	+
Nifedipine	☺	☹	☺	☺	☺	☺	☺	☺	+	☺
Verapamil	☺	☹	☺	☺	☺	☺	☺	☺	+	☺

Immunosuppressants/Chemotherapeutic agents

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Azathioprine	+	+	+	+	☺[1]	+	+	+	+
Carboplatin	☺	+	☺	☺[2]	☺[1]	+	+	+	+
Ciclosporin	☺	☺	☺	☺[2]	☺	☺[3]	☺[3]	☺[3]	☺
Cisplatin	☺	+	☺	☺[2]	☺[1]	+	+	+	+
Cyclophosph.	☺	☺	☺	☺	☺[1]	☺	☺	☺	+
Cytarabine	+	+	+	+	☺[1]	+	+	+	+
Daunorubicin	+	+	+	+	☺[1]	+	+	+	☺
Docetaxel	+	+	+	+	+	☺	☺	☺	☺
Doxorubicine	+	+	+	+	☺[1]	☺	☺	☺	+
Etoposide	+	+	+	+	☺[1]	☺	☺	☺	+
Fluorouracil	+	☺	☺	☺	☺[1]	+	+	+	+
Gemcitabine	+	+	+	+	☺[1]	+	+	+	+
Interferon α	+	+	+	+	☺[1]	+	+	+	+
Interleukin 2	+	+	+	☺[2]	☺[1]	+	+	+	+
Irinotecan	+	+	+	+	☺[1]	☺	☺	☺	+
Methotrexate[5]	☺	☺	☺	☺[2]	☺[1]	☺	☺	☺	☺
Mycophenolat Oxaliplatin	+	+	+	+	+	+	+	+	+
Paclitaxel	☺	☺	☺	☺	☺[1]	+[4]	+[4]	+[4]	+

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Sirolimus	+	+	+	+	+	☺³	☺³	☺³	+
Tacrolimus	☺	+	☺	☺²	+	☺³	☺³	☺³	☺
Tamoxifen	+	+	+	+	+	☺	☺	☺	+
Vinblastine	☺	+	☺	☺	☺	☺	☺	☺	+
Vincristine	☺	+	☺	☺	☹	☺	☺	☺	+

[1] AZT: Hematotoxicity, avoid if possible   [2] Additive nephrotoxicity possible   [3] Immunosuppressants ↑−↓, always TDM and dose adjustments!   [4] Paclitaxel ↓   [5] Dose of MTX in rheumatology (betw. 15–25mg/wk) less nephro- and hematotoxic than hematologic dose.

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Azathioprin	+	+	+	+	+	+	+	+	+	+
Carboplatin	+	+	+	+	+	+	+	+	+	+
Ciclosporin¹	☺	☺	☹	☺	☺	☺	☺	☺	+	+
Cisplatin	+	+	+	+	+	+	+	☺	+	+
Cyclophosph.	+	+	+	+	+	+	☺	☺	+	☺
Cytarabine	+	+	+	+	+	+	+	+	+	+
Daunorubicin	☺	+	+	☺	☹⁶	☺	+	+	+	+
Docetaxel	☺	☺	☺	☺	☺	☺	☺	☺	+	+
Doxorubicine	☺³	☺³	☺³	☺³	☺³	+	☺	+	+	☺
Etoposide	☺³	☺³	☺³	☺³	☺³	☺³	☺	☺	+	☺
Fluorouracil	+	+	+	+	+	+	+	+	+	+
Gemcitabine	+	+	+	+	+	+	+	+	+	+
Interferon α	+	+	+	+	+	+	+	+	+	+
Interleukin 2	+	+	+	+	+	+	+	☺	+	+
Irinotecan	☹²	☺²	☺²	☺²	☺²	☺⁴	+	☺	+	+
Methotrexate⁶	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
Mycophenol.⁷	+	+	+	+	+	+	+	+	+	+
Oxaliplatin	+	+	+	+	+	+	+	+	☺	+
Paclitaxel	☺⁵	☺⁵	☺⁵	☺⁵	☺⁵	☺⁵	☺	☺	☺	☺
Sirolimus¹	☺	☺	☺	☺	☺	☺	+	☺	+	+
Tacrolimus¹	☺	☺	☺	☺	☺	☺	+	☺	+	+
Tamoxifen	☺	☺	☺	☺	☺	☺	☺	☺	+	+
Vinblastine	☺	☺	☺	☺	☺	☺	☺	☺	☺	
Vincristine	☺	☺	☺	☺	☺	☺	+	☺	+	+

[1] Cyclosporine, sirolimus and tacrolimus ↑-↑↑↑ in combination with PIs, always TDM, dose adjustments if necessary!   [2] Irinotecan toxicity may be increased   [3] PIs-drug levels vary in this combinations, TDM!   [4] Irinotecan ↓   [5] Paclitaxel ↓   [6] Some manufacturers state that use of methotrexate is contraindicated in HIV+ patients, if used, monitor closely.   [7] Coadministration of inducers or inhibitors of glucuronidation, e.g. some PIs could alter mycophenolate levels. TDM of MMF recommended

Contraception

The serum levels of both ethinylestradiol and norethindrone can be very fluctuation especially in combination with (boosted) PIs. Therefore the use of oral contraceptives containing these hormones might be unsafe. Furthermore their levels can be fluctuating if combined with efavirenz and nevirapine. Combination with etravirine is usually safe. For these reasons as well as for STD and HIV transmission prophylaxis oral contraceptives should always be combined with an additional method of contraception, preferably a condom.

Antimalarials/Antiprotozoals

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Artemisin	+	+	+	+	☺[1]	☺	☺	☺	☺
Atovaquone	+	+	+	+	☺[1]	☺	+	+	☺
Quinine	+	+	+	+	+	☺	☺	☺	☺
Choroquine	+	+	+	+	+	+	+	+	☺
Halofantrine	+	+	+	+	+	☺	☺	☺	☺
Lumefantrine	☺	☺	☺	☺	☺	+	+	+	☺
Mefloquine	+	+	+	+	+	+	+	+	+
Pentamidine	+	+	+	☺[2]	+	☺	+	+	☺
Primaquine	+	+	+	+	+	☺	☺	☺	☺
Proguanil	+	+	+	+	+	+	+	+	+
Pyrimethamin	☺	+	☺	☺	☺[3]	+	+	+	+

[1] AZT ↑, monitor for toxicity   [2] Caveat: Nephrotoxicity   [3] Caveat: Hematotoxicity

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Artemisin	☺	☺	☺	+	☺	☺	☺	☺	+	+
Atovaquone	☺	+	+	☺	+	☺	+	+	+	+
Quinine	☺	☺	☺	☺	☺	☺	☺	☺	+	+
Chloroquine	+	+	+	+	+	+	+	+	+	+
Halofantrine	☹	☹	☹	☹	☹	☹	+	☺	+	+
Lumefantrine	☺	☺	☺	☺	☹	☹	+	☺	+	+
Mefloquine	+	+	+	+	+	+	+	☺	+	+
Pentamidine	+	+	+	+	+	+	+	☺	+	+
Primaquine	+	+	+	+	+	+	+	+	+	+
Proguanil	+	+	+	+	+	+	+	+	+	+
Pyrimethamine	+	+	+	+	+	+	+	+	+	+

Phosphodiesterase type 5 inhibitors

Combinations of most PDE5 inhibitors (e.g. sildenafil, tadalafil and vardenafil) with PIs can cause severe increase in PDE5 serum levels. Thus they should be started carefully with a reduced (usually half) dose every 48 to 72 hours. In combination with NNRTIs the levels of PDE5 inhibitors are fluctuating strongly, TDM and individual dosing are recommended (etravirine and sildenafil can be combined, sometimes sildenafil needs to be increased). There are no known relevant interactions of PDE5 inhibitors with NRTIs, T-20, maraviroc and raltegravir. If PDE5 inhibitors are prescribed for pulmonary arterial hypertension alternatives such as endothelin receptors inhibitors should be evaluated.

Due to an FDA warning sildenafil is contraindicated for treatment of PAH in combination with a PI. Tadalafil and bosentan need to be adjusted if prescribed as treatment for PAH in combination with a PI. Coadministration of bosentan and atazanavir (without ritonavir booster) is not recommended.

Statins/Lipid lowering drugs

The combination of NRTIs, entry inhibitors and integrase inhibitors with statins is generally possible, but the combination with PIs can cause problems.

PIs/NNRTIs/STB

	ATV	DRV	FPV	LPV	SQV	TPV	EFV	ETV	NVP	RPV
Atorvastatin	☺[1]	☺[1]	☺[1]	☺[1]	☺[1]	☹[1]	☺[2]	☺[2]	☺[2]	+
Clofibrat	+	+	+	+	+	+	+	+	+	+
Ezetimibe	☺	+	+	+	+	+	+	+	+	+
Fenofibrate	+	+	+	+	+	+	+	+	+	+
Fish oils	+	+	+	+	+	+	+	+	+	+
Fluvastatin	+	+	+	+	+	+	+	☺	+	+
Gemfibrozil	+	+	+	☺	+	+	+	+	+	+
Lovastatin[3]	☹	☹	☹	☹	☹	☹	☺	☺	☺	+
Pravastatin	+	☹[3]	+	+	☺	+	☺	☺	+	+
Rosuvastatin	☺	☺	☺	☺	☺	☺	+	☺	+	+
Simvastatin	☹[3]	☹[3]	☹[3]	☹[3]	☹[3]	☹[3]	☺	☺	☺	☺

[1] Atorvastatin ↑ if combined with PIs, low dosing! Consider alternatives: e.g. Pravastatin
[2] Atorvastatin ↓, increase dose if applicable or chose alternatives: e.g. Fluvastatin / Pravastatin
[3] Statin levels severely increased, avoid these combinations!

Comment: All statins should be started low-dose if combined with PIs!

Anti-addictive drugs

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Buprenorphine	+	+	+	+	+	☺[1]	☺	☺	+
Naloxone	+	+	+	+	+	+	+	+	☺
Methadone	+	☺[2]	+	+	☺[3]	☺[2]	+	☺[2]	+

[1] Buprenorphine ↓, increase dose if necessary   [2] Methadone ↓, increase dose if necessary
[3] AZT ↑, relevance unclear

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Buprenorphine	☺[1]	+[3]	☺	☺	☺	☺	+	+	+	+
Naloxone	+	+	+	+	+	+	+	+	+	+
Methadone	☺[2]	☺[2]	☺[2]	☺[2]	+[2]	+[2]	+	+	+	+

[1] Buprenorphine ↑-↑↑, reduce dose if necessary   [2] Methadone (↓), adjust dose if necessary
[3] Buprenorphine ↓, adjust dose if necessary

Antiviral drugs

There are no known relevant interactions between PIs/NNRTIs and antiviral drugs. Little data exists on interactions with CCR5 inhibitors or integrase inhibitors.

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Aciclovir	+	+	+	☺[1]	+[2]	+	+	+	+
Adefovir	+	+	☺	☹[1]	+	+	+	+	+
Cidofovir	☺	+	☺	☺[1]	☺	+	+	+	+
Daclatasvir	+	+	+	+	+	😐	😐	😐	+
Entecavir[5]	+	+	+	+	+	+	+	+	+
Famciclovir	+	+	+	☺	+	+	+	+	+
Foscarnet	☺	+	☺	☺[1]	☺[2]	+	+	+	+
Ganciclovir	☹	😐	☹	☺[1]	☺[2]	+	+	+	+
Ledipasvir/ Sofosbuvir	+	+	+	☺	+	☺	+	+	+
Oseltamivir	+	+	+	+	+	+	+	+	+
Ribavirin	☺	☺[4]	+	+	☹[2]	+	+	+	+
Simeprevir	+	+	+	+	+	☹[3]	☹[3]	☹[3]	+
Sofosbuvir	+	+	+	+	+	+	+	+	+
Valaciclovir	+	+	+	☺[1]	+	+	+	+	+
Valganciclovir	☺	☺	☺	☺	☺[2]	+	+	+	+
Zanamivir	+	+	+	+	+	+	+	+	+

[1] Caveat: Nephrotoxicity, increased levels through tubular secretion   [2] Hematotoxicity increased
[3] Simeprevir decreased in these combinations, choose alternative regimen   [4] Possible antagonism (controversial)   [5] Caveat: Possible resistance (M184V), sparse data on combination with HIV-NRTIs

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Aciclovir	+	+	+	+	+	+	+	😐	+	+
Adefovir	+	+	+	+	+	+	+	☹	+	+
Cidofovir	+	+	+	+	+	+	+	😐	+	+
Daclatasvir	😐	+	😐	+	😐	😐	+	😐	+	+
Entecavir[5]	+	+	+	+	+	+	+	+	+	+
Famciclovir	+	+	+	+	+	+	+	+	+	+
Foscarnet	+	+	+	+	+	+	+	😐	+	+
Ganciclovir	+	+	+	+	+	+	+	😐	+	+
Ledipasvir/ Sofosbuvir	+	+	+	+	+	☹	+	😐	+	+
Oseltamivir	+	+	+	+	+	+	+	+	+	+
Ribavirin	😐[1]	+	+	+	+	+	+	+	+	+
Simeprevir	😐[2]	😐[2]	😐[2]	😐[2]	😐[2]	😐[2]	+	😐[2]	+	+
Sofosbuvir	+	+	+	+	+	☹		+	+	+
Valacyclovir	+	+	+	+	+	+	+	😐	+	😐
Valgancyclovir	+	+	+	+	+	+	😐	😐	+	😐
Zanamivir	+	+	+	+	+	+	+	+	+	+

[1] Increased hyperbilirubinemia / jaundice cobicistat, avoid these combinations   [2] Altered simeprevir concentration with boosted PI/r or

Others

In the following additional drugs are listed in alphabetical order, which are of interest for HIV clinicians. This group does not represent single categories of drugs.

NRTIs/NNRTIs

	3TC	ABC	FTC	TDF	AZT	EFV	ETV	NVP	RPV
Alendronic acid	☺	☺	☺	☺	☺	☺	☺	☺	☺
Allopurinole	+	+	+	+	+	+	+	+	+
Ambrisentan	+	+	+	+	+	☺	☺	☺	☺
Bosentan	+	+	+	+	+[1]	☺	☺	☺[2]	☺
Budesonide	+	+	+	+	+	+	+	+	+
Cholecalciferol	+	+	+	+	+	+	+	+	+
Clopidogrel	+	+	+	+	+	☺	☺	☺	+
Dabigatran	+	+	+	+	+	☺	☺	☺	+
Dexamethason	+	+	+	+	+	+	☺	☺	☺[4]
Ibandronaic acid	+	+	+	+	+	+	+	+	☺
Iloprost	+	+	+	+	+	+	+	+	☺
Pamidronic acid	+	+	+	+	+	+	+	+	☺
Raloxifene	+	+	+	+	+	+	+	+	☺
Prasugrel	+	+	+	+	+	+	+	+	+
Phenprocoumon	☺	☺	☺	☺	☺	☺	☺[3]	☺	+
Prednisone	+	+	+	+	+	☺	☺	☺	+
Rivaroxaban	+	+	+	+	+	☺	☺	☺	+
Sitaxsentan	+	+	+	+	+	+	+	+	☺
Strontium	+	+	+	+	+	+	+	+	☺
Theophylline	☺	☺	☺	☺	☺	+	+	+	☺
Ticagrelor	+	+	+	+	+	☺	☺	☺	+
Torasemide	+	+	+	+	+	☺	☺	☺	+
Warfarin	+	+	+	+	+	☺	☺	☺	+

[1] Caveat: Hematotoxicity   [2] Caveat: Hepatotoxicity   [3] Phenprocoumon can be ↑   [4] RPV decreased

PIs/EIs/INSTIs

	ATV	DRV	FPV	LPV	SQV	TPV	MVC	STB	DTG	RAL
Alendronat	+	+	+	+	+	+	+	☺	☺	+
Allopurinol	+	+	+	+	+	+	+	+	+	+
Ambrisentan	+	+	+	+	+	+	+	+	+	+
Bosentan[1]	☺[2]	☺	☺	☺	☺	☺	☺	☺	+	+
Budesonide	☺	☺	☺	☺	☺	☺	+	☺	+	+
Cholecalciferol	+	+	+	+	+	+	+	+	+	+
Clopidogrel	☺	☺	☺	☺	☺	☺	+	☺	+	+
Dagibatran	☺	☺	☺	☺	☺	☺	+	☺	+	+
Dexamethasone	☺	☺	☺	☺	☺	☺	+	☺	+	+
Ibandronat	+	+	+	+	+	+	+	+	+	+
Iloprost	+	+	+	+	+	+	+	+	+	+
Pamidronat	+	+	+	+	+	+	+	+	+	+
Phenprocoumon	☺	☺	☺	☺	☺	☺	☺	☺	+	☺
Prasugrel	+	+	+	+	+	+	+	+	+	+
Prednisone	☺	☺	☺	☺	☺	☺	+	☺	+	+
Raloxifen	+	+	+	+	+	+	+	+	+	+
Rivaroxaban[3]	☺	☺	☺	☺	☺	☺	+	☺	+	+
Sitaxentan	☺	☺	+	+	☺	+	☺	+	+	+
Strontium	+	+	+	+	+	+	+	+	+	+
Theophylline	☺	☺	+	☺	+	☺	+	+	+	+
Ticagrelor[4]	☺	☺	☺	☺	☺	☺	+	☺	+	+
Torasemide	☺	☺	☺	☺	☺	☺	☺	☺	+	+
Warfarin	☺	☺	☺	☺	☺	☺	+	☺	+	+

[1] Because of an FDA warning bosentan needs to be dose adjusted if combined with a PI
[2] Bosentan is contraindicated in combination with unboosted ATV [3] Combination with PI, or cobicistat increases rivaroxaban substanvcially.   [4] Combination with PI or cobicistat increases ticagrelor substantially

References

Bartlett JG; Pocket Guide Adult HIV/AIDS Treatment 2008-09. www.hopkins-hivguide.org

Drug information: Aptivus®, Atripla®, Celsentri®, Combivir®, Emtriva®, Edurant®, Epivir®, Eplivera® Fuzeon®, Intelence®, Invirase®, Isentress®, Kaletra®, Kivexa®, Prezista®, Retrovir®, Reyataz®, Stribilt®, Sustiva®, Telzir®, Tivicay®, Truvada®, Triumeq®, Tybost®, Viramune®, Viread®, Ziagen®.

Pocket guide to pharmacokinetic interaction profiles of ritonavir boosted PIs; October 2008, Boehringer Ingelheim

Pocket guide to pharmacokinetic interaction profiles of ritonavir boosted PIs; October 2008, Boehringer Ingelheim

https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv-guidelines/26/hiv-hcv

www.clinicaloptions.com/inPractice/HIV/Antiretroviral%20Therapy/ch19_pt2_Drug-Drug_Interactions.aspx

www.dosing-gmbh.de Wechselwirkungs-Check (Dosing-GmbH)

www.fda.gov (FDA 04/2010)

www.hiv-druginteractions.org

www.hivinsite.org

www.ifi-interaktions-hotline.de

www.uptodate.com (Lexi-Comp Online™ Interaction Analysis)

33. Drug Profiles

CHRISTIAN HOFFMANN

3TC (lamivudine)

Manufacturer: ViiV Healthcare. Several generics available!

Indications and trade names: HIV infection, for both naïve and pretreated patients. The lower dosage of 3TC which is approved for Hepatitis B (Zeffix®) is not recommended. 3TC is a component of the following:

- Epivir® tablets, 150 mg or 300 mg 3TC.
- Epivir® oral solution, 10 mg per ml 3TC.
- Fixed-dose combinations:
- Combivir® film-coated tablets, 150 mg 3TC + 300 mg AZT
- Dutrebis® film-coated tablets, 150 mg 3TC + 300 mg raltegravir
- Kivexa® (USA: Epzicom®) film-coated tablets, 300 mg 3TC+ 600 mg ABC
- Trizivir® film-coated tablets, 150 mg 3TC + 300 mg AZT+ 300 mg ABC
- Triumeq® film-coated tablets, 300 mg 3TC + 600 mg ABC+ 50 mg dolutegravir

Dosage: 300 mg QD or 150 mg BID. Children receive 4 mg/kg with a maximum of 150 mg BID. Dose adjustment is required with reduced creatinine clearance. Below 30 kg, only the oral solution should be used.

Creat. Clearance (ml/min)	Initial Dose	Maintenance dosage
30–49	150 mg (15 ml)	150 mg (15 ml) QD
15–29	150 mg (15 ml)	100 mg (10 ml) QD
5–14	150 mg (15 ml)	50 mg (5 ml) QD
<5	50 mg (5 ml)	25 mg (2.5 ml) QD

Side effects: mild and uncommon. Fatigue, nausea, vomiting, diarrhea, headache, insomnia and myalgia are usually due to the other drugs in the combination (see AZT and ABC). Polyneuropathy, pancreatitis, anemia and lactic acidosis are rare.

Comments: well-tolerated, often-prescribed NRTI, available in different dosages and fixed-dose combinations. Resistance to 3TC develops quickly but impairs viral fitness. 3TC is also effective against hepatitis B (caution: development of HIV resistance can be quite fast when used as HBV monotherapy).

For detailed information see page: 74

Abacavir (ABC)

Manufacturer: ViiV Healthcare.

Indication and trade names: HIV infection, as component of a combination ART for both naïve and pretreated patients. Abacavir is a component of the following:

- Ziagen® film-coated tablets, 300 mg ABC. Oral solution, 20 mg per ml
- Kivexa® (US: Epzicom®) film-coated tablets, 600 mg ABC+300 mg 3TC
- Trizivir® film-coated tablets, 300 mg ABC+150 mg 3TC+300 mg AZT
- Triumeq® film-coated tablets, 600 mg ABC+300 mg 3TC+50 mg dolutegravir

Dosage: 300 mg BID or 600 mg QD, with or without food. Although mainly metabolized by the liver, abacavir should be avoided in patients with severe renal insufficiency (GFR <20 ml).

Side effects: abacavir causes a hypersensitivity syndrome (HSR) in about 2 to 8% of patients. HSR usually occurs within the first six weeks. Pruritus and rash are common, but may also be absent. HSR may present as just fever and slowly developing malaise. Gastrointestinal complaints (nausea, diarrhea, abdominal pain) and fatigue are also possible, but not necessarily linked to the HSR. Elevated liver function tests, insomnia, dizziness, breathlessness, sore throat or cough are rare. Before starting abacavir, a test for the HLA-B*5701 allele is strongly recommended. HLA testing reduces the HSR risk considerably, but not completely. Rechallenge after suspected HSR may be fatal and is contraindicated. A slighty elevated risk of myocardial infarction has been reported. The mechanism for this is not clear.

Comments: abacavir is an NRTI (guanosine analog) with good CNS penetration. Mainly used in FDCs. Abacavir is usually well-tolerated and has little mitochondrial toxicity. The main problem is HSR which can be avoided by prior HLA testing.

For detailed information see page: 72

Acyclovir

Manufacturer and trade names: diverse manufacturers, several trade names such as Aciclobeta®, Aciclostad®, Acyclovir®, Zovirax®.

Indications: herpes zoster, as well as prophylaxis of serious herpes simplex infections in immunosuppressed adults.

Dosage: For **herpes zoster** 800 mg orally five times a day for one week. In cases of disseminated or complicated herpes zoster, 10 mg/kg IV TID. Reduce dosage in patients with renal insufficiency at a creatinine clearance of 25–10 ml/min, 800 mg TID, and at <10 ml/min, 800 mg BID. For **genital HSV infection**, 400 mg five times a day. In severe cases (ulcerating genital herpes) intravenous treatment with 5–10 mg/kg IV TID. For HSV encephalitis or HSV esophagitis 10 mg/kg IV TID. Initiation of treatment for HSV infections should be within the first 24 hours after appearance of symptoms (HZV infection within the first 4 days).

Side effects: uncommon. Headache, nausea and elevation of creatinine may occur. Phlebitis can occur with intravenous dosing.

Comments: approved and well tolerated HZV/HSV medicine. Generics are significantly cheaper than the originally introduced formulation, Zovirax®. Newer studies reported on a moderate but significant effect on HIV replication.

For detailed information see page: 264

Agenerase®, see Amprenavir.

Amphotericin B

Manufacturer: Bristol-Myers Squibb (Amphotericin B®), Gilead (Ambisome®), Dermapharm (Ampho-Moronal®).

Indications and trade names: amphotericin B® is indicated for organ mycoses and generalized mycoses, primarily candidiasis, aspergillosis, cryptococcosis and histoplasmosis. AmBisome® (expensive!) is indicated only if conventional Amphotericin B® is contraindicated due to kidney dysfunction or intolerance. The indication also

applies to visceral leishmaniasis. Suspension and tablets are only licensed for oral candidiasis. Amphotericin is a component of the following:

• Amphotericin B® injection vial, 50 mg powder
• AmBisome® injection vial, 50 mg dry agent
• Ampho-Moronal® suspension, 100 mg/ml
• Ampho-Moronal® lozenges, 10 mg

Dosage: when using Amphotericin B®, always apply test dose first (see below). For aspergillosis 1.0–1.5 mg/kg per day, for other mycoses 0.5–1 mg/kg usually suffices. Maximum dose is 1.5 mg/kg. In case of overdosage, respiratory and cardiac arrest can occur. Dose of Ambisome®: initial 1 mg/kg QD, if necessary may be gradually increased to 3 mg/kg.

Side effects: nephrotoxicity, hypokalemia and gastrointestinal complaints. Frequent: fever, chills, and hypotension approximately 10–20 min after starting infusions. Thrombophlebitis (non-liposomal amphotericin B only via a central venous line). Side effects are generally less severe with Ambisome®.

Comments: daily monitoring of electrolytes, creatinine, BUN, ALT, blood count. A central venous line is always necessary due to hypokalemia and the usually required potassium substitution. Sodium should be kept at normal levels. Do not combine with other nephrotoxic drugs.

Always prehydrate with 1000 ml 0.9% NaCl. Always test first dose with 5 mg in 250 ml 5% glucose over 30–60 min with strict monitoring of blood pressure and pulse for the first hour. If the test dose is tolerated, then half of the planned maintenance dose may subsequently be given on the same day. In cases of fever or chills (can be very impressive), the following may be repeated after 30 min: 50 mg pethidine (e.g., Dolantin®) IV plus 1 ampule clemastine (e.g., Tavegil®), steroids only if complaints persist (prednisolone 1 mg/kg).

If side effects are severe, then switch to Ambisome®, which is probably not more effective than conventional amphotericin B but significantly better tolerated and less nephrotoxic (no test dose, no prehydration, no central line necessary). Never mix amphotericin infusions, and always protect from light. Infuse slowly. The longer the infusion time (>3 hours), the better the tolerability. Always use 5% glucose as a diluting agent.

Amprenavir (Agenerase®), replaced by fosamprenavir in 2008.

Atazanavir

Manufacturer: Bristol-Myers Squibb.

Indications and trade names: HIV infection, as part of a combination, adults and children >6 years of age, for both pretreated and ART-naïve patients. Atazanavir is a component of the following:

• Reyataz® capsules, 150 mg, 200 mg, 300 mg
• Reyataz® oral powder for oral suspension, 50 mg packet
• Evotaz® film-coated tablets, 300 mg plus 150 mg cobicistat

Dosage: 300 mg atazanavir QD combined with 100 mg ritonavir (instead of ritonavir, cobicistat may also be used as a booster). If ritonavir is not tolerated, atazanavir can be given 400 mg QD, without booster (combination with tenofovir should then be avoided). If atazanavir is combined with efavirenz (even if boosted), increase

dosage to 400 mg. The capsules should be swallowed (not chewed) and taken with a meal. Recommended dosage of atazanavir/r in pediatric patients as follows: Children less than 15 kg: not recommended; 15–20 kg: 150/100 mg; 20–40 kg: 200/100 mg; at least 40 kg: 300/100 mg.

Side effects: very often hyperbilirubinemia (up to 50%), also with jaundice; rarer elevated transaminases. Diarrhea, nausea, vomiting, headache, insomnia and abdominal pain are also relatively rare. In contrast to other PIs, there is less dyslipidemia. The effect on lipodystrophy remains unknown. Rarely nephrolithiasis.

Interactions, warnings: do not combine with indinavir. Caution with impaired liver function. Atazanavir is contraindicated in patients with Child-Pugh B and C. Combinations with the following pharmaceuticals are contraindicated: cisapride, midazolam, triazolam, simvastatin, lovastatin, ergotamines, calcium antagonists. Life-threatening interactions may occur with concomitant administration of amiodarone, lidocaine (systemic dosing), tricyclic anti-depressants and quinidine (measure plasma levels). Do not combine boosted atazanavir with clarithromycin. It should not be given with rifampin. Reduce the rifabutin dose by 75% (instead of 300 mg daily, give only 150 mg every other day or three times per week). Be careful with proton pump inhibitors (PPI) and antacids!

Comments: PI with a favorable lipid profile. The most important side effect is hyperbilirubinemia, which often presents as jaundice. There are some relevant interactions – primarily with proton pump inhibitors and antacids, but also with tenofovir, efavirenz, nevirapine and ddI.

For detailed information see page: 92

Atovaquone

Manufacturer: GlaxoSmithKline.

Indications and trade names: mild or moderate PCP in cases of hypersensitivity to cotrimoxazole; in combination with proguanil for the treatment and prophylaxis of malaria. Off-label, can be used as PCP prophylaxis (as reserve) and as acute treatment of cerebral toxoplasmosis.

Atovaquone is a component of the following:
• Wellvone® suspension, 750 mg atovaquone/5 ml
• Malarone® film-coated tablets, 250 mg atovaquone and 100 mg proguanil

Dosage: as therapy for acute PCP (or toxoplasmosis): 750–1500 mg BID (i.e., 1–2 measuring spoons of 5 ml BID) for 21 days. For prophylaxis 750 mg BID (i.e., 1 measuring spoon of 5 ml BID) or 1500 mg QD.

Side effects: nausea, vomiting and diarrhea are frequent (but often mild), as are rashes, which occur in approximately 20% of patients. Less common are headache and insomnia. Elevated liver enzymes, amylase. Anemia, leukopenia (rare).

Interactions, warnings: atovaquone should be taken with meals, ideally with fatty dishes, as this improves absorption. Rifampin and possibly also rifabutin lower plasma levels of atovaquone by 50%. Fluconazole probably increases levels.

Comments: nowadays, only rarely used. Atovaquone is considerably more expensive than other drugs for PCP prophylaxis.

Atripla®

Manufacturer: co-manufactured by Gilead Sciences, Bristol-Myers Squibb and MSD.

Indications and trade name: adult HIV-infected patients. It should be noted that in Europe, approval for Atripla® is more strict than in the US. The EMA has only approved the use of Atripla® in patients who have already achieved virologic suppression to below 50 copies/ml on their current ART for at least three months. Furthermore, patients must not have experienced virologic failure with an earlier treatment combination or be known to have resistance to any of the drugs in Atripla®.

• Atripla® film-coated tablets with 600 mg EFV, 200 mg FTC, 300 mg TDF

Dosage: one tablet daily in the evening, unchewed, on an empty stomach.

Comments: the first complete ART in one single tablet per day. In Europe, the above-mentioned limitation of the indication applies. For side effects, see sections on tenofovir (caution with renal function), efavirenz (CNS side effects) and FTC.

For detailed information see page: 189

Azithromycin

Manufacturer and trade names: diverse, therefore several trade names, such as Azithromycin®, Zithromax®, Ultreon®.

Indications: treatment and prophylaxis of MAC infection. Infections of the respiratory tract, otitis media. Uncomplicated gonorrhea, genital infections with *Chlamydia trachomatis*, chancroid. Azithromycin is a component of the following:

• Ultreon® film-coated tablets, 600 mg
• Zithromax® film-coated tablets, 250 mg and 500 mg
• Zithromax®, dry suspension, 200 mg per 5 ml

Dosage: primary prophylaxis of disseminated MAC infection: 1200 mg azithromycin once weekly (2 tablets Ultreon® 600 mg per week). MAC treatment: 1 tablet Ultreon® 600 mg QD, only in combination with ethambutol and rifabutin. Infections of the respiratory tract: 500 mg QD for 3 days. Uncomplicated gonorrhea, uncomplicated genital infections with chlamydia (not LGV!): 1000 mg azithromycin may be given as a single dose.

Side effects: gastrointestinal with stomach cramps, nausea, vomiting, and diarrhea. Rarely, elevated transaminases, cholestatic jaundice. Reversible ototoxicity with high doses. Rarely, taste disturbances, discoloration of the tongue. Allergies.

Comments: this macrolide antibiotic has a long half-life and good tissue penetration. In some genital infections, a single dose is sufficient. For respiratory tract infections, azithromycin should be given for 3-5 days. In HIV infection, azithromycin has been often used as prophylaxis or treatment of MAC infections.

AZT (zidovudine)

Manufacturer: ViiV Healthcare. Generics available!

Indications and trade names: HIV infection, as component in a combination ART for both naïve or pretreated patients. Prevention of maternal-fetal HIV transmission. AZT is a component of the following:

• Retrovir® hard capsules, 100 mg AZT and 250 mg AZT
• Retrovir® film-coated tablets, 300 mg AZT
• Retrovir® oral solution, 100 mg AZT per 10 ml
• Retrovir® concentrate, 10 mg AZT per ml (5 injection vials 200 mg each)
• Combivir® film-coated tablets, 300 mg AZT+300 mg 3TC
• Trizivir® film-coated tablets, 300 mg AZT+150 mg 3TC+300 mg abacavir

Dosage: 250 mg BID (in Combivir® and Trizivir® 300 mg BID). In patients with serious renal impairment (creatinine clearance below 20 ml/min, hemodialysis) 300 mg daily. With severe hepatic impairment 100 mg TID.

Side effects: nausea, vomiting, abdominal discomfort, headache, myalgia, and dizziness. Macrocytic anemia (MCV almost always elevated), rarely neutropenia. Elevations in LDH, CPK and transaminases may occur. Episodes of lactic acidosis are rare.

Interactions, warnings: do not combine with d4T. There is increased myelotoxicity if used with other myelosuppressive drugs. Ribavirin antagonizes the antiviral activity of AZT *in vitro* (combination should be avoided).
Initially monthly monitoring of blood count, transaminases, CPK and bilirubin. Gastrointestinal complaints can be treated symptomatically and usually subside after a few weeks. Anemia can develop even after months.
AZT should always be a component of transmission prophylaxis.

Comments: the first NRTI (thymidine analog) on the market and the oldest HIV drug of all (registered in 1987). Still part of some ART therapies. However, due to numerous toxicities (myelotoxicity, mitochondrial toxicity) AZT is prescribed considerably less frequent than previously. Comprehensive data, good penetration of the blood-brain barrier. Generics abound.

For detailed information see page: 73

Boceprevir

Manufacturer: MSD.

Indications and trade name: Chronic hepatitis C, genotype 1, plus PEG+RIBA.

• Victrelis® hard capsules, 200 mg.

Dosage: 800 mg administered orally TID (four capsules every 7-9 hours) with food (a meal or light snack).

Side effects: Nausea, fatigue, headache, dysgeusia (specific!).

Interactions, warnings: Boceprevir is a strong CYP3A inhibitor, and numerous interactions must be considered prior to and during therapy.

Comments: In 2011 the first HCV PI on the market, boceprevir is no longer used due to the introduction of second-generation DAAs. MSD has announced that they will stop the manufacture and distribution by December 2015.

Caelyx®, see Doxorubicin, liposomal.

Cidofovir

Manufacturer: Gilead Sciences.

Indications and trade name: CMV retinitis in patients without renal dysfunction, mainly in cases of resistance/contraindications to gancyclovir or foscavir. Some experts use cidofovir for PML (off-label use), although efficacy is uncertain.

• Vistide® injection vial, 375 mg per 5 ml (= 75 mg/ml)

Dosage: induction dose 5 mg/kg IV weekly, by day 21 maintenance therapy with 5 mg/kg IV every two weeks. A precise treatment plan (see below) is necessary.

Side effects: renal failure, which can occur even after 1 dose of cidofovir. Less frequent: neutropenia, dyspnea, alopecia, decreased intraocular pressure, iritis, uveitis. Fever, chills, headache, rash, nausea and vomiting are usually caused by probenecid and should subside within 12 hours. Complaints may be lessened with food intake, antipyretics, or antiemetics.

Warnings: renal function (serum creatinine, electrolytes, proteinuria) should be checked before each dose of cidofovir. If serum creatinine increases by more than 0.3 mg/dl, reduce dose to 3 mg/kg. If serum creatinine increases by more than 0.5 mg/dl above levels prior to treatment, discontinue cidofovir. Cidofovir is always contraindicated at serum creatinine levels >1.5 mg/dl or creatinine clearance below 55 ml/min or proteinuria >100 mg/dl. Always ensure sufficient hydration. Cidofovir should be given according to the following scheme:

−3 h	2 g probenecid (4 tablets of 500 mg), prior to that 20-30 drops metamizole plus 50 mg prednisolone
−3 to −1 h	1000-2000 ml 0.9% NaCl
0 to + 2 h	Cidofovir in 500 ml 0.9% NaCl over 1-2 h. Concurrently 1000 ml 0.9% NaCl.
+4 h	1 g probenecid (2 tablets of 500 mg), prior 20 drops metamizole
+10 h	1 g probenecid (2 tablets of 500 mg), prior 20 drops metamizole

Potentially, nephrotoxic drugs such as aminoglycosides, amphotericin B, foscarnet, IV pentamidine or vancomycin must be avoided or discontinued at least 7 days prior to treatment with cidofovir. Probenecid is necessary to reduce nephrotoxicity. Probenecid has drug interactions with acetaminophen, acyclovir, angiotensin converting enzyme inhibitors, aminosalicylic acid, barbiturates, benzodiazepines, bumetanide, clofibrate, methotrexate, famotidine, furosemide and theophylline.

Comments: Reserve drug in severe CMV infections. Rarely used due to considerable nephrotoxicity. The effect in PML is more than questionable.

Clarithromycin

Manufacturer and trade names: diverse manufacturers, therefore several trade names, such as Clarithromycin-CT®, Klacid®, Mavid®

Indications: prophylaxis and treatment of MAC disease. Infections of the respiratory tract and the skin. Clarithromycin is a component of the following (selection):

• Mavid® film-coated tablets, 500 mg
• Klacid® film-coated tablets, 250 mg

Dosage: with MAC 500 mg BID, both for primary prophylaxis and for maintenance therapy. 50% dose reduction if creatinine clearance is below 30 ml/min. For respiratory tract infections 250 mg BID will suffice.

Side effects: mainly gastrointestinal complaints such as nausea, vomiting, abdominal discomfort and diarrhea; in addition, allergic skin reactions, headache, elevated transaminases, alkaline phosphates and bilirubin.

Interactions, warnings: no concurrent treatment with rifampin, carbamazepine, cisapride, terfenadine, pimozide and other macrolide antibiotics such as erythromycin or azithromycin. Lopinavir and ritonavir increase clarithromycin levels. If administered concurrently, oral treatments with clarithromycin and AZT should be taken 1–2 hours apart.

Comments: macrolide antibiotic with a shorter half-life than azithromycin. The daily dose should not exceed 500 mg BID.

Clindamycin

Manufacturer and trade names: diverse manufacturers, therefore several trade names, such as Aclinda®, Clindabeta®, Clindamycin-ratiopharm®, Sobelin®.

Indications: for HIV-infected patients, mainly cerebral toxoplasmosis. Also for serious infections by anaerobes, staphylococci (because of good tissue and bone penetration also used in dentistry).

Dosage: 600 mg IV QID or 600 mg oral QID (always with pyrimethamine and leucovorin). Half dose for (oral) maintenance therapy. In renal failure, reduce dose to a quarter or a third of the normal dose.

Side effects: diarrhea in 10-30% of patients. Allergies are also frequent and often require discontinuation. In cases of infection with *Clostridium difficile* "Pseudomembranous colitis", the clinical spectrum ranges from mild watery stool to severe diarrhea with blood and mucous, leukocytosis, fever and severe abdominal cramps which may progress to peritonitis, shock and toxic megacolon.

Warnings: clindamycin is contraindicated in inflammatory bowel disease and antibiotic-induced colitis. Caution with reduced hepatic or renal function and in asthma. No concurrent administration of antiperistaltics. With occurrence of diarrhea on clindamycin, discontinue and give metronidazole (or vancomycin).

Comments: still used in patients with cerebral toxoplasmosis. Several side effects, caution with colitis.

Cobicistat

Manufacturer: Gilead Sciences.

Indications and trade names: HIV infection, as a pharmacoenhancing drug in combination with elvitegravir, atazanavir and darunavir.

• Tybost® tablets, 150 mg
• Evotaz® tablets, 150 mg plus 300 mg atazanavir
• Rezolsta® (US: Prezcobix®) tablets, 150 mg plus 800 mg darunavir
• Stribild® tablets, 150 mg plus 150 mg elvitegravir+200 mg FTC+300 mg TDF

Dosage: 150 mg QD. No dose adjustment in patients with renal insufficiency. However, combination with tenofovir is not recommended in patients who have an estimated creatinine clearance below 70 mL/min.

Side effects: increase of serum creatinine (often less than 0.4 mg/dl) by inhibiting its renal active tubular secretion without affecting the glomerular filtration rate. Nausea. In combination with atazanavir, the risk for hyperbilirubinemia seems to be higher than with ritonavir.

Interactions, warnings: cobicistat is a potent inhibitor of CYP3A which acts as a boosting agent. Thus, many interactions have to be considered. Among many others, carbamazepine, sildenafil, rifampicin, ergotamines, lovastatin and simvastatin are contraindicated. Do not combine with efavirenz, nevirapine, etravirine and PIs other than atazanavir or darunavir.

Comments: cobicistat is approved as a boosting agent for elvitegravir, atazanavir and darunavir. It has no antiviral activity. Many interactions have to be considered.

For detailed information see page: 91, 102

Combivir®

Manufacturers: ViiV Healthcare. Several generics available.

Indications and trade name: HIV infection, as a component in combined therapy for ART naïve or pretreated patients.

• Combivir® film-coated tablets, 300 mg AZT+150 mg 3TC

Dosage: 1 tablet BID. In cases of reduced renal function (creatinine clearance below 50 ml/min) and anemia, Combivir® should be replaced with the individual drugs to allow for adjustment of 3TC and AZT doses. For side effects, see AZT and 3TC.

Comments: the first fixed-dose combination in HIV medicine (1998). For a long time one of the most used drugs. While it is prescribed less, it remains an alternative in certain circumstances. See AZT for side effects.

For detailed information see page: 77

Complera® (Europe: Eviplera®)

Manufacturer: Gilead Sciences and Janssen-Cilag.

Indications and trade name: adult treatment-naïve patients with HIV RNA less than or equal to 100,000 copies/mL, and in virologically suppressed adult patients on a stable antiretroviral regimen in order to replace their current regimen.

• Complera®/Eviplera® film-c. tablets with 25 mg RPV, 200 mg FTC, 300 mg TDF

Dosage: 1 tablet QD. Must be taken with a meal including some fat. Nutritional drinks are not enough for proper absorption. In cases of reduced renal function (creatinine clearance below 50 mL/min), Complera® should be avoided.

Side effects: usually well tolerated. Rash, mostly mild. For side effects, see sections on tenofovir (caution with renal function, Fanconi syndrome), rilpivirine and FTC.

Interactions, warnings: for interactions, see also sections on tenofovir, rilpivirine and FTC. Complera® should not be coadministered with the following drugs, as significant decreases in rilpivirine plasma concentrations may occur due to CYP3A enzyme induction or gastric pH increase: carbamazepine, phenytoin, rifabutin, rifampin, proton pump inhibitors (PPIs), St. John's wort. In patients with HIV-1 RNA greater than 100,000 copies/mL, the virologic failure rate conferred a higher rate of overall treatment resistance and cross-resistance to NNRTIs compared to Atripla®.

Comments: the second fixed-dose combination (FDC) was approved in 2011 as a complete ART regimen. Should not be used in highly viremic patients, due to high resistance rates. This well-tolerated FDC can be difficult to take because of its food requirement and drug interactions. Excellent adherence is critical.

For detailed information see page: 190

Co-trimoxazole (trimethoprim-sulfamethoxazole)

Manufacturer and trade names: diverse manufacturers, therefore several trade names, such as Cotrim-ratiopharm®, Cotrimstada®, Eusaprim®.

Indications: prophylaxis and treatment of Pneumocystis pneumonia (PCP). Prophylaxis and treatment (second-line) of cerebral toxoplasmosis. Also for other infections, for example urinary tract infections.

• Cotrim 960® or forte® tablets, 160/800 mg TMP/SMX
• Cotrim 480® tablets, 80/400 mg TMP/SMX
• Bactrim® liquid suspension, adults (16/80 mg/ml), children (8/40 mg/ml)
• Bactrim® ampule, 80/400 mg TMP/SMX

Dosage: as PCP prophylaxis: 80/400 mg QD or 160/800 mg 3 x/week. As PCP therapy: 5 mg/kg (based on TMP) orally or IV q 8 h x 21 days, therefore usually 5 to 6 ampules each 80/400 mg TID. Toxoplasmosis prophylaxis: 160/800 mg QD. With reduced renal function, use half-dose with creatinine clearance of 15 to 50 ml/min. Co-trimoxazole is contraindicated below 15 ml/min.

Side effects: allergies. High intravenous doses also cause myelotoxicity (anemia, neutropenia), nausea, vomiting, headache, raised transaminases. Treatment can often be continued in cases of mild allergy.

Comments: caution with sulfonamide allergy. Suspension for children can be used for desensitization. Increase the dose slowly over six days from 12.5, 25, 37.5, 50 and 75 to 100% of the 480 mg tablet dose. Co-trimoxazole can increase levels of anticoagulants and phenytoin and reduce the efficacy of oral contraceptives.

Crixivan®, see Indinavir.

Cymeven®, see Ganciclovir.

d4T (stavudine)

Manufacturer: Bristol-Myers Squibb.

Indications and trade name: HIV infection. In view of the side effects seen with the drug, the EMA recommended in February 2011 that the drug "should only be used when there are no appropriate alternatives, and for the shortest possible time".

• Zerit® hard capsules, 20, 30 and 40 mg
• Zerit® powder for preparation of an oral solution, 1 mg/ml

Dosage: 40 mg or 30 mg BID for body weight >60 kg or <60 kg. On empty stomach or with a light meal. In renal failure dosage must be reduced.

Side effects: Lipoatrophy. Peripheral neuropathy, especially in combination with ddI (up to 24%). Less frequent: diarrhea, nausea, headache, hepatic steatosis and pancreatitis. Very rare, but potentially fatal are lactic acidosis, which occurs mostly in combination with ddI (especially in pregnancy).

Comments: this thymidine analog was long-considered an important alternative to AZT. Due to the mitochondrial toxicity, the use of d4T is no longer recommended. Since 2011, use is severely restricted in both adults and children.

For detailed information see page: 74

Daclatasvir

Manufacturer: Bristol-Myers Squibb.

Indications and trade name: chronic hepatitis C, used in different combinations depending on the genotype (GT) being targeted. Approval differs between Europe and US! Europe: GT1 or GT4 without cirrhosis: daclatasvir + sofosbuvir 12 weeks (cirrhosis 24 weeks, shortening to 12 weeks may be considered for previously untreated patients with low baseline viral load). GT3 with compensated cirrhosis and/or treatment experienced: daclatasvir + sofosbuvir + ribavirin 24 weeks. In the US, daclatasvir is approved for GT3 only (+ sofosbuvir, 12 weeks).

• Daklinza® film-coated tablets, 30 mg and 60 mg

Dosage: 60 mg QD, to be taken with or without meals. Dosage should be reduced to 30 mg QD with regimens containing ritonavir or cobicistat and increased to 90 mg QD with NNRTIs except rilpivirine. No dose adjustment is required for patients with renal impairment.

Side effects: usually well tolerated. Fatigue, headache, and nausea.

Interactions, warnings: duration and combination depend on prior treatment, liver function and HCV genotypes. Dose adjustments required in combination with boosted PIs, cobicistat, and several NNRTIs. Coadministration with strong CYP3A4 inducers and P-glycoprotein transporters should be avoided. These include but are not limited to phenytoin, carbamazepine, rifampicin, rifabutin, and the herbal product St John's wort.

Comments: this pan-genotypic NS5A replication complex inhibitor was approved in 2014. Efficacy in HIV-coinfected patients was shown in the ALLY-2 trial. Should be initiated and monitored by a physician experienced in the management of HIV/HCV coinfection. Dose adaptations with ART have to be kept in mind.

For detailed information see page: 459

Dapsone

Manufacturer: Fatol.

Indications: rarely used reserve drug for prophylaxis of PCP and cerebral toxoplasmosis. Other (rare) areas of application are in dermatology (bullous pemphigoid), rheumatology and leprosy.

• Dapson-Fatol® tablets, 50 mg

Dosage: 100 mg daily. Alternative: 50 mg QD + pyrimethamine 50 mg/week + folinic acid 30 mg/week.

Side effects: allergies (pruritus, rash), fever. Frequently hemolytic anemia (with almost obligatory elevation of LDH), hepatitis.

Comments: dapsone is contraindicated in severe anemia and must be used with caution in *G6PD* deficiency. Not to be taken simultaneously with ddI, antacids or H2 blockers (to be taken at least two hours apart). Rifabutin and rifampin lower dapsone levels.

Daraprim®, see Pyrimethamine.

Darunavir

Manufacturer: Janssen-Cilag.

Indications and trade names: to be used in either ART-naïve or pretreated HIV patients, adults and children.

• Prezista® tablets, 400, 600 and 800 mg (75 and 150 mg as pediatric formulations)
• Prezista® oral suspension, 100 mg/ml (pediatric formulation)
• Rezolsta® (US: Prezcobix®) tablets, 800 mg plus 150 mg cobicistat

Dosage: 800 mg QD (2 tablets of 400 mg) + 100 mg ritonavir QD, with or shortly after meals. In patients with extensive pretreatment (and/or limited resistance mutations), it is recommended to use 600 mg BID (1 tablet of 600 mg) + 100 mg ritonavir BID. Instead of ritonavir, cobicistat may also be used. In 2009, darunavir was also approved for children aged 6 years and older. Recommended dosage is 375/50 mg BID (Wt ≥20 kg to <30 kg), 450/60 BID (Wt ≥30 kg to <40 kg). At ≥40 kg, adult dosage.

Side effects: the usual PI side effects, with (moderate) gastrointestinal complaints and dyslipidemia. The dyslipidemia may not be as pronounced as with other PIs. Data on lipodystrophy is lacking. Rash (7%) within the first 2 weeks, usually mild.

Interactions, warnings: caution for sulfonamide allergy. Since darunavir is metabolized by the cytochrome P450 system, some interactions have to be taken into account. Lopinavir and saquinavir lower the plasma levels of darunavir and should be avoided. Also do not combine with St. John's wort, astemizole, cisapride, midazolam, ergotamine derivatives, rifampicin, phenytoin, and carbamazepine. Use atorvastatin instead of pravastatin at the lowest dose (10 mg). Dosage adjustments may be required with efavirenz (decreased darunavir levels and increased efavirenz levels), rifabutin (dose should be reduced to 150 mg every two days), calcium antagonists (increased levels), methadone (reduced levels). Interactions with contraceptives may occur. Maximum doses of PDE5 inhibitors when combined with darunavir, 10 mg Cialis® in 72 hours; 2.5 mg Levitra® in 72 hours; 25 mg Viagra® in 48 hours.

Comments: Well-tolerated and broadly applicable HIV protease inhibitor that has considerable activity against PI-resistant viruses. Needs to be boosted with ritonavir. Different dosages as well as interactions have to be taken into account.

For detailed information see page: 93

Dasabuvir

Manufacturer: AbbVie.

Indications and trade name: In combination with ombitasvir + paritaprevir + ritonavir (Viekirax®) for adult patients with chronic hepatitis C, genotype 1.

• Exviera® film-coated tablets, 250 mg dasabuvir

Dosage: 250 mg BID with food. No dose adjustment is required even for patients with severe renal impairment.

Side effects: the most common side effects are fatigue and nausea.

Interactions, warnings: genotype 1 only. Combination 12 weeks with Viekirax® in genotype 1b (cirrhosis plus ribavirin), with Viekirax® plus ribavirin in genotype 1a (cirrhosis duration 24 weeks). Numerous drug interactions especially with ritonavir which is provided as part of Viekirax®. Do not combine with efavirenz, nevirapine,

etravirine, beware of rilpivirine (higher levels, QT prolongation). HIV PIs should be given only unboosted, the fixed-dose lopinavir/r and cobicistat containing regimens are contraindicated.

Comments: non-nucleoside NS5B polymerase inhibitor for hepatitis C GT1. Efficacy data in HIV-coinfected patients is limited. Numerous interactions with ART have to be considered as dasabuvir is usually given with the ritonavir-boosted HCV PI paritaprevir (see Vikierax®).

For detailed information see page: 458

Daunorubicin (liposomal)

Manufacturer: Gilead Sciences (Galen Limited), Fresenius

Indications and trade name: AIDS-associated Kaposi sarcoma with <200 CD4 T cells/µl and severe mucocutaneous or visceral involvement.

• DaunoXome® injection vial, 50 mg (25 ml)

Dosage: 40 mg/m² in 250 ml 5% glucose solution intravenously over 30-60 minutes. Repeat after 2-3 weeks.

Side effects: during infusion: back pain, flushing (up to 14%). Symptoms usually occur during the first minutes and resolve when the infusion is slowed or stopped. Fatigue, headaches, chills. Myelosuppression, cardiomyopathy. Beware of paravasation!

Interactions, warnings: liposomal doxorubicin is contraindicated in decompensated cardiomyopathy, severe myelosuppression (neutrophils <1,000/µl, platelets <50,000/µl). Cardiological examination is important (ECG, echocardiography: left ventricular ejection fraction, LVEF) before initiation of treatment and at periodic intervals during treatment.

Comments: compared to pegylated liposomal doxorubicin, treatment with liposomal daunoribicin yields to slightly lower remission rates of KS. However, as capacity constraints for Caelyx® were seen in the past years, DaunoXome® represents an alternative for KS treatment.

ddC (Zalcitabine), manufacturing and distribution was stopped in 2006.

ddI (didanosine)

Manufacturer: Bristol-Myers Squibb.

Indications and trade name: HIV infection, in combination with other antiretroviral agents.

• Videx® hard capsules, 125, 200, 250 and 400 mg.
• Videx® pediatric powder for oral solution, 2 g (must be reconstituted with purified water by the pharmacist)

Dosage: 400 mg QD (body weight >60 kg) or 250 mg QD (body weight <60 kg). ddI must be taken on an empty stomach, 2 hours after or 1 hour before meals.

Side effects: diarrhea, nausea, headache. Pancreatitis, even after longer periods of treatment. Peripheral polyneuropathy. Rarely lactic acidosis, especially in combination with d4T and ribavirin.

Interactions, warnings: acute and chronic pancreatitis are contraindications, as well therapy with ribavirin. The following drugs should be used with caution: d4T, ethambutol, cisplatin, disulfiram, INH, vincristine (peripheral neuropathy). Concurrent dosing with tenofovir should be avoided because it increases the AUC of ddI by 44%. Treatment with indinavir, dapsone, ketoconazole, itraconazole, or tetracyclines should be given 2 hours before or after ddI. Initially, monthly monitoring of amylase, blood count, transaminases and bilirubin. ddI should be discontinued if there is clinical suspicion for pancreatitis with no future rechallenge.

Comments: due to considerable toxicity (pancreatitis, polyneuropathy, mitochondrial toxicity) this old NRTI is rarely used today.

For detailed information see page: 73

Delavirdine

Manufacturer: ViiV Healthcare (Pfizer).

Indications and trade name: HIV infection. Not licensed in Europe.

• Rescriptor® tablets, 100 mg and 200 mg

Dose: 400 mg TID. The tablets can be dissolved in water.

Side effects: rash, usually occurring within the first six weeks of treatment. In uncomplicated cases, give antihistamines. Discontinue if systemic effects such as fever, conjunctivitis, and myalgia occur. Nausea, elevated transaminases.

Interactions, warnings: delavirdine is contraindicated for concurrent treatment with rifabutin, rifampin, carbamazepine, phenytoin, alprazolam, astemizole, phenobarbital, cisapride, midazolam, terfenadine and triazolam.
Delavirdine interacts with numerous drugs via reduction of CYP3A activity. It increases the AUC of some PIs (saquinavir, nelfinavir), sildenafil, dapsone, clarithromycin, quinidine and warfarin. Delavirdine levels are lowered by ddI, H_2 blockers, carbamazepine, phenytoin and antacids.

Comments: this NNRTI was never approved in Europe. It is rarely used anywhere (i.e., US or Canada) due to high pill burden and drug interactions.

For detailed information see page: 83

Diflucan®, see Fluconazole.

Dolutegravir

Manufacturer: ViiV Healthcare.

Indications and trade names: in combination with other antiretroviral agents, to be used in either ART-naïve or pretreated HIV+ patients, adults and children (12 years of age and older, weighing at least 40 kg).

• Tivicay® film-coated tablets, 50 mg
• Triumeq® film-coated tablets, 50 mg plus 600 mg ABC + 300 mg 3TC

Dosage: 50 mg QD, with or without food. May be increased to 50 mg BID, in the case of INI resistance mutations or comedication. No adjustment for Tivicay® in patients with renal insufficiency.

Side effects: headaches, nausea, diarrheas (usually mild). Hypersensitivity reactions have been reported and were characterized by rash, constitutional findings, and sometimes organ dysfunction, including liver injury (<1%).

Interactions, warnings: dolutegravir is metabolized by UGT1A1 with some contribution from CYP3A. Some interactions should be considered. When combined with efavirenz, fosamprenavir/r and tipranavir/r (and rifampicin), adjust dose of dolutegravir to 50 mg BID. If dosage was already increased due to INSTI resistances, these combinations should be avoided! Coadministration with etravirine without boosted PIs is not recommended. Nevirapine, phenytoin, carbamazepine should not be combined. Administer dolutegravir 2 hours before or 6 hours after taking medications containing polyvalent cations (antacids). When used as Triumeq®, abacavir HSR must be considered (HLA testing).

Comments: first integrase strand transfer inhibitor (INSTI) which can be administered once daily, without boostering. Very potent, seems to have a higher resistance barrier than other INSTIs. Since its approval in 2014, dolutegravir is now broadly used in many countries. It is also available as a component of a fixed-dose combination with abacavir and 3TC.

For detailed information see page: 100

Doxorubicin (liposomal)

Manufacturer: Janssen-Cilag (Johnson & Johnson).

Indications and trade name: AIDS-associated Kaposi sarcoma with <200 CD4 cells/µl and severe mucocutaneous or visceral involvement.

• Caelyx® (or Doxil®) injection vial, 10 ml (20 mg) and 25 ml (50 mg)

Dosage: 20 mg/m^2 in 250 ml 5% glucose solution intravenously over 30 minutes. Repeat after 2-3 weeks.

Side effects: myelosuppression, cardiomyopathy, stomatitis (rarely severe), palmar-plantar erythrodysesthesia (PPE or hand-foot syndrome – erythematous rash which may be very painful. Treatment: cool affected areas). Be careful with extravasations (never SC or intramuscular, never give a bolus).

Interactions, warnings: doxorubicin is contraindicated in decompensated cardiomyopathy, severe myelosuppression (neutrophils <1,000/µl, platelets <50,000/µl). Cardiological examination is important (ECG, echocardiography: left ventricular ejection fraction, LVEF) before initiation of treatment and at periodic intervals during treatment. If the cumulative dose of 450 mg/m^2 is exceeded, then an echocardiography is necessary before each further cycle. It is important to inform patients of PPE (may be induced by sweating, pressure, friction – i.e., no tight gloves, no sun, no long warm showers). Cool drinks are helpful.

Comments: Treatment of choice for KS requiring chemotherapy. Expensive!

Dutrebis®, see Raltegravir or 3TC

Edurant®, see Rilpivirine.

Efavirenz

Manufacturer: BMS (Sustiva®); MSD (Stocrin®); Gilead/BMS/MSD (Atripla®). Several generics available!

Indications and trade names: HIV infection.

• Sustiva® film-coated tablets, 600 mg, in some countries known as Stocrin®
• Sustiva® hard capsules, 50 mg, 200 mg
• Sustiva® solution for oral administration as 30 mg/ml (180 ml = 5.4 g)
• Atripla® film-coated tablets, 600 mg efavirenz +200 mg FTC +300 mg tenofovir

Dosage: 600 mg daily preferably before going to bed on an empty stomach.

Side effects: CNS symptoms occur frequently in the first weeks. Nightmares, confusion, dizziness, depression, somnolence, impaired concentration, insomnia and depersonalization. A generally mild rash (15%) may also occur in the first weeks, and continued treatment is usually possible. Elevation of liver function tests and biliary enzymes. Dyslipidemia. Occasionally painful gynecomastia.

Interactions, warnings: contraindicated in pregnancy. Caution with women of childbearing age. Efavirenz should not be taken with fatty meals (possibly higher absorption and side effects).
Contraindicated for concurrent administration with ergotamines, astemizole, cisapride, midazolam, terfenadine and triazolam. Should not be combined with contraceptives. Dose increases may be necessary for lopinavir/r (to 3 tablets BID), atazanavir/r (400/100 mg), rifabutin (450 mg), methadone (20-30%) and maraviroc (600 mg BID if no boosted PI is given).

Comments: efavirenz is still a frequently used NNRTI. However, it has some CNS side effects. Further disadvantages as with the other members of this drug class include drug interactions, a low resistance barrier and cross-resistance.

For detailed information see page: 83

Elvitegravir

Manufacturer: Gilead Sciences.

Indications and trade names: in combination with a boosted PI in antiretroviral treatment-experienced adults (see different indication for Stribild®)

• Vitekta® film-coated tablets, 85 mg and 150 mg
• Stribild® film-coated tablets, 150 mg plus 150 mg cobicistat, 200 mg FTC, 300 mg tenofovir DF

Dosage: Once daily, with food. Dosage depends on the boosted PI! 85 mg elvitegravir QD (plus atazanavir/r 300/100 mg QD or lopinavir/r 400/100 mg BID) or 150 mg elvitegravir QD (plus darunavir/r 800/100 mg QD or fosamprenavir/r 700/100 BID). In case of a BID PI, elvitegravir should be taken with the first dosage. No dose adjustment with renal impairment required (see Stribild®).

Side effects: diarrhea. Also nausea, headache, usually mild.

Interactions, warnings: The combination of elvitegravir with a cobicistat-boosted PI (insufficient data) or with other integrase strand transfer inihbitors (INSTIs) is not recommend. Elvitegravir is metabolized by CYP3A. CYP3A inducer are expected to increase the clearance of Elvitegravir. Coadministration with efavirenz, nevirapine, but also rifampicin, carbamazepine and St. John's wort is not recommended. It is recommended to separate antacid administration by at least 2 hours.

Comments: INSTI which requires boosting. When prescribed as a single agent, combination with boosted PI (and different dosages) are required. Much more commonly used as a component in the fixed-dose combination Stribild®.

For detailed information see page: 101

Emtricitabine (FTC)

Manufacturer: Gilead (Emtriva®, Truvada®, Stribild®); Gilead+BMS+MSD (Atripla®); Gilead+Janssen-Cilag (Complera®).

Indications and trade name: HIV infection.

• Emtriva® hard capsules, 200 mg FTC. Solution, 10 mg FTC per ml
• Truvada® film-coated tablets, 200 mg FTC + 300 mg tenofovir
• Atripla® film-coated tablets, 200 mg FTC + 300 mg TDF + 600 mg efavirenz
• Complera® film-coated tablets, 200 mg FTC + 300 mg TDF + 25 mg rilpivirine
• Stribild® film-coated tablets, 200 mg FTC + 300 mg TDF + 150 mg elvitegravir + 150 mg cobicistat

Dose: 200 mg QD (solution recommended dose 240 mg = 24 ml). At reduced creatinine clearance (Cr Cl), FDCs should be avoided. FTC is adapted as follows: 200 mg FTC every 2 days (30-49 ml/min), every 3 days (15-29 ml/min) or every 4 days (below 14 ml/min or dialysis).

Side effects: mild headache, nausea, diarrhea, rash. Possibly hyperpigmentation.

Comments: FTC is a well-tolerated cytidine analog which has the same resistance profile but has a significantly longer half-life than 3TC. FTC is mainly used as a component in different fixed-dose combinations. Effective against HBV, beware of viral rebounds after discontinuing FTC.

For detailed information see page: 74

Emtriva®, see Emtricitabine.

Enfuvirtide®, see T-20.

Epivir®, see 3TC.

Eremfat®, see Rifampicin.

Ethambutol

Manufacturer: among others Riemser Several generics.

Indications and trade names: tuberculosis, MAC infection.

• EMB-Fatol® tablets, 100 mg
• EMB-Fatol® film-coated tablets, 250 mg, 400 mg and 500 mg
• EMB-Fatol® injection solution, 1 g in 10 ml
• Myambutol® film-coated tablets, 100 mg and 400 mg
• Myambutol® injection solution, 400 mg/4 ml and 1000 mg/10 ml

Dosage: 15 to 25 mg/kg (maximum 2 g) daily, usually 3 x 400 mg tablets QD.

Dose reduction in renal failure as follows:

Cr Cl	Dose
Above 75 ml/min	25 mg/kg
40–75 ml/min	15 mg/kg
30–40 ml/min	15 mg/kg every second day
<30 ml/min	Measurement of serum levels required (should be within the range of minimal inhibitory concentration 2–5 µg/ml after 2–4 hours)

Side effects: ethambutol can lead to optical neuritis with impaired vision (decreased acuity, restricted fields, loss of red-green color discrimination). It is usually reversible if ethambutol is discontinued immediately. Other side effects: nausea, vomiting, abdominal pain, headache, dizziness, pruritus, arthralgia, elevated serum uric acid (possible acute gout attacks), abnormal liver function tests.

Interactions, warnings: ethambutol is contraindicated with pre-existing optical nerve damage. Ophthalmologic examination before initiation of treatment and subsequently at 4-week intervals (color discrimination, field of vision, acuity). Immediate discontinuation to prevent optical atrophy if vision impairment occurs. Patients should be informed that impairment of vision may occur and to immediately report this to the treating physician. Aluminum hydroxide reduces absorption of ethambutol; ethambutol should therefore be taken at least one hour before antacids. Monitor liver values and uric acid levels at monthly intervals.

Etravirine

Manufacturer: Janssen-Cilag.

Indications and trade name: in combination with a boosted PI and other antiretroviral agents for the treatment of HIV-1 infection in antiretroviral treatment-experienced adult patients and in children aged 6 years or older.

• Intelence® tablets, 200 mg

Dosage: 200 mg (2 x 1 pill) BID after a meal. The tablets are soluble in water.

Side effects: mostly mild rash, nausea is rare. With mild exanthema, which usually appears in the second week, treatment can usually be continued, immediately stopping at a serious exanthema. Rarely Stevens-Johnson syndrome. In October 2009, the company published a Dear Doctor letter, reporting on three cases of TEN.

Interactions, warnings: etravirine is a substrate of the CYP450 enzyme system as well as an inducer of CYP3A4 and an inhibitor of CYP2C9, therefore, some interactions are to be anticipated. Etravirine reduces the serum concentrations of atazanavir, maraviroc and raltegravir and increases fosamprenavir levels. On the other hand, etravirine levels are considerably reduced by tipranavir, efavirenz and nevirapine (moderately by darunavir, saquinavir and tenofovir). Lopinavir and delavirdine increase the levels of etravirine.
Etravirine should not be combined with the following: atazanavir, fosamprenavir, tipranavir, unboosted PIs or other NNRTIs. Avoid rifampicin, carbamazepine, phenobarbital, phenytoin and St. John's wort as well.

Comments: etravirine is the first second-generation NNRTI that was licensed in 2008 for pre-treated patients. It is well-tolerated and effective against some (but not all) NNRTI-resistant HIV strains. Should be combined with a boosted PI (preferably darunavir, due to the lack of data with other PIs).

For detailed information see page: 84

Eviplera®, see Complera.

Evotaz®, see Atazanavir and Cobicistat

Exviera®, see Dasabuvir.

Fluconazole

Manufacturers and trade names: Pfizer and many other companies, therefore several trade names, such as Diflucan®, Fluconazole CT®/Stada, or Flucobeta®.

Indications: *Candida* infection, cryptococcal meningitis and some rare mycoses.

• Fluconazole® capsules, 50 mg, 100 mg, 200 mg. Suspension, 50 mg per 10 ml
• Fluconazole® IV for injections, 100, 200 and 400 mg

Dosage: for oral candidiasis, 100 mg QD orally; for *candida esophagitis* 200 mg QD for 7–10 days. Double the dose on the first day. An attempt with a higher dose (up to 800 mg daily) may be made if there is persistent candidiasis after 10 days.
Cryptococcal meningitis: Acute therapy for 6 weeks with 400–800 mg daily, combined with flucytosine and amphotericin B if possible. Then maintenance therapy with 200 mg fluconazole daily. Renal insufficiency: half the dose with creatinine clearance of 10 to 50 ml/min; reduce to 25% below 10 ml/min.

Side effects: Rarely gastrointestinal complaints and elevated transaminases. Reversible alopecia in approximately 10% of cases with more than 400 mg daily.

Interactions, warnings: long-term treatment may lead to development of *candida*-resistant strains. Fluconazole is not effective against *C. krusei* or aspergillus. In cases of *C. glabrata* infection higher doses are required (sensitivity is dose-dependent). Fluconazole levels are reduced with concurrent administration of rifabutin/rifampin. Fluconazole increases the serum levels of rifabutin, atovaquone, clarithromycin, theophylline, opiates, coumarin derivatives, benzodiazepines, cyclosporine, tacrolimus, phenytoin and anti-convulsive drugs as well as AZT.

Comments: fluconazole is the first choice for HIV-associated candidiasis and for the secondary prophylaxis of cryptococcosis (also as component of acute therapy). The tablets have good absorption. Infusions (more expensive) are only required in cases of non-adherence, severe mucositis, and/or problems with absorption.

Fortovase ®, see Saquinavir.

Fosamprenavir

Manufacturer: ViiV Healthcare.

Indications and trade names: HIV infection, for both treatment-naïve and experienced patients (for limitations, see below). US trade name: Lexiva®.

• Telzir® film-coated tablets, 700 mg. Suspension, 50 mg/ml (225 ml bottle)

Dosage in treatment-naïve patients:
700 mg BID + 100 mg ritonavir BID (2 x 2 pills, normal dose)
1400 mg BID (without ritonavir, not approved in Europe)
1400 mg QD + 200 mg ritonavir QD (1 x 4 pills; not approved in Europe)

Dosage in PI-experienced patients:
700 mg BID + 100 mg ritonavir BID (2 x 2 pills)

Side effects: most frequently diarrhea, may be severe in some cases. Also nausea, vomiting, rash (up to 20%). Rarely Stevens-Johnson syndrome (<1%).

Interactions, warnings: Fosamprenavir can be taken on an empty stomach or with a meal. Contraindicated: cisapride, midazolam, ergotamines, flecainide and propafenone. There may be life-threatening interactions upon concurrent administration of amiodarone, lidocaine (systemic), tricyclic anti-depressants and quinidine. Do not use together with rifampin, delavirdine or St. John's wort; use cautiously with simvastatin, lovastatin, sildenafil, vardenafil. Carbamazepine, phenobarbital, and phenytoin can lower plasma levels of amprenavir.
Rifabutin: dose reduction of rifabutin by at least 50%. If fosamprenavir is boosted with ritonavir, a 75% reduction of the rifabutin dose is required (instead of 300 mg daily, only 150 mg every other day, or 150 mg three times per week). An increased methadone dose may be required.
Efavirenz seems to lower plasma levels significantly (probably clinically relevant). However, this is not the case if fosamprenavir is boosted with ritonavir. If fosamprenavir/r is administered once daily, then the ritonavir dose should be increased to 300 mg. Caution in combination with lopinavir – plasma levels of both drugs are reduced. If fosamprenavir is boosted with ritonavir, ketoconazole and itraconazole maximum dose 200 mg daily. Caution in patients with sulfonamide allergy or with reduced liver function.

Comments: Except for diarrhea, this PI is well-tolerated. However, fosamprenavir currently does not play an important role in HIV medicine. One advantage of the drug is that there are no restrictions with respect to food intake.

For detailed information see page: 94

Foscarnet

Manufacturer: AstraZeneca.

Indications and trade name: reserve drug for induction and maintenance therapy of CMV retinitis. Severe acyclovir-resistant herpes or varicella zoster infections.

• Foscavir® IV, 250 ml with 24 mg/ml

Dosage: 90 mg/kg IV over at least 2 hours BID for induction therapy (2–3 weeks) of CMV retinitis. 90–120 mg/kg over 2 hours QD for maintenance therapy.
HSV and HZV infections: 60 mg/kg IV BID for 2 weeks.

Side effects: nephrotoxicity, usually reversible after discontinuation. Electrolyte changes (hypocalcemia, hypokalemia) are also common. More rarely, anemia, neutropenia, fever, rash, headache, nausea, vomiting, diarrhea. Often painful penile ulcerations (washing recommended after every urination).

Interactions, warnings: good hydration. At least 2.5 l fluids daily. To prevent hypocalcemia give one ampule of 10% calcium solution in 100 ml 5% glucose immediately prior to infusion of foscarnet. Give 500–1000 ml 5% glucose before or after foscarnet dose. Do not mix infusions.
Initial monitoring of Na, K, Ca, creatinine, blood count at least 3 x week.
No concurrent treatment with other nephrotoxic drugs.
Adjust dose in renal insufficiency. See prescribing information.

Comments: since the approval of valgancyclovir, foscarnet is used only rarely. However, it can be useful in some resistance situations (herpes viruses).

Foscavir®, see Foscarnet.

Fuzeon®, see T-20.

Ganciclovir

Manufacturer: Hoffmann-La Roche.

Indications and trade name: CMV retinitis.

• Cymeven® IV, 500 mg

Dosage: initial treatment with normal renal function: 5 mg/kg BID as an IV infusion for one hour. Treatment duration, 14 to 21 days.
Maintenance therapy: 6 mg/kg IV QD, 5 x week.

Side effects: leukopenia, anemia and thrombocytopenia are dose limiting. Nausea, vomiting or CNS symptoms such as confusion or headache are rare.

Interactions, warnings: monitor blood count every other day. Discontinue drug when below 500/µl (G-CSF if necessary). Contraindicated in neutropenia <500/µl, thrombocytopenia <25,000/µl and concurrent chemotherapy (KS, NHL). Do not combine with AZT and ddI (increased toxicity). Gancyclovir is a potential teratogen. Dose adjustment is necessary in renal insufficiency.

Comment: since the approval of valgancyclovir, gancyclovir is only used rarely.

Harvoni®

Manufacturer: Gilead Sciences.

Indication and trade name: This fixed-dose combination of sofosbuvir, an HCV nucleotide analog NS5B polymerase inhibitor, and ledipasvir, an HCV NS5A inhibitor, is indicated for chronic hepatitis C, GT1, GT3, GT4 infection in adults (Europe; approval differs in the US). Duration in HIV/HCV+ patients is usually 12 weeks. Hard-to-treat patients (prior non-responders with HCV GT1a and cirrhosis) may either extend to 24 weeks or include ribavirin (doubling the duration raises the costs while adding ribavirin increases side effects, please refer to country-specific approvals and restrictions).

• Harvoni® film-coated tablets, 400 mg sofosbuvir and 90 mg ledipasvir

Dosage: one tablet QD, with or without food. No dose recommendation can be given for patients with severe renal impairment (eGFR <30 mL/min) due to higher exposures of the predominant sofosbuvir metabolite.

Side effects: headache and fatigue common (usually mild), nausea (usually mild).

Interactions, warnings: coadministration with amiodarone may result in serious symptomatic bradycardia and is contraindicated! Sofosbuvir and ledipasvir are not metabolized by the cytochrome P450 enzyme system and, therefore, can be combined with most antiretroviral drugs except for the P-gp inducers tipranavir (and rifampin, St. John's wort). The coadministration of ledipasvir/sofosbuvir and tenofovir may increase exposure to tenofovir, especially in combination with a boosted PI – alternatives can be considered. In the ION trial, tenofovir and FTC were given with efavirenz, rilpivirine and raltegravir or rilpivirine (Complera®).

Comments: Well-tolerated fixed-dose combination for hepatitis C which has shown high efficacy and excellent tolerability in HIV/HCV+ patients with GT1 and GT4 (ION trial). GT3 use (approved in Europe) is not recommended by current guidelines (weaker potency, costs).

Hivid®, see ddC, no longer manufactured.

Incivek®, see Telaprevir.

Indinavir

Manufacturer: MSD.

Indications and trade name: HIV infection.

• Crixivan® hard capsules, 200 mg and 400 mg

Dosage: Ususally 800 mg BID plus 100 mg ritonavir BID. Dose reduction is often possible with TDM. Dose without ritonavir not recommended.

Side effects: nephrolithiasis (in up to 25%). Less frequently, nephrotoxicity with elevated serum creatinine. Diarrhea, nausea, vomiting. Hyperbilirubinemia. A sicca syndrome occurs relatively frequently (dry skin, mouth, eyes); ingrown toenails and paronychia; rarely alopecia. Lipodystrophy (originally called Crix belly), dyslipidemia, disorders of glucose metabolism.

Interactions, warnings: At least 2 l of fluid should be consumed daily to prevent nephrolithiasis. The occurrence of nephrolithiasis and probably skin problems too, correlates with plasma levels. No concurrent administration with ddI. The concurrent use of rifampin, astemizole, terfenadine, cisapride, triazolam, ergotamines, simvastatin, lovastatin, or St. John's wort is contraindicated.
When using IDV/r, 150 mg rifabutin every 2 days or three times a week. Keto/itraconazole: 600 mg IDV TID. Sildenafil: maximum 25 mg sildenafil/48h.

Comments: Was one of the first PIs on the market in 1996. Due to toxicity and need for hydration, etc, indinavir does not play a role any longer in HIV medicine.

For detailed information see page: 94

Intelence®, see Etravirine.

Interferon α-2a/2b

Manufacturers: Essex (interferon α-2b as Intron A®, pegylated as PegIntron®) and Roche (interferon α-2a as Roferon®, pegylated as Pegasys®).

Indications and trade name: chronic hepatitis C (IFN α-2b and IFN α-2a), chronic hepatitis B (α-2a). Kaposi's sarcoma with good immune status (>250 CD4 cells/µl); pegylated interferons are not licensed for KS in Europe.

• PegIntron® injector, 50, 80, 100, 120, 150 µg in 0.5 ml
• Pegasys® prefilled syringe, 135, 180 µg
• Roferon-A® prefilled syringe, 3, 4.5, 9, 18 Mio IU
• Intron A® pens, 18, 30, 60 Mio IU

Dosage: PegIntron®, 1.5 µg/kg body weight 1 x/week. Pegasys®, 180 µg 1 x/week Standard interferons: 6 Mio IU 3 x/week. Interferon is injected subcutaneously. Duration depends on success of treatment of KS, on HCV genotype and success of treatment.

Side effects: frequent side effects. Influenza-like symptoms such as fever and myalgia. Depression, even suicidal tendencies, fatigue, sleeping disorders, personality changes. Anemia, thrombocytopenia and leukopenia. Autoimmune thyroiditis. Reversible hair loss. Possibly impaired vision.

Interactions, warnings: influenza-like symptoms usually occur a few hours after dosing and can be reduced with paracetamol (take 1000 mg in advance). All side effects are usually reversible. Contraindications are severe liver or renal dysfunction, severe heart disease, bone marrow disorders, CNS disorders (e.g., epilepsy, severe depression), uncompensated thyroid disorders.
Monitor blood count every two weeks initially, and then later, monthly with standard laboratory tests. TSH every three months. Interferons must be kept refrigerated.

Comments: In 2015, many guidelines do not further recommend interferon for treatment of chronic hepatitis C. Can be considered for treatment of acute HCV and in some KS cases.

Intron A®, see Interferon.

Invirase®, see Saquinavir.

Isentress®, see Raltegravir.

Isoniazid (INH)

Manufacturers and trade name: isoniazid is made by various manufacturers and has many trade names.

Indications: part of combination therapy for tuberculosis. Prophylactic treatment after tuberculin conversion.

• Isozid comp® film-coated tablets, 200, 300, 400 mg INH and 40, 60, 80 mg vitamin B6 (pyridoxin-HCl)
• Also in various combination preparations (see rifampicin).

Dosage: 200 to 300 mg QD (4 to 5 mg/kg, maximum 300 mg) orally, IV only in severe cases during the first two weeks of therapy. For prophylaxis of neuropathy

100 mg pyridoxine orally QD. Pyridoxine is contained in the dosage of 20 mg per 100 mg of isoniazid in Isozid comp®.

Pediatric dose: 6 (to 10) mg/kg QD, maximum 300 mg.

Side effects: toxic hepatitis, more frequent in older patients, and patients with chronic liver disease and alcohol abuse. Peripheral neuropathy. Discontinue isoniazid in severe cases and treat for several weeks with pyridoxine and vitamin B-12. Psychosis, CNS symptoms. Fever, rash, nausea, vomiting, anemia, leukopenia, thrombocytopenia.

Interactions, warnings: INH should not be used in acute hepatitis and patients with a history of INH-associated hepatopathy or severe febrile reactions, peripheral neuropathy, macrohematuria. Always combine with vitamin B6. Diverse interactions with barbiturates, cycloserine, theophylline, phenytoin and rifampin; doses of these drugs should be reduced due to CNS disorders.

Reduced absorption if taken concurrently with aluminum-based antacids. Do not combine with ddI (peripheral neuropathy!). Caution with alcohol during treatment.

Initially, biweekly monitoring of blood count, transaminases, bilirubin, and renal function. Discontinue isoniazid with elevation of transaminases of more than 3-fold initial levels and symptoms; or with a 5-fold elevation in the absence of symptoms.

Itraconazole

Manufacturers and trade name: diverse, with several trade names.

Indications: histoplasmosis, aspergillosis, treatment-resistant *Candida* infections (second-line). Also for onychomycosis.

• Sempera® capsules, 100 mg
• Sempera Liquid® juice, 10 mg/ml (150 ml)

Dosage: Fluconazole-resistant *Candida* infections: 100 mg QD to 100 mg BID (up to 200 mg BID) ideally as itraconazole oral solution. Histoplasmosis, aspergillosis 200 mg BID.

Side effects: nausea, vomiting, rash, dizziness. Toxic hepatitis.

Interactions, warnings: To achieve maximum absorption, the capsules should be taken immediately after a full meal. Acidic drinks such as coke and orange juice may increase absorption.
No concurrent administration of itraconazole capsules with ddI, H2 blockers, omeprazole, antacids. No concurrent administration (of capsules or oral solution) with rifampin, rifabutin, carbamazepine, phenytoin, phenobarbital, simvastatin, lovastatin and isoniazid (these lower the bioavailability of itraconazole).
Itraconazole increases serum levels of cyclosporine, calcium antagonists, digoxin, lovastatin, simvastatin and indinavir. Itraconazole has a negative inotropic effect and should not be given to patients with heart failure.

Comments: Due to numerous interactions and unreliable plasma levels, administration of itraconazole is problematic. However, in contrast to fluconazole, it is effective for many non-albicans strains, aspergillosis, and histoplasmosis.

Kaletra®, see Lopinavir/r.

Kivexa® (US: Epzicom)

Manufacturer: ViiV Healthcare

Indications and trade name: HIV infection.

• Kivexa® film-coated tablets, 600 mg abacavir and 300 mg 3TC

Dosage: 1 tablet QD. Replace Kivexa® with the individual drugs if kidney function is impaired (creatinine clearance below 50 ml/min), in order to adjust the 3TC dose.

Side effects: hypersensitivity reaction due to abacavir (see abacavir). Controversial data on a potentially (slightly) enhanced risk of myocardial infarction in patients with an elevated risk of cardiovascular events. Otherwise well-tolerated.

Comments: frequently-used fixed-dose combination (FDC) and NRTI backbone in numerous ART regimens. Since 2014, it is also available in a single tablet FDC Triumeq®. The abacavir HSR can be prevented by prior HLA testing.

For detailed information see page: 76

Klacid®, see Clarithromycin.

Lamivudine, see 3TC.

Ledipasvir, see Harvoni.

Lexiva®, see Fosamprenavir.

Lopinavir/r

Manufacturer: AbbVie.

Indications and trade name: HIV infection, treatment naïve or pretreated patients, adults or pediatric patients (14 days or older).

• Kaletra® tablets, 200 mg lopinavir + 50 mg ritonavir
• Aluvia® tablets, 100 mg lopinavir + 25 mg ritonavir
• Kaletra® solution, 80 mg lopinavir + 20 mg ritonavir per ml

Dosage: 2 tablets BID (400 mg lopinavir/100 mg ritonavir) or 5 ml solution BID, taken with food. In the US, 4 tablets QD is approved in patients with less than 3 PI key resistance mutations. In Europe QD approval is restricted to adult patients new to HIV therapy.

Side effects: mainly diarrhea, nausea, dyslipidemia. More rare: headaches, and elevated transaminases.

Interactions, warnings: The solution (not the tablets) should be kept in the refrigerator. Numerous drug interactions. All drugs metabolized by the CYP3A or CYP2D6 enzyme systems are contraindicated: flecainide, propafenone, terfenadine, ergotamines, cisapride, midazolam, triazolam. Rifampin and St. John's wort reduce the levels of lopinavir. In combination with efavirenz (perhaps also nevirapine) increase dose to 3 tablets BID or 6.5 ml solution BID. Measure plasma levels.
Caution with: lovastatin, simvastatin (myopathy, rhabdomyolysis), carbamazepine, phenobarbital, phenytoin or sildenafil (hypotension), amiodarone, warfarin, lidocaine, tricyclic antidepressants, quinidine, cyclosporine, tacrolimus. Measure plasma levels in patients with reduced liver function tests.

If lopinavir is combined with ddI, ddI must be taken one hour before or two hours after lopinavir. Lopinavir solution contains alcohol, therefore no comedication with disulfiram or metronidazole. Caution with contraception – not safe. Increasing the methadone dose may be necessary. When used with rifabutin, the rifabutin dose should be reduced by 75% (i.e., to 1 x 150 mg every two days).

Comments: Effective PI for both ART-naïve and pretreated patients and the only PI with a fixed-dose of a ritonavir booster. Disadvantages include gastrointestinal side effects (diarrhea) and the often significant dyslipidemia. As with all PIs, various drug interactions should be considered.

For detailed information see page: 94

Maraviroc

Manufacturer: ViiV Healthcare.

Indications and trade name: In Europe, maraviroc is approved only for pretreated adult HIV-infected patients with CCR5-tropic HIV strains (R5). In November 2009, FDA has expanded use to ART-naïve patients with R5 viruses.

• Celsentri® or Selzentry® tablets, 150 mg and 300 mg

Dosage: 300 mg BID with or without food. Depending on the comedication, multiple dosage adjustments of maraviroc are recommended.

Combined Drugs	Maraviroc dose adjustment
Nevirapine, tenofovir, other NRTIs	none
Efavirenz + no protease inhibitors or other strong CYP3A4 inhibitors	600 mg BID
Rifampicin + no concurrent CYP3A4 inhibitor	600 mg BID
Boosted PIs and Elvitegravir/c (exception: tipranavir/r and fosamprenavir/r → standard dosage)	150 mg BID
Efavirenz + simultaneous PI therapy (exception: fosamprenavir/r)	150 mg BID
Rifabutin + concurrent administration of PIs (exception: with tipranavir/r or fosamprenavir/r → standard dosage)	150 mg BID
Itraconazole, ketoconazole, clarithromycin, telithromycin	150 mg BID

In combination, the dosage varies according to the PI; when both an inhibitor and an inducer are given, the inhibitor dominates.

The following adjustments are recommended to reduce creatinine clearance:

Cr Cl (ml/min)	Without comedication of a strong CYP3A4 inhibitor or together with tipranavir/r	Concurrent treatment with a strong CYP3A4 inhibitor, e.g., lopinavir/r, darunavir/r, atazanavir/r, ketoconazole	Concurrent treatment with saquinavir/r
50–80	no adjustment of dosage interval	every 24 hours	every 24 hours
30–49			every 48 hours
<30			every 72 hours

Side effects: well-tolerated, rare headaches, dizziness, fatigue, nausea. In high doses orthostatic hypotension. Occasional reports of CK increases, mycositis.

Interactions, warnings: the concurrent administration of maraviroc and rifampicin plus efavirenz is not recommended. St. John's wort can lower maraviroc levels. It is required to have a valid tropism test indicating the presence of R5 viruses.

Comments: The first CCR5 antagonist and the first oral entry inhibitor that was licensed for HIV therapy. The coreceptor tropism has to be determined prior to treatment. Well tolerated but complex dosage regulations.

For detailed information see page: 110

Mavid®, see Clarithromycin.

Mycobutin®, see Rifabutin.

Nelfinavir

Manufacturers: ViiV Healthcare.

Indications and trade name: HIV infection.
- Viracept® film-coated tablets, 625 mg (not in Europe)
- Viracept oral powder®, 50 mg/g

Dosage: 1250 mg BID (5 tablets) or 750 mg TID (3 tablets) with meals. Boosting with ritonavir is not useful.

Side effects: diarrhea >20%, meteorism, nausea, flatulence. Lipodystrophy, dyslipidemia, reduced glucose tolerance.

Interactions, warnings: contraindicated for comedication with rifampin, the contraceptive pill, astemizole, terfenadine, cisapride, triazolam, ergotamines, simvastatin, lovastatin, and St. John's wort. With rifabutin, 150 mg rifabutin QD and increase nelfinavir to 1250 mg BID or 1000 mg TID. If withdrawal symptoms occur while on methadone dose may be increased. Sildenafil maximum dose 25 mg in 48 h. Diarrhea can often be controlled with loperamide (maximum 16 mg/day).

Comments: Nelfinavir is less potent than boosted PIs or NNRTIs. No longer manufactured in Europe.

For detailed information see page: 95

Neupogen®, see G-CSF.

Nevirapine

Manufacturers: Boehringer-Ingelheim. Generics available (not for XR)!

Indications and trade name: HIV infection. ART-naïve patients with a good immune status (women >250, men >400 CD4 T cells/µl) should avoid nevirapine because of an elevated risk of hepatotoxicity (see below).

- Viramune® XR™ extended-release tablets, 400 mg
- Viramune® tablets, 200 mg (for pediatric patients also 100 mg, 50 mg). Several generics available
- Viramune Suspension®, 10 mg/ml

Dosage: 400 mg per day (1 XR tablet QD or 1 tablet BID). Always start with lead-in dosing (1 tablet 200 mg QD for 2 weeks) to reduce the frequency of rash. May be taken on an empty stomach or with meals.

Side effects: Elevation of transaminases (TAs) 10-15%, rash. Caution is needed when both appear simultaneously (see below). Less frequently, fever, nausea, drowsiness, headache, myalgia. These side effects may occur with or without hepatotoxicity and/or rash. GGT elevation on nevirapine is almost always the rule.
To detect hepatotoxicity (an TA increase to at least three times the upper limit of normal), liver function tests should be monitored biweekly for the first two months. Thereafter, monthly tests are necessary. If hepatotoxicity does occur, treatment must be interrupted until liver function tests have returned to initial levels. Treatment is restarted with 200 mg QD. The dose may be increased to 200 mg BID only after a prolonged period of observation. If liver enzymes increase again, nevirapine should be permanently discontinued. The risk is greater with good immune status (women >250 CD4 T cells/µl, 12-fold; men >400 CD4 T cells/µl, 5-fold). This elevated risk applies probably only to ART-naïve patients.
A mild rash, usually occurring within the first weeks of treatment, can be treated with antihistamines (e.g., Fenistil retard® 1 x 1 tablet) if mucous membranes are not involved and if transaminases are normal. Nevirapine must be discontinued if a severe rash or other systemic symptoms (fever, conjunctivitis, myalgia, arthralgia, malaise) occur; in these cases, steroids are recommended (e.g., prednisolone 1 mg/kg for 3-5 days). If the rash occurs during the first two weeks of treatment, then the dose should not be increased until the rash has resolved completely. Prophylactic treatment (steroids, antihistamines) is not advised. Nevirapine has a favorable long-term profile with regard to lipid levels. GGT is almost always increased during long-term treatment (values of up to 150 U/l can be tolerated).

Interactions, warnings: cautious use in hepatic dysfunction (TDM). No concurrent treatment with rifampin, ketoconazole, and St. John's wort. Dose adjustments with methadone (methadone dose increase may be required) and lopinavir/r (increase Kaletra® to 3 capsules BID). Nevirapine should not be given for post-exposure prophylaxis. The inactive tablet matrix is eliminated in the feces (patients should be informed).

Comments: In some countries, nevirapine remains a frequently prescribed NNRTI. As with all NNRTIs, a single point mutation is sufficient for high-level resistance. During the first weeks of treatment, nevirapine is saddled with allergies and hepatotoxicity (start with lead-in dosing). However, long-term tolerance is good.

For detailed information see page: 85

Norvir®, see Ritonavir.

Olysio®, see Simeprevir.

Ombitasvir, see Viekirax®.

Paritaprevir, see Viekirax®.

Pegasys®, see Interferon.

PegIntron®, see Interferon.

Pentacarinat®, see Pentamidine.

Pentamidine

Manufacturer: Sanofi-aventis/GlaxoSmithKline.

Indications and trade name: treatment and secondary prophylaxis of Pneumocystis pneumonia if cotrimoxazole is contraindicated (hypersensitivity, resistance to treatment). Also for visceral leishmaniasis.

• Pentacarinat® injection vials, 300 mg

Dosage: for treatment, 200–300 mg Pentacarinat® QD IV for five days (4 mg/kg), then halve the dose. In very mild cases, daily inhalations with 300 mg. In renal failure and creatinine clearance of 10 to 50 ml/min: 4 mg/kg q 24–36 h; below 10 ml/min: 4 mg/kg q 48 h. Prophylaxis: inhalation of 300 mg 1–2 x month.

Side effects: frequent with intravenous dosing. Nausea, vomiting, metallic taste; nephrotoxicity (increased creatinine in the second week of treatment) up to renal failure. Hypo- or hyperglycemia (possible even months after end of treatment), hypotension, arrhythmia, pancreatitis. Leukopenia and thrombocytopenia. Inhalation may induce cough, rarely asthma attacks.

Interactions, warnings: For inhalation, pentamidine as an aerosol is contraindicated in asthma. Inhalation is ineffective with several pulmonary diseases. Prior inhalation of a ß-mimetic (e.g., Berotec®) is desirable.

For infusions, caution with liver or renal failure, hyper- or hypotension, hyperglycemia, cytopenia. Always ensure sufficient intake of electrolytes and fluids. No concurrent administration of other nephrotoxic drugs (e.g., aminoglycosides, amphotericin B, foscarnet). Patient should remain in supine position before, during and after infusions of pentamidine (caution: hypotension). Pentamidine should be infused slowly over at least 2 hours. Daily monitoring of renal function, blood count, fasting blood glucose, urinalysis and serum electrolytes, weekly monitoring of bilirubin, alkaline phosphatase, transaminases.

Pyrimethamine

Manufacturer: GlaxoSmithKline.

Indications and trade name: prophylaxis and treatment of cerebral toxoplasmosis. Prophylaxis of *Pneumocystis pneumonia*.

• Daraprim® tablets, 25 mg

Dosage: treatment of toxoplasmosis, Daraprim® 2 tablets (50 mg) BID (for 3 days, then 1 tablet BID) + Leucovorin® 3 x 1 tablets/week each 15 mg + either sulfadiazine, clindamycin or atovaquone (second choice).
For PCP prophylaxis in combination with dapsone, Daraprim® 2 tablets (50 mg) per week + Dapsone® 1 tablet (50 mg) QD + Leucovorin® 2 tablets (30 mg) per week.

Side effects: nausea, colic, vomiting, diarrhea, leukopenia, anemia or thrombocytopenia. Rarely seizures, tremor or ataxia.

Interactions, warnings: pyrimethamine is contraindicated in megaloblastic anemia resulting from folic acid deficiency. Caution in patients with seizures, renal failure, asthma or G6PD deficiency. All patients taking pyrimethamine should receive folinic acid (not folic acid) to decrease risk of myelosuppression. Initial monitoring of blood count at weekly intervals.

Raltegravir

Manufacturer: MSD.

Indications and trade names: treatment-naïve and pretreated HIV+ patients.

• Isentress® film-coated tablets, 400 mg
• Dutrebis® film-coated tablets, 300 mg plus 150 mg 3TC

Dosage: 1 tablet of 400 mg BID with or without food. In patients with renal or moderate hepatic impairment, no dose adjustment is required. The lower dosage in Dutrebis® is possible due to a new formulation.

Side effects: raltegravir is very well tolerated – in studies, there have generally been no more adverse events than seen with placebo. At a frequency of 1% to 10%, dizziness, stomach ache, flatulence, obstipation, hyperhidrosis, arthralgia, tiredness, weakness. CK elevations. Recent FDA warning on suicidal thoughts. Case reports on rhabdomyolysis, hepatitis, insomnia.

Interactions, warnings: raltegravir is eliminated via UGT1A1-mediated glucuronidation, so that relevant interactions with other antiretroviral agents are unlikely. Strong inducers of UGT1A1 like rifampicin reduce plasma levels of raltegravir. If a combination is unavoidable, raltegravir dose should be doubled. Omeprazole or other gastric acid inhibitors may increase the plasma levels of raltegravir.

Comments: first-in-class integrase strand transfer inhibitor. Well-tolerated, effective in the setting of multiple resistances as well as in ART-naïve patients. Low interaction potential. Relatively low resistance barrier, BID dosing required (new once daily formulation currently under investigation).

For detailed information see page: 102

Rebetol®, see Ribavirin.

Rescriptor®, see Delavirdine.

Retrovir®, see AZT.

Reyataz®, see Atazanavir.

Rezolsta®, see Darunavir.

Ribavirin

Manufacturers: Roche and Essex. Several generics available.

Indications and trade names: chronic hepatitis C, only in combination with interferon and with some DAAs. In Europe, the license for HIV/HCV-coinfected patients only applies to Copegus®.

• Copegus® film-coated tablets, 200 mg, 400 mg
• Rebetol® hard capsules, 200 mg. Solution, 40 mg/ml

Dosage: daily dose 800 mg for body weight <65 kg, 1000 mg for 65–85 kg, 1200 mg for >85 kg. Capsules are divided into two daily doses and taken with meals. Treatment duration depends on the genotype and success of treatment.

Side effects: the most frequent side effect is hemolytic anemia (Hb decrease by at least 2 g/dl obligatory), gastrointestinal complaints, headache and fatigue may also occur. Rarely lactic acidosis, pancreatitis in combination with NRTIs.

Interactions, warnings: ribavirin is contraindicated in severe coronary disease, renal failure, decompensated liver cirrhosis, and hemoglobinopathy. It is also contraindicated in pregnancy (teratogenicity).

Dose reduction for hemoglobin <10 g/dl. Reduce dose to 600–800 mg/day. Discontinue ribavirin at hemoglobin values <8.5 g/dl. Before reducing or discontinuing ribavirin, however, consider erythropoietin and transfusions. Avoid concurrent treatment with other myelosuppressive drugs (AZT).

Ribavirin can lead to lactic acidosis in combination with other NRTIs. Most importantly, ddI should be avoided while care should be taken with all other NRTIs like d4T. Possible antagonism with abacavir (mechanism unclear). Efavirenz-induced depression may worsen on ribavirin.

Monitoring of lab values (blood count, ALT, amylase, lipase) initially at biweekly intervals and then monthly. Measure lactate if unspecific symptoms occur.

Comments: still used with some DAA combinations for HCV therapy. Caution with hematotoxicity.

Rifabutin

Manufacturer: Pfizer.

Indications and trade name: infections with *Mycobacterium avium* complex (MAC) in combination with other drugs (usually ethambutol and azithromycin). Also for patients with tuberculosis, when rifampicin is contraindicated.

• Mycobutin® (previously, Alfacid®) capsules, 150 mg.

Dosage: 300 mg rifabutin daily (+ azithromycin + ethambutol).

Renal failure, dose reduction by 50% for creatinine clearance <30 ml/min.

Dose adjustments for concurrent dosing with antiretroviral drugs:

Drug	Recommendation
Atazanavir/r, darunavir/r, fosamprenavir/r, indinavir/r, lopinavir/r, saqui-navir/r, tipranavir/r	Rifabutin: 150 mg every other day or three times per week (see product information)
Nelfinavir	Nelfinavir 1250 mg BID + rifabutin 150 mg/day
Efavirenz	Increase rifabutin to 450 mg/day or 600 mg twice or three times weekly
Nevirapine	Standard dose

Side effects: Nausea, vomiting, elevation of liver enzymes, jaundice. Uveitis usually only with a daily dose >300 mg and concurrent treatment with clarithromycin or fluconazole. Red discoloration of urine, skin and body secretions (inform patients about this).

Interactions, warnings: Rifabutin should not be used in thrombocytopenia and severe hepatic dysfunction. Monitor blood count and liver enzymes initially biweekly and then monthly.

Rifabutin can decrease the efficacy of the following drugs: analgesics, anticoagulants, corticosteroids, cyclosporine, digitalis (except digoxin), dapsone, oral antidiabetics, oral contraceptives, narcotic analgesics, phenytoin and quinidine. Erythromycin, different azoles can increase plasma levels of rifabutin. Antacids should be taken at least three hours after rifabutin.

Rifampin

Manufacturers: several companies, also part of several FDCs (see below).

Indications and trade names: tuberculosis. Use only in combination.

• Rifa® tablets, 150, 300, 450, 600 mg rifampicin
• Eremfat® syrup, 20 mg rifampicin per ml
• Eremfat® IV, 300 mg and 600 mg
• Rifinah® or Tebesium duo® film-coated tablets, 300 mg rifampicin +150 mg INH
• Rifater® or Tebesium trio® sugar-coated film-coated tablets, 120 mg rifampicin + 50 mg isoniazid + 300 mg pyrazinamide

Dosage: 600 mg daily (body weight >50 kg) or 450 mg (body weight <50 kg). Ideally taken in the morning on an empty stomach.

Side effects: toxic hepatitis (up to 20%), cholestatic changes. Red discoloration of urine and other body fluids (inform patients of this). Soft contact lenses may permanently stain red. Allergies are frequent. Nausea, vomiting, diarrhea.

Interactions, warnings: caution in patients with chronic liver disease. Discontinue rifampin if ALT >100 U/l or with elevated bilirubin (careful on re-exposure, gradually increasing doses is possible after normalization of values), and with patients who experience severe and persistent diarrhea (pseudomembranous colitis).
Rifampin should be avoided if concurrent NNRTIs or PIs are necessary. Rifampin increases metabolism of numerous drugs, reducing their efficacy if administered concurrently. These drugs include atovaquone, warfarin, barbiturates, benzodiazepines, beta blockers, clarithromycin, contraceptives, steroids, oral antidiabetics, cyclosporine, dapsone, digitalis, doxycycline, erythromycin, haloperidol, ketoconazole, methadone, phenytoin, theophylline, trimethoprim, verapamil.
Combination with ketoconazole or voriconazole is contraindicated. Antacids, opiates and anticholinergics reduce the bioavailability of orally administered rifampin, if given simultaneously. To avoid this interaction, rifampin should be given several hours before these drugs. Not for use in pregnancy. Blood count and liver values should be monitored every two weeks.

Rilpivirine

Manufacturer: Janssen-Cilag.

Indications and trade names: ART naïve patients with a plasma viremia of less than 100,000 copies/ml, since 2013 also for preteated patients with virological suppression and without resistance mutations.

• Edurant® film-coated tablets, 25 mg rilpivirine (RPV)
• Complera® film-coated tablets, 25 mg RPV + 200 mg FTC + 300 mg tenofovir

Dosage: 25 mg QD, always to be taken with a meal (at least 400 calories, with fat).

Side effects: Headache, insomnia. CNS symptoms occur less frequently than with efavirenz. A generally mild rash may occur in the first weeks (continued treatment is usually possible). Prolongation of the cardiac QTc interval was observed in studies of HIV-uninfected subjects given supratherapeutic doses of rilpivirine.

Interactions, warnings: An acidic gastric environment is necessary for absorption – PPIs should not be given to persons taking rilpivirine. Rilpivirine is a substrate of hepatic cytochrome P450 3A, so drugs that induce or inhibit the action of this isoenzyme may alter serum rilpivirine levels. Rifamycins, anticonvulsants (eg,

carbamazepine and phenytoin), and St. John's wort may substantially decrease rilpivirine concentrations and should be avoided. Macrolides, azole antifungals and PIs may increase rilpivirine levels.

Comments: Rilpivirine is a NNRTI which was approved in 2011. It is mostly used in the single tablet regimen Complera/Eviplera®. In untreated patients, rilpivirine is restricted to patients with low viremia. As with all NNRTIs, drug interactions and a low resistance barrier have to be considered. Food intake is important!

For detailed information see page: 86

Ritonavir

Manufacturer: AbbVie.

Indications and trade names: HIV infection. Also a component of Kaletra® and of the anti-HCV drug Viekirax®.

• Norvir® tablets, 100 mg. Soft gel capsules, 100 mg
• Norvir® oral solution, 80 mg per ml (7.5 ml = 600 mg)

Dosage: in rare cases, when ritonavir is used as a single PI, the dose is 600 mg BID (increase dose over two weeks: 300 mg BID on day 1–2, 400 mg BID on day 3–5, 500 mg BID on day 6-13). However, ritonavir should ideally be used only for boosting of other PIs. Daily doses in combination with:
• Atazanavir (Reyataz®, 300 mg QD), 100 mg ritonavir QD
• Darunavir (Prezista®, 600 mg BID), 100 mg ritonavir BID
• Darunavir (Prezista®, 800 mg QD), 100 mg ritonavir QD
• Fosamprenavir (Telzir®, 700 mg BID), 100 mg ritonavir BID, also 1400 mg
   fosamprenavir QD + 200 mg QD (US only, naive patients)
• Indinavir (Crixivan®, 800 mg BID), 100 mg ritonavir BID
• Lopinavir (Kaletra®) fixed-dose combination, see lopinavir/r
• Saquinavir (Invirase®, 1000 mg BID), 100 mg ritonavir BID
• Tipranavir (Aptivus®), 200 mg ritonavir BID
• HCV: Ombitasvir and Paritaprevir, fixed-dose combination, see Viekirax®

Side effects: depending on dosage, frequent, nausea, vomiting, diarrhea, perioral paresthesia and electric sensations on arms and legs. Elevated transaminases and GGT, dyslipidemia, lipodystrophy and rarely diabetes mellitus.

Interactions: even the low boosting doses have multiple drug interactions. The following drugs are contraindicated: rifampin, amiodarone, astemizole, bepridil, terfenadine, encainide, flecainide, cisapride, triazolam, ergotamine, simvastatin, lovastatin, quinidine and St. John's wort. Sildenafil should be avoided.
Caution should be taken and plasma levels measured for both ritonavir and (if possible) the following comedications: methadone, immunosuppressants (cyclosporine, tacrolimus), macrolide antibiotics, steroids, calcium antagonists, tricyclic antidepressants, other antidepressants, neuroleptics (haloperidol, risperidone, thioridazine), antimycotic drugs (ketoconazole, itraconazole), carbamazepine, tolbutamide, rifabutin, theophylline, and warfarin.

Comments: One of the first PIs. The dosage required to inhibit HIV replication is too toxic. Today ritonavir should only be used as booster for other antiretroviral drugs (mainly PIs). Numerous interactions.

For detailed information see page: 95

Saquinavir

Manufacturer: Hoffmann-La Roche. Generics available!

Indications and trade names: HIV infected adults.

• Invirase 500® film-coated tablets, 500 mg saquinavir

Dosage: 1,000 mg saquinavir BID + 100 mg ritonavir BID, with a meal.

Side effects: diarrhea, nausea, abdominal discomfort, meteorism. Rarely elevation of transaminases or γGT, headache. As with other PIs, lipodystrophy, dyslipidemia and reduced glucose tolerance may occur with long-term treatment. QT and PR interval prolonging.

Interactions, warnings: contraindicated with rifampin, astemizole, terfenadine, cisapride, triazolam, ergotamine, simvastatin, lovastatin, and St. John's wort. Use with caution in patients with arrhythmias or certain heart diseases (QT prolongation, torsades de points). ECG monitoring is required in all patients prior to initiation and at 3–4 days on therapy (for detailed information, see package insert).

Comments: saquinavir was the first PI to be licensed for HIV therapy in 1995. High pill burden, gastrointestinal problems and QT prolongation hamper its use.

For detailed information see page: 95

Sempera®, see Itraconazole.

Simeprevir

Manufacturer: Janssen-Cilag.

Indication and trade name: Together with sofosbuvir, in patients with chronic hepatitis C GT1 (US label) or GT1 or GT4 infection (EU label). Recommended treatment duration is 12 (24) weeks for patients without (with) cirrhosis.

• Olysio® hard capsules, 150 mg

Dosage: 150 mg QD, with food. The capsule should be swallowed whole.

Side effects: Rash (including photosensitivity, mostly mild or moderate severity), pruritus, nausea, myalgia, dyspnea. Most side effects occurred in the first 4 weeks of treatment. Hyperbilirubinemia.

Interactions, warnings: In patients infected with GT1a, HCV-screening for the NS3 Q80K polymorphism is recommended as efficacy is reduced when used with pegIFN/RBV (combination with sofosbuvir in IFN-free regimens may mitigate the negative effect Q80K has on simeprevir). Simeprevir is not recommended in patients with moderate/severe hepatic impairment and in patients who have previously failed therapy with other HCV PIs (resistance testing).
Simeprevir is a substrate and inhibitor of CYP3A4 and other enzymes, and therefore may have significant interactions with many antiretroviral agents that are metabolized by the same pathways. Coadministration with efavirenz, nevirapine, etravirine, boosted PIs and elvitegravir/c is not recommended. Preferred antiretroviral agents are maraviroc, raltegravir, dolutegravir, rilpivirine, NRTIs.

Comments: A new HCV PI which was approved in 2014. Limited data in HIV coinfected patients, numerous interactions have to be considered.

For detailed information see page: 459

Sofosbuvir

Manufacturer: Gilead Sciences.

Indication and trade names: chronic hepatitis C, genotypes (GT) 1-4. Numerous regimens, depending on HCV genotype and patient population (pretreatment and liver function). GT1 or GT4: Combination with other DAAs (ledipasvir – see Harvoni®, but also daclatasvir, simeprevir). Without other DAAs in GT2: Plus ribavirin 12 weeks (extension to 16 weeks in cirrhotics), GT3: plus ribavirin 24 weeks.

• Sovaldi® film-coated tablets, 400 mg
• Harvoni® film-coated tablets, 400 mg plus 90 mg ledipasvir

Dosage: 400 mg QD, with or without food. For patients with severe renal impairment (<30 mL/min), dose recommendation cannot be made

Side effects: Well-tolerated, fatigue, headache, nausea, insomnia. Bradycardia with amiodarone coadministration!

Interactions, warnings: Coadministration of amiodarone is not recommended. Bradycardia may occur, particularly in patients also receiving beta blockers, or those with underlying cardiac comorbidities and/or advanced liver disease. As sofosbuvir is a substrate of drug transporter P-gp, P-gp inducers may decrease plasma concentration: coadministration with tipranavir, carbamazepine, phenytoin, phenobarbital, rifampicin, rifabutin or St. John's wort is not recommended. No dose adjustment with NRTIs, rilpivirine, efavirenz, darunavir/r or raltegravir.

Comments: Potent HCV nucleotide analog NS5B polymerase inhibitor which was approved in 2014. Used in various regimens, only limited interactions with ART. Frequently used in HIV/HCV coinfected patients.

For detailed information see page: 459

Sovaldi®, see Sofosbuvir.

Stavudine, see d4T.

Stocrin®, see Efavirenz.

Stribild®

Manufacturer: Gilead Sciences.

Indications and trade name: adult HIV+ patients who are treatment-naïve or who are virologically suppressed on a stable antiretroviral regimen for at least 6 months with no history of treatment failure and no known substitutions associated with resistance to the individual components.

• Stribild® film-coated tablets with 150 mg elvitegravir, 150 mg cobicistat, 200 mg FTC, 300 mg TDF.

Dosage: one tablet daily in the evening, unchewed, on an empty stomach.

Side effects: Usually well tolerated. Nausea (mild), diarrhea (slightly more frequently than with raltegravir), ALT elevation (less than with raltegravir). A modest elevation in serum creatinine (0.1–0.2 mg/dl) and decrease in estimated creatinine clearance (CrCl 10-15 ml/min) is to be expected in most patients due to a cobicistat-related inhibition of tubular creatinine secretion. Actual GFR is not affected.

Interactions, warnings: Data on interactions are limited. Do not use in patients with renal impairment (CrCl <70 ml/min). Other nephrotoxic agents should be avoided. Routine monitoring of estimated creatinine clearance, urine glucose, and urine protein should be performed in all patients.

Comments: The third complete ART in one single tablet per day (STR = single tablet regimen), the first including an integrase inhibitor. For side effects, see also sections on tenofovir (caution with renal function) and cobicistat. A new formulation with tenofovir-alafenamide (TAF) is expected for the end of 2015.

For detailed information see page: 193

Sulfadiazine®

Manufacturer: Heyl, among many others.

Indications and trade name: treatment and prophylaxis of cerebral toxoplasmosis, only in combination with pyrimethamine.

• Sulfadiazin-Heyl® tablets, 500 mg

Dosage: For treatment, 2–3 500 mg tablets QD (= daily dose 4–6 g). For prophylaxis, halve the dose (500 mg QD). Renal insufficiency: creatinine clearance 10–50 ml/min: halve dose, <10 ml/min: one third of the dose.

Side effects: very frequently allergies with pruritus, fever and urticaria, often treatment-limiting. Rarely Stevens-Johnson syndrome. Nausea, vomiting, diarrhea. Renal problems with renal failure, crystalluria, nephrolithiasis in up to 7%. Anemia, leukopenia, thrombocytopenia. Elevated liver enzymes.

Interactions, warnings: sulfadiazine is contraindicated in sulfonamide hypersensitivity in G6PD deficiency, renal failure, severe hepatic disease or dysfunction (e.g., acute hepatitis) and during pregnancy and breastfeeding.
Sulfadiazine can increase the effect of sulfonylurea urea (oral antidiabetics), anticoagulants, diphenylhydantoin. Concurrent use of antacids reduces absorption of sulfadiazine (separate administration by 1–2 hours). Ensure sufficient intake of fluids (at least 2 l daily). Initially, monitor blood count, ALT, creatinine, and BUN at least weekly. Monitor urine. In case of crystalluria, alkalize urine.

Sustiva®, see Efavirenz.

T-20 (enfuvirtide)

Manufacturer: Hoffmann-La Roche.

Indications and trade name: treatment of patients with evidence of HIV replication despite ongoing ART with at least one PI, any NRTI or NNRTI.

• Fuzeon® 90 mg/ml powder and solvent

Dosage: 90 mg subcutaneously BID.

Side effects: generally well-tolerated. However, almost all patients have local injection site reactions: erythema, inflammation, induration, rash. Possibly an increased risk of bacterial pneumonia. It is important to be particularly vigilant in patients with risk factors for pneumonia (low baseline CD4 counts, high viral load, IV drug users, smokers, history of pulmonary disease).

Hypersensitivity reactions with rash, fever, nausea, chills, hypotension or elevated transaminases are rare (<1%).

Interactions, warnings: interactions are not known. Injection sites – upper arm, ventral hip, and abdomen. Change injection sites often. On the back, possibly fewer irritations. Do not inject at sites with inflammatory signs from previous injections. Do not inject at sites with birth marks, scars or disrupted skin integrity.

Comments: T-20 is an entry inhibitor used for heavily treatment-experienced patients. T-20 must be injected subcutaneously BID. Rarely used. Expensive, may double the price of ART.

For detailed information see page: 113

Telaprevir

Manufacturer: Janssen-Cilag/Vertex.

Indications and trade name: in combination therapy with peg-interferon alfa and ribavirin for patients with chronic hepatitis C, genotype 1. Response-guided regimen, depending on viral response and prior response status.

• Incivek® film-coated tablets, 375 mg (Europe: Incivo®)

Dosage: 750 mg taken 3 times a day (7–9 hours apart) with food (not low fat).

Side effects: Nausea (try haloperidol), vomiting, fatigue, diarrhea, pruritus, anemia. Mild skin rashes are common, leading to discontinuation of the drug in up to 7%.

Comments: Released to much fanfare in 2011, this HCV NS34A protease inhibitor had a rapid rise and fall. Facing new and better options for hepatitis C, Vertex announced in 2014 the discontinuation of development and sales of telaprevir.

Tenofovir

Manufacturer: Gilead Sciences.

Indications and trade names: HIV infection, chronic hepatitis B.

• Viread® film-coated (fc) tablets, 300 mg tenofovir disoproxil fumarate
• Truvada® fc tablets, 300 mg + 200 mg FTC
• Atripla® fc tablets, 300 mg + 200 mg FTC + 600 mg efavirenz
• Complera® fc tablets, 300 mg + 200 mg FTC + 25 mg rilpivirine
• Stribild® fc tablets, 300 mg + 200 mg FTC + 150 mg elvitegravir + 150 mg cobicistat

Dosage: 300 mg QD, to be taken with a meal. Dose adjustments in patients with renal impairment are required. Double dosage interval (every 48 hours) at moderate kidney dysfunction (creatinine clearance 30–49 ml/min, below 30 ml/min it should be avoided). In hemodialysis patients, every 7 days following completion of hemodialysis.

Side effects: generally well-tolerated. Rarely, renal side effects (renal tubulopathies including Fanconi's syndrome, nephrogenic diabetes insipidus). Bone loss, osteomalacia. Rarely, elevation of liver enzymes. CK rises observed in up to 48% (macro CK, relevance is unclear).

Interactions, warnings: patients with existing renal disease should either not receive tenofovir or, with no alternatives, reduce the dose. Check creatinine clearance and serum phosphate before starting therapy, during the first year of treatment every four weeks and thereafter every three months. When serum phosphate is <1.5 mg/dl (0.48 mmol/l) or creatinine clearance <50 ml/min check renal function again within one week. Simultaneous determination of blood glucose and potassium, as well as glucose in the urine. Interruption of therapy may be necessary, if creatinine clearance is <50 ml/min or serum phosphate is <1.0 mg/dl (0.32 mmol/l).

Creatinine clearance in ml/min is calculated as follows:
Women: (1.04 x (140 – age) x kg) / creatinine (µmol/l)
Men: (1.23 x (140 – age) x kg) / creatinine (µmol/l)

Concurrent administration of tenofovir and drugs that are also eliminated via active tubular secretion can lead to increased serum concentrations of both drugs: cidofovir, acyclovir, valacyclovir, ganciclovir, valganciclovir.
Do not combine with ddI, comedication with tenofovir increases the AUC of ddI by 44%. Atazanavir and lopinavir increase tenofovir levels. Tenofovir lowers the plasma levels of atazanavir (always boost with 100 mg of ritonavir).

Comments: one of the most frequently used drugs in HIV medicine. Part of several fixed-dose regimens. However, potential nephrotoxicity has to be taken into account as well as some interactions. A less toxic compound (tenofovir alafenamide) is in development.

For detailed information see page: 75

Tipranavir

Manufacturer: Boehringer Ingelheim.

Indications and trade name: HIV-infected adults who are either highly treatment-experienced or who have multiple PI resistance.

• Aptivus® capsules, 250 mg

Dosage: 500 mg BID + 200 mg BID ritonavir with meals.

Side effects: Frequent side effects are gastrointestinal, diarrhea and nausea. Elevated transaminases in at least 6%, with clinical hepatitis and liver failure in rare cases. More frequent than with other PIs dyslipidemia (20%). Rare rash (urticarial or maculopapular). Occasional reports (and FDA warning) of intracranial bleedings (causality unclear).

Interactions, warnings: Tipranavir is a substrate, activator and inhibitor of cytochrome CYP3A and both a substrate and inhibitor of the P-glycoprotein. Consequently, various interactions have to be taken into account. Several antiarrhythmics, antihistamines, ergotamines and sedatives (midazolam) should be avoided. Tipranavir reduces the serum level of other PIs, so a double PI regime is not applicable. Fluconazole and clarithromycin increase the serum level of tipranavir. Antacids reduce tipranavir levels by 30% (administer separately).
Rifampicin reduces tipranavir levels by 80% (avoid). Dose reduction by at least 75% for rifabutin: 150 mg every other day or three times per week. Tipranavir/r increases the serum levels of atorvastatin by 8-10 fold (use another statin like pravastatin or fluvastatin). Tipranavir should not be used in patients with moderate to severe hepatic impairment. Use cautiously in patients with HBV or HCV coinfection. Determine transaminases monthly during the first 6 months.

Comments: Tipranavir has been the first non-peptidic PI and may be helpful in some salvage settings. Tipranavir has to be boosted with elevated ritonavir doses. Numerous interactions have to be taken into account.

For detailed information see page: 96

Tivicay®, see Dolutegravir.

Triumeq®

Manufacturer: ViiV Healthcare.

Indications and trade name: HIV infection, adults

• Triumeq® film-coated tablets, 300 mg 3TC + 300 mg ABC + 50 mg dolutegravir

Dosage: one tablet OD, with or without food. In cases of impaired renal function (creatinine clearance less than 50 ml/min), the individual drugs should be given separately to allow for dose adjustment of 3TC. When Triumeq® is used with other antiretroviral agents (in salvage settings), coadministration with efavirenz or tipranavir/r or integrase resistance mutations may require an additional dolutegravir 50 mg tablet, intake separated by 12 hours.

Side effects: usually well-tolerated, see also individual drugs, especially abacavir (HSR and cardiovascular events).

Warnings: Should not be used in patients with previous hypersensitivity reaction to abacavir.

Comments: First single-tablet regimen (STR) on the market that does not contain tenofovir (+FTC) as a backbone. Highly effective, high resistance barrier. HLA screening for abacavir HRS is required prior to initiation.

For detailed information see page: 193

Trizivir®

Manufacturer: ViiV Healthcare.

Indications and trade name: HIV infection.

• Trizivir® film-coated tablets, 150 mg 3TC + 300 mg AZT + 300 mg ABC

Dosage: 1 tablet BID. In cases of impaired renal function (creatinine clearance less than 50 ml/min), the individual drugs should be given separately to allow for dose adjustment of 3TC.

Side effects: mostly gastrointestinal, see individual drugs. Abacavir HSR (see abacavir). There are possibly additive effects with regard to mitochondrial toxicity. Possibly an increased risk for cardiovascular events (see abacavir).

Comments: Triple-NRTI, less effective than multi-class combinations. Further disadvantages include mitochondrial toxicity, abacavir HSR. QD dosing is not possible. Thus, Trizivir® is only indicated in individual cases.

For detailed information see page: 197

Truvada®

Manufacturer: Gilead (Truvada®)

Indications and trade name: HIV infection.

• Truvada® film-coated tablets, 300 mg tenofovir + 200 mg FTC.

Dosage: 1 tablet QD. Caution in patients with renal dysfunction. If there are no alternatives, with a reduced creatinine clearance of 30–49 ml/min, then it is recommended to reduce the dose to 1 tablet every two days (<30: avoid Truvada®). Absorption of Truvada® not affected by food intake.

Side effects: monitoring of renal parameters, see tenofovir.

Interactions, warnings: see tenofovir. In patients coinfected with chronic hepatitis B, Truvada® is preferred. Exacerbation of hepatitis may occur after discontinuing Truvada®.

Comments: NRTI combination pill consisting of tenofovir and FTC. To date, one of the most frequently prescribed HIV drugs. Well-tolerated. However, renal dysfunction and bone loss may occur (see tenofovir).

For detailed information see page: 76

Tybost®, see Cobicistat.

Valganciclovir

Manufacturer: Hoffmann-La Roche. Generics available!

Indications and trade name: induction and maintenance therapy of CMV retinitis.

• Valcyte® or Valganciclovir® tablets, 450 mg

Dosage: for induction therapy 900 mg BID for 3 weeks (or until scar formation of CMV lesions), then suppressive therapy with 900 mg QD. Should be taken with a meal. The following doses should be used for renal failure:

Cr Cl (ml/min)	Induction therapy	Suppressive therapy
≥ 60	900 mg BID	900 mg QD
40–59	450 mg BID	450 mg QD
25–39	450 mg QD	450 mg q 48 h
10–24	450 mg q 48 h	450 mg 2 x week

Side effects: frequent leukopenia, also thrombocytopenia, anemia. Gastrointestinal complaints such as nausea, vomiting and diarrhea are more frequent than with intravenously-administered ganciclovir.

Warnings: monitoring of blood count at least 2–3 x week during induction. Discontinue if neutrophils below 500/µl (G-CSF if needed). Contraindicated in neutropenia <500/µl, thrombocytopenia <25,000/µl and concurrent chemotherapy. Caution when concurrent dosing with ddI, as valgancyclovir can double levels of ddI (increased toxicity). Valganciclovir is potentially teratogenic and carcinogenic; reliable contraception is required. The drug is expensive. It should be discontinued when sufficient immune reconstitution has been reached (see chapter on OIs).

Comments: Valganciclovir was the first effective anti-CMV drug to be administered orally. It is a prodrug of ganciclovir and has a similar toxicity profile, including neutropenia, anemia and thrombocytopenia.

Victrelis®, see Boceprevir.

Videx®, see ddI.

Viekirax®

Manufacturer: AbbVie.

Indications and trade name: In combination with ribavirin and/or dasabuvir for the treatment of chronic hepatitis C in adults (GT1 and GT4, details below).

• Viekirax® film-coated tbl, 12.5 mg ombitasvir, 75 mg paritaprevir, 50 mg ritonavir

Dosage: Two tablets QD with food. Patients should be instructed to swallow the tablets whole (do not chew, break or dissolve the tablets). No dose adjustment is required for patients even with severe renal impairment. Contraindicated in patients with severe hepatic impairment (Child-Pugh C).

Side effects: Well-tolerated. The most common side effects are fatigue and nausea. ALT elevations.

Interactions, warnings: Combination with ribavirin in genotype 4, with dasabuvir in genotype 1b without cirrhosis, with dasabuvir and ribavirin in genotype 1a and in cirrhotic patients with 1b. Duration 12 weeks, 24 weeks only in cirrhotic patients with genotype 1a or 4. Given that several CYP enzymes and drug transporters are involved in the metabolism of ombitasvir, paritaprevir, ritonavir (and dasabuvir), complex drug-drug interactions are likely, especially in the setting of HIV coinfection. When either ritonavir or cobicistat is used, the boosting agent should be discontinued during HCV therapy. In HIV+ patients not on ART, other HCV options should be considered because ritonavir has low activity against HIV (risk of resistance!).
Atazanavir or darunavir (should be taken in the morning at the same time, without ritonavir, since ritonavir 100 mg once daily is provided as part of Viekirax®) can be used. Other PIs are contraindicated. Raltegravir exposure is increased (2-fold), no adjustment required. Rilpivirine exposure is increased (3-fold). Due to its potential for QT-prolongation, rilpivirine should be used cautiously, in the setting of repeated ECG monitoring. NNRTIs other than rilpivirine (efavirenz, etravirine and nevirapine) are contraindicated.

Comments: Second-generation DAAs in a fixed-dose combination for hepatitis C, containing ritonavir as a booster. Good data for genotypes 1 and 4. In HIV+ patients, data is limited and complex interactions with ART have to be considered.

Viracept®, see Nelfinavir.

Viramune®, see Nevirapine.

Viread®, see Tenofovir.

Vistide®, see Cidofovir.

Vitekta®, see Elvitegravir.

Zerit®, see d4T.

Ziagen®, see Abacavir.

Zidovudine, see AZT.

Zovirax®, see Acyclovir.
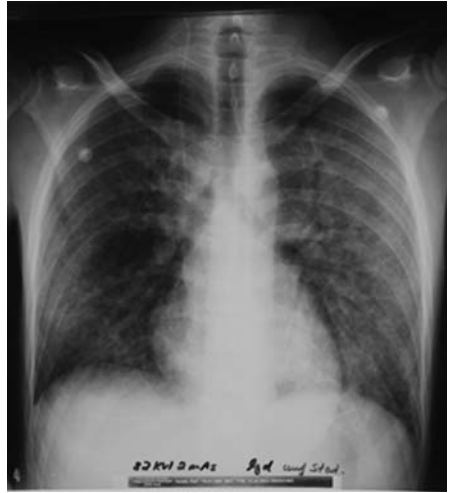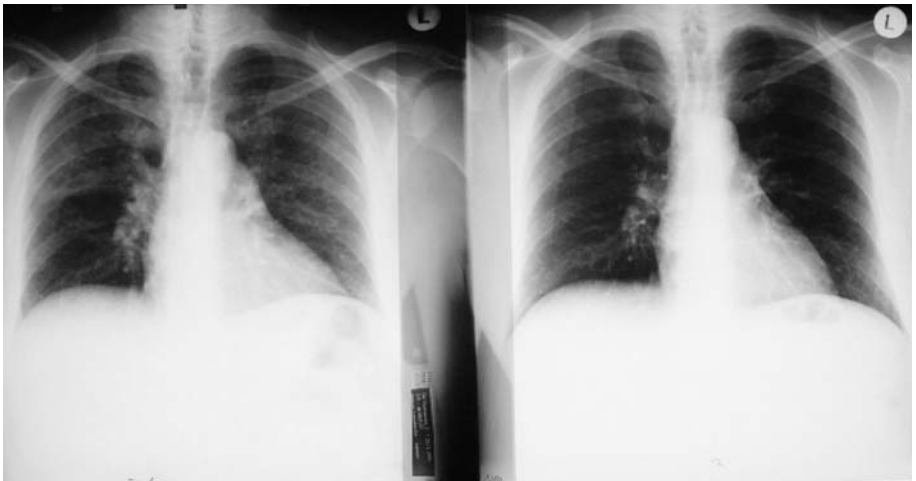
1. Pneumocystis pneumonia (PCP), chest x-ray

1. Interstitial infiltrates. Endotracheal intubation.

2. Typical PCP findings. Fine reticular interstitial pulmonary pattern

3. PCP, before and three weeks after treatment with co-trimoxazole.

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2. Pneumocystis pneumonia (PCP), CT scans
Ground-glass pattern predominantly involving perihilar and mid zones. Figure 3 shows also
several KS lesions (in the setting of an IRIS)
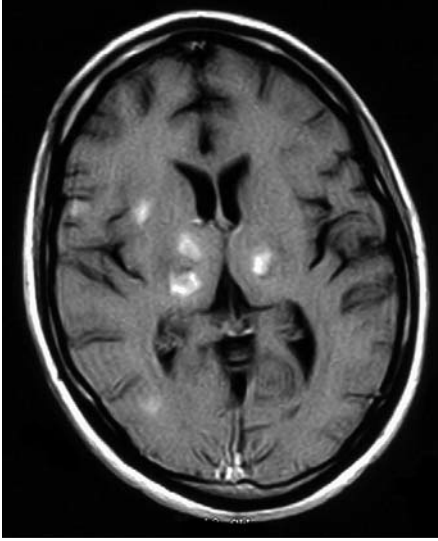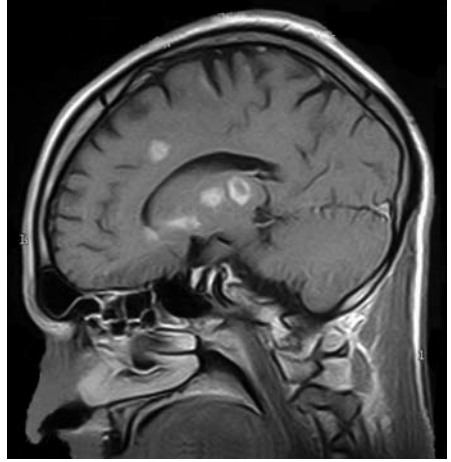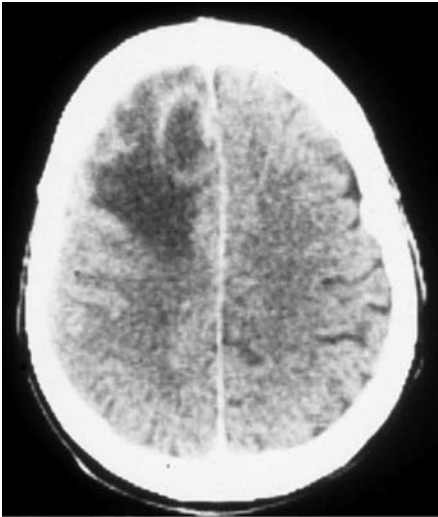
3. Cerebral toxoplasmosis (TE)
1. and 2. MRI scan of the same patient, multiple, small TE lesions.
3. Solitary TE lesion with typical ring enhancement (CT scan).
4. Cerebral CT scan with a large, solitary lesion and extensive edema.
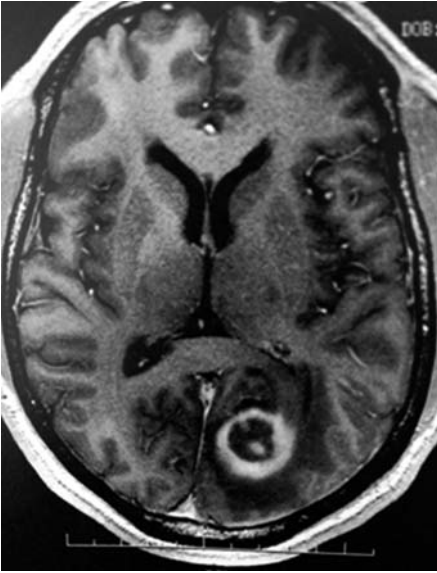
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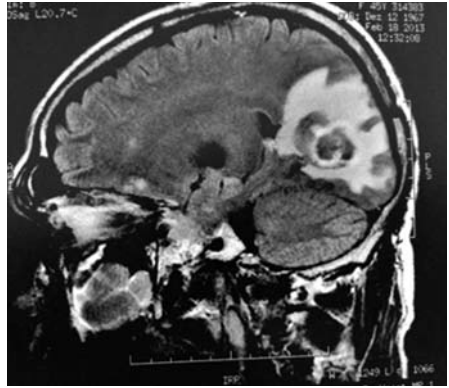
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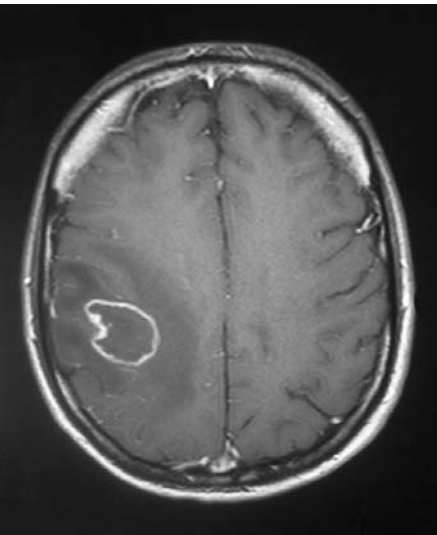
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4. Cerebral toxoplasmosis (TE), further images
1. and 2. Large lesion, occipital
3. Typical ring enhancement
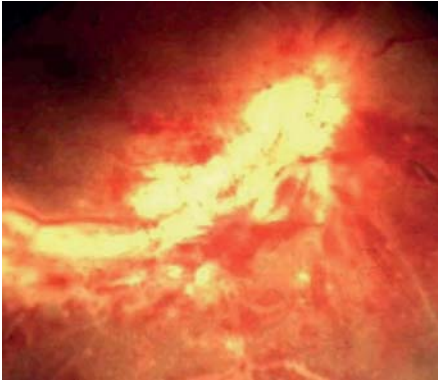
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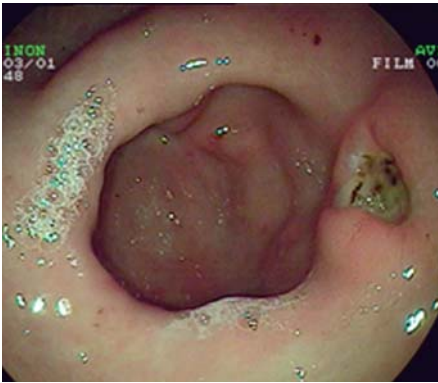
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5. Cytomegalovirus (CMV) diseases

1. Typical funduscopy findings, CMV retinitis
2. Large CMV ulcer on the tongue, severe immune deficiency
3. CMV-associated gastric ulcer
4. Esophageal CMV ulcers in which CMV was detected
5. Extensive CMV colitis
6. CMV proctitis

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6. Herpes simplex virus (HSV) infections

1. und 2. Refractory HSV-infection in a patient with massive immune deficiency (1), lesions completely resolved after weeks of foscarnet treatment (2).

3. HSV esophagitis

4. Large anal HSV ulcer

1

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4

7. Herpes zoster virus (HZV) infections

1. Zoster lesions at the right arm, hemorrhagic.

2. Zoster ophtalmicus

3. and 4. Zoster lesions at the upper back, dermatomes C7 and C8, prior and three weeks after therapy

1a

1b
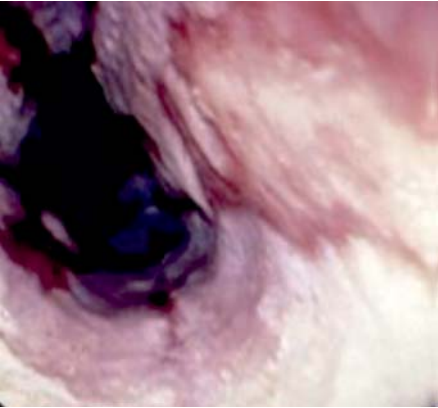
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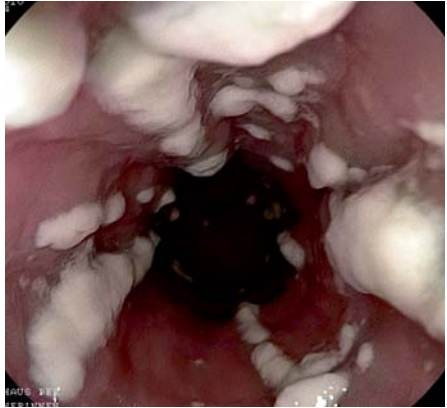
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8. Candidiasis, OHL
1a,b. Oral candidiasis (oral thrush).
2. and 3. Esophageal candidiasis, endoscopic pictures
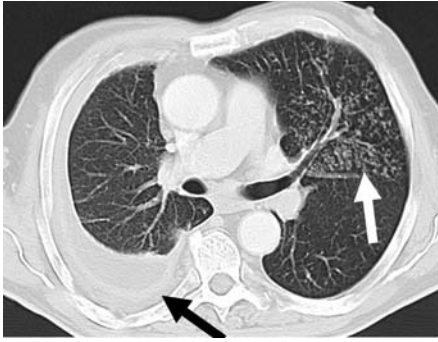
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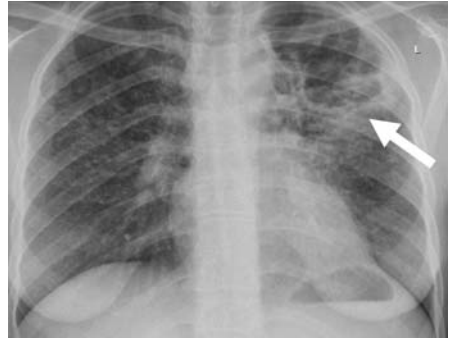
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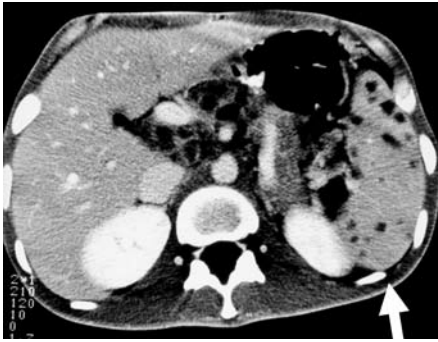
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9. Tuberculosis, different manifestations

1. Tuberculosis pleuritis with right sided effusion. Left sided "Tree-in-bud" phenomenon as seen in bronchial spread of tuberculosis.
2. Tuberculosis cavities in the left upper lobe. Right sided fine miliar nodules.
3. Tuberculosis, involvement of the spleen.

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10. Lymph node tuberculosis
Cervical and supraclavicular abscesses due to M. tuberculosis. In all patients, TB became manifest in the setting of an immune reconstitution syndrome (IRIS), shortly after initiation of ART.

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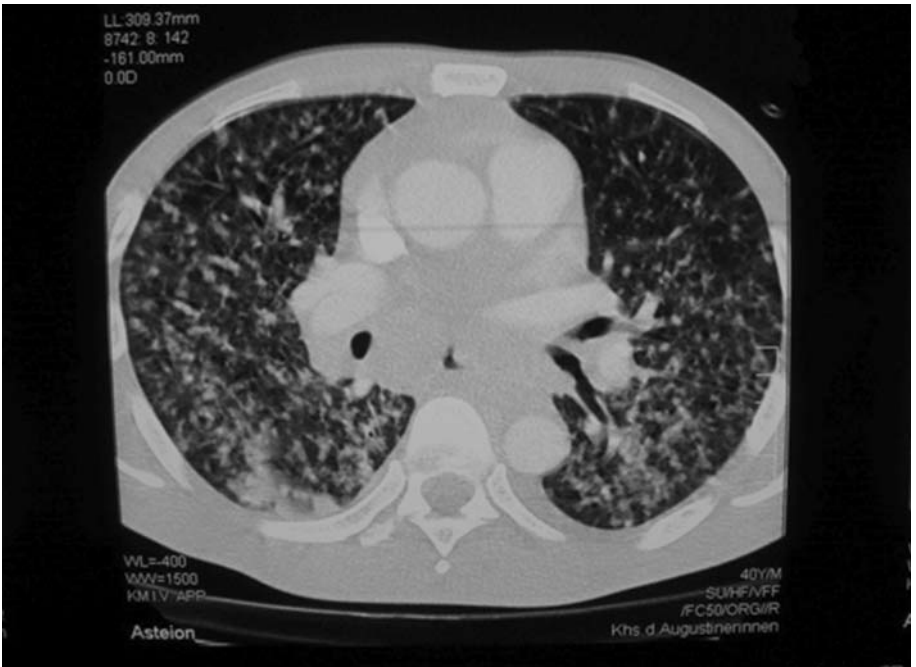
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11. Miliar tuberculosis, chest x-ray (above) and CT scan
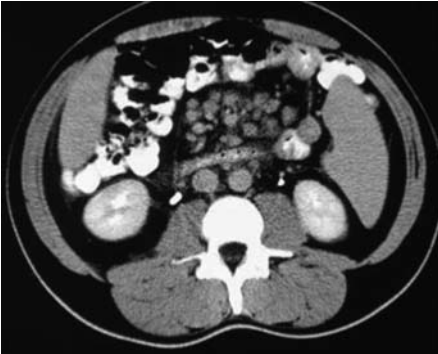
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12. Atypical mycobacterial infections
1. CT scan of the abdomen with multiple lymph nodes, infection with M. avium complex (MAC)
2. Abscess, detection of *M. xenopi* (manifestation as IRIS)
3. and 4. Intestinal infection of MAC
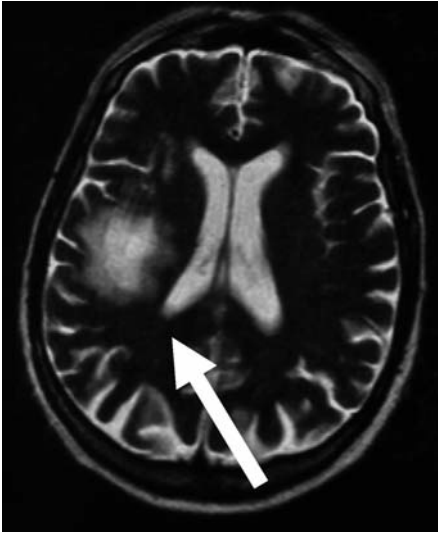
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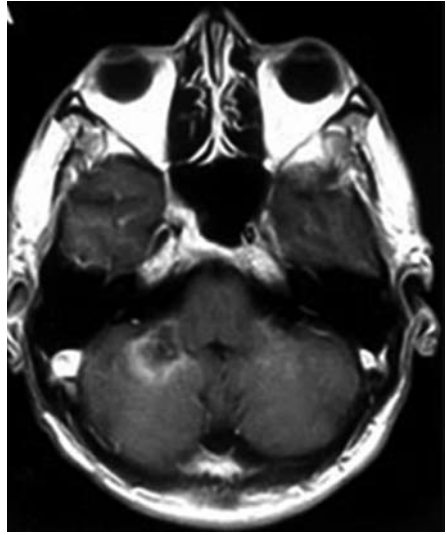
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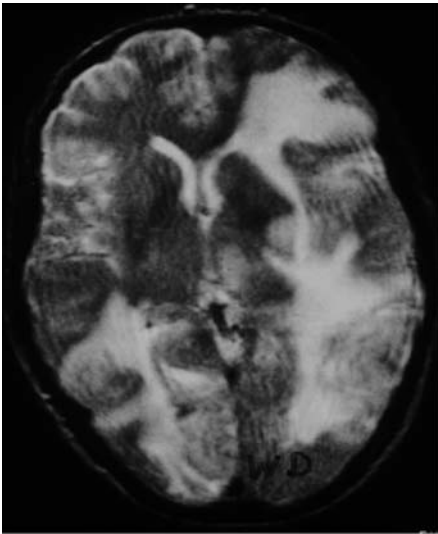
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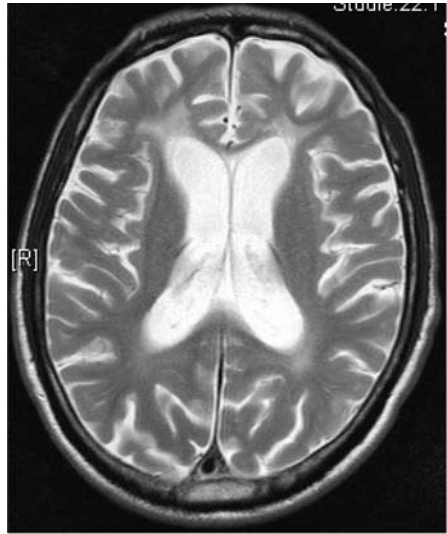
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13. PML and HIV-associated neurological deficit (HAND)

1. MRI with relatively discrete PML lesions (JCV was detected in the CNS)
2. Cerebellar involvement
3. MRI with extensive PML lesions
4. Axial T2-weighted MRI scan of a 60-year-old patient with HAND. Moderate atrophy, hyperintense lesions at the rostral and caudal ends of the cella media of lateral ventricles. This is typical but not specific for HAND
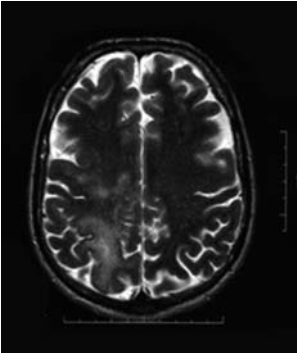
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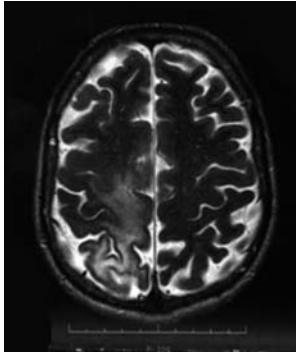
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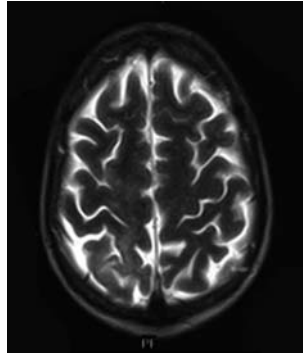
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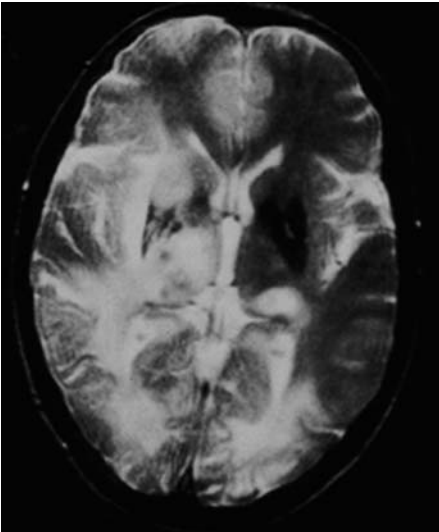
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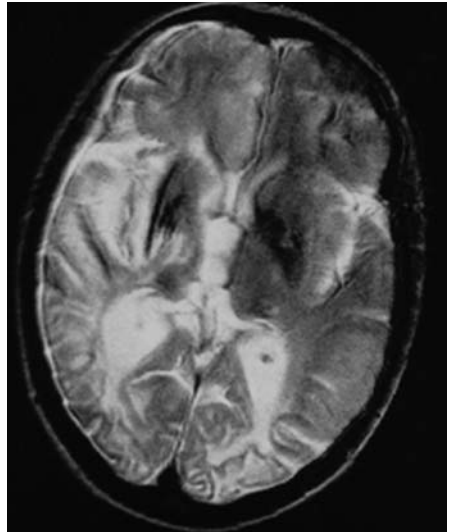
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14. PML

1.–3. PML during antiretroviral therapy. Picture 1 shows an occipital lesion two months after ART inititation (IRIS), picture 2 shows progression of the lesion a further two months later. Picture 3 with regression three months later, after immune reconstitution. No specific therapy for PML was given.

4. and 5. Extensive Lesions before and six months after initiation of ART. Partial regression (T2-weighted scan)
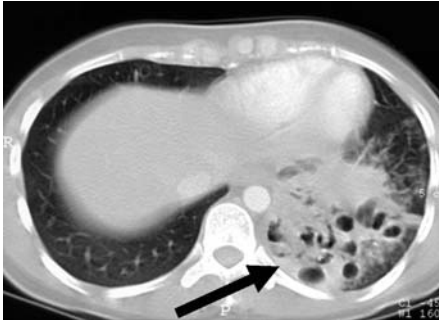
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15. Mycotic infections

1. CT scan of the lungs of a Thai patient with pulmonary cryptococcosis.
2. Chest x-ray, pulmonary cryptococcoma
3. CT scan, pulmonary aspergilloma (and CMV pneumonia)
4. and 5. Cutaneous infection with *Penicillium marneffi* (non-AIDS-defining, but common infection in HIV+ patients from South East Asia)

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16. Kaposi's sarcoma (KS), cutaneous manifestations
All lesions were biopsy proven

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1b

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17. Kaposi's sarcoma (KS), mucocutaneous and visceral manifestations.

1. Involvement of hard palate, before (1a) and after four cylces of chemotherapy with liposomal doxorubicine (1b)
2. Penile KS lesion
3. Conjunctival KS cycles
4. Visceral KS
5. Pulmonary KS (plus PCP)
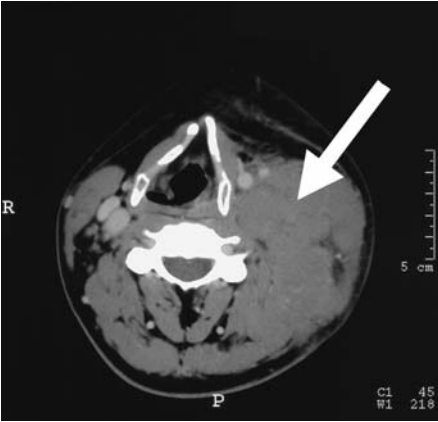
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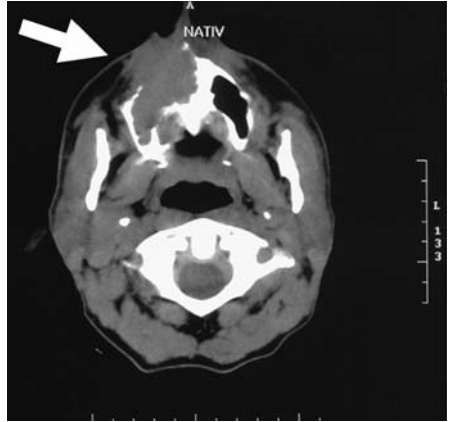
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18. Malignant Non Hodgkin lymphomas

1. Burkitt's lymphoma, cervical localization, rapidly growing.
2. Centroblastic Non-Hodgkin's Lymphoma (NHL), destruction of the nasal area.
3. Plasmablastic lymphoma of the oral cavity (rare subtype, almost exclusively occurring in HIV+ patients).
4. Primary CNS lymphoma, large solitary lesion with contrast enhancement.
5. Same patient, complete remission after radiation therapy.
6. Same patient, three years later, now with atrophy (clinical dementia) which was probably a long-term sequela of radiation therapy.
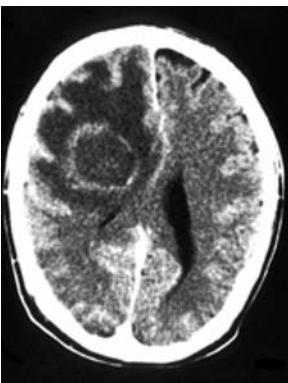
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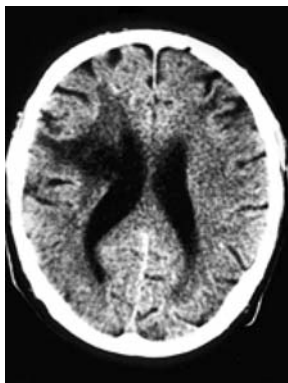
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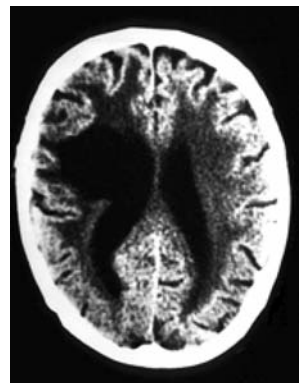
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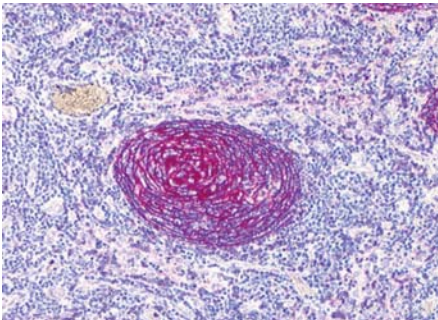
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**19. Hodgkin's disease (HD), Multicentric
Castleman's Disease (MCD)**

1. and 2. HD with cervical manifestation,
   before and after ABVD chemotherapy
   (complete remission).
3. MCD with hepatosplenomegaly (CT scan of
   the abdomen).
4. MCD, large swollen spleen (autopsy finding).
5. MCD, histological findings, germinal center
   with a typical "onion-skin" pattern.

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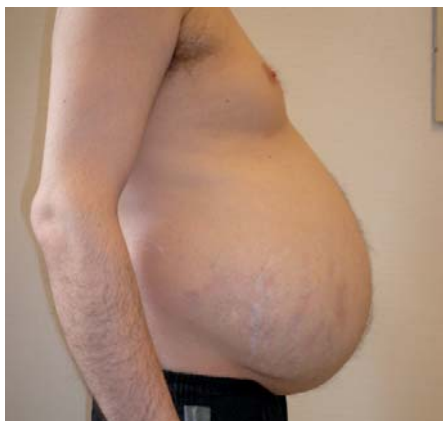
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20. Adverse events during ART. Lipodystrophy, hypertrophic manifestation
1. and 2. Lipodystrophy with dorsocervical fat pads ("buffalo hump")
3.–5. Abdominal visceral fat accumulation

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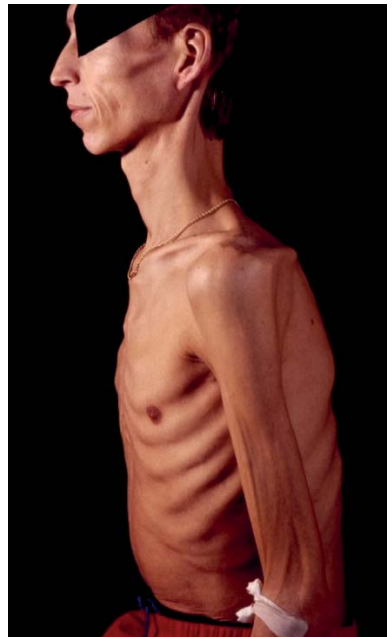
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21. Adverse events during ART. Lipoatrophy

1. Loss of buccal fat.
2. Due to subcutaneous fat loss, a portacath system (used for CMV treatment) is visible at the clavicle.
3. and 4. Loss of subcutaneous fat and bulging veins at the legs, after years of NRTI treatment ("d-drugs" such as d4T and ddI)
5. Wasting syndrome (AIDS-defining illness, untreated patient, no lipoatrophy!)
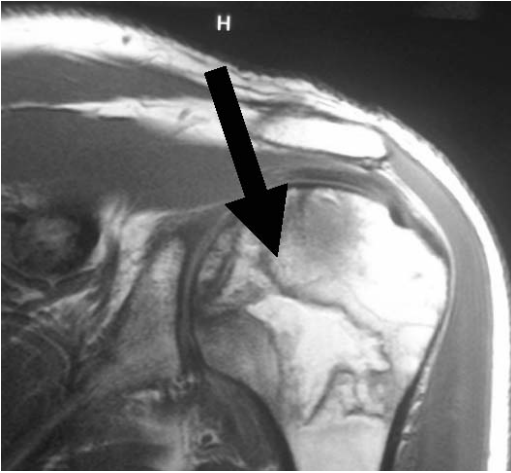
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22. Adverse events during ART, different findings

1. Avascular necrosis of the humeral head, possibly due to PI therapy
2. Blood sample of a patient with a hypertriglyceridemia of > 3,000 mg/dl which was caused by PI therapy.
3. Ergotism with gangrene as a result of vasoconstriction induced by ergotamine in a patient receiving lopinavir and ritonavir. The effects of ergotamine may be potentiated by cytochrome P450 3A4 inducers such as ritonavir

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23. Adverse events during ART, allergic reactions

1.–3. Rash in patients treated with nevirapine (one to three weeks after initiation)

4. Rash, occurring in a patient who started darunavir/r

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24. Different cutaneous manifestations in HIV+ patients

1. Macular exanthema in the setting of an acute HIV infection
2. Seborrheic dermatitis (indicator disease!) with flaky to form on oily areas
3. Cutaneous porphyria (extrahepatic manifestation of HCV coinfection, resolving after successfull HCV treatment)
4. Scabies infection
5. Sideeffects of "chemsex" in an HIV+ MSM. Injecting mephedrone (and other agents) may cause severe skin erosions and limb abscesses

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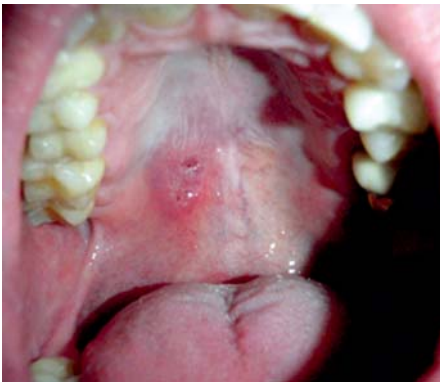
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25. Syphilis and Lymphogranuloma Venereum (LGV)

1.–3. Primary syphilis with ulcers at different sites.

4. LGV, painful penile ulcer (L3 serotype of *Chlamydia trachomatis*) and swollen lymph nodes (arrow).

5. Multiple ulcers, primary syphilis, painless

6. Very painful penile ulcer, LGV (syphilitic ulcers are ususally painless)

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26. Secondary Syphilis
Variable dermatological findings. Syphilis is an important indicator disease!

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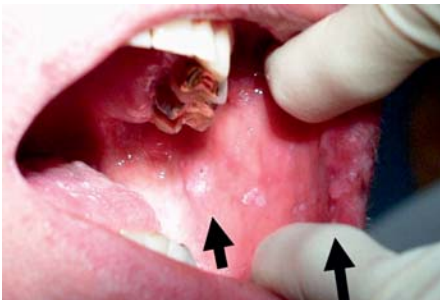
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27. Oral manifestations in HIV+ patients (oral thrush see candidiasis)

1. and 2. Oral hairy leukoplakia (OHL) with typical plaques on the sides of the tongue which cannot be scraped off
3. Solitary oral wart on tongue
4. Solitary wart on oral mucosa
5. Plaques muqueuses, oral manifestation of syphilis
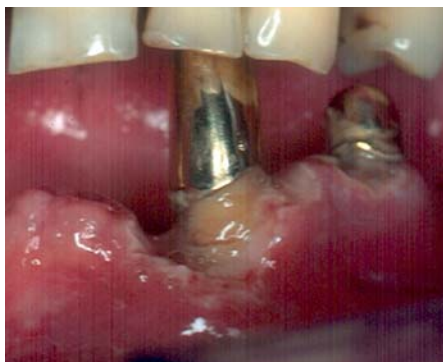
28. Other oral manifestations in HIV+ patients
1. Necrotizing ulcerative parodontosis on teeth 33 and 35.
2. Linear gingival erythema on vestibular gingiva.
3. Generalized chronic parondontosis.
4. Disseminated oral warts on vestibular gingiva.
5. Mouth ulcer vestibular on tooth 37

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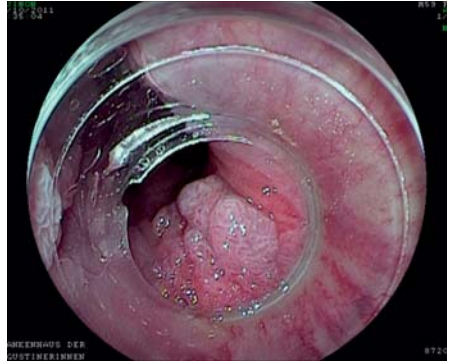
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29. Condylomata acuminata and differential diagnosis
1. and 2. Anal condylomata acuminata.
3. Genital condylomata acuminata.
4. Bowenoid papulosis.
5. Perianal infection with *Molluscum contagiosum virus* (no HPV involved)
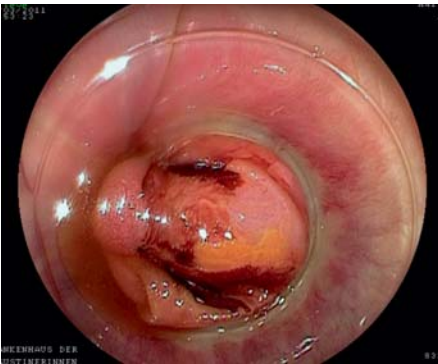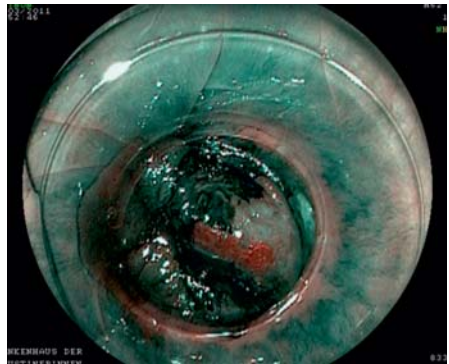
30. Anal intraepithelial neoplasia (AIN)
1. In all lesions, AIN III was found
2. Arrow shows AIN III (green Seton drainage of a fistula)
3. Anal dysplasia (yellow)
4. Anal dysplasia (red, FICE)

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31. Anal cancer and differential diagnosis

1. HIV+ female patient with "hemorroids". Diagnosis: Invasive anal cancer, stage T2
2. Verrucous carcinoma of the anus.
3. HIV+ patient with a three-week history of a growing tumour: Chlamydial proctitis.
4. and 5. Painful ulcer due to LGV (chlamydia L3 serotype), firstly misinterpretated as anal cancer, before and after three weeks of doxycycline

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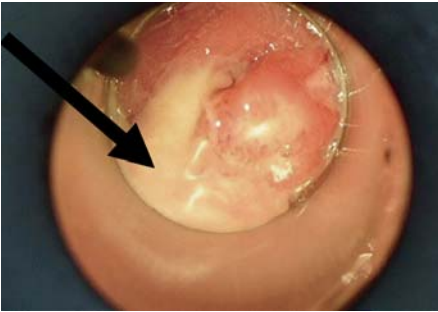
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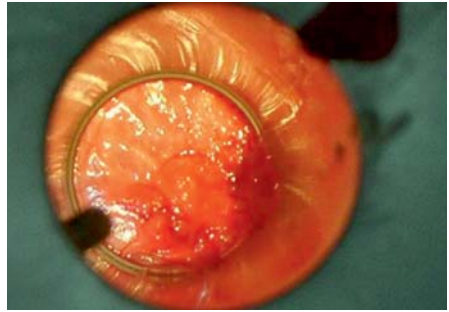
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32. Other proctological findings
1. Anal CMV infection, extremely painful ulcers.
2. Anal HSV infection, also extremely painful ulcers.
3. Purulent gonococcal proctitis.
4. Very painful purulent proctitis (detection of both gonoccoccus and chlamydia L3 serotype)

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