CASPER ROKX

HIV: Treatment and Comorbidity

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HIV: Behandeling en Comorbiditeit

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Jules Deelder

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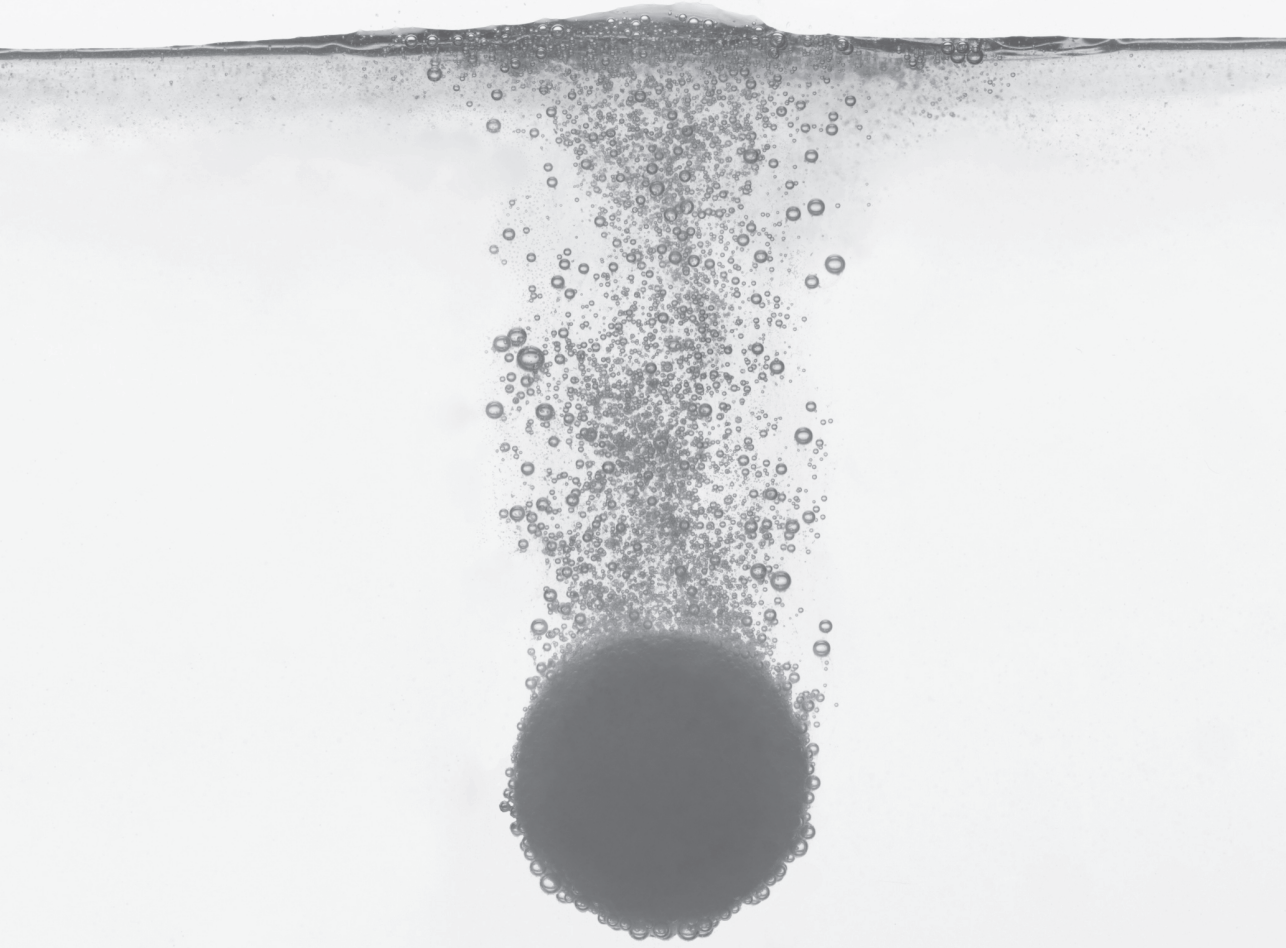


Chapter 1

General introduction and outline of the thesis

HIV: FROM PAST TO PRESENT

The human immunodeficiency virus type 1 (HIV) can establish a permanent infection in its host which results in the acquired immune deficiency syndrome (AIDS) and death without treatment. HIV has been responsible for over 75 million confirmed human infections worldwide since its discovery in 1983.[1, 2]

The identification of the causative virus of AIDS came nearly 60 years after the cross-species transmission of the simian immunodeficiency virus (SIV) from primates, primarily chimpanzees (*pan troglodytes troglodytes*), to humans. The first forward transmission networks in humans were formed in the Congo River basin.[3] The epicenter of the epidemic has been remarkably well defined and tracked down to Kinshasa, capital of the Democratic Republic of Congo. The virus could spread inland to the cities Kisangani, Mbuji-Mayi and Lubumbashi and to the coastal city of Pointe-Noire by transportation networks over the Congo river and railroads during a 40 year period. Apart from the pandemic group M, three other HIV lineages (group N, O, and P) have emerged from autonomous cross-species transmission events. The lineages N, O, and P are substantially less disseminated globally.[4] Independent onward transmission by iatrogenic interventions and sexual behaviour changes took place and gave rise to the divergent HIV subtypes. Both the HIV subtype C, accounting for 50% of all HIV infections primarily in Africa, and the HIV subtype B, the most widespread subtype, originated from HIV group M as distinct monophyletic clusters. HIV subtype B is dominant in the Western world and was exported from Africa to Haiti around 1966.[5] A single virus migration to the United States of America in 1969 is thought to have caused HIV's global dispersion. The virus remained unrecognised until the first reports of alarming medical conditions associated with decreased cellular immunity in young homosexual men in San Francisco, New York City and Los Angeles in 1981.[6, 7]

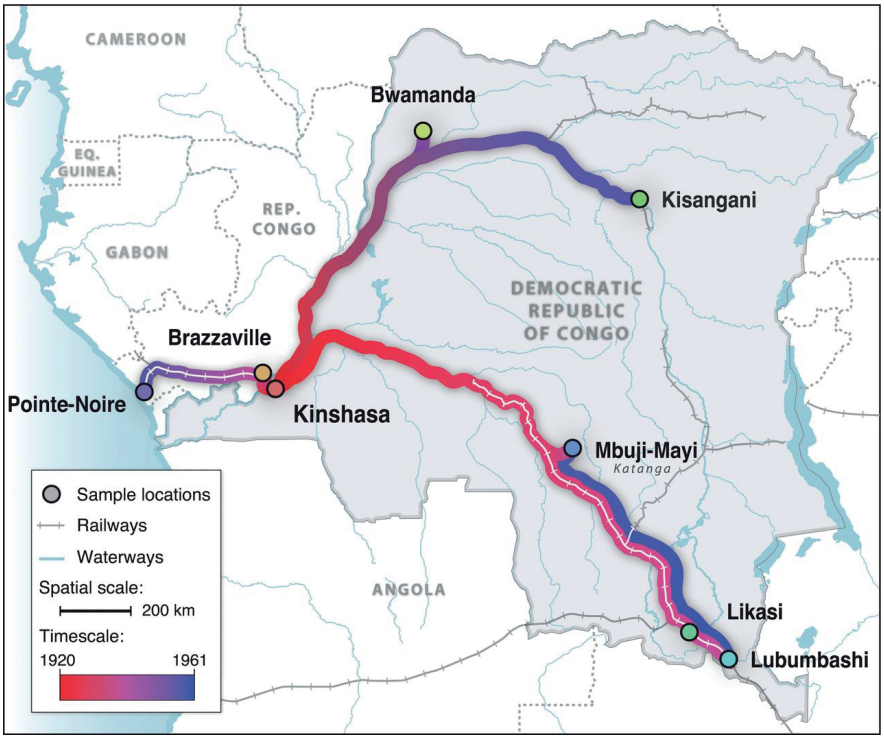


Figure 1. Early HIV dissemination networks through railway and waterway transportation networks. Adapted from Faria et al.[3]

Nowadays, HIV has spread worldwide creating a pandemic since the inception of the early dissemination networks. An estimated 36.9 million people are HIV infected in 2015.[8] Approximately 22 million people infected with HIV do still not have access to lifesaving combination antiretroviral therapy (cART). Seventeen million of these people are unaware of their infection due to lacking testing facilities or fear of stigmatization. Nonetheless, the global response and cART upscale since 2000, when virtually no treatment was available in resource limited countries, has already averted an estimated 30 million new HIV infections and almost 8 million deaths related to AIDS.

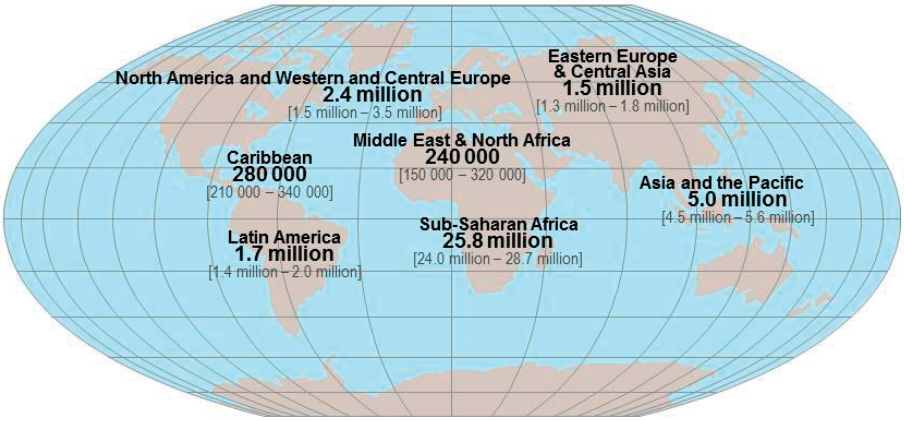


Figure 2. The global HIV epidemic in 2015. Adapted from UNAIDS.[8]

THE VIRUS

The lentivirus HIV was identified to cause AIDS in patients and belongs to the family *retroviridae*. Retroviruses have the ability to transcribe viral ribonucleic acid (RNA) in deoxyribonucleic acid (DNA) by the enzyme reverse transcriptase. A HIV particle is approximately 100 nm in diameter and consists of several components: the outer envelope with integrated matrix, the capsid, and the nucleocapsid which surrounds the core with two copies of single stranded genomic RNA molecules, enzymes and accessory viral proteins. The HIV genome, flanked by 2 long terminal repeat (LTR) regions, consists of around 9700 nucleotides encoding for structural (gag, pol, env), regulatory (tat, rev), and accessory (nef, vif, vpr, vpu) proteins.[9]

The gag gene encodes for the matrix protein (MA, p17) which forms the inside of the viral membrane, the capsid (CA, p24) which both counteracts the host restriction factor TRIM5α that destabilises the viral capsid and covers the nucleocapsid (NC, p7) that surrounds the viral RNA genome,[10] and p6 which is involved in viral membrane binding. The reading frame of the gag gene overlaps with the pol gene. The pol proteins are synthesized by ribosomal frameshifting of the gag-pol fusion product. These precursor proteins are cleaved by viral protease in the viral enzymes protease, integrase (p31) and the heterodimer reverse transcriptase (p51/p66, RT). RT is only

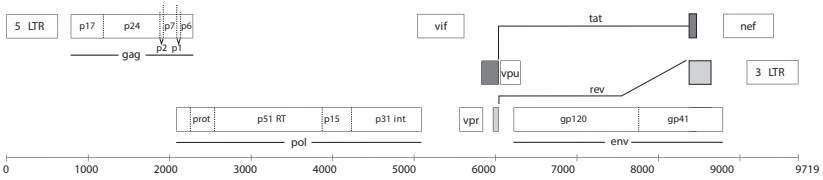


Figure 3. HIV genome. Adapted from Muesing et al.[9]

enzymatically active after dimerization in the enzymetically inactive subunit p51, and p66 which contains the active polymerase sites and the ribonuclease H (RNase H) domain.[11] The env gene encodes for the glycoprotein (gp)160 which is cleaved into the surface (SU) gp120 and transmembrane (TM) gp41 which are involved in target cell membrane receptor-virus fusion.[12]

Viral gene transcription is controlled by the transactivator of transcription (tat) and the regulator of expression of virion (rev) proteins which directly bind HIV RNA transcripts. Tat has multiple functions and relieves the repression of integrated HIV provirus LTR regions. The LTR region contains the enhancer and promotor elements and tat interacts with chromatin remodelling complexes and histone modifying enzymes.[13, 14] Tat also binds the transcriptional activation region (TAR) located at the 5' region of all initiated HIV RNA transcripts thereby inhibiting the premature abortion of HIV DNA transcription by RNA polymerase II. Both factors enhance the efficiency of full length transcription of viral messenger RNA (mRNA). The rev protein binds to the rev responsive element (RRE) on viral RNA in the nucleus.[15] RRE is present on unspliced HIV RNA and singly-spliced HIV RNA that encodes for env and accessory proteins. This promotes the translocation of unspliced or singly-spliced RNA to the cytoplasm as mRNA and genomic RNA for assembly and inclusion in the virion.[16]

The accessory genes are nef (negative factor), vif (virion of infectivity factor), vpr (viral protein R), and vpu (viral protein U). These genes promote disease progression. Nef enhances HIV replication through downregulation of membrane receptors including cluster of differentiation (CD)4.[17, 18] The nef mediated CD_4 decline aids env incorporation in virions and virus release from the cell. Furthermore, nef inhibits human leucocyte antigen (HLA) class I expression on the cell membrane which hinders the cytotoxic mediated killing of infected cells.[19] The vif protein degrades the host cellular restriction factor APOBEC3. APOBEC3 causes lethal mutations by converting

cytosine to uracil in the negative strand of viral complementary DNA (cDNA).[20] The vpr protein helps the transport of the HIV DNA pre-integration complex into the nucleus and virus production in terminally differentiated cells.[21] The host restriction factor tetherin can capture virions at the cell surface and is counteracted by vpu. Apart from this role in the release of HIV from infected cells, vpu also downregulates newly formed CD4.[22]

A HIV infection is the result of the successful integration of intact replication competent viral HIV DNA into the host genome, which can be transcribed to produce a viral particle containing functional HIV RNA that is able to infect other cells. Circulating infectious HIV virions in the host can then be transferred to an uninfected individual following the transmission of body fluids with free virus, either through sexual intercourse, direct blood contact, vertical transmission (i.e. mother to child), or via breastfeeding.

NATURAL HISTORY

Viral transmission and infection

The large majority of patients are infected with HIV through sexual contact. HIV transmission between serodiscordant couples without access to cART varies with sexual practices and the integrity of the genital mucosal barrier. The HIV transmission risk is influenced by the height of the viral load in body fluids of the HIV infected person.[23] The risk per coital act ranges from 0.04% for female-to-male HIV transmission to 1.4% with unprotected anal intercourse.[24, 25] HIV transmission risks are increased through direct blood-blood contact by sharing needles in intravenous drug users or transfusion. Mother to child transmission can occur in utero, peripartum or by breastfeeding. These routes are important transmission causes worldwide.

Successful host infection following HIV transmission during sexual intercourse can be established by the transmission of only one infectious virus particle.[26] Infection requires HIV integration in the host DNA of the CD4+ T lymphocytes. T lymphocytes are rarely present in the genital mucosa. The virus therefore invades the antigen presenting cells (APC) of the genital mucosa, known as Langerhans cells (LC) in the epithelium and dendritic cells (DC) in the subepithelium. LC are the first targets and bind HIV through C-type lectin Langerin. This transmembrane protein forms

the Birbeck granules in which the virus is degraded under normal circumstances.[27] Langerin can be saturated by high HIV loads. Inflammation increases tumor necrosis factor α (TNFα) levels. Elevated TNFα and genital coinfections that trigger toll like receptor (TLR)1/2 on LC can enhance HIV transmission.[28] The immature LC is transformed in an APC able to transfect CD4+ T lymphocytes.[29] Langerin might also be inhibited by gene mutations which reduce the HIV binding capacity of LC and increase subepithelial DC uptake.[30] Notably, LC and thus Langerin are absent in the rectal mucosa.[31] The DC are innate immune cells that encounter HIV in the subepithelium if the epithelial mucosa is breached. The DC specific intercellular adhesion molecule (ICAM)-3-grabbing-nonintegrin (DC-SIGN) can bind HIV gp120 and internalise the captured virus.[32] DC present the intact virus to the CD4+ T lymphocyte where viral transfer is mediated by DC-SIGN. The APC migrate to the lymphoid tissue within 3 to 5 days. The permanent infection of the host is completed after viral transfer with viral DNA integration in the CD4+ T lymphocyte. The virus can now reproduce, infect other CD4+ T lymphocytes and disseminate through the human body.

HIV cannot enter all human cells but only specific immune cells with a CD4 receptor. CD4 is expressed on T lymphocytes, macrophages, monocytes and DC.[12] HIV exploits two other coreceptors on the cell membrane, CCR5 or CXCR4.[33, 34] Tropism is based on the ability to use CCR5 (R5 strains), CXCR4 (R4 strains), or both (R5X4 strains). Viral gp120 interacts with CD4 and one of the coreceptors. The final step involves the insertion of gp41 in the cell membrane, creating a fused pore with viral uncoating and virus release in the cytoplasm.[35-37] A tropism shift from R5 to X4 is caused by one mutation in the V3 loop at position 322 of gp120.[38] R5 strains are preferentially replicated in newly infected patients even if both strains are present in the donor.[26, 39] During early HIV infection, CCR5+ CD4+ T lymphocytes are predominantly found in the gastrointestinal associated lymphoid tissue (GALT) and the blood.[40, 41] A homozygous 32 base pair deletion in exon 1 of the CCR5 gene prevents HIV from entering CD4+ T lymphocytes. Circa 1% of the Caucasians is homozygous carrier of the CCR5Δ32 mutation resulting in resistance against HIV.[42, 43]

Viral dynamics in humans

The intracellular viral life cycle in the CD4+ T lymphocyte starts after successful fusion and cell entry. The single stranded RNA template needs to be converted into DNA for integration in the human genome. The crucial enzyme RT for this process

contains three enzymatic functions to ensure proper HIV DNA formation: RNA dependent DNA polymerase, RNase H, and DNA dependent DNA polymerase.[44, 45] The two single stranded HIV RNA molecules are reversily transcribed by RT into cDNA in the cytoplasm. The RNA template from the RNA-DNA complex is degraded by RNase H, and DNA polymerase produces sense DNA from the antisense cDNA forming double stranded DNA. This pre-integration complex is transferred to the nucleus where HIV DNA is integrated in the host genome by integrase. HIV DNA integration does not appear to be a random process.[46] Hotspots for integration favor active genes,[47] including genes associated with cancer.[48, 49] The HIV DNA template is transcribed in activated HIV infected cells to produce viral transcripts necessary for viral protein production and progeny HIV RNA. Cellular transcription factors in activated HIV infected cells can further enhance HIV gene expression.

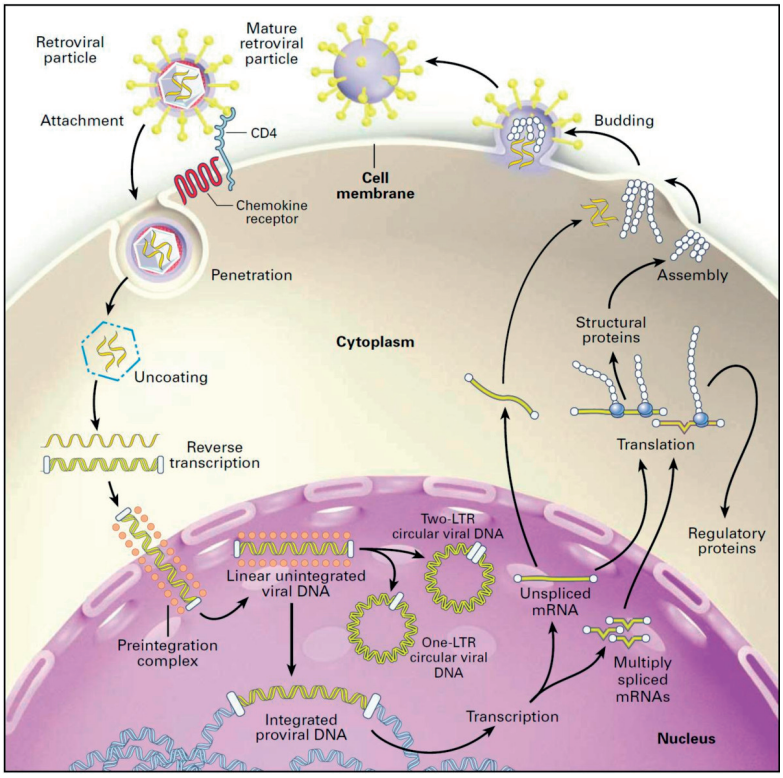


Figure 4. HIV replication life cycle. Adapted from Furtado et al.[134]

A subset of infected CD4+ T lymphocytes have integrated HIV DNA that is transcriptionally silent by cell reversal to a resting memory state. This is the latent HIV infected reservoir.[50] In transcriptionally activated infected cells, the HIV RNA is exported from the nucleus to the cytoplasm. Gag and the gag-pol polyproteins direct the transfer of gag, gag-pol, vpr, and HIV RNA to the cell membrane. The env protein, gag protein, gag-pol polyproteins, and 2 HIV RNA strain buds from the host cell. Tetherin prevents viral budding but is counteracted by vpu.[51] The gag and gag-pol proteins are cleaved in the structural components and enzymes in the immature virion by protease during the budding process. The virion is now mature and can infect CD4+ T lymphocytes by cell cell transfer or cell free spread in extracellular fluids including blood.

Modelling studies have provided insight in HIV dynamics in humans. The life span of a single HIV infected cell is approximately 2.2 days and the virions released from one cell are in the plasma for 0.3 days.[52] The time from virion release until infection with release of new virus from another cell is around 2.6 days. After viral release from the first cell, HIV spreads exponentially. The plasma viral load doubles every 0.65 days.[53] The basic reproductive number (R_o) of HIV is approximately 8; 1 infected cell infects 8 other cells on average. Exponantial growth is followed by a viral load setpoint which is interindividually variable and reflects the balance between viral production by activated CD4+ T lymphocytes and decay of these infected cells.[54] The total number of HIV infected CD4+ T lymphocytes producing HIV is approximately 0.5×10^9, with every single productive HIV infected cell able to produce 10^3 virions daily.[55-57]

All these viruses have the similar error prone rapid replication process after successful infection. HIV mutations occur during reverse transcription of HIV RNA and do not generally occur as an erroneous transcription of integrated HIV DNA. Mutants will develop because RT is prone for base substitution, insertion, or addition at a rate per incorporated nucleotide of 1/1700.[58] The (pro)viral population in HIV infected patients is heterogeneic, consisting of genetically linked quasispecies to one transmitted founder virus.[59] The 'wild-type' virus refers to the variant that is the most replication fit and predominant strain in the population.

Clinical stages of HIV

A patient will not notice the initial viral transmission and early HIV dissemination. This is called the eclipse phase of the acute, or primary, HIV infection and lasts 10

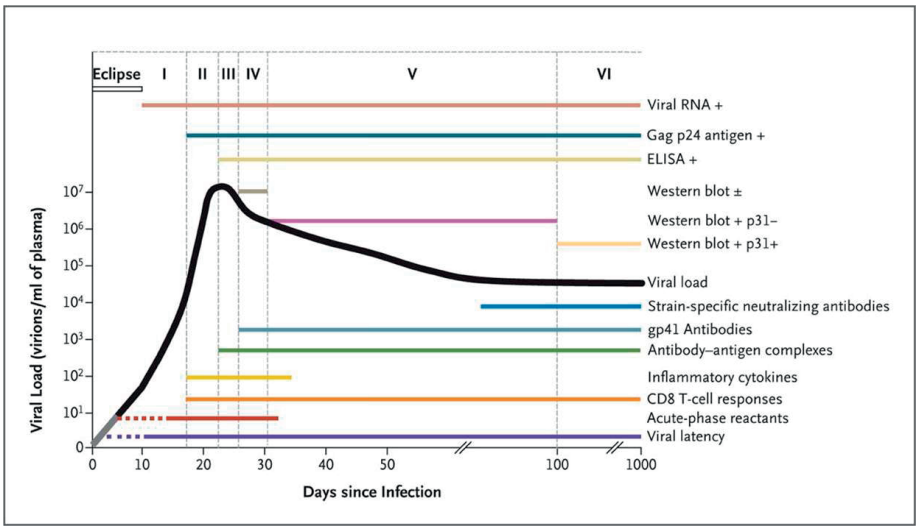


Figure 5. The stages of acute HIV. Adapted from Cohen et al.[489]

days. The Fiebig stages are the clinical stages of acute HIV infections.[60] The appearance of plasma HIV RNA classifies a patient in Fiebig stage I. The virus spreads hematogeneously through the body, including the GALT, which results in permanent damage during acute HIV.[61] P24 detection in plasma denotes Fiebig stage II. Fiebig stage III is the detection of HIV antibodies by enzyme linked immuno sorbent assays. Seroconversion means the detection of HIV antibodies. The subsequent Fiebig stages IV-VI include HIV antibody detection using Western blots. The detection of the p31 antibody signals the transition from acute to chronic HIV. At 3 to 4 weeks after infection the plasma viral load often peaks to above 10^6 HIV RNA copies per mililiter (copies/mL). Most patients have flu-like signs and symptoms at peak viremia: the acute retroviral syndrome.[62, 63] An estimated 50% of all HIV transmissions occur by undiagnosed patients with acute HIV infections because infectivity is highest at peak viremia.[64, 65] The majority of non productive infected CD4+ T lymphocytes die through pyroptosis, not apoptosis, in acute HIV infections.[66] Pyroptosis causes cytokine release and inflammation.[67] At the end of the acute infection, a dynamic equilibirum is achieved with a stable plasma viral load 'setpoint'. The persistent viremia indicates a suboptimal immune response.

Patients are often asymptomatic during chronic HIV but they remain infectious. Cellular immunity declines over 1 to 20 years until AIDS develops, the disease progresses

and the patient dies. The CD4+ T lymphocyte count is directly linked to HIV related morbidity. A CD4+ T lymphocyte count below 200 cells per mm^3 indicates a severe immunocompromised state which is known as AIDS. AIDS related diseases are classified as class C events in the Clinical Staging and Disease Classification System (CDC). They include opportunistic infections (herpesviridae, mycobacteria, pneumocystis jiroveci, toxoplasma and others), malignancies (aggressive B cell lymphoma, morbus Castleman, Kaposi sarcoma), AIDS dementia, and wasting.[68] AIDS is diagnosed if either the CD4+ T lymphocyte count drops below 200 cells per mm^3 or a CDC class C event occurs.

HIV PERSISTENCE

Immune response and reservoir formation

The innate and adaptive immune responses are insufficient to eradicate HIV. Crucial immunological insights in HIV pathogenesis have been derived from natural and non-natural host primates. Natural hosts do not have chronic immune activation or progression despite persistent viremia.[69, 70] Non-natural hosts have persistent viremia, chronic immune activation and progressive disease.[71]

The cells of the innate immune system are the first defence against HIV. These cells detect HIV with pattern recognition receptors.[72] Natural killer (NK) cells are early effector cells.[73] NK cells bind HLA class I proteins of infected cells with activating or inhibitory NK immunoglobulin-like receptors (KIR).[74] Macrophages and DC present HIV peptides by HLA class I to the T cell receptor (TCR) of the CD8+ T lymphocytes or by HLA class II to the TCR of the CD4+ T lymphocytes.[75, 76] This results in a cellular immune response against infected cells that present HIV peptides on HLA class I. Certain HLA class I, including B*27 and B*57, and a persistent immunodominant response give better cytotoxic T cell (CTL) responses and slower disease progression.[77-81] B lymphocytes can produce HIV specific antibodies as part of the humoral immune response. Neutralizing antibodies bind the virus particle and impair the function of HIV proteins (e.g. env). Non-neutralizing antibodies can trigger other immune cells. The breadth of HIV antibodies relates to the percentage of HIV strains that can be neutralized. CD4+ T lymphocytes are not only the target cells of HIV, but also have a role in the immune response.[82-84] CD4+ T lymphocytes can modulate the function of HIV specific CTL.[85] Furthermore, CD4+ T helper 17 cells

produce interleukin 17 that protect mucosal barriers and CD4+ follicular T helper cells might aid B lymphocytes.[86, 87] Last, chemokines are produced that compete with HIV for coreceptor binding.[88]

HIV is persistent and evades the immune system. HIV variance and immunological pressure select for viral strains that preferentially bind inhibitory KIR on NK cells. Regulatory T lymphocytes expand which downregulate the immune response.[89] Selection of HIV escape mutations impair TCR binding and cellular immunity.[90-92] The breadth of HIV antibodies is often insufficient with the development of antibody escape mutations.[93-95] Together, this results in a dysregulated immune system with chronic immune activation.[96] Intestinal bacterial products are translocated and contribute to immune activation.[97] The level of immune activation is correlated with mortality.[98] Eventually, T lymphocytes become exhausted and depleted.[99, 100] All factors combined cause HIV persistence, comorbidities and death.[101]

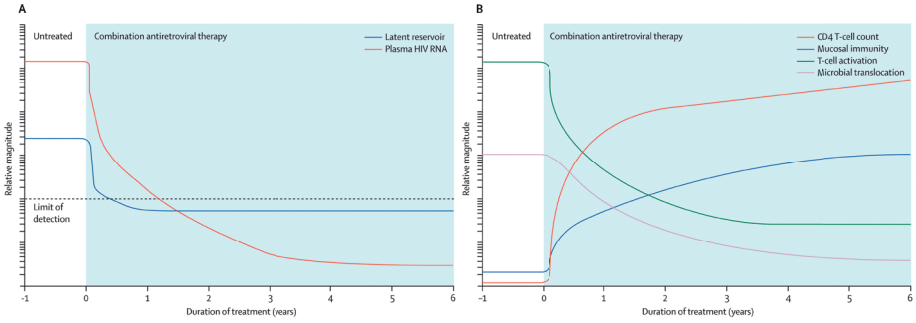


Figure 6. HIV persistence over time. **A.** Viral reservoir. **B.** Immune response. Adapted from Volberding et al.[574]

The persistence of HIV is also the result of the latent HIV reservoir. This reservoir is the major barrier to cure and cannot be eradicated by current treatments.[102] All HIV infected patients have a reservoir in a subset of their CD4+ T lymphocytes.[103] Approximately 1 in every 10^6 central memory CD4+ T lymphocytes differentiates in a resting latently HIV infected cell.[104] Their integrated HIV DNA is transcriptionally silent and often defective but can be replication competent.[105] HIV DNA silencing is multifactorially established.[106] Reservoir cells decay slowly ($T_{½}$ 44 months) and are responsible for low-level viremia during treatment.[107, 108] It allows for swift HIV reactivation after treatment interruption resulting in viral rebound and infection of new cells. Early treatment can restrict the reservoir size.[109] However, treatment is

unable to sufficiently penetrate all reservoir sites, including lymphoid tissue.[110] A sterilizing cure for HIV has not yet been discovered. Antiretroviral treatment is the only method for most patients to interrupt the viral replication cycle and to preserve their immune function. Therapy efficacy and safety is therefore pivotal since HIV treatment is still lifelong.

HIV control in the absence of antiretroviral treatment

A small subset (~5%) of patients can control HIV replication without treatment. These patients are called long term non-progressors (LTNP).[111, 112] LTNP have low level viremia below 1000 c/mL with slow or no disease progression. A subset are the elite controllers (EC) who remain aviremic. A third group of non-progressors are patients with low level viremia after treatment interruption. Post-treatment controllers (PTC) are selected by treatment initiation during acute HIV. The 14 PTC of the Visconti cohort are extensively studied.[113] Mechanisms for spontaneous HIV control are increasingly understood. Differences exist between LTNP and PTC. LTNP appear to have a favorable host genetic composition including alterations in HLA class I and II alleles, NK KIR expression, CCR5Δ32 carriers, antiviral cytokines, and viral factors associated with lower viral fitness. LTNP have cross-reactive CTL with activity against multiple strains and directed against vital conserved viral regions. This results in markedly decreased viral fitness following escape mutations and less susceptible individuals for the effects of regulatory CD4+ T lymphocytes.[114-116]. CTL perforin and granzymes are increased in LTNP partly due to the upregulated T-bet transcription factor which enhances their production.[117] The role of neutralizing and non-neutralizing antibodies appears limited. The effects of antibody dependent cellular cytotoxicity in spontaneous viral control is debated.[118-121] In contrast, PTC do not have overexpression of protective HLA class I alleles, have weak CTL responses and low T lymphocyte activation.[113] HIV control might be related to their reduced HIV reservoir size.[122] An unique PTC is the Berlin patient. This patient has been off treatment after surviving acute myeloid leukemia by reinduction chemotherapy, total body irradiation and allogeneic transplantation of stemcells from a donor with a homozygous CCR5Δ32 mutation.[123]

The broader clinical implications of these controllers are still limited. LTNP can still progress. A minority of patients is diagnosed and started on cART during acute HIV. PTC cannot be predicted and late rebounds occur. Stem cell transplantations have significant mortality and should only be used in patients with hematological malig-

nancies. With the exception of some unique individuals, cART remains lifesaving for HIV patients until a cure is discovered.

TREATMENT STRATEGIES

The persistence of HIV in populations and individuals is remarkable. The virus has proven to be perseverant and adaptible. It spreads by exploiting a long clinically latent period which outweights its relatively low transmission rate. Eventually, an infected individual will die from HIV without treatment. The introduction of cART, or highly active antiretroviral therapy (HAART), has revolutionised therapy by reducing HIV related morbidity and mortality.[124] Treatment interferes with the HIV replication

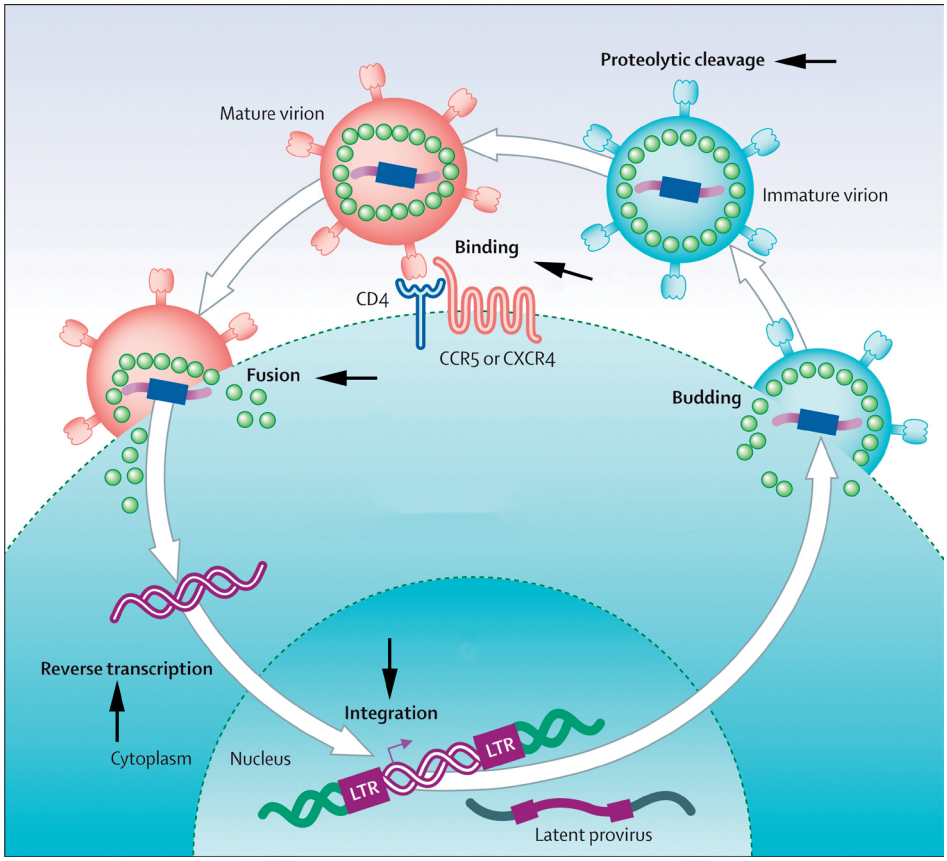


Figure 7. Antiretroviral treatment. Black arrows indicate where antiretroviral drugs interfere with the viral replication cycle. Adapted from Volberding et al.[574]

cycle by blocking pivotal viral mechanisms. Entry inhibitors prevent the interaction between cell surface receptors and viral particles. This class includes CCR5 antagonists and attachment inhibitors that bind gp120. Fusion inhibitors bind gp41 and block viral fusion with the cell membrane. Nucleoside reverse transcriptase inhibitors (NRTI) compete with nucleosides to inhibit the HIV RNA transcription in cDNA. NRTI are nucleoside analogues of thymidine, cytidine, adenosine, guanosine and act as chain terminators blocking DNA synthesis.[125] Non-nucleoside reverse transcriptase inhibitors (NNRTI) bind and inhibit RT directly. Integrase inhibitors (INI) prevent HIV DNA integration in the host genome. Protease inhibitors (PI) interact with protease in immature virions, preventing proteolysis of gag and gag-pol precursor proteins.

Antiretroviral treatment serves to stop the HIV replication cycle. Plasma HIV RNA decreases in two phases after treatment initiation. The first decline is rapid due to prompt replication interruption with decay of active HIV infected cells. The second decline is slower due to the decay of long-lived HIV infected CD4+ T lymphocytes that contain functional virus.[52] The ultimate treatment goal is to halt the CD4+ T lymphocyte decline with recovery of the cellular immunity. An optimally treated patient has a well suppressed plasma viral load. This has additional benefits for the population in which a HIV infected patient lives. Successful treatment of HIV infected individuals restricts further viral dissemination in a population,[126] and prevents HIV transmission in serodiscordant couples.[127-129] This important concept is known as *treatment as prevention*.

Plasma HIV RNA suppression below the level of detection by quantitative polymerase chain reaction (qPCR) assays, usually 50 or 20 copies/mL, is considered adequate. Ultrasensitive single copy assays can often detect residual plasma viremia below the level of detection. The clinical relevance is unclear because it may reflect HIV RNA release from the reservoir instead of ongoing replication cycles.[130-132] Current cART does not affect already integrated active HIV DNA. Therefore, a stable amount of cell-associated (CA) HIV RNA persists despite adequate treatment and plasma aviremia. CA HIV RNA is not routinely measured in clinical practice.[133, 134] Modest therapy non-adherence may already promote ongoing replication cycles, infection of new cells, and lead to therapy failure.[135] Intermittant plasma viral load blips above 50 copies/mL are related to the level of residual viremia below 50 copies/mL.[136] An ongoing low level viremia above 50 though below 1000 copies/mL is linearly associated with failure.[137-139]

Treatment failure and selection of resistance

The initial cART in an ART naïve HIV infected patient is the first-line treatment. Second line is initiated after first-line treatment failure. Salvage therapy is reserved for those with extensive pretreatment and selection of resistance against multiple drug classes.

The definition of virological failure differs between clinical studies and clinical practice. In clinical studies, a detectable plasma viral load above the level of detection at 1 timepoint within a restricted time window (usually week 48 ±2 after treatment initiation) defines virological failure. Patients who fail to reach this window for any reason are also failures in pre-marketing trials. In clinical practice, a persistent increase in plasma viremia on more than 1 timepoint defines virological failure. This often reflects therapy incompliance rather than insufficient cART potency. Virological failure is nonetheless confirmed if resistant HIV strains have been selected that severely reduce HIV's susceptibility to the antiretroviral drugs used at that moment. Treatment failure can be defined by a broader composite outcome that includes regimen switches due to virological failure, toxicity, the occurrence of AIDS associated illnesses despite cART, or death. A combined approach is preferable in clinical settings to guide antiretroviral strategies: both a consistent detectable viral load despite optimal adherence (especially with resistance) and HIV or ART related clinical endpoints despite aviremia should define treatment failure. The clinical studies in this thesis cover both the timepoint dependent definition of virological failure, and the composite definition of treatment failure.

Resistant HIV strains emerge during treatment by 2 mechanisms. The rapid viral turnover and the error prone RT process cause single- or multidrug-class resistent viral strains even before therapy initiation. A pre-therapy drug-resistant strain gains a survival advantage by ART selection pressure.[140] Based on the RT nucleotide misincorporation rate, the likelihood that a virus with 1 to 3 basepair mutations is present in an infected cell is around 1 in every 10^3 to 1 in at least 10^9 cells.[141] Second, resistant mutants are generated and selected de novo during treatment in the setting of suboptimal adherence, insufficient antiviral cART potency, low drug levels, or incomplete drug penetrance. The incomplete suppression of HIV replication cycles results in the generation and selection of drug-resistant viral strains in these situations.

Most mutant viruses arise at a fitness cost compared to wild type viruses.[142] Only in the presence of antiretroviral drugs may these mutant strains have survival advantages, often at the cost of lower replicative capacities. The fitness cost of resistant mutations can be (partly) compensated by the acquisition of compensatory mutations. The probability of selecting a replicative competent mutant virus, resistant to an antiretroviral drug, is a drug's genetic barrier. Resistance will occur if mutants arise that profoundly increase the drug's inhibitory concentration (IC). The trough plasma concentration (C_{trough}) and the $IC_{50/90}$ define a drug's robustness by its inhibitory quotient (C_{trough}/IC_{50}). Resistance is prevented when good drug tolerability permits the necessary high drug plasma levels. The genetic barrier of individual drugs used as monotherapy is generally too low to prevent selection of resistant strains. Only 1 or a few basepair alterations are sufficient for most drugs to lose their antiviral potential. Therefore, antiretroviral drugs are nearly always used in combinations during the period prior to plasma viral suppression -the induction phase- and thereafter -the maintenance phase-. Single drug induction-maintenance therapy might be possible if a drug's genetic barrier is high enough. Whether monotherapy is feasible in all patients with the current drugs is still debated.

Basepair mutations that reduce drug susceptibility are classified as primary, or major, drug resistance mutations (DRM). Secondary, or minor/accessory, DRM further reduce susceptibility in combination with primary DRM, or arise to increase the replication fitness of mutant viruses. This results in evolution pathways towards greater resistance depending on the involved drug. Additionally, a phenomenon known as resistance cross-reactivity exists; one mutation induces resistance against multiple antiretrovirals.[143] On the other hand, certain mutations that decrease the susceptibility to one drug can paradoxically increase the susceptibility to another from the same class. Amino-acids substitutions are indicated by a consensus wildtype amino-acid and its position, followed by the altered amino-acid detected. A mixture of detected amino acids at one position is possible if more strains circulate within the total viral population.

Adherence to cART is directly related to the probability of virological failure and selection of resistance.[144] Sequential intermittent poor adherence to individual drugs in antiretroviral regimens poses the patient at the highest risk for selection of resistance.[145] Single tablet regimens (STR) might improve adherence and thus lower failure rates.[146] Furthermore, selection of resistance results in virological failure which

diminishes the effect of treatment as prevention. This promotes forward transmission with drug resistant strains within a population which could reduce the treatment susceptibility to initial regimens.[147, 148]

Most drug resistant strains are thought to have lower transmission efficacy.[149] Nonetheless, these viruses can establish persistent infections albeit their replicative competence may be impaired.[150] Resistant viruses, selected in a HIV patient on cART, tend to revert back to wild type in the absence of drug-pressure. In the setting of transmitted drug resistant strains, this will only occur if the replicative capacity of the transmitted mutated virus is severely compromised and the reversion is not hindered by fixation through compensatory mutations.[151-153] The presence of transmitted drug resistance (TDR) increases the risk of virological failure.[154] Especially NNRTI based regimens may fail in the presence of TDR regardless of the predicted effect of the TDR on the NNRTI that was detected with population sequencing. This might be due to minority resistance mutations to NNRTI which are not detected by standard population sequencing.[155, 156]

Antiretroviral treatment

Although an effective treatment for HIV is available today, many patients have died prior to its introduction. No treatment was available in the first years after the discovery of HIV. The first drug, 3'-deoxy-3'-azido-thymidine (AZT), was discovered in 1987. AZT monotherapy quickly selected resistant strains and failed to offer lasting survival benefits.[157, 158] Sequential therapy after AZT failure with either cytidine or adenosine analogues, and initiating duotherapy had transient effects.[159, 160] Resistance selection was slower but eventually all patients progressed. The concept of triple drug containing cART comprises the use of at least 2 drug classes, one being a NRTI. The simultaneous initiation of all drugs resulted in lasting plasma HIV RNA suppression without selection of resistance.[161, 162] However, long-term suppressive cART did not result in eradication of the reservoir.[103] Nonetheless, cART with at least 3 active drugs, usually a dual drug NRTI backbone with another drug class, has been the cornerstone of antiretroviral therapy since its introduction in 1996. The essence of cART is its ability to suppress all possible viral variants.

Treatment guidelines can aid in clinical HIV management. Five guidelines are extensively used worldwide.[163-167] The recommended first-line regimens, definitions and management of virological failure vary slightly between them. Current recommended

initial treatment strategies include INI, PI or NNRTI based cART in combination with a NRTI backbone. This backbone consists of emtricitabine (FTC) or lamivudine (3TC) with tenofovir disoproxil-fumarate (TDF), abacavir (ABC), or AZT. Due to cross-resistance and toxicity, only a limited number of drug combinations are possible despite a higher theoretical number. Current and future recommended regimens should have proven their efficacy in phase III registration randomized clinical trials (RCT) prior to inclusion in the guidelines. This is true for all recommended first-line cART in current guidelines, except for the assumed clinical equivalence of FTC and 3TC when either one is used in combination with TDF as NRTI backbone in NNRTI, PI, or INI based cART.

The first generation INI are raltegravir (RAL) and cobicistat boosted elvitegravir (EVG/c). The first generation NNRTI are nevirapine (NVP) and efavirenz (EFV). The second generation NNRTI rilpivirine (RPV) and etravirine (ETR) do not have fully overlapping cross-resistance patterns with NVP and EFV. The first generation INI, NNRTI and RPV are still widely used but prone for resistance due to low genetic barriers. First generation PI are no longer used because of toxicity and lower efficacy compared to ritonavir boosted PI. The ritonavir boosted PI darunavir (DRV/r), atazanavir (ATV/r), and lopinavir (LPV/r) and the second generation INI dolutegravir (DTG) have the highest genetic barriers to resistance of the current approved antiretroviral drugs.

The level of resistance can be predicted by drug resistance algorithms.[168] Multiple mutations have complex effects on drug susceptibility. Mutations in RT associated with significant resistance to recommended NRTI include K65R for TDF, and L74V/I, Y115F and K65R for ABC. M184V/I is the main mutation for FTC and 3TC, decreases ABC susceptibility but increases TDF efficacy. The thymidine analogues AZT and stavudine (d4T) select for multiple thymidine analog mutations (TAM). TAM accumulate on failing regimens in 2 partly overlapping patterns. Type 1 includes M41L, L201W, and T215Y, whereas type 2 includes D67N, K70R, T215F, and K219Q/E. Multiple TAM are directly related to increased cross-resistance to ABC or TDF. Pathways to multidrug resistance (MDR) mutations for NRTI exist. The Q151M usually occurs with accessory mutations at positions 62, 75, 77, or 116. This pathway causes high resistance to AZT and ABC, and intermediate resistance to 3TC, FTC and TDF if at least 2 accessory mutations are present. The other MDR pathway uses distinct mechanisms by insertion or deletion of basepairs in the β3-β4 region (positions 62 to

78) of RT that binds the deoxyribose nucleoside triphosphates (dNTP). A 6 basepair insertion at position 69 (T69Ins) results in T69S-SS or T69S-SG and usually concur with multiple TAM. An amino-acid deletion can develop at the positions 67 and 69 usually in combination with multiple TAM and either Q151M (T67del) or K65R (T69del).[169, 170] These mutations make RT less susceptible to modified dNTP and are associated with MDR.

High level resistance mutations to EFV and NVP are found at positions 100, 101, 103, 106, 188, 190 and 230. K103N is often selected by EFV. Y181C is often selected by NVP. E138K is a major mutation for RPV. RPV and ETR might be more effective to strains with mutations in RT at positions 103, 106 and 190. High level resistance to PI is the consequence of combinations of multiple mutations in protease at positions 50, 84, and 99 for ATV and at positions 47, 76, 82 for LPV. Mutations at positions 47, 50, 54, 76, and 82 result in moderate resistance to DRV. Mutations at positions 66, 92, 138, 140, 143, 147, 148, and 155 are associated with intermediate to high levels of resistance to first generation INI. Significant mutations are Q148R or N155H for EVG and RAL, and E92Q for EVG. Many integrase mutations do not impact DTG susceptibility.[171]

Nowadays, all guidelines recommend immediate initiation of HIV treatment for all patients upon their HIV diagnosis. The benefits of immediate treatment initiation and earlier HIV diagnosis instead of deferring treatment initiation to later disease stages have been shown.[172-175] This important treatment paradigm is called *test and treat*.

Comorbidities and switch strategies

Patients have to commit themselves to lifelong cART. Antiretroviral therapy efficacy optimalisation, management of comorbidity, and avoidence of cART toxicity is therefore pivotal as long as cure remains an utopy. Long-term cART can only be successful if well-tolerated by an adhering patient. Treatment nowadays focuses on prolonged survival with prevention of toxicities and comorbidities. Several pioneer antiretroviral drugs have already been withdrawn from the market due to unacceptable toxicity. Toxicity and virological failure still result in alterations of initial cART in approximately 50% of patients because it remains impossible to predict which patient will develop toxicity.[176] Antiretroviral regimens are also frequently switched to prevent drug-drug interactions (DDI), and when novel regimens are introduced which are associated with a more beneficial risk profile or a lower pill burden.

Switching antiretroviral drugs in suppressed HIV patients can be more complex than anticipated due to several factors. First, the ongoing effects of the interrupted regimen might have a profound influence on the new regimen. For instance, drugs that significantly induce cytochrome P450 (CYP), a major drug metabolizing system, may result in CYP induction that persists after drug interruption. This could increase failure due to lower initial C_{trough} of newly started drugs that are CYP metabolized. Second, switching cART can be difficult if resistance is acquired. Although within drug class switches can theoretically work based on absent evidence of cross-resistance in vitro, this does not necessarily imply evidence of absence in vivo. Third, virological efficacy of new drug regimens should be weighed against drug toxicities such as cardiovascular disease (CVD). The effects of cART on CVD are incompletely characterised but should be taken into account, especially since CVD emerges as a prominent cause of death in HIV patients. Fourth, regimen switches in well suppressed HIV patients might be necessary to prevent drug toxicity or DDI. In multimorbid patients with numerous comedication, avoiding DDI or increased risks on comorbidities can be challenging. Antiretroviral monotherapy with a robust drug could be promising in these patients. Experience with monotherapy is available for the PI class only but trial results give rise to concerns on potency and DDI. The INI DTG might be a monotherapeutic alternative, but has not yet been studied.

Not only the proportion of antiretroviral drug switches, but also the proportion non-AIDS related deaths increases over time.[177] Drugs can either contribute to comorbidities as a consequence of toxicities or prevent them. Toxicity is frequently the result of cART cross-reactivity with human metabolism, such as nucleoside analogues with human polymerases and PI with human proteases. This results in a spectrum of potential toxicities in which individual antiretroviral drugs have their strengths and limitations.

CVD, renal disorders, and malignancies occur more frequently in the ageing HIV population compared to matched uninfected individuals.[178] The optimal preventive and therapeutic strategies for these comorbidities remain to be defined. An increased CVD risk has been signaled for ABC and certain PI.[179, 180] PI related dyslipidemia can only partly explain this association. Other NRTI, INI and NNRTI, especially NVP, do not cause dyslipidemia and this possibly ameliorates the CVD risk. Treatment with TDF or PI may result in acute or chronic renal disorders in HIV infected patients. Kidney injury is reflected by increased glomerular filtration rate (GFR)

declines, dysfunctional handling of solutes in the tubules, or both.[181, 182] AIDS and non-AIDS related malignancies are seen at increased rates in HIV patients.[183] A distinct type of malignancies in HIV is related to a coinfection with the human herpes virus type 8 (HHV8). This virus causes Kaposi sarcoma (KS) and multicentric Castleman's disease (MCD) in HIV patients. Antiretroviral treatment is essential in decreasing the mortality of these diseases. Despite cART use, MCD incidence has been reported to increase. cART can also be insufficient for some HIV patients with KS.[184, 185] Additional treatment usually requires chemotherapy which is associated with significant toxicity. Immunomodulatory therapy is another approach for KS and MCD but the experience with these treatments in HIV patients is limited.

RESEARCH AIMS

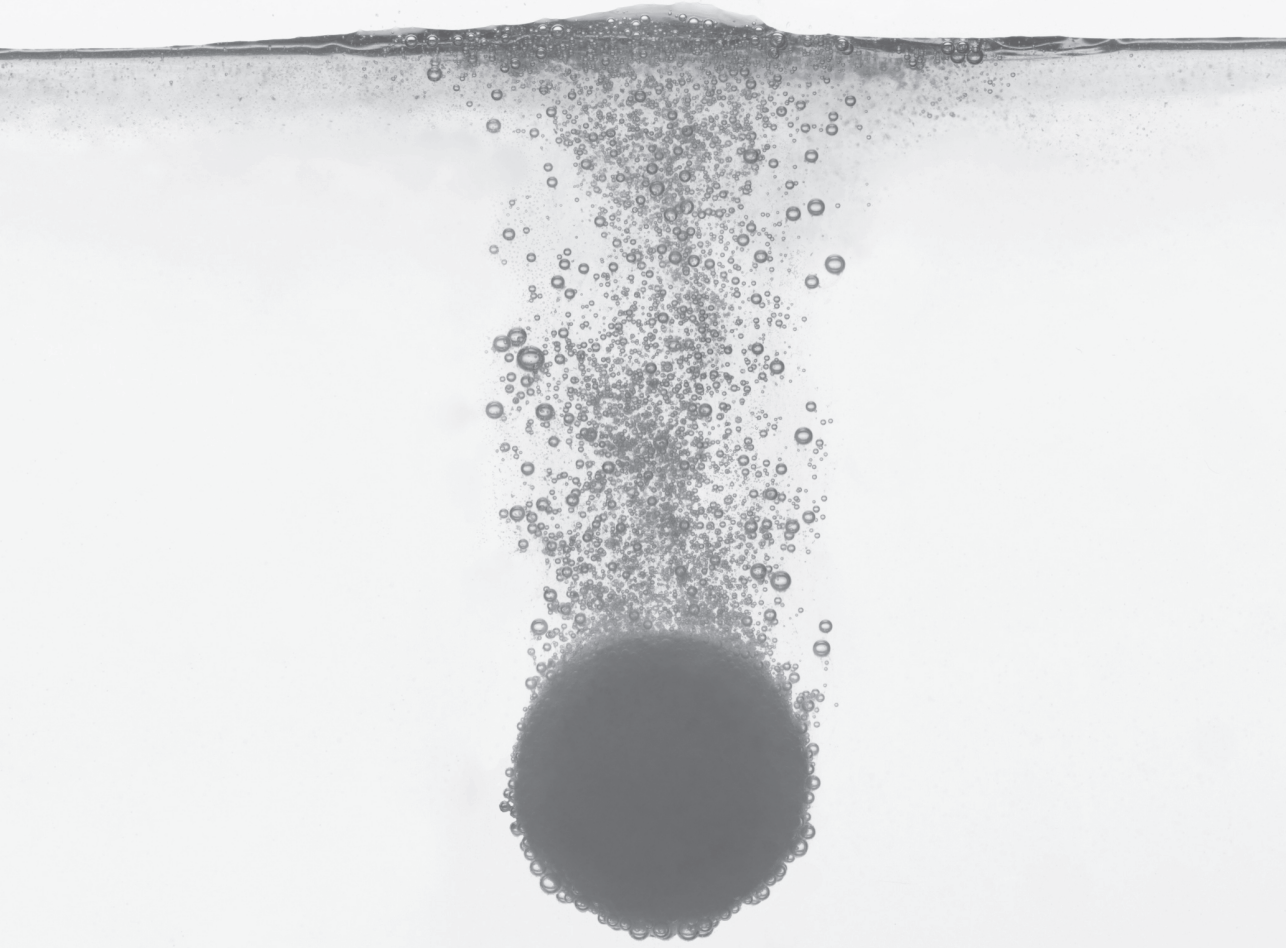
The central aim of this thesis is to evaluate the safety and effectiveness of the drugs used in lifelong cART for HIV infected patients. This can contribute to the 'test and treat' and 'treatment as prevention' strategies. The studies center on four issues.

Part 1 concentrates on the efficacy of two central drugs in first-line HIV antiretroviral treatment, 3TC and FTC. The current evidence is insufficient to justify their firm recommendation as clinical equivalent drugs. The efficacy of these drugs is studied in the recommended combinations for first-line treatment including TDF and either a first-generation NNRTI or a PI in **Chapter 2** and **Chapter 3**.

Part 2 highlights the safety of novel antiretroviral treatment switch strategies. In **Chapter 4**, we study the safety of a switch from the CYP inducer NVP to CYP metabolized RPV. We have conducted two pilot studies on pioneering antiretroviral treatment strategies. The within class switch to RPV in patients with an isolated K103N mutant strain selected on previous first generation NNRTI regimens is discussed in **Chapter 5**. The robustness of DTG as monotherapy in well suppressed HIV patients is tested in a proof of concept study in **Chapter 6**.

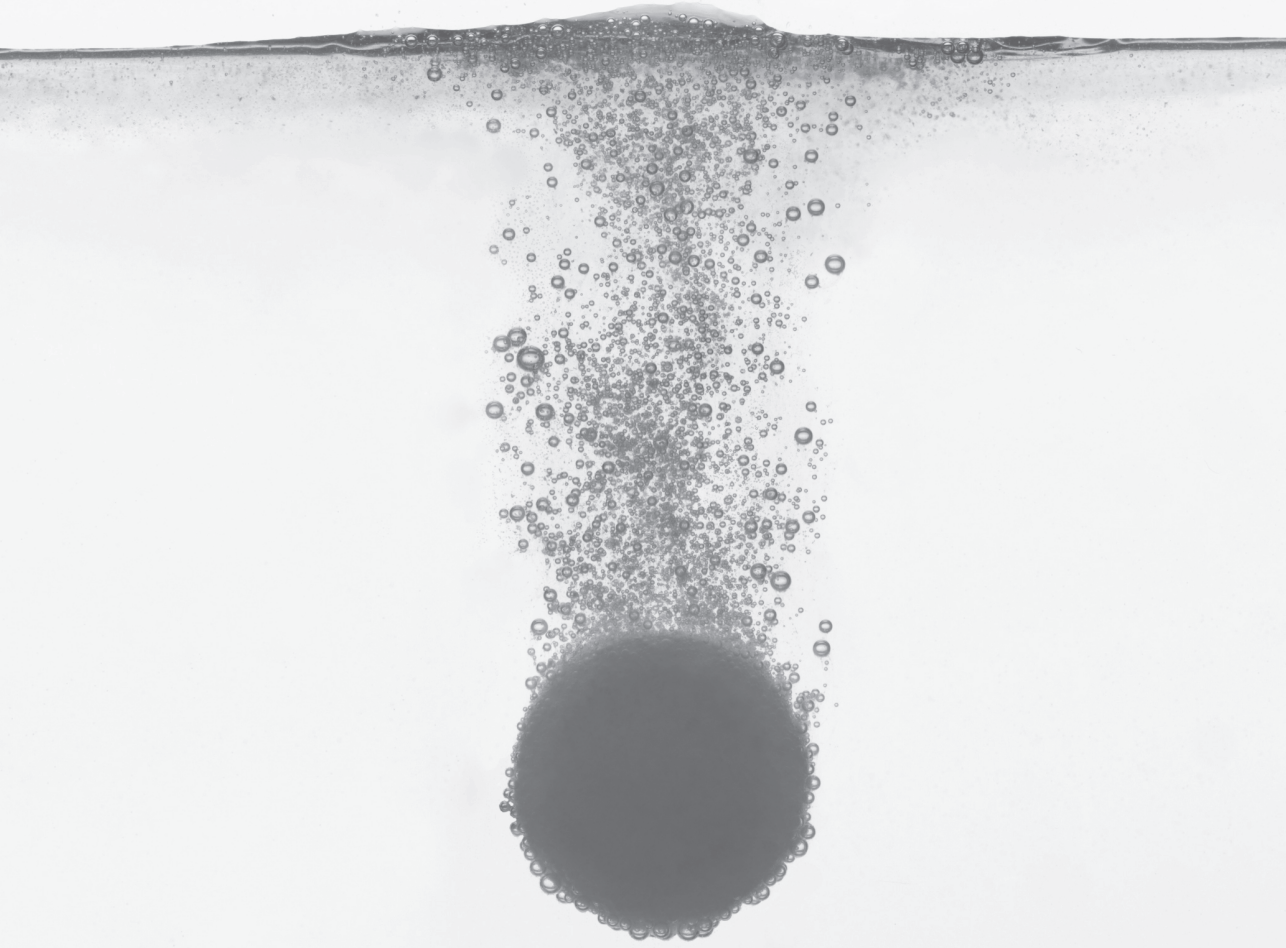
Part 3 focuses on the occurrence and management of comorbidities arising during antiretroviral treatment. In **Chapter 7**, we study the prevalence of renal disease in HIV patients treated with recommended TDF containing antiretroviral regimens and investigate a potential novel risk-factor of TDF related kidney disease. Alterations in dyslipidemia and CVD risk following the discontinuation of NVP are investigated in **Chapter 8**. Immunomodulatory treatments for the HHV8 related diseases KS and MCD are investigated in **Chapter 9** and **Chapter 10**.

Part 4 describes collaborative propositions in **Chapter 11** on the future management of HIV with insights how to investigate the reservoir and evade HIV persistence. The results and implications of the studies are summarized and discussed in **Chapter 12**.



Part 1

Efficacy of first-line antiretroviral treatment



Chapter 2

Increased virological failure in naive HIV-1-infected patients taking lamivudine compared with emtricitabine in combination with tenofovir and efavirenz or nevirapine in the Dutch nationwide ATHENA cohort.

C. Rokx, A. Fibriani, D.A.M.C. van de Vijver, A. Verbon, M. Schutten, L. Gras, B.J.A. Rijnders; on behalf of the AIDS Therapy Evaluation in the Netherlands (ATHENA) National Observational Cohort.

Clin Infect Dis. 2015. Jan;60(1):143-53.

Editorial and correspondence open access online

Comparative efficacy of lamivudine and emtricitabine: comparing the results of randomized trials and cohorts.

N. Ford, A. Hill, M. Vitoria, E.J. Mills.

Clin Infect Dis. 2015. Jan;60(1):154-6.
http://cid.oxfordjournals.org/content/60/1/154.long

Evidence gathered from randomized clinical trials and observational studies on the equivalence of emtricitabine and lamivudine.

C. Rokx, B.J.A. Rijnders.

Clin Infect Dis. 2015. Jun;60(11):1732-3.
http://cid.oxfordjournals.org/content/60/11/1732.1.long

ABSTRACT

Introduction

Guidelines for treatment of human immunodeficiency virus type 1 (HIV-1) infection consider lamivudine and emtricitabine to be interchangeable components in first-line combination antiretroviral therapy (cART). The evidence for their clinical equivalence in cART is inconsistent. The primary aim of this study was to evaluate the virological responses to lamivudine and emtricitabine in recommended cART.

Methods

This was an observational study using data from the AIDS Therapy Evaluation in the Netherlands (ATHENA) nationwide HIV cohort. The virological responses to lamivudine and emtricitabine were compared by multivariable adjusted logistic regression and Cox proportional hazard models. Sensitivity analyses included propensity score-adjusted models.

Results

Therapy-naive HIV-1-infected patients without baseline resistance (n = 4740) initiated lamivudine or emtricitabine with efavirenz/tenofovir or nevirapine/tenofovir. The use of lamivudine was associated with more virological failure at week 48 compared to emtricitabine with efavirenz/tenofovir (10.8% vs 3.6%; adjusted odds ratio [AOR], 1.78; 95% confidence interval [CI], 1.11-2.84) and nevirapine/tenofovir (27% vs 11%; AOR, 2.09; 95% CI, 1.25-3.52) in on-treatment analysis. Propensity score-adjusted models and intent-to-treat sensitivity analyses gave comparable results. The adjusted hazard ratio of virological failure at week 240 using lamivudine instead of emtricitabine was 2.35 (95% CI, 1.61-3.42) with efavirenz and 2.01 (95% CI, 1.36-2.98) with nevirapine. The inclusion of lamivudine or emtricitabine in cART did not influence the time to virological suppression within 48 weeks or the probability of virological rebound after successful virological suppression.

Conclusion

The use of emtricitabine instead of lamivudine as part of cART was associated with better virological responses. These findings are relevant for settings with extensive use of lamivudine and for settings where generic lamivudine will be available.

INTRODUCTION

Treatment guidelines for human immunodeficiency virus type 1 (HIV-1) consider the nucleoside reverse transcriptase inhibitors (NRTIs) lamivudine and emtricitabine to be interchangeable components in recommended combination antiretroviral therapy (cART).[163, 164, 167] Emtricitabine is frequently used as part of first-line cART with efavirenz and tenofovir in resource-rich settings, whereas lamivudine is more frequently combined with nevirapine and tenofovir in resource-limited settings. Major HIV-1 therapy-related cost savings are possible as generic lamivudine has become available in resource-rich settings.[186] However, the use of generic lamivudine instead of emtricitabine should be cautiously considered if these components do not have comparable effectiveness in clinical use.

The relative effectiveness of lamivudine vs emtricitabine in cART for HIV-1 infection is unclear. Comparisons by randomized trials have suggested lower virological responses in patients on lamivudine-containing NRTI backbone regimens, especially at higher baseline viral loads,[187-193] with increased rates of acquired drug resistance.[194-196] Other randomized trials observed no difference in virological responses to lamivudine- vs emtricitabine-containing regimens.[197-199] The available evidence therefore remains inconclusive. The main reason for the presumed clinical equipoise is that not only lamivudine and emtricitabine but also the second NRTI differed in the treatment arms of most clinical trials. As such, the use of NRTI coformulations (with abacavir, zidovudine, or tenofovir) remains a confounder in determining the possible lower potency of lamivudine.

The aim of this study is to compare the virological responses to lamivudine and emtricitabine as part of first-line cART with efavirenz/tenofovir or nevirapine/tenofovir for HIV-1 in ART-naive patients without baseline resistance.

METHODS

Data Source and Regulatory Approval

HIV-infected individuals in the Netherlands are registered in the nationwide cohort maintained by the HIV Monitoring Foundation (Stichting HIV Monitoring), known as the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort. ATHENA

has collected data of individuals in HIV care since January 1996 from the 26 HIV treatment centers in the Netherlands.[200] HIV patients can opt out from the ATHENA cohort after being informed by their treating physicians on the purpose of data collection. The data collection in the ATHENA cohort is part of standard HIV care and no ethical approval of institutional review boards is needed. The study protocol was peer reviewed and is registered under Stichting HIV Monitoring number I13018.[201]

Study Population

By 31 December 2012, 21 012 HIV-infected individuals were registered in the Netherlands; 20 676 (98.4%) patients consented to inclusion in the ATHENA cohort and subsequent structured prospective data collection.[202] Recorded data included demographics, comorbidities, initial cART, antiretroviral therapy (ART) switches, and clinical, immunological, and virological parameters. The reasons for switching ART were registered by the treating physicians and included, among other reasons, virological failure and toxicity. For this study, we identified HIV-infected adults from ATHENA who initiated lamivudine or emtricitabine in cART with either efavirenz/tenofovir or nevirapine/tenofovir between 1 January 2002 and 31 January 2012. ART-experienced patients and patients with baseline resistance (at least low level) according to the Stanford Database to any component of cART were identified and excluded from the analyses of outcomes.

Study Outcomes

We used a clinical approach to evaluate 5 outcomes. First, virological failure at week 48 after cART initiation was analyzed. Any HIV RNA ≥400 copies/mL within the 48 ± 10-week window defined virological failure. Patients without any HIV RNA in this window were not included in the analysis of this outcome. In addition, all cART discontinuations for registered virological failures or for deaths while the last HIV RNA level was ≥400 copies/mL prior to 48 ± 10 weeks were considered virological failures. HIV RNA copies/mL ≥400 was considered a "viral blip" if preceded and followed by HIV RNA <400 copies/mL. Patients without any HIV RNA levels recorded after cART initiation were considered lost to followup. The second outcome was virological suppression and was defined by the time from cART initiation to the first of 2 consecutive HIV RNA levels <400 copies/mL within 48 ± 10 weeks. Third, we analyzed the time to registered virological failure within 240 weeks after the initiation of cART. The time to virological failure was defined by the time from cART initiation to cART switches for registered virological failure or death while HIV RNA was ≥400

copies/mL. Fourth, the time from cART initiation to virological failure within 240 weeks was evaluated after achieving HIV RNA <400 copies/mL first on initial cART. These virological failures after an HIV RNA level <400 copies/mL were defined as rebounds. Last, the HIV-1 reverse transcriptase sequences at cART initiation and failure were evaluated and compared regarding mutations that resulted in at least low-level resistance according to the Stanford Resistance Database.[168]

Data were collected on cART, previous ART, drug resistance, age at cART initiation, sex, region of origin, HIV-1 transmission route, hepatitis B /C virus coinfection (HBV/HCV), treatment hospital, last HIV RNA level (continuous until ≥100 000), and CD4 count prior to initiation of cART. The presence of HBV surface antigen, HCV RNA or, if unavailable, HCV antibody defined HBV and HCV coinfection. Missing baseline HIV RNA (3.5% of total) and CD4 counts (3.9%) were imputed and estimated by age, sex, region of origin, transmission route, HCV, and cART initiation year.

Statistical Analysis

Data were described as means, medians, or numbers with percentages. Adjusted logistic regression models were used to estimate odds ratios (ORs) with 95% confidence intervals (95% CIs) on virological failure at week 48. These models included cART, HIV RNA, CD4 count, region of origin, and all covariates with $P < .1$ in unadjusted analysis of virological failure as fixed effects. The treatment hospitals were included as random covariate. The primary outcome was virological failure at week 48 by on-treatment (OT) analysis. In this analysis, patients with registered cART switches for other reasons than virological failure or loss to follow-up were not considered failures. The ratio of the patients with virological failure (numerator) and the OT population (denominator) defined the virological failure rates. Three sensitivity analyses were used to evaluate virological failure at week 48. First, any HIV RNA >50 copies/mL instead of ≥400 copies/mL within 48 ± 10 weeks was defined as virological failure. Second, all patients lost to follow-up and all patients who switched cART for other reasons while HIV RNA was ≥400 copies/mL were considered virological failures in modified intent-to-treat (ITT) analyses. Third, propensity score-adjusted models were used to correct for selection bias.[203] The probability of initiating lamivudine or emtricitabine was calculated by all measured covariates in logistic regression models. The propensity scores were included as covariates and inverse weights with cART in logistic regression models to evaluate virological failure within 48 weeks.

Multivariable Cox regression models and Kaplan-Meier estimates were used for the analysis of (1) time to virological suppression within 48 weeks, (2) time to a cART switch for virological failure within 240 weeks, and (3) time to a rebound within 240 weeks. Hazard ratios (HRs) were adjusted for CD4 count and HIV RNA. Patients were censored at cART switches, last HIV RNA, or the end of the study period at week 48 after 31 January 2012. The analyses were done using SPSS software version 21.0 and GraphPad Prism version 5.0.

RESULTS

Cohort Characteristics

A total of 4836 HIV-1-infected patients initiated lamivudine- or emtricitabine-containing cART between 2002 and 2012. Baseline genotyping was available in 2267 patients and 39 patients (1.7%) had at least low-level resistance. Fifty-seven of 4836 patients (1.2%) were ART experienced. The characteristics of the 4740 naive HIV-1-infected patients are provided in Table 1. The patients initiated lamivudine/efavirenz/tenofovir (n = 535), emtricitabine/efavirenz/tenofovir (n = 3343), lamivudine/nevirapine/tenofovir (n = 193), or emtricitabine/nevirapine/tenofovir (n = 669). The mean age in the cohort was 40 years. Overall, patients on emtricitabine compared with lamivudine were more frequently men (88.0% vs 76.4%) having sex with men (69.2% vs 47.0%), from Western countries (70.0% vs 53.7%). The median cART initiation year was 2004 for lamivudine and 2009 for emtricitabine regimens. Patients on emtricitabine had higher median CD4 cell counts (260 vs 184 cells/μL) and lower median HIV RNA (82 173 vs 100 000 copies/mL) than those on lamivudine.

Virological Responses

At week 48, 100 of 4740 patients (2.1%) were lost to follow-up and 831 (17.5%) discontinued cART prior to 48 ± 10 weeks for other reasons than virological failure, predominantly ART toxicity (supplementary data).[204] Three hundred sixty-nine patients (7.8%) without HIV RNA recorded in the 48 ± 10-week window were equally distributed among the 4 groups (P = .077). The majority of these patients had HIV RNA <400 copies/mL (96.2%) or <50 copies/mL (80.5%) prior to this window. These patients were not included in the OT population, which consisted of 3440 patients.

Table 1. Baseline characteristics of therapy-naive HIV-1 infected patients (n=4740) in the ATHENA cohort.

	Efavirenz/Tenofovir					Nevirapine/Tenofovir				
	Lamivudine (n=535)		Emtricitabine (n=3343)			Lamivudine (n=193)		Emtricitabine (n=669)		
	No.	(%)	No.	(%)	P	No.	(%)	No.	(%)	P
Gender, male sex	412	(77.0)	2965	(88.7)	<.001	144	(74.6)	565	(84.5)	.002
Age, mean (SD)	39	(10)	41	(10)	.001	40	(10)	40	(10)	.906
Start year, median (IQR)	2005	(2004 - 2006)	2009	(2008 - 2010)	<.001	2004	(2003 - 2005)	2009	(2008 - 2010)	<.001
Documented HIV-1 wild-type	111	(20.8)	1732	(51.8)	<.001	54	(28.0)	321	(47.9)	<.001
Treatment hospital										
<500 patients	24	(4.5)	308	(9.2)		6	(3.1)	36	(5.4)	
500 - 2000 patients	216	(40.4)	1827	(54.7)		29	(15.0)	329	(49.2)	
>2000 patients	295	(55.1)	1208	(36.1)	<.001	158	(81.9)	304	(45.4)	<.001
Hepatitis B										
Positive	68	(12.7)	237	(7.1)		8	(4.1)	36	(5.4)	
Negative	445	(83.2)	2980	(89.1)		181	(93.8)	611	(91.3)	
Unknown	22	(4.1)	126	(3.8)	<.001	4	(2.1)	22	(3.3)	.527
Hepatitis C										
Positive	36	(6.7)	246	(7.4)		15	(7.8)	50	(7.5)	
Negative	458	(85.6)	2936	(87.8)		164	(85.0)	601	(89.8)	
Unknown	41	(7.7)	161	(4.8)	.021	14	(7.3)	18	(2.7)	.012

Table 1. Baseline characteristics of therapy-naive HIV-1 infected patients (n=4740) in the ATHENA cohort. (continued)

	Efavirenz/Tenofovir					Nevirapine/Tenofovir				
	Lamivudine (n=535)		Emtricitabine (n=3343)			Lamivudine (n=193)		Emtricitabine (n=669)		
	No.	(%)	No.	(%)	P	No.	(%)	No.	(%)	P
Transmission										
MSM	260	(48.6)	2316	(69.3)		82	(42.5)	460	(68.8)	
Heterosexual	212	(39.6)	822	(24.6)		82	(42.5)	164	(24.5)	
Intravenous drug use	16	(3.0)	32	(1.0)		11	(5.7)	13	(1.9)	
Other	6	(1.1)	22	(0.7)		2	(1.0)	5	(0.7)	
Unknown	41	(7.7)	151	(4.5)	<.001	16	(8.3)	27	(4.0)	<.001
Region of origin										
Western Countries	278	(52.0)	2351	(70.3)		113	(58.5)	457	(68.3)	
Sub-Saharan Africa	116	(21.7)	337	(10.1)		36	(18.7)	63	(9.4)	
Asia	31	(5.8)	134	(4.0)		4	(2.1)	21	(3.1)	
Latin America	52	(9.7)	238	(7.1)		22	(11.4)	53	(7.9)	
Caribbean	30	(5.6)	130	(3.9)		7	(3.6)	38	(5.7)	
Other	28	(5.2)	153	(4.6)	<.001	11	(5.7)	37	(5.5)	.004
HIV-RNA, copies/mL										
<1000	12	(2.2)	71	(2.1)		2	(1.0)	8	(1.2)	
1000 - 9,999	34	(6.4)	310	(9.3)		13	(6.7)	89	(13.3)	
10,000 - 99,999	212	(39.6)	1414	(42.3)		86	(44.6)	312	(46.6)	
≥100,000	277	(51.8)	1548	(46.3)	.043	92	(47.7)	260	(38.9)	.037

Table 1. Baseline characteristics of therapy-naive HIV-1 infected patients (n=4740) in the ATHENA cohort. (continued)

	Efavirenz/Tenofovir					Nevirapine/Tenofovir				
	Lamivudine (n=535)		Emtricitabine (n=3343)			Lamivudine (n=193)		Emtricitabine (n=669)		
	No.	(%)	No.	(%)	P	No.	(%)	No.	(%)	P
CD4 count, cells/mm³										
<100	149	(27.9)	441	(13.2)		45	(23.3)	71	(10.6)	
100 - 199	147	(27.5)	528	(15.8)		50	(25.9)	108	(16.1)	
200 - 349	208	(38.9)	1672	(50.0)		83	(43.0)	366	(54.7)	
≥350	31	(5.8)	702	(21.0)	<.001	15	(7.8)	124	(18.5)	<.001

Data are presented as No. (%) unless otherwise specified. Comparisons were done by χ^2 tests, independent T tests or Mann-Whitney U tests. Abbreviations: ATHENA, AIDS therapy evaluation in the Netherlands; cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men having sex with men; SD, standard deviation.

By week 48, 38 of 352 patients (10.8%) on lamivudine/efavirenz/tenofovir had virological failure compared to 88 of 2437 patients (3.6%) on emtricitabine/efavirenz/tenofovir (OR, 3.23; 95% CI, 2.17-4.81; P < .001). Most patients (n = 91, 72.2%) met the definition of virological failure because of registered virological failure before the 48 ± 10-week window. Thirty-five patients were considered to have virological failure because they died with HIV RNA ≥400 copies/mL (n = 9; median baseline CD4 count, 90 cells/μL) or had HIV RNA ≥400 copies/mL in the 48 ± 10-week window (n = 26), including 24 patients on emtricitabine. Twenty-three of these 24 patients had HIV RNA <50 copies/mL with emtricitabine/efavirenz/tenofovir after this window. With nevirapine/tenofovir, 43 of 159 patients on lamivudine (27.0%) and 54 of 492 patients on emtricitabine (11.0%) had virological failure (OR, 3.00; 95% CI, 1.92-4.72; P < .001). Most patients were considered failures because of registered virological failure (n = 83 [85.6%]). Fourteen patients were considered failures because they died with HIV RNA ≥400 copies/mL (n = 7; median baseline CD4 count, 80 cells/μL) or had HIV RNA ≥400 copies/mL at 48 ± 10 weeks (n = 7), including 4 patients on emtricitabine. These 4 patients on emtricitabine achieved HIV RNA <50 copies/mL on initial cART.

The multivariable adjusted ORs on virological failure for patients on lamivudine compared to emtricitabine were 1.78 (95% CI, 1.11-2.84; P = .016) with efavirenz/tenofovir and 2.09 (95% CI, 1.25-3.52; P = .005) with nevirapine/tenofovir (Table 2). These ORs were adjusted for CD4 count, HIV RNA, region of origin, and all other covariates associated with virological failure in univariable analyses (supplementary data).[204] Sensitivity analyses showed consistent virological failure rates if HIV RNA >50 copies/mL at the 48 ± 10-week window defined virological failure (OT), in analyses by ITT and in propensity score-adjusted models (supplementary data).[204] Only 6 of 119 patients with an HIV RNA level >50 copies/mL but <400 copies/mL at 48 ± 10 weeks had subsequent registered virological failure. Compared to emtricitabine, the propensity score-adjusted OR (OT) for lamivudine with efavirenz/tenofovir on virological failure was 1.89 (95% CI, 1.20-2.97; P = .006). The OR on virological failure for lamivudine with nevirapine/tenofovir was 1.65 (95% CI, .99-2.77; P = .057). Similar results were obtained by weighed propensity score models.

The time to virological suppression within 48 weeks was not significantly influenced by including lamivudine or emtricitabine in cART (Figure 1A); adjusted HRs were 1.04 (95% CI, .93-1.15; P = .498) with efavirenz and 0.96 (95% CI, .79-1.17; P = .680)

Table 2. Multivariable adjusted logistic regression analysis on the association between virological failure and cART in therapy naive HIV-1 patients in the ATHENA cohort (OT population: n=3440).

	Efavirenz/Tenofovir (n=2789)			Nevirapine/Tenofovir (n=651)		
	OR	**(95% CI)**	**P**	**OR**	**(95% CI)**	**P**
cART						
Lamivudine	1.78	(1.11 - 2.84)	.016	2.09	(1.25 - 3.52)	.005
Emtricitabine[a]	1			1		
HIV-RNA, copies/mL						
≥100,000	1.89	(1.24 - 2.89)	.003	2.35	(1.43 - 3.86)	.001
<100,000[a]	1			1		
CD4 count, cells/mm³						
<100	3.45	(1.75 - 6.79)	<.001	9.33	(3.56 - 24.45)	<.001
100 - 199	1.46	(0.72 - 2.97)	.300	2.56	(0.98 - 6.70)	.055
200 - 349	0.69	(0.35 - 1.35)	.276	1.42	(0.58 - 3.49)	.440
≥350[a]	1			1		
Age, year increase	0.97	(0.96 - 0.99)	.013	-		

[a] Reference categories within covariates.

Abbreviations: ATHENA, AIDS therapy evaluation in the Netherlands; cART, combination antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; OR, odds-ratio.

with nevirapine. The HRs for virological failure at <240 weeks were higher on lamivudine vs emtricitabine with efavirenz (2.35; 95% CI, 1.61-3.42) or nevirapine (2.01; 95% CI, 1.36-2.98; Figure 1B). However, if HIV RNA <400 copies/mL was achieved on initial cART, no significant differences were observed in rebounds between lamivudine and emtricitabine with efavirenz (P = .090) or nevirapine (P = .255; Figure 1C). The Kaplan-Meier estimate of the percentage of patients still on initial regimen after 240 weeks was 50%: an estimated 26% of patients had switched because of toxicity, 5% because of virological failure, and 19% for other reasons (Figure 2).

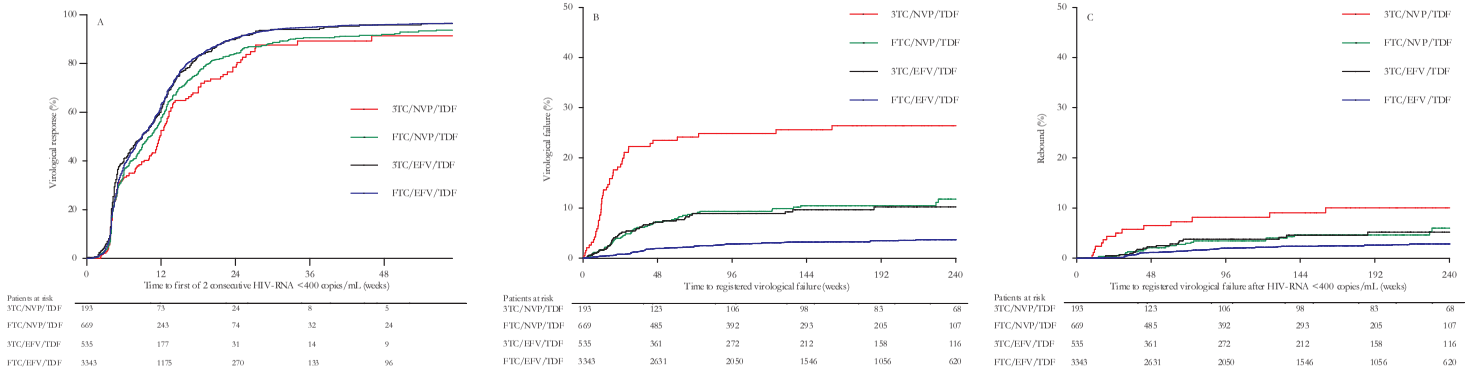


Figure 1. Kaplan–Meier curves of the virological responses to lamivudine (3TC) or emtricitabine (FTC) with efavirenz (EFV)/tenofovir (TDF) (black and blue lines) or nevirapine (NVP)/TDF (red and green lines) in 4740 antiretroviral therapy (ART)–naive human immunodeficiency virus (HIV) type 1–infected patients from the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort. Hazard ratios (HRs) are adjusted for baseline CD4 cell count and HIV RNA < or ≥100 000 copies/mL. **A.** Time to virological suppression, defined as the first of 2 consecutive HIV RNA levels <400 copies/mL within 48 weeks on initial combination ART (cART). The adjusted HR on virological suppression were not significantly different between 3TC and FTC with EFV/TDF (1.04; 95% con-fidence interval [CI], .93–1.15; P = .498) and NVP/TDF (0.96; 95% CI, .79–1.17; P = .680). **B.** The time to cART switches for registered virological failure in the ATHENA cohort within 240 weeks after initiating cART. The adjusted HR of cART switches for virological failure were significantly increased for patients on 3TC with EFV/TDF (2.35; 95% CI, 1.61–3.42; P = .001) and NVP/TDF (2.01; 95% CI, 1.36–2.98; P < .001). **C.** Time to cART switches for registered virological failure in the ATHENA cohort following successful virological suppression to HIV RNA <400 copies/mL first on initial cART (rebounds). No significant differences in adjusted HR on rebounds within 240 weeks were observed between 3TC with EFV/TDF (1.60; 95% CI, .93–2.76; P = .090) and NVP/TDF (1.48; 95% CI, .75–2.90; P = .255) once HIV RNA was suppressed to <400 copies/mL first.

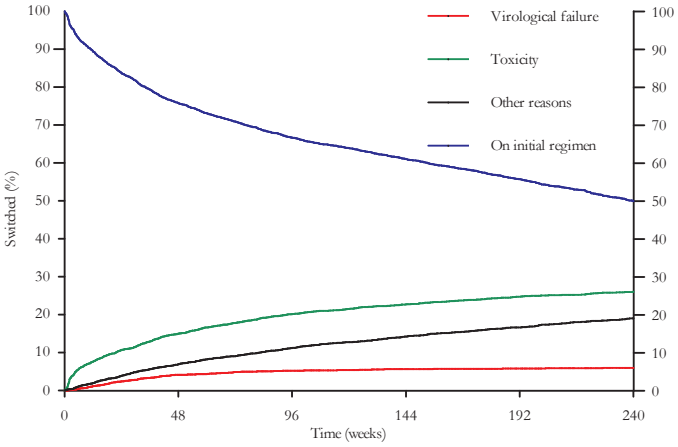


Figure 2. Kaplan–Meier estimates of the percentages of 4740 human immunodeficiency virus type 1–infected patients from the AIDS Therapy Evaluation in the Netherlands cohort who remained on initial lamivudine- or emtricitabine-containing regimens (blue line) and who switched combination antiretroviral therapy (cART) for any reason (red, green, and black lines) at week 240 after cART initiation.

Resistance Associated Mutations

Acquired resistance to reverse transcriptase was evaluated in 267 of 4740 HIV-1-infected patients, including 234 patients with registered virological failure within 240 weeks and 33 patients with HIV RNA ≥400 copies/mL at week 48. At failure, patients on lamivudine regimens had a higher median HIV RNA level of 49 231 copies/mL compared with HIV RNA of 4230 copies/mL on emtricitabine regimens ($P < .001$). Sixty-four of 267 patients had HIV RNA <1000 copies/mL and their genotyping results, if available, were not used. Of these 64 patients, 57 patients (89.1%) were on emtricitabine-containing regimens. Of 203 patients with HIV RNA ≥1000 copies/mL at virological failure, the HIV-1 genotyping results were available for 123 patients. Baseline genotypes that did not show resistance were available in 88 of 123 patients, and these patients were used for analysis of acquired resistance. At least 1 low- or higher level resistance mutation was found in 80 of 88 (90.9%) patients, including 40 of 44 patients on lamivudine and 40 of 44 patients on emtricitabine (Table 3). The proportion of resistance against both NRTIs and NNRTIs was not different between lamivudine-containing (84.1%) and emtricitabine-containing (84.1%) regimens nor was the prevalence of the primary resistance mutations M184V/I and K65R.

Table 3. Acquired at least low level resistance according to Stanford HIV Resistance Database to any component of cART in reverse transcriptase of HIV-1 patients experiencing virological failure with HIV-RNA ≥1000 copies/mL and genotyped baseline wild-type HIV-1 (n=88).

	Efavirenz/Tenofovir				Nevirapine/Tenofovir				Overall			
	Lamivudine (n=9)		Emtricitabine (n=16)		Lamivudine (n=35)		Emtricitabine (n=28)		Lamivudine (n=44)		Emtricitabine (n=44)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
NRTI RAM												
K65R	2	(22.2)	3	(18.8)	13	(37.1)	10	(35.7)	15	(34.1)	13	(29.5)
K70E	0	(0)	0	(0)	0	(0)	1	(3.6)	0	(0)	1	(2.3)
Y115F	0	(0)	0	(0)	0	(0)	2	(7.1)	0	(0)	2	(4.5)
M184I/V	4	(44.4)	9	(56.2)	23	(65.7)	21	(75.0)	27	(61.4)	30	(68.2)
Non-NRTI RAM												
A98G	1	(11.1)	1	(6.2)	0	(0)	0	(0)	1	(2.3)	1	(2.3)
K101E	1	(11.1)	1	(6.2)	2	(5.7)	3	(10.7)	3	(6.8)	4	(9.1)
K103N	2	(22.2)	10	(62.5)	6	(17.1)	5	(17.9)	8	(18.2)	15	(34.1)
V106A/M	1	(11.1)	1	(6.2)	6	(17.2)	2	(7.1)	7	(15.9)	3	(6.8)
Y181C	0	(0)	0	(0)	20	(57.1)	20	(71.4)	20	(45.5)	20	(45.5)
Y188C/L	2	(22.2)	2	(12.5)	4	(11.4)	1	(3.6)	6	(13.6)	3	(6.8)
G190A/E/S	3	(33.3)	2	(12.5)	5	(14.3)	4	(14.3)	8	(18.2)	6	(13.6)
P225H	0	(0)	3	(18.8)	0	(0)	0	(0)	0	(0)	3	(6.8)
F227L	0	(0)	0	(0)	2	(5.7)	0	(0)	2	(4.5)	0	(0)
K238T	0	(0)	1	(6.2)	0	(0)	0	(0)	0	(0)	1	(2.3)
Y318F	0	(0)	0	(0)	1	(2.9)	0	(0)	1	(2.3)	0	(0)

Table 3. Acquired at least low level resistance according to Stanford HIV Resistance Database to any component of cART in reverse transcriptase of HIV-1 patients experiencing virological failure with HIV-RNA ≥1000 copies/mL and genotyped baseline wild-type HIV-1 (n=88). (continued)

	Efavirenz/Tenofovir				Nevirapine/Tenofovir				Overall			
	Lamivudine (n=9)		Emtricitabine (n=16)		Lamivudine (n=35)		Emtricitabine (n=28)		Lamivudine (n=44)		Emtricitabine (n=44)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Resistance Patterns												
No RAM	2	(22.2)	2	(12.5)	2	(5.7)	2	(7.1)	4	(9.1)	4	(9.1)
≥1 (non-)NRTI RAM	7	(77.8)	14	(87.5)	33	(94.3)	26	(92.9)	40	(90.9)	40	(90.9)
≥1 NRTI and ≥1 non-NRTI RAM	6	(66.7)	12	(75.0)	31	(88.6)	25	(89.3)	37	(84.1)	37	(84.1)

Data are presented as No. (%).

Abbreviations: cART, combination antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; RAM, resistance associated mutation.

DISCUSSION

This study compared the virological responses to lamivudine with emtricitabine as part of first-line cART with efavirenz/tenofovir or nevirapine/tenofovir. The use of lamivudine in both regimens was significantly associated with more virological failure within 48 and 240 weeks of cART. Patients on lamivudine-containing cART had higher HIV RNA levels at virological failure. However, the time to virological suppression and the probability of rebound after successful virological suppression were comparable regardless of including lamivudine or emtricitabine in initial cART. This study is the largest to date to directly compare lamivudine with emtricitabine in ART-naive patients and the first from a resource-rich setting. In vitro observations of lamivudine's lower efficacy against HIV-1 preceded a limited number of clinical studies on this subject to date.[205-207] The high virological failure rate we observed in patients on lamivudine/nevirapine/tenofovir is consistent with the results of 2 prematurely terminated prospective trials. In these studies, a 25%-30% virological failure rate was observed on this regimen, although only 59 patients were included in both studies combined.[208, 209] To our knowledge, only 3 studies compared lamivudine with emtricitabine as part of otherwise identical cART for ART-naive patients.[210-212] Their generalizibility to other HIV-1 populations, like ATHENA, remains questionable because these 3 studies were all conducted in sub-Saharan populations of resource-limited settings (South-Africa, Nigeria, Zambia). Only 2 were randomized clinical trials, of which 1 included stavudine, an NRTI that should no longer be used, in cART.[210] The other study was a prospective open-label randomized clinical trial on 332 predominantly female Zambians that did not show a significant difference in virological failure between lamivudine and emtricitabine with efavirenz/tenofovir. However, the power to detect a clinically significant difference in virological failure with the included number in this study is problematic.[212] The only randomized trial from a resource-rich setting that directly compared lamivudine with emtricitabine was done in already HIV RNA-suppressed patients.[213] All other randomized trials that included lamivudine or emtricitabine in the treatment arms for ART-naive patients had other NRTI variations as well.[187-192, 197, 198] Given the limitations of the trials that have directly compared lamivudine with emtricitabine, an adequately powered, double-blind randomized clinical trial is needed and should directly compare lamivudine with emtricitabine as part of currently recommended cART regimens. In certain resource-limited settings, this often still includes nevirapine.

Our study has several strengths. We used the data of an ongoing nationwide cohort with a well-established infrastructure and data collection. The diminished virological responses to lamivudine in this large cohort were consistently found in all models and sensitivity analyses. Our study methods support the intended clinical relevance of the study. Virological failure was primarily analyzed by OT instead of ITT analysis because we considered an ITT analysis a method that is too conservative to evaluate drug effectiveness outside the context of a clinical trial. Nonetheless, the included sensitivity analysis by ITT showed comparable differences in virological failure. In our opinion, the use of HIV RNA ≥400 copies/mL instead of lower thresholds to evaluate virological failure improved the interpretation of the drugs' clinical effectiveness. Detectable HIV RNA 50-399 copies/mL could represent other situations (eg, temporary incompliance) rather than true virological failure. This is supported by the observation that the large majority of patients with HIV RNA 50-399 copies/mL at week 48 do not have virological failure in follow-up but resuppressed <50 copies/mL on initial regimens. Last, the decreased effectiveness with lamivudine appears to be independent of the NNRTI background regimens; all patients on efavirenz received once-daily cART regardless of lamivudine or emtricitabine.

Several limitations should also be noticed. First, we realize that treatment guidelines have changed during the study period, particularly on CD_4 counts at cART initiation.[214] In the multivariable models, we adjusted for CD_4 counts and other observed differences in patient characteristics. Second, the calendar year at the start of cART only minimally overlapped between the lamivudine and emtricitabine groups and as such could not be corrected for in the multivariable models. During the studied time frame, differences between treatment centers or between physicians may have influenced the results. Two factors make the influence of these potential confounders less likely: HIV care in the Netherlands is highly organized using internationally accepted guidelines, and the treatment centers have been accounted for in the models. Third, an observational study cannot correct for unmeasured confounders or balance known and unknown baseline differences. These factors can only be controlled for in a randomized trial. Furthermore, no data on medication adherence were available and adherence could have differed, as no single-tablet regimen exists that includes tenofovir and lamivudine. However, the large majority of patients in our cohort did not initiate emtricitabine with efavirenz/tenofovir as a single-tablet regimen but started Truvada with efavirenz. Therefore, the pill count differed by only 1 tablet (2 vs 3) in a once-daily regimen. As the observed virological responses were consistent on efavirenz

and nevirapine, we do not consider adherence to be a major explanatory factor of our observations. Finally, resistance data were available in only 50% of patients at baseline and at the time of virological failure and should be interpreted cautiously with respect to selection bias for resistance testing at time of failure.

Our study could have important implications. The presumed clinical equivalence of lamivudine and emtricitabine in treatment guidelines could have a significant impact on HIV-1 care, as generic lamivudine has become available. The observed increased virological failure rate on recommended cART that includes generic lamivudine instead of emtricitabine could result in additional morbidity and costs. Whether these additional costs will exceed initial savings by using generic lamivudine is unknown. From a public health perspective, in particular in settings without routine HIV RNA monitoring, transmission of resistant HIV-1 may be another consequence. On the other hand, as we observed no difference in virological failure once HIV RNA was <400 copies/mL, a switch to lamivudine once patients are virologically suppressed on an emtricitabine-based regimen may be acceptable.

In conclusion, our findings add to the evidence that lamivudine and emtricitabine may not be interchangeable in recommended first-line cART. The use of emtricitabine was associated with better virological responses compared with lamivudine. As the potential implications are substantial, a randomized clinical trial is urgently needed.

SUPPLEMENTARY DATA CHAPTER 2

Table 1. The characteristics of the entire study cohort (n=4836) of HIV-1 infected patients. Patients with baseline resistance, prior ART experience, without HIV-RNA in the 48 ±10 week window and patients lost to follow up or who discontinued cART for other reasons than virological failure prior to week 48 are shown.

	Efavirenz/Tenofovir				**Nevirapine/Tenofovir**			
	Lamivudine (n=546)		**Emtricitabine (n=3391)**		**Lamivudine (n=207)**		**Emtricitabine (n=692)**	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**
Baseline resistance to (non-)NRTI	1	(0.2)	28	(0.8)	2	(1.0)	8	(1.2)
ART experienced	10	(1.8)	20	(0.6)	12	(5.8)	15	(2.2)
No HIV-RNA in week 48 window	51	(9.5)	267	(8.0)	10	(5.2)	41	(6.1)
Lost to follow up	9	(1.6)	76	(2.2)	3	(1.4)	12	(1.7)
cART switch <week 48 window								
Overall	123	(22.5)	563	(16.6)	21	(10.1)	124	(17.9)
Toxicity	73		450		14		97	
Pregnancy	11		6		1		1	
Patient's discretion	8		27		3		9	
cART simplification	19		1		2		4	
Study participation	0		7		0		1	
Drug-drug interactions	0		9		1		2	
Other/unknown	12		63		0		10	

Abbreviations: cART, combination antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor.

Supplementary Table 2. The unadjusted odds ratios (OR) with 95% confidence intervals (95% CI) of covariates on virological failure (n=126) within 48 weeks in patients initiating efavirenz/tenofovir regimens (OT population: n=2789).

	Virological Failure		
	OR	**(95% CI)**	**P**
Lamivudine	3.23	(2.17 - 4.81)	<.001
Emtricitabine[a]	1		
Gender, male sex	0.66	(0.41 - 1.07)	.093
Female sex[a]	1		
Age, year increase	0.97	(0.96 - 0.99)	.003
Hepatitis B			
Positive	1.47	(0.52 - 4.17)	.468
Negative	0.88	(0.35 - 2.20)	.782
Unknown[a]	1		
Hepatitis C			
Positive	0.48	(0.19 - 1.24)	.130
Negative	0.48	(0.25 - 0.92)	.026
Unknown[a]	1		
Transmission			
MSM	0.37	(0.19 - 0.75)	.005
Heterosexual	0.99	(0.49 - 2.00)	.984
Intravenous drug use	0.51	(0.06 - 4.17)	.529
Other	0.56	(0.07 - 4.59)	.586
Unknown[a]	1		
Region of Origin			
Western Countries	0.71	(0.30 - 1.68)	.436
Sub-Saharan Africa	2.33	(0.95 - 5.75)	.066
Asia	0.93	(0.28 - 3.13)	.909
Latin America	1.58	(0.60 - 4.20)	.354
Caribbean	1.02	(0.30 - 3.46)	.968
Other[a]	1		
HIV-RNA, copies/mL			
≥100.000	2.70	(1.83 - 3.98)	<.001
<100,000[a]	1		
CD4 count, cells/mm^3			
<100	6.21	(3.36 - 11.49)	<.001
100 - 199	2.17	(1.10 - 4.27)	.025
200 - 349	0.81	(0.42 - 1.57)	.528
≥350[a]	1		

[a] Reference categories within covariates.

Abbreviation: MSM, men having sex with men; OT, on treatment.

Supplementary Table 3. The unadjusted odds ratios (OR) with 95% confidence intervals (95% CI) of covariates on virological failure (n=97) within 48 weeks in patients initiating nevirapine/tenofovir regimens (OT population: n=651).

	Virological Failure		
	OR	**(95% CI)**	**P**
Lamivudine	3.00	(1.92 - 4.72)	<.001
Emtricitabine[a]	1		
Gender, male sex	0.59	(0.35 - 1.02)	.058
Female sex[a]	1		
Age, year increase	1.00	(0.97 - 1.02)	.798
Hepatitis B			
Positive	2.19	(0.98 - 4.85)	.055
Negative[a]	1		
Unknown	-		
Hepatitis C			
Positive	1.06	(0.24 - 4.69)	.943
Negative	1.12	(0.32 - 3.86)	.861
Unknown[a]	1		
Transmission			
MSM	0.38	(0.17 - 0.85)	.019
Heterosexual	0.58	(0.25 - 1.38)	.221
Intravenous drug use	0.76	(0.20 - 2.94)	.693
Other	0.67	(0.07 - 6.80)	.732
Unknown[a]	1		
Region of Origin			
Western Countries	1.64	(0.49 - 5.56)	.423
Sub-Saharan Africa	2.41	(0.64 - 9.10)	.196
Asia	1.25	(0.19 - 8.26)	.817
Latin America	1.70	(0.42 - 6.94)	.458
Caribbean	3.33	(0.80 - 13.89)	.099
Other[a]	1		
HIV-RNA, copies/mL			
≥100.000	4.05	(2.55 - 6.41)	<.001
<100,000[a]	1		
CD4 count, cells/mm³			
<100	20.00	(7.41 - 52.63)	<.001
100 - 199	4.41	(1.60 - 12.20)	.004
200 - 349	1.90	(0.72 - 5.05)	.197
≥350[a]	1		

[a] Reference categories within covariates. Hepatitis B reference was set at “negative” as no patient with unknown Hepatitis B serology had virological failure.

Abbreviation: MSM, men having sex with men; OT, on treatment.

Supplementary Table 4a. Sensitivity analysis of virological failure within 48 weeks in ART naive HIV-1 patients of the ATHENA cohort. Any HIV-RNA >50 at 48 ±10 week window equals virological failure (OT population: n=3440).

	Virological Failure				Virological Failure		
	No.	**(%)**	**P**		**No.**	**(%)**	**P**
Efavirenz/Tenofovir				**Nevirapine/Tenofovir**			
Lamivudine (n=352)	44	(12.5)		Lamivudine (n=159)	46	(28.9)	
Emtricitabine (n=2437)	171	(7.0)	<.001	Emtricitabine (n=492)	81	(16.5)	.001

Data are numbers (%) and compared by χ^2 tests.

Abbreviations: ART, antiretroviral therapy; ATHENA, AIDS therapy evaluation in the Netherlands; OT, on-treatment.

Supplementary Table 4b. Sensitivity analysis of virological failure by ITT within 48 weeks in ART naive HIV-1 patients of the ATHENA cohort (n=4371). Patients lost to follow up and patients with ART switches for other reasons with last HIV-RNA ≥400 copies/mL were considered virological failures.

	Virological Failure				Virological Failure		
	No.	**(%)**	**P**		**No.**	**(%)**	**P**
Efavirenz/Tenofovir				Nevirapine/Tenofovir			
Lamivudine (n=484)	102	(21.1)		Lamivudine (n=183)	54	(29.5)	
Emtricitabine (n=3076)	387	(12.6)	<.001	Emtricitabine (n=628)	145	(23.1)	.076

Data are numbers (%) and compared by χ^2 tests.

Abbreviations: ART, antiretroviral therapy; ATHENA, AIDS therapy evaluation in the Netherlands; ITT, modified intent-to-treat.

Propensity score adjusted logistic regression model: test statistics and covariate balance.

The propensity scores for therapy-naive HIV-1 patients for receiving lamivudine were calculated for efavirenz/tenofovir and nevirapine/tenofovir containing cART separately. The data from all 4740 therapy-naive HIV-1 infected patients were used to calculate the propensity scores.

Of the 3878 patients who were initiated on efavirenz/tenofovir, 535 were exposed to lamivudine. Their propensity scores ranged from 0.31 to 0.99. The Hosmer-Lemeshow goodness of fit test was 5.236 (P=0.732). The median propensity score for lamivudine/efavirenz/tenofovir was 0.80 (interquartile range: 0.69 - 0.88) and for emtricitabine/efavirenz/tenofovir the median propensity score was 0.90 (interquartile range: 0.83 - 0.94).

Of the 862 patients who were initiated on nevirapine/tenofovir, 193 were exposed to lamivudine. The propensity scores ranged from 0.15 to 0.99. The Hosmer-Lemeshow goodness of fit test was 9.805 (P=0.279). The median propensity score for lamivudine with nevirapine/tenofovir was 0.65 (interquartile range: 0.47 - 0.77) and for emtricitabine with nevirapine/tenofovir the median propensity score was 0.87 (interquartile range: 0.74 - 0.95).

Categorical covariates' balances and virological failure per quintile were analyzed (Supplementary Table 5 and Table 6). The covariates' balances were checked by Mann-Whitney U tests for age and by χ^2 tests for categorical data. A P value < .05 defined an imbalanced covariate. For efavirenz/tenofovir, the treatment hospital was not balanced in quintile 1, hepatitis C and CD4 count were imbalanced in quintile 2, age was imbalanced in quintile 3, region of origin and transmission route and CD4 count were imbalanced in quintile 5. For nevirapine/tenofovir, only hepatitis C was imbalanced in quintile 3. Supplementary Table 7 shows the results of the propensity score adjusted logistic regression models, the double robust propensity score adjusted analysis (in which the imbalanced covariates are also included in the adjusted logistic regression model) and the inverse weighed propensity score adjusted analysis.

Supplementary Table 5. Distribution of baseline covariates per propensity score quintile in lamivudine or emtricitabine exposed HIV-1 infected patients on nevirapine/tenofovir (n=862).

	Propensity Score (Range) Quintiles									
	1 (0.15 - 0.61)		2 (0.62 - 0.77)		3 (0.77 - 0.87)		4 (0.87 - 0.95)		5 (0.95 - 0.99)	
	3TC n=89 %	FTC n=83 %	3TC n=53 %	FTC n=119 %	3TC n=29 %	FTC n=144 %	3TC n=17 %	FTC n=155 %	3TC n=5 %	FTC n=168 %
Gender, male sex	68.5	62.7	83.0	79.0	72.4	81.2	76.5	89.0	100	97.6
Age (median)	39	39	41	39	39	39	39	38	38	42
Treatment hospital										
<500 patients	0	1.2	1.9	4.2	10.3	6.2	11.8	11.0	0	2.4
500-2000 patients	1.1	4.8	1.9	5.9	31.0	26.4	76.5	77.4	100	95.2
>2000 patients	98.9	94.0	96.2	89.9	58.6	67.4	11.8	11.6	0	2.4
Hepatitis B										
Positive	2.2	0.0	3.8	5.9	6.9	3.5	11.8	5.2	0	7.7
Negative	97.8	94.0	92.5	93.3	89.7	92.4	82.4	92.3	100	86.9
Unknown	3.6	2.4	3.8	0.8	3.4	4.2	5.9	2.6	0	5.4
Hepatitis C										
Positive	12.4	6.0	7.5	5.0	0	10.4	0	7.1	0	7.7
Negative	77.5	80.7	90.6	94.1	86.2	86.8	100	92.3	100	91.7
Unknown	10.1	13.3	1.9	0.8	13.8	2.8	0	0.6	0	0.6
Transmission										
MSM	16.9	15.7	67.9	60.5	62.1	69.4	47.1	73.5	100	95.8
Heterosexual	58.4	60.2	24.5	33.6	37.9	25.0	35.3	20.6	0	3.6
Intravenous drug use	11.2	7.2	1.9	1.7	0	2.1	0	1.3	0	0
Other	1.1	1.2	1.9	1.7	0	0.7	0	0.6	0	0
Unknown	12.4	15.7	3.8	2.5	0	2.8	17.6	3.9	0	0.6
Region of origin										
Western Countries	48.3	54.2	71.7	61.3	58.6	63.2	64.7	76.1	80.0	77.4
Sub-Saharan Africa	29.2	21.7	7.5	10.1	10.3	16.7	17.6	5.2	0	0.6
Asia	1.1	2.4	1.9	2.5	3.4	0.7	5.9	5.2	0	4.2
Latin America	13.5	13.3	7.5	15.1	13.8	6.9	5.9	2.6	20.0	6.0
Caribbean	1.1	3.6	5.7	5.0	10.3	5.6	0	5.2	0	7.7
Other	6.7	4.8	5.7	5.9	3.4	6.9	5.9	5.8	0	4.2
HIV-RNA, copies/mL										
<100,000	40.4	44.6	54.7	60.5	79.3	68.1	47.1	45.8	100	78.0
≥100,000	59.6	55.4	45.3	39.5	20.7	31.9	52.9	54.2	0	22.0
CD4 count, cells/mm^3										
<100	42.7	27.7	9.4	17.6	3.4	9.7	5.9	7.7	0	0.6
100 - 199	28.1	33.7	26.4	26.1	24.1	8.3	23.5	20.6	0	3.0
200 - 349	27.0	37.3	58.5	51.3	51.7	56.9	58.8	60.6	60.0	58.3
≥350	2.2	1.2	5.7	5.0	20.7	25.0	11.8	11.0	40.0	38.1

Data are % unless otherwise specified.

Abbreviations: 3TC, lamivudine; FTC, emtricitabine; MSM, men having sex with men.

Supplementary Table 6. Virological failure per propensity score quintile in lamivudine or emtricitabine exposed HIV-1 infected patients on efavirenz/tenofovir or nevirapine/tenofovir in the OT population (n=3440).

	Propensity Score Quintile									
	1		2		3		4		5	
Efavirenz/Tenofovir (n=2789)	**3TC n=158 %**	**FTC n=348 %**	**3TC n=74 %**	**FTC n=480 %**	**3TC n=64 %**	**FTC n=523 %**	**3TC n=31 %**	**FTC n=543 %**	**3TC n=25 %**	**FTC n=543 %**
Virological Failure	16.5	9.8	2.7	4.0	6.2	3.3	12.9	1.3	8.0	2.0

	Propensity Score Quintile									
	1		2		3		4		5	
Nevirapine/Tenofovir (n=651)	**3TC n=72 %**	**FTC n=60 %**	**3TC n=45 %**	**FTC n=91 %**	**3TC n=24 %**	**FTC n=107 %**	**3TC n=13 %**	**FTC n=102 %**	**3TC n=5 %**	**FTC n=132 %**
Virological Failure	40.3	26.7	22.2	12.1	4.2	9.3	23.1	11.8	0	3.8

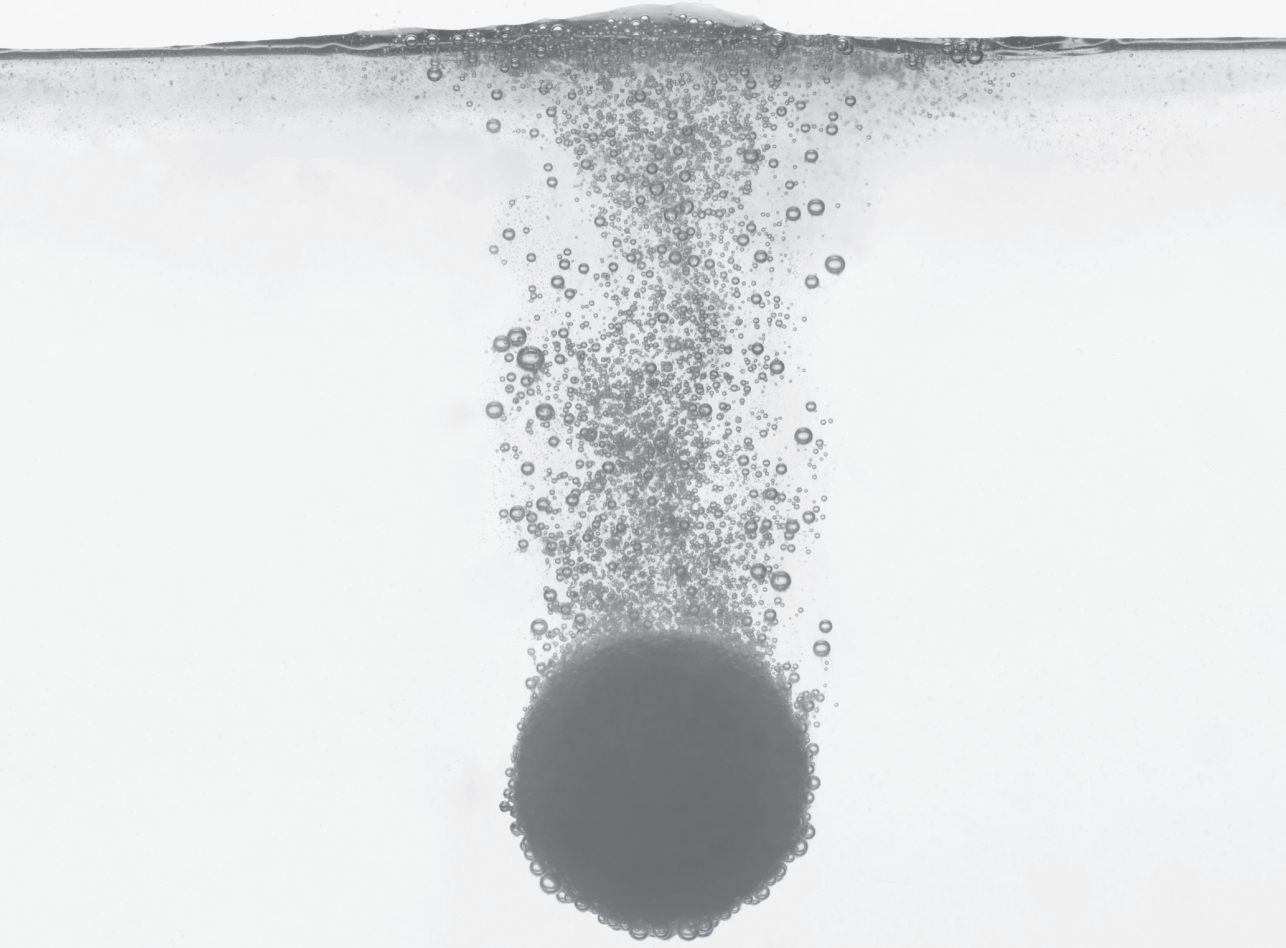
Abbreviations: 3TC, lamivudine; FTC, emtricitabine; OT, on-treatment.

Supplementary Table 7. Propensity score adjusted logistic regression models on the association between virological failure and antiretroviral regimens in naive HIV-1 patients (OT population: n=3440).

	Efavirenz/Tenofovir (n=2789)			Nevirapine/Tenofovir (n=651)		
	Virological Failure			Virological Failure		
	OR	(95% CI)	P	OR	(95% CI)	P
Propensity score adjusted models.						
cART						
Lamivudine	1.89	(1.20 - 2.97)	.006	1.65	(0.99 - 2.77)	.057
Emtricitabine[a]	1			1		
Double robust propensity score adjusted models.						
cART						
Lamivudine	1.96	(1.23 - 3.14)	.005	1.59	(0.94 - 2.68)	.081
Emtricitabine[a]	1			1		
Inverse weighed propensity score adjusted models.						
cART						
Lamivudine	1.88	(1.09 - 3.25)	.023	1.79	(1.07 - 2.99)	.027
Emtricitabine[a]	1			1		

[a] Reference categories within covariates.

Abbreviations: cART, combination antiretroviral therapy; CI, confidence interval; OR, odds ratio.



Chapter 3

Virological responses to lamivudine or emtricitabine when combined with tenofovir and a protease inhibitor in treatment-naïve HIV-1-infected patients in the Dutch AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort.

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ABSTRACT

Introduction

Lamivudine (3TC) and emtricitabine (FTC) are considered interchangeable in recommended tenofovir disoproxil-fumarate (TDF)-containing combination antiretroviral therapies (cART). This statement of equivalence has not been systematically studied. We compared the treatment responses to 3TC and FTC combined with TDF in boosted protease inhibitor (PI)-based cART for HIV-1-infected patients.

Methods

An observational study in the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort was carried out between 2002 and 2013. Virological failure rates, time to HIV RNA suppression < 400 copies/mL, and time to treatment failure were analysed using multivariable logistic regression and Cox proportional hazard models. Sensitivity analyses included propensity score-adjusted models.

Results

A total of 1582 ART-naïve HIV-1-infected patients initiated 3TC or FTC with TDF and ritonavir-boosted darunavir (29.6%), atazanavir (41.5%), lopinavir (27.1%) or another PI (1.8%). Week 48 virological failure rates on 3TC and FTC were comparable (8.9% and 5.6%, respectively; P = 0.208). The multivariable adjusted odds ratio of virological failure when using 3TC instead of FTC with TDF in PI-based cART was 0.75 (95% confidence interval (CI) 0.32-1.79; P = 0.51). Propensity score-adjusted models showed comparable results. The adjusted hazard ratio (HR) for treatment failure of 3TC compared with FTC was 1.15 (95% CI 0.58-2.27) within 240 weeks after cART initiation. The time to two consecutive HIV RNA measurements <400 copies/mL within 48 weeks (HR 0.94; 95% CI 0.78-1.16) and the time to treatment failure after suppression <400 copies/mL (HR 0.94; 95% CI 0.36-2.50) were not significantly influenced by the use of 3TC in TDF/PI-containing cART.

Conclusion

The virological responses were not significantly different in treatment-naïve HIV-1-infected patients starting either 3TC/TDF or FTC/TDF and a ritonavir-boosted PI.

INTRODUCTION

The nucleoside reverse transcriptase inhibitors (NRTIs) lamivudine (3TC) and emtricitabine (FTC) are considered interchangeable in recommended combination antiretroviral therapy (cART) in current HIV-1 guidelines.[163, 164, 167] Both can be used in combination with tenofovir disoproxil-fumarate (TDF) and a nonnucleoside reverse transcriptase inhibitor (NNRTI), boosted protease inhibitor (PI) or integrase inhibitor (INI) as first-line cART for treatment-naïve HIV-1-infected patients.

The use of 3TC instead of FTC with TDF has been associated with increased rates of virological failure and acquired resistance in NNRTI-based cART for ART-naïve HIV-1 patients,[193, 195, 196, 215, 216] although others observed no difference in failure rates.[199, 217] Whether the use of 3TC instead of FTC with TDF and a boosted PI for treatment-naïve patients is associated with decreased clinical effectiveness is unknown. The limited number of randomized trials in ART-naïve patients showed inconsistent responses to 3TC or FTC in combination with a boosted PI. These trials were all confounded because the second NRTI combined with either 3TC or FTC also differed (TDF, abacavir or zidovudine).[218-222] The evidence to support the equivalent recommendation of 3TC and FTC with TDF in HIV-1 treatment guidelines appears insufficient. This unresolved issue has become more relevant with the introduction of generic 3TC, which could be used instead of FTC to reduce costs.[186]

This study aimed to evaluate the responses to 3TC and FTC as part of the recommended TDF-containing NRTI backbone in combination with a boosted PI for the treatment of ART-naïve HIV-1-infected patients in a nationwide HIV-cohort.

METHODS

Regulatory approval and study design

The Dutch HIV Monitoring Foundation [Stichting HIV Monitoring (SHM)] has registered all HIV-infected patients in a nationwide cohort. This cohort is known as the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort. Data obtained from patients in clinical care in or after 1996 in any of the 27 HIV treatment centres in the Netherlands have been systematically collected as part of routine care.[223] Patients can choose to be excluded from prospective data collection in ATHENA. A total of

21 858 HIV-infected patients were registered in ATHENA by 31 December 2013, of whom 21 485 (98.3%) had consented to data collection.[224]

For this study, we included all ART-naïve HIV-1-infected patients, aged 18 years or older at inclusion in ATHENA, who started either 3TC or FTC in combination with TDF and a boosted PI between 1 January 2002 and 1 June 2013. Data collection included ART history, baseline and acquired drug resistance when available, age, sex, country of birth, HIV-1 transmission route, treatment hospital, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, CD4 cell count, and all HIV RNA measurements prior to and after cART initiation. HBV infection was defined by the presence of HBV surface antigen and HCV infection was defined by presence of HCV RNA or, if HCV RNA was not available, by a positive HCV antibody test. All ART switches and reasons for treatment alterations have been registered in ATHENA by the patients' HIV physicians. The reasons for discontinuation of each drug were identified, and these reasons included virological failure, toxicity, pregnancy and drug-drug interactions. Within PI class changes were considered continued PI use.

Five clinical outcomes were analysed, as previously described.[215] First, we assessed virological failure at week 48 after cART initiation. Failure was defined as (1) any HIV RNA measurement ≥400 HIV-1 RNA copies/mL within a week 48 ±10 window, (2) any ART switch for documented virological failure or because of new Centers for Disease Control and Prevention (CDC) B/C events, and (3) deaths from any cause while the last HIV RNA measurement was ≥400 copies/mL. Patients without HIV RNA measurements available within 48 ±10 weeks after cART initiation were considered lost to follow-up. Secondly, we evaluated the time from cART initiation to the first of two consecutive measurements of successfully suppressed HIV RNA <400 copies/mL within 48 weeks. Furthermore, we compared the time from cART initiation to treatment failure up to 240 weeks. Any ART discontinuation for documented virological failure, new CDC B/C events or death defined the event treatment failure. Fourthly, we evaluated the time to treatment failure following successful suppression <400 copies/mL. Lastly, the presence of at least low-level baseline and acquired resistance upon failure in HIV-1 reverse transcriptase and protease sequences was evaluated.[168]

Statistical analysis

Data are described as means with 95% confidence intervals (95% CIs), medians with interquartile ranges (IQRs) or numbers with percentages. The primary outcome week

48 virological failure was analysed on treatment (OT); ART switches for reasons other than failure, and loss to follow-up did not define failure and were excluded from this analysis. The virological failure rate was calculated as the number of patients with failure divided by the OT population. The adjusted odds ratios (aOR) of failure on 3TC compared with FTC was evaluated in a multivariable logistic regression model. The fixed effects in this model were baseline HIV RNA, CD4 cell count, region of origin (Western Europe/North America/Australia, sub-Saharan Africa, Latin America, and other), initial PI (darunavir, atazanavir, lopinavir and other) and all covariates associated ($P < 0.1$) with failure in univariable analysis. The treatment hospitals were included as random effects.

The time to virological suppression within 48 weeks, the time to treatment failure within 240 weeks, and the time to treatment failure following successful suppression within 240 weeks were evaluated in multivariable Cox regression models and Kaplan-Meier graphs. Hazard ratios (HRs) were adjusted for baseline CD4 cell count, HIV RNA, and initial PI in cART. Censoring occurred at ART switches, at the end of the study period at week 48 or 240 after cART initiation, or at the last HIV RNA measurement. For those with missing baseline HIV RNA or CD4 cell counts (7.3% of total), we imputed 5 times an HIV RNA value or CD4 cell count based on age, sex, region of origin, HIV-1 transmission route, HCV infection and cART initiation year. These data sets were then analysed as described above and estimates were subsequently combined using methods for multiple imputation.[225]

We used three sensitivity analyses to evaluate the primary outcome: (1) any HIV RNA measurement >50 copies/mL (instead of 400 copies/mL) within week 48 ±10 defined virological failure, (2) modified intent-to-treat analysis (ITT) considered loss to follow-up and ART switches while HIV RNA was >400 copies/mL as failures, and (3) propensity score-adjusted models were used. Propensity scores can partially adjust for selection or treatment allocation bias in cohorts.[203] The individual probabilities of receiving 3TC- or FTC-containing cART were estimated based on the measured baseline variables. These propensity scores on treatment with 3TC or FTC were used as covariates, and inverse weights in logistic regression models to evaluate week 48 failure on cART. SPSS v. 22.0, Graphpad Prism v. 5.0, and R v. 3.0.1 were used for the analyses.

RESULTS

Patient characteristics

A total of 1614 HIV-1-infected patients initiated 3TC or FTC with TDF and a boosted PI between 2002 and 2013. Thirty-two of 1614 (2.0%) had previously received antiretroviral drugs. Table 1 shows the baseline characteristics of the 1582 included HIV-1-infected patients who initiated 3TC-containing (n = 142) or FTC-containing (n = 1440) regimens. HIV-1 subtype data were available for 510 patients (32.2%) and, of those, 79.8% had subtype B. The median age was 41 years. Patients were predominantly male (85.1%) from Western countries (69.1%). Patients on 3TC were less likely to be HIV-1 infected through sexual contact with men, and initiated cART at earlier calendar years (median 2005) compared with patients on FTC (median 2010). The principal initial PIs in cART were imbalanced and included atazanavir or lopinavir for patients on 3TC, and atazanavir or darunavir for patients on FTC. The median HIV RNA was higher (5.3 and 5.1 log copies/mL, respectively) and the median CD4 cell count was lower (159 and 250 cells/lL, respectively) at baseline for patients on 3TC- compared with FTC-containing regimens.

Virological responses to cART

At week 48, 272 patients (17.2%) had switched ART for reasons other than virological failure (supplementary data). Important reasons for these premature discontinuations were toxicity (40.8%), ART simplification (18.4%), or to avoid drug-drug interactions (6.6%). Sixty patients (3.8%) were lost to follow-up after cART initiation. In addition, 130 patients (8.2%) did not have HIV RNA measurements in the week 48 window. Of these patients, 120 had HIV RNA <400 copies/mL (92.3%) and 103 had HIV RNA <50 copies/mL (79.2%) prior to this window. These patients were not included in the OT population, which consisted of 1120 patients.

No significant difference in failure rates was observed between 3TC- and FTC-containing regimens at week 48. Virological failure rates were eight of 90 (8.9%) for 3TC and 58 of 1030 (5.6%) for FTC (OR 1.64; 95% CI 0.76-3.54; P = 0.208). A total of 66 failing patients were considered to have virological failure: 18 patients had documented virological failure, 31 patients had an HIV RNA measurement ≥400 copies/mL in the week 48 window, and 17 patients (with a median CD4 count of 50 cells/lL) had new CDC B/C events (n = 3) or died (n = 14) while their last HIV RNA measurement was ≥400 copies/mL. Twenty-one of the 31 patients (27 on FTC and

Table 1. Baseline characteristics of antiretroviral naïve HIV-1 infected patients (n=1582) initiating lamivudine or emtricitabine with tenofovir disoproxil-fumarate and a boosted protease inhibitor in the ATHENA cohort.

	Lamivudine/Tenofovir (n=142)		Emtricitabine/Tenofovir (n=1440)	
	No.	**(%)**	**No.**	**(%)**
Male sex	117	(82.4)	1230	(85.4)
Age (median, IQR)	39	(34 - 47)	41	(33 - 48)
Start year (median, IQR)	2005	(2004 - 2006)	2010	(2008 - 2011)
Treatment hospital				
<500 patients	7	(4.9)	161	(11.2)
500 - 2000 patients	83	(58.5)	809	(56.2)
>2000 patients	52	(36.6)	470	(32.6)
Hepatitis B				
Positive	14	(9.9)	100	(6.9)
Negative	119	(83.8)	1282	(89.0)
Unknown	9	(6.3)	58	(4.0)
Hepatitis C				
Positive	24	(16.9)	138	(9.6)
Negative	106	(74.6)	1219	(84.7)
Unknown	12	(8.5)	83	(5.8)
HIV-1 transmission				
MSM	73	(51.4)	920	(63.9)
Heterosexual	41	(28.9)	398	(27.6)
Intravenous drug use	7	(4.9)	30	(2.1)
Other	2	(1.4)	14	(1.0)
Unknown	19	(13.4)	78	(5.4)
Region of origin				
Western Countries	98	(69.0)	995	(69.1)
Sub-Saharan Africa	18	(12.7)	151	(10.5)
Asia	2	(1.4)	53	(3.7)
Latin-America	5	(3.5)	121	(8.4)
Caribbean	9	(6.3)	42	(2.9)
Other	10	(7.0)	78	(5.6)

Table 1. Baseline characteristics of antiretroviral naïve HIV-1 infected patients (n=1582) initiating lamivudine or emtricitabine with tenofovir disoproxil-fumarate and a boosted protease inhibitor in the ATHENA cohort. (continued)

	Lamivudine/Tenofovir (n=142)		Emtricitabine/Tenofovir (n=1440)	
	No.	**(%)**	**No.**	**(%)**
Boosted protease inhibitor				
Darunavir	2	(1.4)	466	(32.4)
Atazanavir	45	(31.7)	611	(42.2)
Lopinavir	89	(62.7)	339	(23.5)
Other	6	(4.2)	24	(1.7)
HIV-RNA (copies/mL)				
<1000	4	(2.8)	42	(2.9)
1000 - 9,999	6	(4.2)	128	(8.9)
10,000 - 99,999	36	(25.4)	491	(34.1)
≥100,000	96	(67.6)	779	(54.1)
CD4 count (cells/mm^3)				
<100	55	(38.7)	330	(22.9)
100 - 199	29	(20.4)	224	(15.6)
200 - 349	42	(29.6)	535	(37.2)
≥350	16	(11.3)	351	(24.4)

Data are presented as No. (%) unless otherwise specified.

Abbreviations: ATHENA, AIDS therapy evaluation in the Netherlands; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men having sex with men; SD, standard deviation.

four on 3TC) with HIV RNA >400 copies/mL in the week 48 window achieved HIV RNA < 50 copies/mL on continued PI regimens with their initial NRTI backbones after week 48. These 21 patients consisted of 20 of the 27 (74.1%) patients on FTC and one of the four (25%) patients on 3TC.

The aOR of virological failure for the use of 3TC compared with FTC with TDF and a boosted PI was 0.75 (95% CI 0.32-1.79; P = 0.51), and was adjusted for HIV RNA, CD4 cell count, region of origin, initial PI in cART and HIV-1 transmission route in a multivariable logistic regression model (Table 2). Associations with failure were observed for an HIV RNA measurement ≥100.000 (aOR 2.45; 95% CI 1.30-4.62; P = 0.006) and for nonheterosexual or MSM HIV-1 transmission (aOR: 2.68; 95% CI 1.25-5.74; P = 0.01). A trend towards better responses with darunavir was observed

Table 2. Multivariable logistic regression analysis of virological failure of using lamivudine/tenofovir disoproxil-fumarate or emtricitabine/tenofovir disoproxil-fumarate with a protease inhibitor in antiretroviral naive HIV-1 patients of ATHENA (OT population: n=1120).

	Virological Failure (n=66)		
	OR	**(95% CI)**	**P**
cART			
3TC/TDF/PI	0.75	(0.32 - 1.79)	0.51
FTC/TDF/PI[a]	1		
HIV-RNA (copies/mL)			
≥100,000	2.45	(1.30 - 4.62)	0.006
<100,000[a]	1		
CD4 count (cells/mm^3)			
<100	1.54	(0.65 - 3.64)	0.32
100 - 199	0.57	(0.18 - 1.81)	0.33
200 - 349	0.82	(0.32 - 2.10)	0.68
≥350[a]	1		
Region of origin			
Sub-Saharan Africa	1.17	(0.51 - 2.70)	0.71
Latin-America	0.83	(0.35 - 1.94)	0.66
Other	0.28	(0.06 - 1.21)	0.09
Western[a]	1		
HIV-1 Transmission			
Heterosexual	1.34	(0.68 - 2.62)	0.40
Other	2.68	(1.25 - 5.74)	0.01
MSM[a]	1		
Boosted PI			
Darunavir	0.21	(0.04 - 1.11)	0.07
Atazanavir	0.50	(0.10 - 2.41)	0.38
Lopinavir	0.90	(0.18 - 4.38)	0.89
Other[a]	1		

[a] Reference categories within covariates.

Treatment hospitals included as random effect.

Abbreviations: 3TC, lamivudine; 95%CI, 95% confidence interval; ATHENA, AIDS therapy evaluation in the Netherlands; cART, combination antiretroviral therapy; FTC, emtricitabine; OR, odds-ratio; OT, on-treatment; PI, protease inhibitor; TDF, tenofovir disoproxil-fumarate.

(aOR of failure: 0.21; 95% CI 0.04-1.11; P = 0.07). Other measured covariates were not associated (P < 0.1) with virological failure in univariable analysis, and were not included in the multivariable model (supplementary data).[226]

The virological failure rates with 3TC and FTC in cART were consistent in the sensitivity analyses. At week 48, 22.2% (n = 20) of patients on 3TC and 20.1% (n = 207) of patients on FTC had HIV RNA >50 copies/mL (OR 1.14; 95% CI 0.68-1.91; P = 0.631). Of 161 patients with week 48 HIV RNA >50 to <400 copies/mL, only eight (4.9%) had subsequent documented treatment failure during 240 weeks of follow-up. The virological failure rate with 3TC was higher compared with FTC in ITT analysis (15.5% versus 9.1%, respectively; P = 0.014). This difference was driven by more ART switches for reasons other than failure (e.g. cART simplification) when the last HIV RNA measurement was >400 copies/mL with 3TC. The propensity score-adjusted models consistently showed similar risks of virological failure for 3TC and FTC in TDF- and boosted PI-containing regimens (supplementary data).[226]

The time to virological suppression was not significantly different between 3TC and FTC when combined with TDF in boosted PI-containing cART (HR 0.94; 95% CI 0.78-1.16; P = 0.589; Fig. 1a), treatment failure within 240 weeks (HR 1.15; 95% CI 0.58-2.27; P = 0.690; Fig. 1b), or treatment failure within 240 weeks in those with initial successful suppression <400 copies/mL first (HR 0.94; 95% CI 0.36-2.50; P = 0.91; Fig. 1c). All Cox models were adjusted for baseline HIV RNA, CD4 cell count and initial PI in cART. A total of 222 patients (14.0%) had within PI class changes without NRTI backbone alteration for reasons other than virological failure during follow-up. The predominant reasons for these within PI class changes were toxicity and patients' preferences (50.5%), and 138 out of these 222 patients did not have subsequent PI class discontinuations or within NRTI class changes. At week 240, 22.9% of patients on 3TC and 61.3% of patients on FTC were on initial regimens. An estimated 43.6% of all patients had switched away from the PI class or had within NRTI class changes for any reason within 240 weeks (Fig. 2). The estimated percentage of these switchers was 15.4% for toxicity, 5.1% for virological failure, and 23.1% for other reasons.

Baseline resistance genotyping data were available in 787 of 1582 patients at a median of 92 days (IQR 19-628 days) prior to cART initiation. No protease mutations associated with at least low-level resistance to PI were detected in these patients. Eighteen patients had a combination of two to four thymidine-associated mutations (M41L,

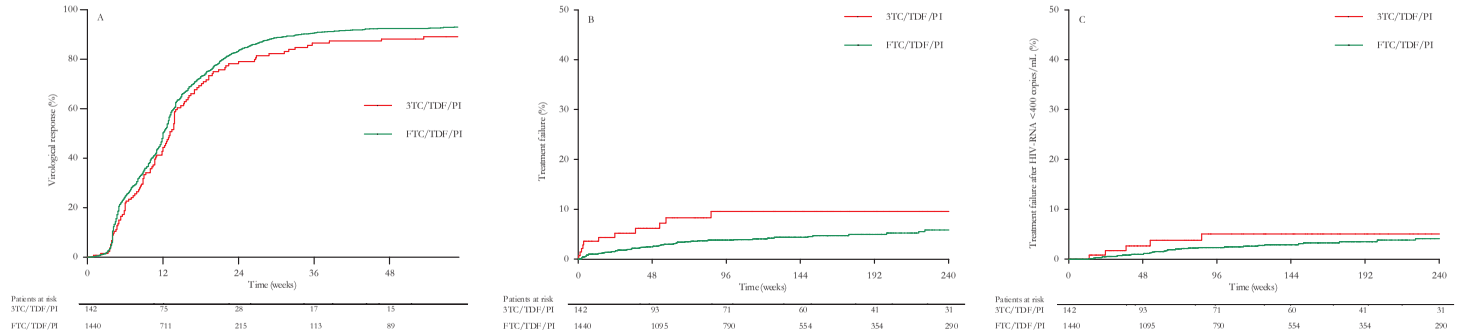

Figure 1. Kaplan–Meier estimates of the virological responses to lamivudine (3TC) (red) or emtricitabine (FTC) (green) with tenofovir disoproxil-fumarate (TDF) in nucleoside reverse transcriptase inhibitor (NRTI) backbones as part of protease inhibitor (PI)-containing combination antiretroviral therapy (cART) in 1582 ART-naïve HIV-1-infected patients in the Dutch AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort. All Cox proportional hazard models were adjusted for baseline CD4 cell count, HIV RNA, and initial PI in cART. **A.** The time to first of two consecutive HIV RNA measurements < 400 copies/mL within 48 weeks on cART. The adjusted hazard ratio (HR) of virological suppression on 3TC compared with FTC was 0.94 (95% confidence interval 0.78–1.16) with TDF/PI. **B.** The time to ART switches for treatment failure in ATHENA within 240 weeks after the initiation of cART. The adjusted HR of treatment failure on 3TC compared with FTC was 1.15 (95% CI 0.58–2.27) with TDF/PI. **C.** The time to ART switches for treatment failure in ATHENA after virological suppression to HIV RNA < 400 copies/ mL on cART. The adjusted HR of treatment failure following suppression on 3TC compared with FTC was 0.94 (95% CI 0.36–2.50) with TDF/PI.

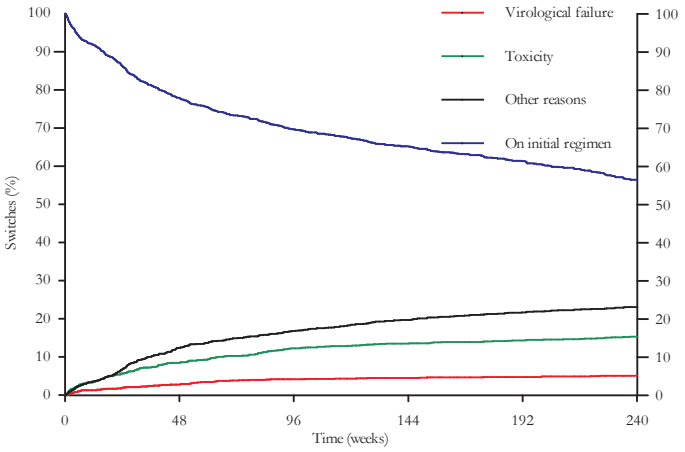

Figure 2. Kaplan–Meier estimates for the 1582 antiretroviral therapy (ART)-naive HIV-1-infected patients in the Dutch AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort who remained on initial lamivudine (3TC) or emtricitabine (FTC) with tenofovir disoproxil-fumarate (TDF)/protease inhibitor (PI) (blue line), and who switched therapy for any reason (red, green and black) up to week 240 after combination antiretroviral therapy (cART) initiation.

D67G, T69S, L210E/F/W/Y, T215C/D/E/L/S and K219E/R) associated with low-level resistance against TDF. One patient had combined low-level resistance against TDF and FTC (K65N and K219R). We evaluated resistance in the 94 patients identified as failures because they had either HIV RNA >400 copies/mL within the 48 ±10 week window or switched cART for documented virological failure, death or new CDC B/C events within 240 weeks. Their median HIV RNA at these events was 467 copies/mL on 3TC and 613 copies/mL on FTC. Baseline resistance was available in 49 of 94 failing patients; 47 patients had wild-type HIV-1, and two patients had M41L/L210W/T215D or D67G/T215C/K219E. For 17 of those failing patients (three on 3TC), the genotypic testing results were available during follow-up. No acquired resistance to protease inhibitors of at least low level was detected. Thirteen patients had wild-type viruses at failure. One patient with baseline M41L/L210W/T215D had virological failure without additional acquired resistance mutations. Three patients with wild-type HIV-1 at baseline acquired significant resistance; one patient on 3TC had K65R/V108I/Y181C/M184V/H221Y and two patients on FTC had V179D/M184VI or K70Q/M184V resistance patterns at failure.

DISCUSSION

We compared the virological responses to 3TC with those to FTC in HIV-1-infected patients starting TDF and a boosted PI. The use of 3TC instead of FTC in these regimens was not significantly associated with decreased virological responses over 5 years of follow-up. This study is the first to support their assumed equivalence in HIV guidelines in TDF/PI regimens. These results may indicate that 3TC and FTC are interchangeable with TDF as NRTI backbones in boosted PI-based first-line cART specifically. Direct comparisons of 3TC with FTC when combined with TDF and a boosted PI in the context of a randomized clinical trial have never been made. Our current results are in contrast to the increased failure that we recently observed in patients initiating 3TC or FTC with TDF in an NNRTI-containing regimen.[215] Their equivalence in the context of boosted PI-containing cART may be a reflection of the higher genetic barrier to resistance of boosted PIs compared with NNRTIs.

The possibility that 3TC has a lower antiretroviral potency against HIV-1 was first hypothesized based on in vitro observations,[205-207] and an early trial in vivo.[187] Subsequent randomized trials in ART-naïve patients compared 3TC-containing regimens with FTC-containing regimens and provided conflicting results. All well-powered trials to date were confounded by variations in the second NRTI of the study treatment arms, and never included both 3TC and TDF. Therefore, FTC/TDF was always compared with 3TC co-formulated with abacavir or zidovudine in boosted PI-based regimens,[188, 197, 218, 220-222] in NNRTI-based regimens,[189, 190, 218] or in INI-based regimens.[227, 228] Several trials found comparable efficacies of FTC and 3TC, but randomized patients when they had already become HIV RNA suppressed on previous cART.[229-233] The use of 3TC in combination with TDF in an NRTI backbone was only evaluated in three small studies in NNRTI-based regimens, including two pilot trials without an FTC-containing comparator arm,[208, 209] and one unpublished underpowered open-label randomized trial.[212] Only two clinical trials have directly compared 3TC and FTC in otherwise identical cART. However, their generalizability to current ART practices is limited by the inclusion of stavudine as second NRTI and only nelfinavir or indinavir as PIs.[210, 213] Notably, three meta-analyses have been conducted to date. These studies came to different conclusions on the equivalence of FTC and 3TC with TDF, although their analyses included the same previously mentioned heterogenic studies.[193, 199, 219]

The present study has certain strengths. The data in the ATHENA cohort are prospectively collected and monitored by trained data managers. The main analyses and sensitivity analyses showed consistent results. The OT analysis supports the clinical significance of the results. Analysis by ITT and using an HIV-RNA <50 copies/mL threshold for virological failure are considered appropriate for the evaluation of new antiretroviral compounds. However, a higher HIV RNA cut-off to classify virological responses may be more suitable in real life, particularly when boosted PI-containing regimens are given to ART-naïve patients. Temporary HIV RNA >50 but <400 copies/mL on PI-containing regimens probably represents nonadherence rather than virological failure as a result of acquired resistance. Our observation that patients with week 48 HIV RNA >50 but <400 copies/mL did not experience virological failure upon follow-up supports the suggestion that a higher HIV RNA cut-off for PI-containing cART may be used.

Several limitations need to be addressed. First, the number of patients on 3TC was small and considerably lower than the number of patients on FTC. This limited the power to detect smaller differences in responses. The absence of any clinically relevant difference should therefore be confirmed in larger cohorts. Secondly, the more recent introduction of FTC than 3TC led to imbalances in covariates, because recommended PIs and CD4 count thresholds for cART initiation changed during the studied period (42). The multivariable models were used to correct for PI backbone and baseline CD4 count in multiple ways and consistently found no difference in responses to 3TC and FTC. Because of the minimal overlap between the year of cART initiation in the two groups, the initiation year was not included in the models. The initial PI was included in the models, although more patients on 3TC had received lopinavir compared with those on FTC, and only a limited number of patients on 3TC had received darunavir. Thirdly, apart from baseline imbalances, differences in clinical care between treatment centres may have biased the results. We aimed to minimize bias by including treatment hospital as a random effect in the models. The structured HIV-1 care in the Netherlands, following international HIV guidelines, further limited the influence of this confounder. Fourthly, adherence might be lower in patients with higher pill counts, and for regimens that have to be used multiple times per day. Although adherence is not systematically recorded in ATHENA, we did correct for once-daily regimens (atazanavir) or twice-daily regimens (lopinavir and darunavir). The observation that the failure rate in patients on 3TC-containing cART, with a higher pill count compared with patients on FTC-containing cART,

was not significantly different suggests that adherence differences are unlikely to have confounded the results. A minor limitation is the nonsystematic determination of genotypic resistance prior to the start of PI-based cART. Finally, we cannot correct for unmeasured confounders. Only an adequately powered randomized clinical trial can give a definite answer on the equivalence of 3TC and FTC with TDF; however, this is unlikely to be performed.

The implications of our study results could be substantial. In contrast to regimens containing TDF and NNRTIs, where conflicting observations on responses to 3TC and FTC have been reported, the assumed clinical equivalence of 3TC and FTC in current guidelines may hold true for TDF- and boosted PI-containing regimens. This may suggest that generic 3TC instead of FTC in these PI regimens may be used without an increased risk of virological failure and possibly subsequent additional costs, morbidity or mortality. This information is relevant in view of the increased focus on HIV care cost containment in settings worldwide.

In conclusion, we did not find evidence that the use of 3TC or FTC with TDF as part of boosted PI-containing regimens results in a different virological response. Despite the limitations of an observational study, the results may indicate that 3TC and FTC have comparable virological responses and are interchangeable with TDF and a boosted PI for treatment-naïve HIV-1-infected patients.

SUPPLEMENTARY DATA CHAPTER 3

Table 1. The characteristics of the entire study cohort of HIV-1 patients (n=1614).

	3TC/TDF/PI (n=151)		**FTC/TDF/PI (n=1463)**		**Total (n=1614)**	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**
ART experienced	9	(6.0)	23	(1.6)	32	(2.0)
Lost to follow up	7	(4.6)	53	(3.6)	60	(3.7)
HIV-RNA missing in week 48 window	9	(6.0)	121	(8.3)	130	(8.1)
ART switch prior to week 48 window	36	(23.8)	236	(16.1)	272	(16.9)
Reasons						
Toxicity	14	(38.9)	97	(41.1)	111	(40.8)
Pregnancy	1	(2.8)	8	(3.4)	9	(3.3)
Patient's discretion	1	(2.8)	17	(7.2)	18	(6.6)
ART simplification	8	(22.2)	42	(17.8)	50	(18.4)
Study participation	1	(2.8)	12	(5.1)	13	(4.8)
Drug-drug interactions	0	(0.0)	18	(7.6)	18	(6.6)
Other/unknown	11	(30.6)	42	(17.8)	53	(19.5)

Abbreviations: 3TC, lamivudine; ART, combination antiretroviral therapy; FTC, emtricitabine; PI, boosted protease inhibitor; TDF, tenofovir disoproxil-fumarate.

Supplementary Table 2. Unadjusted analysis of the association of covariates with virological failure (n=66) within 48 weeks in HIV-1 patients on protease inhibitor/tenofovir disoproxil-fumarate regimens (on treatment population: n=1120).

	Odds-ratio	(95% confidence interval)	P
Lamivudine	1.64	(0.76 - 3.54)	.208
Emtricitabine[a]	1		
Male sex	1.56	(0.66 - 3.67)	.312
Female sex[a]	1		
Age (per year increase)	0.99	(0.97 - 1.01)	.666
Treatment hospital			
<500 patients	1.37	(0.63 - 2.98)	.430
500 - 2000 patients	0.86	(0.50 - 1.50)	.595
>2000 patients[a]	1		
Hepatitis B positive	0.75	(0.20 - 2.81)	.669
Negative	0.60	(0.21 - 1.74)	.345
Unknown[a]	1		
Hepatitis C positive	0.53	(0.16 - 1.72)	.290
Negative	0.54	(0.22 - 1.31)	.175
Unknown[a]	1		
HIV-1 Transmission			
Men having sex with men	0.23	(0.11 - 0.48)	<.001
Heterosexual	0.36	(0.16 - 0.80)	.012
Intravenous drug use	0.25	(0.03 - 2.02)	.192
Other	0.55	(0.06 - 4.76)	.583
Unknown[a]	1		
Region of Origin			
Western Countries	1.88	(0.45 - 7.94)	.532
Sub-Saharan Africa	3.17	(0.67 - 14.93)	.315
Asia	-[b]		
Latin-America	2.51	(0.49 - 12.82)	.399
Caribbean	1.56	(0.21 - 11.63)	.639
Other[a]	1		
Protease inhibitor			
Darunavir	0.23	(0.05 - 1.16)	.075
Atazanavir	0.52	(0.12 - 2.38)	.403
Lopinavir	1.11	(0.25 - 5.05)	.888
Other[a]	1		

Supplementary Table 2. Unadjusted analysis of the association of covariates with virological failure (n=66) within 48 weeks in HIV-1 patients on protease inhibitor/tenofovir disoproxil-fumarate regimens (on treatment population: n=1120). (continued)

	Odds-ratio	(95% confidence interval)	P
HIV-RNA (copies/mL)			
≥100.000	3.19	(1.75 - 5.81)	<.001
<100,000[a]	1		
CD4 count (cells/mm³)			
<100	3.53	(1.64 - 7.63)	.001
100 - 199	1.12	(0.41 - 3.05)	.831
200 - 349	1.47	(0.66 - 3.26)	.345
≥350[a]	1		

[a] Reference categories within covariates. [b] Not calculated no patient experienced virological failure.

Propensity score adjusted logistic regression model: test statistics and covariate balance.

The propensity scores for therapy-naive HIV-1 patients for receiving 3TC instead of FTC in TDF and boosted PI containing regimens were calculated. The data from all 1582 ART-naive HIV-1 infected patients were used to calculate the propensity scores. Of these 1582 patients, 142 received 3TC in their initial cART. The propensity scores ranged from 0.37 to 0.99. The Hosmer-Lemeshow goodness of fit test was 7.067 (P=0.529). The median propensity score for 3TC was 0.82 (interquartile range: 0.73 - 0.90) and for FTC the median propensity score was 0.95 (interquartile range: 0.90 - 0.99).

The covariates' balance and the virological failure rate per quartile were analyzed (Supplementary Table 3). Balance were checked by Mann-Whitney U tests for age and by χ^2 tests for categorical data. P<.05 defined an imbalanced covariate within a quartile. The distribution of boosted protease inhibitors were imbalanced in quartiles 2, 3, and 4 due to low number of boosted darunavir with lamivudine. CD4 count were imbalanced in quartile 1, 2, and 4; HIV-RNA remained imbalanced in quartile 2 and 3. Transmission routes were imbalanced in quartile 1 and 4. Hepatitis C coinfection remained imbalanced in quartile 1 and 3. Failure rates remained comparable for 3TC and FTC per quartile.

Supplementary Table 4 shows the results of the propensity score adjusted logistic regression models, the double robust propensity score adjusted analysis (in which the imbalanced covariates are also included in the adjusted logistic regression model) and the inverse weighed propensity score adjusted analysis.

Supplementary Table 3. Virological failure per propensity score quartile in 3TC or FTC exposed HIV-1 infected patients on TDF and boosted PI in the on-treatment population (n=1120).

	Propensity Score Quartile							
	1		2		3		4	
PI/TDF	**3TC n=24 %**	**FTC n=223 %**	**3TC n=20 %**	**FTC n=266 %**	**3TC n=23 %**	**FTC n=265 %**	**3TC n=23 %**	**FTC n=276 %**
Virological Failure	8.3	12.1	15.0	6.0	8.7	2.6	4.3	2.9

Abbreviations: 3TC, lamivudine; FTC, emtricitabine; PI, protease inhibitor; TDF, tenofovir-DF.

Supplementary Table 4. Propensity score adjusted logistic regression models on the association between virological failure at week 48 and antiretroviral regimens in naive HIV-1 patients (OT population: n=1120).

		Virological Failure		
		OR	**(95% CI)**	**P**
Propensity score adjusted model				
	3TC/TDF/PI	0.74	(0.31 - 1.76)	.493
	FTC/TDF/PI[a]	1		
Double robust propensity score adjusted model				
	3TC/TDF/PI	0.79	(0.32 - 1.91)	.593
	FTC/TDF/PI[a]	1		
Inverse weighed propensity score adjusted model				
	3TC/TDF/PI	1.28	(0.56 - 2.95)	.559
	FTC/TDF/PI[a]	1		

[a] Reference categories within covariates.

Abbreviations: 3TC, lamivudine; CI, confidence interval; FTC, emtricitabine; OR, odds ratio; PI, boosted PI.

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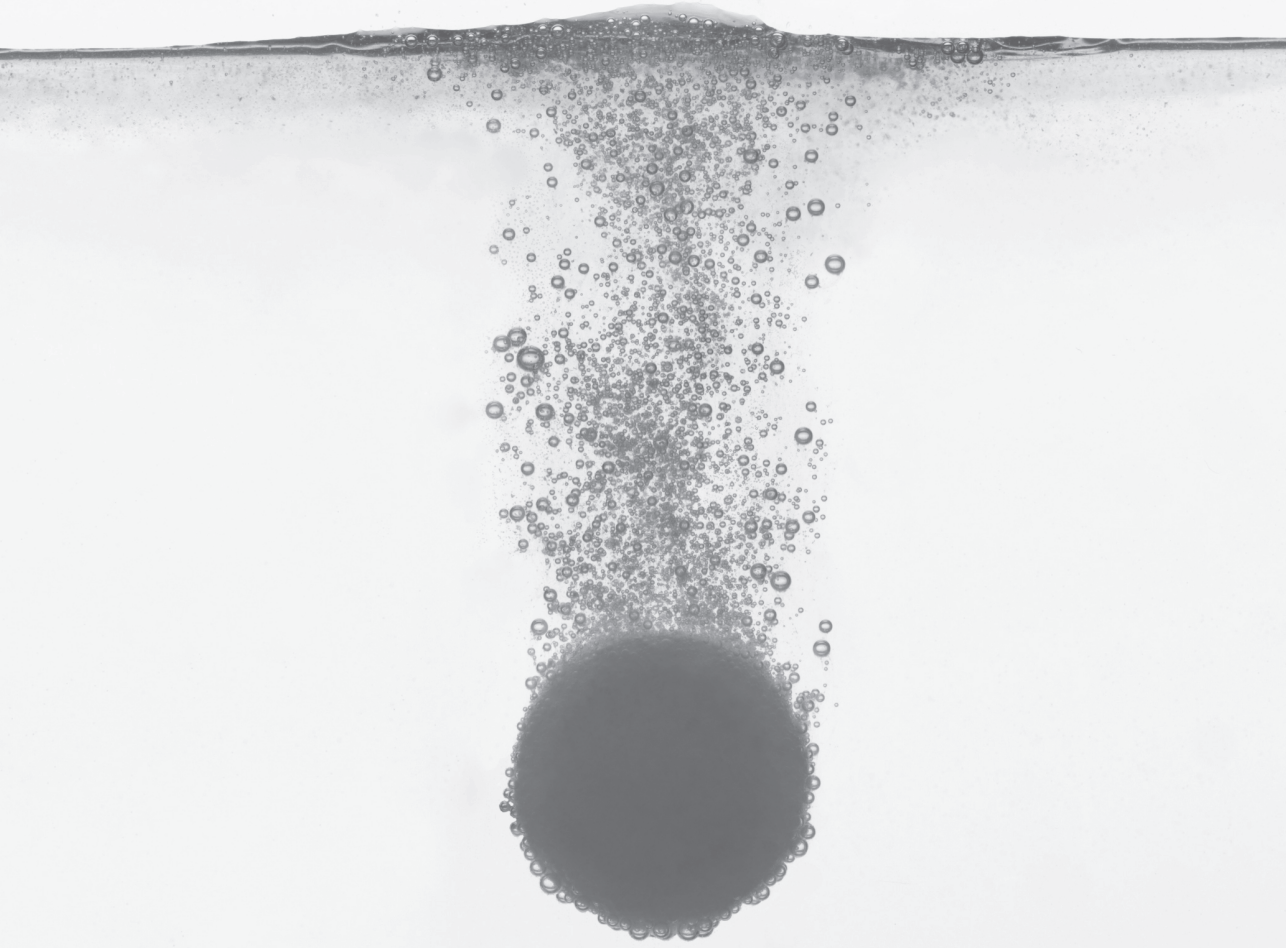
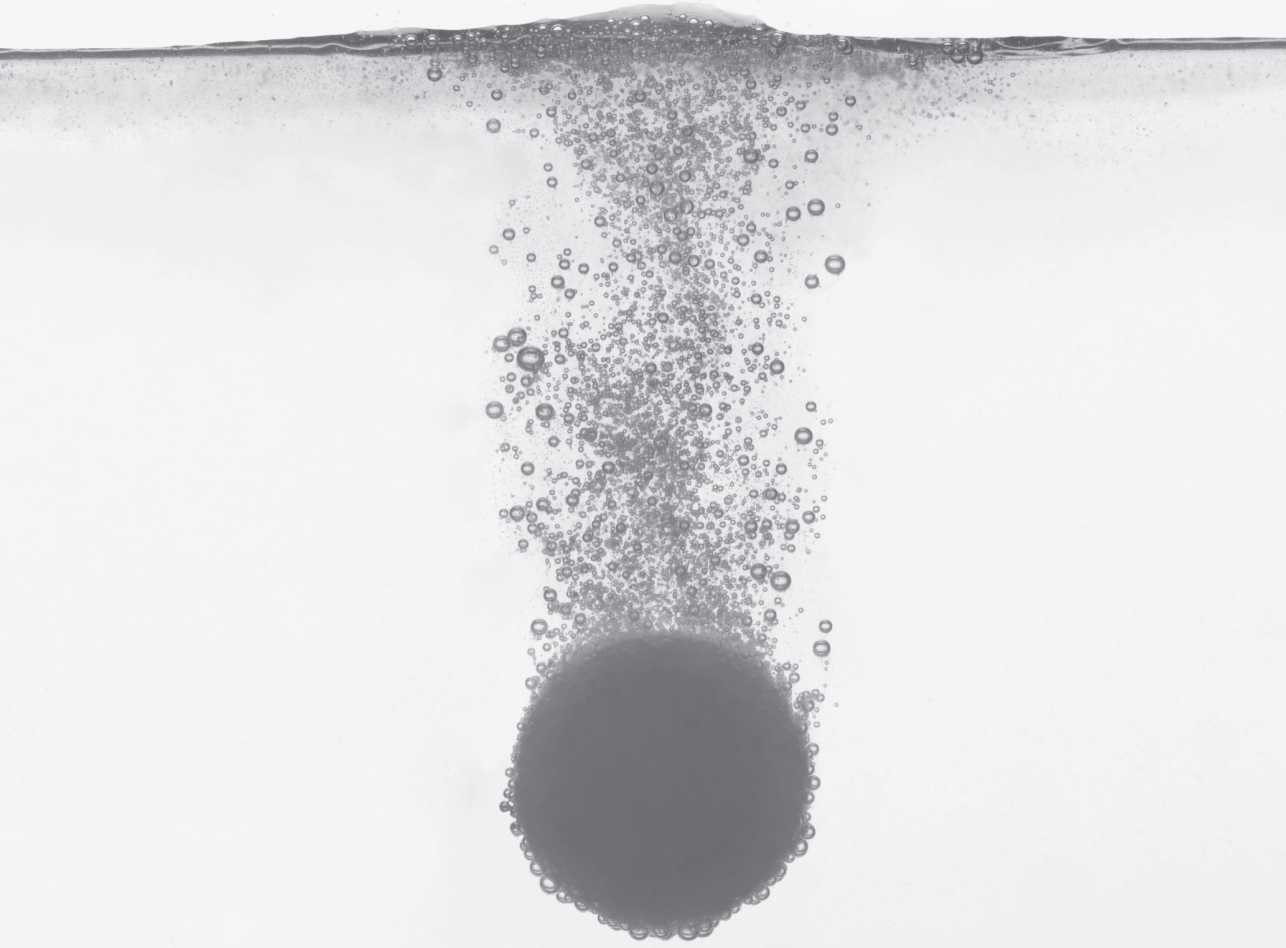


Part 2

Antiretroviral treatment switch strategies



Chapter 4

The efficacy, pharmacokinetics, and safety of a nevirapine to rilpivirine switch in virologically suppressed HIV-1-infected patients.

C. Rokx, M. Blonk, A. Verbon, D. Burger, B.J.A. Rijnders.

J Acquir Immune Defic Syndr. 2015. Jan;68(1):36-9.

ABSTRACT

Introduction

Nevirapine induces cytochrome P450 3A4 by which rilpivirine is metabolized. Switching nevirapine to rilpivirine could result in decreased rilpivirine exposure with subsequent virological failure. This trial evaluated the efficacy, pharmacokinetics, and safety of switching nevirapine to rilpivirine.

Methods

Prospective open label non-randomized controlled trial. HIV-1 patients with HIV-1 RNA <50 copies/mL on once daily nevirapine with emtricitabine/tenofovir switched to single tablet rilpivirine/emtricitabine/tenofovir. Eligible patients on nevirapine with emtricitabine/tenofovir were controls. Primary endpoint was week 12 HIV-1 RNA <50 copies/mL by intention to treat analysis. Secondary endpoints were week 24 HIV-1 RNA <50 copies/mL, nevirapine and rilpivirine pharmacokinetics, and safety.

Results

Of 189 eligible patients, we included 50 rilpivirine switchers and 139 nevirapine controls. Week 12 HIV-RNA was <50 copies/mL in 46/50 switchers (92.0%) which was not different from the hypothesized 90% week 12 suppression rate (P = .431). Forty-four of 50 switchers had week 24 HIV-1 RNA <50 copies/mL compared to 126/139 controls (difference: 2.6%, 95% CI -7.6% to 12.8%, P = .593). Nevirapine plasma concentrations were below the detection level in all at week 3. Mean week 1 rilpivirine trough concentration was 0.083 mg/L and comparable to phase III trial data (P = 0.747). Adverse events occurred in 36 switchers, the majority (82.0%) were grade one. Two switchers discontinued rilpivirine for side effects.

Conclusion

Substituting rilpivirine for nevirapine resulted in ongoing virological suppression and did not have clinically relevant pharmacokinetic effects by cytochrome P450 interactions.

INTRODUCTION

The nonnucleoside reverse transcriptase inhibitor (NNRTI) nevirapine is commonly used in combination antiretroviral therapy (cART) for HIV-1. Although long-term nevirapine use is generally well tolerated, its initiation can be associated with more serious adverse events (AE) and discontinuations compared with other NNRTIs.[234] Replacing nevirapine for other NNRTIs occurs for intolerability, potential drug interactions, or if single-tablet regimens (STR) are desired.[176] The new NNRTI rilpivirine is well tolerated, available as STR, effective,[235, 236] and recommended in first-line cART. Rilpivirine could therefore be a good alternative to nevirapine when a switch is needed.[163-165]

Rilpivirine is predominantly metabolized by cytochrome P450 3A4 (CYP3A4), sharing this metabolic pathway with the first-generation NNRTIs and CYP3A4 inducers nevirapine and efavirenz. CYP3A4 inductive effect of efavirenz influences rilpivirine's pharmacokinetics (PK) for 4 weeks after rilpivirine initiation.[237, 238] Whether the discontinuation of nevirapine results in an ongoing inducing effect of CYP3A4, which lowers rilpivirine's exposure and efficacy, is unknown. This trial evaluates rilpivirine's PK, safety, and virological control over HIV-1 after nevirapine discontinuation.

METHODS

Study Design

This prospective, open-label nonrandomized clinical trial aimed to study the efficacy, PK, and safety of switching once-daily 400 mg nevirapine XR with 200/245 mg emtricitabine/tenofovir disoproxil fumarate (DF) to 25/200/245 mg rilpivirine/emtricitabine/tenofovir DF as STR. Participants were HIV-1-seropositive adults, had HIV-1 RNA <50 copies per milliliter for ≥6 months, and were on nevirapine for ≥9 months. Exclusion criteria were previous virological failure, documented resistance to reverse transcriptase inhibitors, glomerular filtration rate (GFR) <60 mL/min, inadequate intake (<500 kcal), (desired) pregnancy, concomitant proton-pump inhibitors, medicine use with potential interactions, significant comorbidity, substance abuse, or the incapacity to comply with the study. Eligible patients were approached for participation as switchers. Controls were eligible HIV-1 patients on nevirapine who did not want to switch. These non-switchers remained in standard HIV-1 care

and underwent no study procedures. Controls were registered by the Dutch HIV Monitoring Foundation and have consented to the use of their routinely collected data for study purposes. The study was approved by the institutional review board and done in accordance with good clinical practice and the Helsinki Declaration. Switchers provided written informed consent before study procedures. This trial was registered at www.trialregister.nl, number NTR3368.

Endpoints

The primary endpoint was the proportion of switchers with week 12 HIV-1 RNA <50 copies per milliliter (intention to treat). Secondary endpoints were the proportion of switchers with week 24 HIV-1 RNA <50 copies per milliliter compared with controls, plasma nevirapine concentrations and predose rilpivirine trough concentrations (C_{trough}), PK, safety, AE, and treatment satisfaction. The sample size was based on the PK endpoint. With an estimated intrasubject SD for rilpivirine plasma levels of 0.24 and a sample size of 16 switchers who completed each PK time point, the point estimate of rilpivirine's C_{trough} was anticipated to fall within 86% and 116% of the true ratio with 90% confidence.[238] We included 20 switchers in an intensive group for PK analyses and included 50 switchers in total for efficacy analysis. We hypothesized that ≥90% of switchers would remain with HIV-1 RNA <50 copies per milliliter at week 12 and that week 24 suppression rates would be comparable with nonswitchers.

Data Collection

Switchers underwent structured evaluation, including HIV-1 RNA measurements (Cobas AmpliPrep/Cobas Taq-man HIV-1 test v2.0; Roche Diagnostics, Pleasanton, CA) at baseline, weeks 4, 12, and 24 with assessments of meal adequacy,[239] comedications, and reported adherence with pill counts. Liver and renal functions were assessed at baseline and week 24. The Common Terminology Criteria for AE were used.[240] HIV Treatment Satisfaction Questionnaires evaluated overall (0-60 points), clinical, and convenience (0-30 points) satisfaction. The 20 switchers in the PK subgroup had plasma nevirapine and rilpivirine C_{trough} assessments at baseline and weeks 1, 2, 3, 4, 8, and 12 at median of 20 (interquartile range, IQR: 18-21) hours postdose. Nevirapine and rilpivirine concentrations were analyzed by a validated reversed phase ultra-performance liquid chromatographic method with ultraviolet detection. The plasma linear calibration ranged from 0.0063 to 3.75 mg/L for rilpivirine and 0.05 to 16.0 mg/L for nevirapine. Bioanalysis was performed at the laboratory at Radboud University Medical Center and was externally validated through the International

Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma,[241] and by the Proficiency Testing program of the Clinical Pharmacology Quality Assurance and Quality Control (CPQA) program.[242] Controls remained in standard care, including clinical assessments with HIV-1 RNA and renal and liver functions at baseline and week 24.

Statistical Analyses

Descriptive statistics included means with 95% confidence intervals (95% CI), medians with IQR, or numbers (n) with percentages. The binomial test evaluated whether the proportion of switchers with week 12 HIV-1 RNA <50 copies per milliliter was different from the hypothesized 90% suppression rate. The χ^2 test compared week 24 proportions of patients with HIV-1 RNA <50 copies per milliliter in the switch and control groups. Geometric means (GM) with 95% CI were obtained for rilpivirine and nevirapine concentrations at the time points using WinNonlin/Phoenix version 6.3 (Pharsight Corporation, a Certara Company, St. Louis, MO). Rilpivirine GM C_{trough} were compared by the 1-sample T test at the time points with rilpivirine mean C_{trough} from phase 3 trials (0.08 ± 0.037 mg/L SD).[243] Rilpivirine C_{trough} levels <0.05 mg/L have been associated with decreased virological responses.[244] Therefore, we considered a lower bound 95% CI rilpivirine concentration <0.05 mg/L acceptable. Changes in renal and liver functions from baseline to week 24 were analyzed by independent T tests between switchers and controls and within switchers by paired T tests. Differences in treatment satisfaction were evaluated by Wilcoxon signed rank tests. Tests were 2 tailed, and the differences were significant at P <0.05.

RESULTS

Baseline Characteristics

Of 456 screened HIV-1 patients at the Erasmus University Medical Center between November 12, 2012, and September 4, 2013, 267 were ineligible for participation in the study. Of 189 eligible patients, 139 did not want to switch and remained on nevirapine in routine care as controls. Fifty switchers consented to study participation. Switchers and controls were not significantly different (Table 1) and were predominantly Caucasian males (73.0%) and 45 (IQR: 38-52) years of age with HIV-1 transmission through sexual contact with men (57.7%). Patients were HIV-1 suppressed for 31 (IQR: 25-51)

Table 1. Baseline characteristics of virologically suppressed HIV-1 infected patients switching antiretroviral therapy from nevirapine to rilpivirine and controls who remained on nevirapine.

	RPV switch group (n=50)		NVP control group (n=139)		P
	No.	(%)	No.	(%)	
Male gender	41	(82.0)	97	(69.8)	0.095
Age, years (IQR)	45	(39 - 54)	45	(38 - 52)	0.466
HIV-1 transmission					
MSM	31	(62.0)	78	(56.1)	
Heterosexual	18	(36.0)	49	(35.3)	
Intravenous drug use	1	(2.0)	6	(4.3)	
Other	0	(0.0)	6	(4.3)	0.572
Ethnicity					
Caucasian	34	(68.0)	88	(63.3)	
African or African descent	11	(22.0)	37	(26.6)	
Asian	3	(6.0)	6	(4.3)	
Latin-American	2	(2.0)	8	(5.8)	0.833
Months on NVP (IQR)	66	(39 - 127)	51	(32 - 117)	0.267
CD4 cell count per mm^3 (IQR)	565	(470 - 730)	580	(480 - 750)	0.485
Months HIV-1 RNA <50 copies/mL (IQR)	28	(25 - 47)	32	(25 - 53)	0.447
HIV-1 RNA <20 copies/mL	48	(96.0)	130	(93.5)	
HIV-1 RNA 20-50 copies/mL	2	(4.0)	9	(6.5)	0.522
GFR, mL/min (IQR)	79	(70 - 90)	87	(76 - 90)	0.061

Data are presented as No. (%) or median (IQR) and compared by χ^2 or Wilcoxon signed rank tests. GFR were estimated by the MDRD formula. Abbreviations: GFR, glomerular filtration rate; IQR, interquartile range; MSM, men having sex with men; NVP, nevirapine; RPV, rilpivirine.

months, on nevirapine for 57 (IQR: 35-120) months, and had 580 (IQR: 480-730) CD4 cells per cubic millimeter.

Efficacy

Week 12 HIV-1 RNA was <50 copies per milliliter in 46/50 switchers (92.0%), comparable with the hypothesized 90% with HIV-1 suppression (P = 0.431). At week 12, 1 switcher discontinued rilpivirine for related AE and 1 had HIV-1 RNA of 51 copies per milliliter with week 24 HIV-1 RNA <50 copies per milliliter. Another switcher had week 12 HIV-1 RNA of 4820 copies per milliliter without acquired resistance, 100%

pill count, and week 12 rilpivirine C_{trough} of 0.2552 mg/L. This patient's week 4, 16, 18, and 24 HIV-1 RNAs were <50, 1650, <50, and <50 copies per milliliter, respectively. The fourth switcher had HIV-1 RNA of 32,500 copies per milliliter without acquired resistance, 22% pill count, and undetectable week 12 rilpivirine C_{trough}. This patient's participation was terminated and switched to darunavir/ritonavir emtricitabine/tenofovir DF after adherence counseling.

Week 24 virological suppression rates in switchers (88.0%, 95% CI: 79.0 to 97.0) and controls (90.6%, 95% CI: 85.8 to 95.5) were not different (difference: 2.6%, 95% CI: 27.6 to 12.8; P = 0.593). One additional switcher had discontinued rilpivirine for related AE, and 3 switchers had viral blips of HIV-1 RNA of 58, 80, and 228 copies per milliliter, respectively. Week 24 medication adherence was .95% in 47/50 switchers. Thirteen controls did not have week 24 HIV-1 RNA <50 copies per milliliter, 6 were lost to follow-up, 5 had HIV-1 RNA 50-100 copies per milliliter, and 2 had HIV-1 RNA of 2540 and 844 copies per milliliter because of poor therapy adherence without acquired resistance and nevirapine concentrations of 0.47 and 0.20 mg/L, respectively.

Pharmacokinetics

The baseline GM (95% CI) nevirapine concentration was 5.04 mg/L (3.76-6.33, Figure 1). Nevirapine was undetectable in 8/20 switchers at week 1, in 19/20 at week

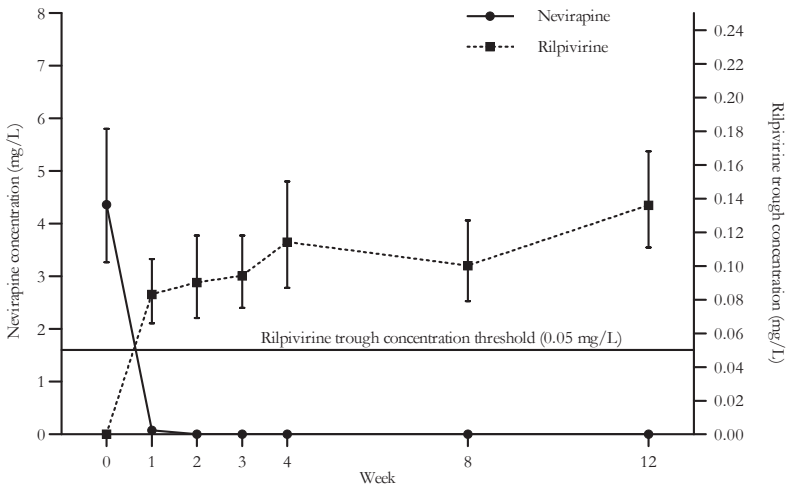


Figure 1. Rilpivirine (RPV) and nevirapine (NVP) pharmacokinetics (geometric mean, 95% confidence interval) after a switch from NVP to RPV.

2, and 20/20 at week 3. The week 1 lower 95% CI border of GM rilpivirine C_{trough} was .0.05 mg/L. Week 1 rilpivirine GM C_{trough} (0.083 mg/L) was comparable with mean rilpivirine C_{trough} (0.008 mg/L) of phase 3 trial data (P = 0.747). Rilpivirine C_{trough} was <0.05mg/L in 17/20 at week 1 and in 18/20 at week 2. Rilpivirine C_{trough} in 2 switchers remained <0.05 mg/L until week 12 with 100% adherence. The baseline nevirapine concentrations in these 2 were 4.50 and 3.26 mg/L and undetectable by week 1. One had HIV-1 RNA <50 copies per milliliter at all time points, and the other had week 4 HIV-1 RNA of 589 copies per milliliter and HIV-1 RNA <50 copies per milliliter at weeks 6, 12, and 24.

Safety and Treatment Satisfaction

The GFR declined 6.7 mL/min in switchers and 2.6 mL/min in controls over 24 weeks (95% CI of difference: 26.5 to 21.8, P , 0.001). Nine switchers with median baseline GFR between 60 and 71 mL/min had grade 2 GFR decline (49-60 mL/min) without urine abnormalities at week 24. No grade 2 or higher alanine transaminase increases were observed in switchers. Switchers had significant changes (all P <0.001) in phosphate (+0.14 mmol/L, 95% CI: 0.09 to 0.19), total bilirubin (+2.3 mmol/L, 95% CI: 1.4 to 3.3), and gamma-GT (-54 U/L, 95% CI: -69 to -39). Thirty-six switchers experienced at least one rilpivirine-related AE, including one serious AE for hospitalization; 82% were grade 1 AE. Three grade 3 AE occurred; 1 switcher with insomnia discontinued rilpivirine, and 2 patients on phenprocoumon had International Normalized Ratio increases without hemorrhages. Ten switchers had related grade 2 sleep disturbances, abdominal complaints, or skin rashes. Apart from one discontinuation for sleep disturbance, all grade 2 AE resolved spontaneously. Baseline and week 24 overall treatment satisfaction was comparable (56/60 vs. 55/60, P = 0.925) without differences in clinical (28/30 vs. 29/30, P = 0.687) or convenience satisfaction (26/30 vs. 27/30, P = 0.530).

DISCUSSION

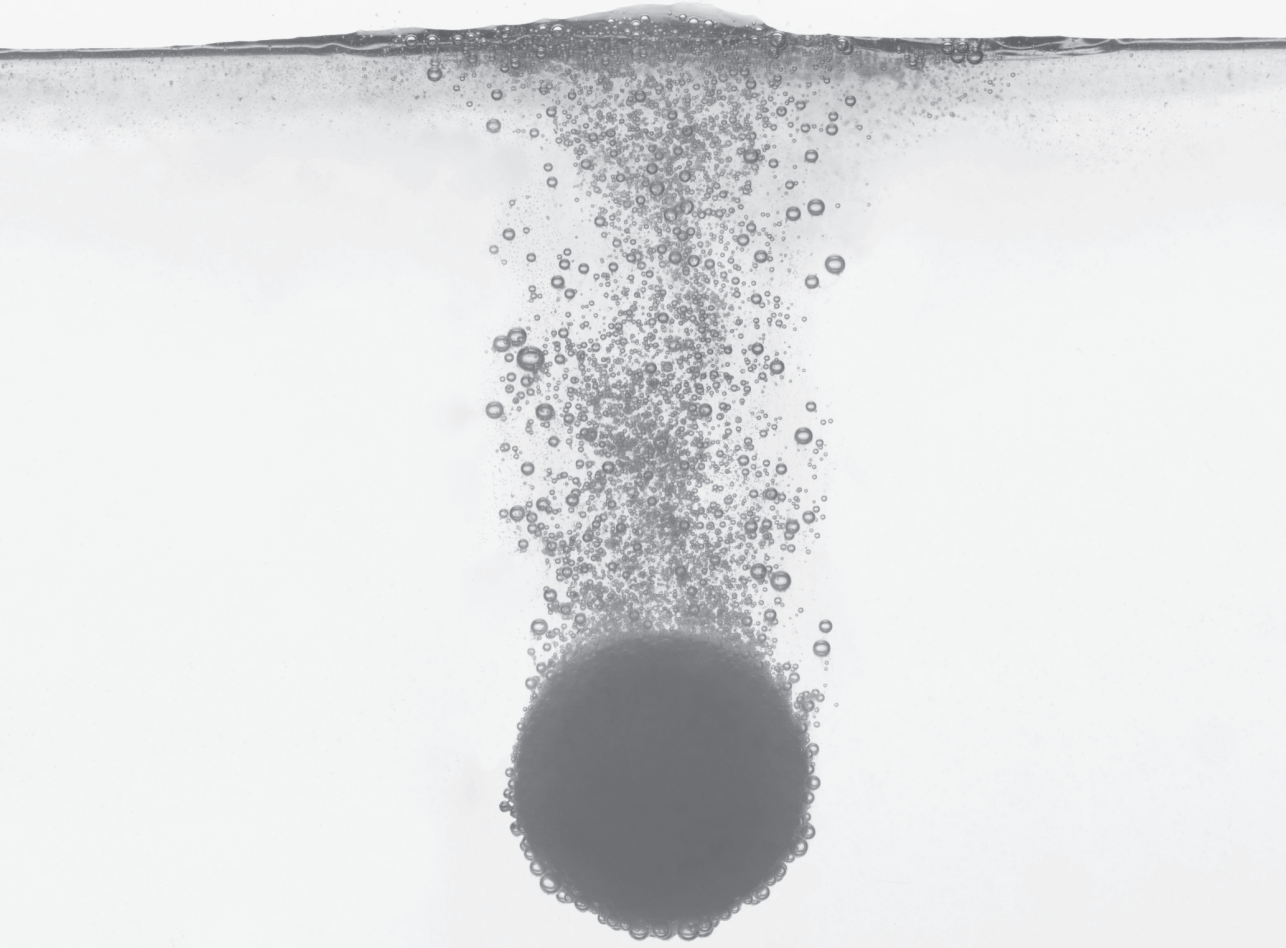
This study shows that replacing nevirapine for rilpivirine in virologically suppressed HIV-1 patients results in an adequate ongoing virological suppression rate over 24 weeks, which is comparable with nevirapine controls. Rilpivirine concentrations were therapeutic at day 7 postswitch in most switchers. The nevirapine levels were negligible by week 2. We can conclude that nevirapine's CYP3A4 induction does not

have a clinically relevant effect on rilpivirine exposure in most patients. A switch from nevirapine to rilpivirine therefore seems to be efficacious and safe without the need for rilpivirine dosage increase, additional HIV-1 RNA measurements, or therapeutic drug monitoring.

This is the first study to evaluate the efficacy and safety of substituting rilpivirine for nevirapine. One previous small study evaluated plasma, seminal, and cerebrospinal fluid rilpivirine exposure in 13 patients after switching nevirapine to rilpivirine.[245] This study was not designed to evaluate the efficacy of this switch, although no virological failure was observed within 60 days. Our observed nevirapine and rilpivirine PK results are comparable with the PK observations in this study. However, our observed plasma rilpivirine concentrations were higher at all time points. An explanation could be that rilpivirine's oral bioavailability is higher when taken as coformulated rilpivirine with high-caloric dinners instead of standardized and possible lighter breakfasts.[239, 246] A comparable study evaluated the safety of switching efavirenz to rilpivirine and found similar suppression rates.[247] This study did not include controls, and rilpivirine C_{trough} was not comparable with phase 3 data until week 2. This PK difference indicates a more moderate CYP3A4 induction by nevirapine than efavirenz. Our findings are relevant for patients who initiate rilpivirine after nevirapine. In contrast to nevirapine, rilpivirine is recommended as part of first-line cART, available as STR, has better tolerability, and lower potential for drug interactions. The GFR decline could be explained by rilpivirine's inhibitory effect on tubular creatinine disposition rather than renal toxicity.[236] From a kidney monitoring perspective, this could confuse clinicians unaware of this insignificant effect. The significant phosphate and gamma-GT changes remain unexplained and their relevance unclear.

Our study has strengths and limitations. This is the largest prospective trial to evaluate the efficacy, PK, and safety of substituting rilpivirine for a first-generation NNRTI. In contrast to other studies, our study design included controls for comparison. Limitations are the nonrandomized design, the potential selection bias of patients who refused participation, the inability to compare the primary endpoint at week 12 between groups, and the sample size calculation was estimated on PK instead of efficacy. These results cannot be generalized to patients failing nevirapine-based regimens, although we recently showed that rilpivirine may be a valid option if only an isolated 103N is acquired and HIV-1 RNA is undetectable on boosted protease inhibitor-based regimens first.[248]

In conclusion, a nevirapine to rilpivirine is safe and results in ongoing virological suppression, and nevirapine's inductive effect does not have a clinically relevant effect on rilpivirine exposure or efficacy in the large majority of patients.



Chapter 5

Successful switch to rilpivirine/tenofovir/emtricitabine in HIV-1-infected patients with an isolated K103N mutation acquired during prior nonnucleoside reverse transcriptase inhibitor therapy

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HIV Medicine. 2014. Nov;15(10):611-4.

ABSTRACT

Introduction

Whether treatment-experienced HIV-1-infected patients with an acquired K103N mutation after failing nonnucleoside reverse transcriptase inhibitor (NNRTI) regimens can be treated with rilpivirine is unknown. The aim of this pilot study was to evaluate the efficacy of rilpivirine/tenofovir/emtricitabine in HIV-1-infected patients with an isolated K103N mutation.

Methods

A prospective study was carried out in HIV-1-infected adults who acquired the K103N mutation on failing NNRTI regimens. No other mutations in reverse transcriptase were allowed. Patients had to be on second-line regimens with HIV-1 RNA <200 copies/mL for ≥6 months. Exclusion criteria were: use of acid-reducing agents, insufficient caloric intake and impaired renal function. Of primary interest was virological success (HIV-1 RNA <200 copies/mL) at weeks 6, 12, 24 and 48.

Results

Of 1550 HIV-1-infected patients at the Erasmus Medical Center Rotterdam, we identified 10 HIV-1-infected patients with an isolated K103N mutation acquired after NNRTI failure. Five patients were not eligible for inclusion in the study, and two patients refused participation. Three African women (23-35 years of age) were included and were switched from boosted protease inhibitor-based second-line therapies to rilpvirine/tenofovir/emtricitabine. HIV-1 RNA was <200 copies/mL at weeks 6, 12, 24 and 48 for all patients. No adverse events were observed. All patients had HIV-1 RNA <200 copies/mL for 6 to 50 months prior to the switch.

Conclusion

This pilot study demonstrates the successful switch of HIV-1-infected patients who acquired an isolated K103N mutation during previous NNRTI therapy to rilpivirine/tenofovir/emtricitabine. In selected patients, single-tablet regimens are also becoming a valid treatment option for second-line HIV-1 therapy

INTRODUCTION

Susceptibility to the first-generation nonnucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz and nevirapine is severely impaired in HIV-1 harbouring a transmitted or acquired K103N mutation. This mutation has no impact on the *in vitro* susceptibility of HIV-1 to the second-generation NNRTI rilpivirine.[249] In the case of a transmitted K103N-containing HIV-1 infection, rilpivirine might still be a first-line treatment option for therapy-naïve patients. This was recently shown for patients with transmitted K103N mutated HIV-1 in the "Switching boosted PI to Rilpvirine in combination with Truvada as single-tablet-regimen" (SPIRIT) trial.[250] In this study, virologically suppressed HIV-1-infected patients on boosted protease inhibitor (PI) regimens switched to single-tablet rilpivirine/tenofovir/emtricitabine. All 18 patients with transmitted K103N mutated HIV-1 infections remained virologically suppressed 24 weeks after the switch.

However, whether treatment-experienced HIV-1-infected patients with acquired K103N-containing HIV-1 infections following NNRTI failure can be effectively treated with a rilpivirine-containing regimen is currently unknown. Drug-resistant minority HIV-1 variants that remain unde-tected during standard genotyping may also impact the treatment response in these patients. A phase II study on the use of rilpivirine in treatment-experienced patients who failed previous NNRTI-containing regimens showed a significant decline in HIV-1 RNA after 7 days of treatment.[251] This observation suggests a potential role for rilpivirine in clinical settings as second-line therapy for HIV-1-infected patients with an acquired K103N mutation. In this pilot study, we prospectively evaluated the virological efficacy of a switch to the single-tablet regimen rilpivirine/tenofovir/emtricitabine as second-line therapy in HIV-1-infected patients with an acquired and isolated K103N mutation who were virological suppressed first by other second-line regimens.

METHODS

Eligible adult HIV-1-infected patients had to be on second-line boosted PI-containing regimens, to which these patients had been switched after failing their previous NNRTI regimens. Apart from the acquired K103N mutation following their failing NNRTI-containing therapies, no other acquired mutations in reverse transcriptase as-

sociated with intermediate or higher resistance levels (according to the Stanford HIV drug resistance database) were allowed.[168] HIV-1 RNA <200 copies/mL for at least 6 months was required for eligibility prior to a potential switch to the single-tablet regimen rilpivirine/tenofovir/emtricitabine. Patients with an impaired renal function, defined as a baseline estimated glomerular filtration rate <60 mL/min (according to the Modification of Diet in Renal Disease formula), were not eligible.

The treating physicians informed the patients of the necessity of an adequate caloric intake and excluded patients with concomitant use of antacids or other disallowed potentially interacting co-medications. It was explained to patients that it was as yet uncertain whether the rilpivirine-based therapy would result in durable HIV-1 suppression and that, in the case of subsequent virological failure, patients would be switched back to their original boosted PI-containing regimens. Treatment adherence was assessed and documented by the treating physicians. All data were obtained from the electronic medical charts. Patients were prospectively evaluated after starting rilpivirine/tenofovir/emtricitabine at 6, 12, 24 and 48 weeks of follow-up on self-reported therapy adherence, clinical and biochemical adverse events and HIV-1 RNA.

The primary outcome was virological success, defined as having HIV-1 RNA <200 copies/mL at weeks 6, 12, 24 and 48 after the switch. HIV-1 RNA >200 copies/mL at weeks 6, 12, 24 and 48 was considered virological failure, which would lead to discontinuation of rilpivirine/tenofovir/emtricitabine and restarting of the prior boosted PI-based second-line regimen.

RESULTS

Ten treatment-experienced HIV-1-infected patients (six women) were identified with an isolated K103N mutation, from approximately 1550 HIV-1-infected patients receiving care at the Erasmus University Medical Center Rotterdam in the Netherlands. The isolated K103N mutations in these patients were acquired following virological failure on either efavirenz- or nevirapine-based regimens. All 10 patients were on boosted PI-containing second-line regimens. One patient was not eligible for a switch to rilpivirine because of concomitant mandatory antacid treatment. Furthermore, two patients did not have HIV-1 RNA <200 copies/mL for at least 6 months and two patients were no longer receiving their medical care at our site. Of the five eligible

patients, two patients were not interested in changing their antiretroviral regimens. Three eligible patients gave informed consent and were included in the pilot study.

Three African women, 23 to 35 years of age, switched their boosted PI regimens to the single-tablet regimen rilpivirine/tenofovir/emtricitabine (Table 1). This resulted in continued plasma HIV-1 RNA <200 copies/mL at weeks 6, 12, 24 and 48 in all three patients. At the start of rilpivirine therapy, two patients (patients 1 and 2) had HIV-1 RNA <50 copies/mL and one patient had HIV-1 RNA of 64 copies/mL. Patients 1 and 2 continued to have HIV-1 RNA <50 copies/mL during 48 weeks of follow-up. The HIV-1 RNA of patient 3 was <50 copies/mL at week 12 and in the 50 to 200 copies/mL range at 24 and 48 weeks. The self-reported drug adherence of patient 3 was 95% during follow-up. No other adherence problems or adverse events were reported during the 48 weeks of follow-up.

HIV-1 infections were diagnosed in 1997 (patient 1), 2006 (patient 2) and 2002 (patient 3) and no resistance to HIV-1 reverse transcriptase was documented at diagnosis. At first presentation, patient 1 had HIV-1 RNA of 59.100 copies/mL, patient 2 of >100.000 copies/mL and patient 3 of 4900 copies/mL. Nadir CD4 cell counts prior to initiation of the first antiretroviral regimens were 60, 120 and 250 cells/μL, respectively. All patients were therapy-naïve when they started antiretroviral treatment with their NNRTI-based regimens and all acquired the isolated K103N mutation at the time of failure of these NNRTI-based regimens. Patient 1 acquired the K103N mutation following virological failure on efavirenz and lamivudine/zidovudine, patient 2 acquired it after failure on nevirapine and tenofovir/emtricitabine and patient 3 acquired it after failure on efavirenz and tenofovir/emtricitabine. HIV-1 RNA at virological failure on the NNRTI regimens was 34.300 copies/mL in patient 1 and >100.000 copies/mL in patients 2 and 3. The total time on the boosted PI-containing second-line therapies prior to the switch to rilpivirine was 154, 66 and 63 months, respectively. The treatment of the included patients with boosted PI regimens had resulted in HIV-1 RNA <200 copies/mL for 50, 37 and 6 months in patients 1, 2 and 3, respectively, and the patients had a minimum CD4 cell count at the initiation of rilpivirine of 550 cells/μL.

Table 1. Characteristics of the HIV-1 patients that switched to rilpivirine/tenofovir/emtricitabine after acquiring an isolated K103N mutation during previous efavirenz or nevirapine based antiretroviral regimens.

						Antiretroviral regimens[1]			HIV-RNA[1]			
Patient	Age[2]	HIV	Subtype	HIV-RNA	CD4 count	Failure regimen	Failure HIV-RNA	ART at switch RPV/TDF/FTC	W0	W12	W24	W48
1	23	1997	CRF01_AE	59,100	60	EFV, 3TC/ ZDV	34,300	LPV/r, ABC/3TC	<50	<50	<50	<50
2	35	2006	CRF02_AG	>100,000	120	NVP, TDF/ FTC	>100,000	ATV/r, TDF/FTC	<50	<50	<50	<50
3	32	2002	CRF02_AG	4,900	250	EFV, TDF/ FTC	>100,000	DRV/r, 3TC/ZDV	64	<50	70	76

HIV-RNA in copies/mL; CD4 count in cells/mm^3.

[1] A "/" symbol represents fixed dose coformulations; "W" represents week of follow up;

[2] Age in years at the switch to rilpivirine/tenofovir/emtricitabine.

Abbreviations: 3TC: lamivudine, ABC: abacavir, ATV/r: atazanavir/ritonavir, ART: antiretroviral therapy, DRV/r: darunavir/ritonavir, EFV: efavirenz, FTC: emtricitabine, LPV/r: lopinavir/ritonavir, NNRTI: non-nucleoside reverse transcriptase inhibitor, NVP: nevirapine, RPV: rilpivirine, TDF: tenofovir, ZDV: zidovudine.

DISCUSSION

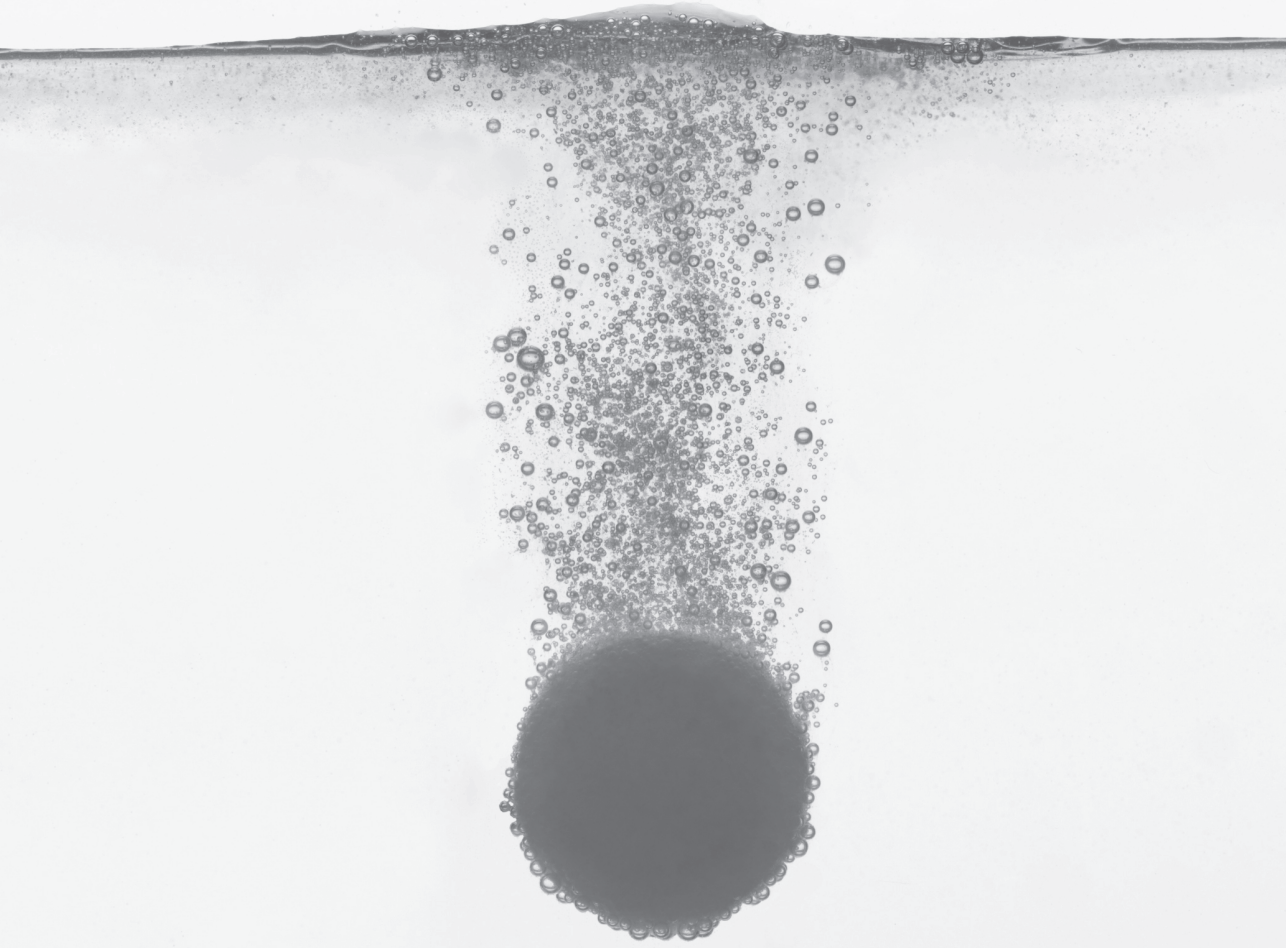
The observations in our pilot study suggest that adult HIV-1-infected patients with an isolated K103N mutation, acquired during a previously failing NNRTI-based therapy, can be safely switched to a single-tablet regimen containing rilpivirine/tenofovir/emtricitabine after long-term HIV-1 RNA suppression has first been achieved on second-line PI-based regimens. This strategy might provide an efficacious NNRTI-based second-line antiretroviral therapy that has a favourable toxicity profile and is available as a single-tablet regimen for a selected but considerable number of HIV-1-infected patients.

We realize that no definite conclusions can be drawn from the observations in this small study, as no comparator arm was included and the follow-up time was limited to 48 weeks. Of importance, and in contrast to a transmitted K103N-harbouring HIV-1 infection, other resistance-associated mutations could have been selected which are not yet detectable by standard genotyping. Failure to successfully suppress the HIV-1 infection with a suboptimal second-line therapy could rapidly induce the emergence of a more resistant virus.[252] Furthermore, *in vitro* data suggest that an isolated K103N mutation could facilitate the development of an E138K mutation following rilpivirine drug pressure.[253] Therefore, it seems imperative that the HIV-1 infection is first controlled by a boosted PI-based regimen prior to starting rilpivirine. If patients are to be switched, frequent monitoring during the first 6 months of therapy is probably necessary to allow for a prompt change in therapy if plasma HIV-1 RNA rebounds.

If confirmed in future studies, the applicability of our approach may be substantial. A recent study showed that only 14.5% of HIV-1-infected patients who failed efavirenz-containing regimens and 25% of HIV-1-infected patients failing nevirapine-containing regimens acquired rilpivirine resistance.[254] Specific major NRTI resistance to emtricitabine or tenofovir could occur after failing first-line therapy. However, the majority of patients treated with a non-thymidine analogue-containing NRTI backbone in combination with efavirenz or nevirapine lack major resistance to tenofovir and emtricitabine.[255, 256] Therefore, the possibility to treat patients with rilpivirine/tenofovir/emtricitabine as a single-tablet regimen could still be preserved in a considerable number of HIV-1-infected patients who acquired an isolated K103N mutation on previous antiretroviral therapies.

To our knowledge, this is the first study in which a single-tablet regimen was used for second-line antiretroviral therapy after previous NNRTI failure. As other single-tablet regimens are being introduced in the near future, the potential role of these co-formulated drugs as suitable second-line therapies is likely to increase substantially. A future prospective and preferably randomized study is needed to confirm our findings and also to evaluate the impact of minor resistance variants on treatment success.

In conclusion, our results indicate that a substantial subgroup of HIV-1-infected patients with an isolated K103N mutation, acquired on previous failing NNRTI regimens, could potentially benefit from rilpivirine in a single-tablet regimen after virological suppression has first been achieved on other second-line regimens.



Chapter 6

Dolutegravir as maintenance monotherapy: first experiences in HIV-1 patients.

C. Rokx, C.A.M. Schurink, C.A.B. Boucher, B.J.A. Rijnders.

J Antimicrob Chemother. 2016. In press.

ABSTRACT

Introduction

Dolutegravir is recommended as part of combination antiretroviral therapy (cART) for HIV-1 infected patients. Toxicities, drug interactions and costs related to cART still warrant the search for improved treatment options. Dolutegravir's high resistance barrier might make it suitable as antiretroviral maintenance monotherapy. The feasibility of this strategy is currently unknown.

Methods

Prospective case series on 5 consecutive HIV-1 infected patients on cART without previous virological failure who switched to dolutegravir monotherapy. All were HIV-RNA suppressed <50 copies/mL and had contraindications to current and alternative combinations of antiretroviral drugs. HIV-RNA was measured at baseline, week 4, 8, 12 and every 6 weeks thereafter. Patients would be switched back to their original cART upon a confirmed HIV-RNA >50 copies/mL.

Results

The five patients had been HIV-RNA suppressed <50 copies/mL for at least 1.5 years prior to the initiation of dolutegravirmonotherapy. All were on NNRTI containing regimens at the switch. The HIV-RNA remained <50 copies/mL at all time-points in four patients. One patient, with end stage renal disease and on calcium supplements, had a pre-cART HIV-RNA 625.000 copies/mL with CD4 nadir of 120 cells/mm^3 and had HIV-RNA 8150 copies/mL at week 30. The dolutegravir C_{trough} was 0.18 mg/L. This patient did not have acquired resistance or evidence of adherence problems and resuppressed after switching to his former cART.

Conclusion

This case series indicates that dolutegravir monotherapy might be a valuable maintenance option in selected HIV infected patients that are well suppressed on cART, if confirmed by future randomized clinical trials.

INTRODUCTION

Dolutegravir is an integrase inhibitor (INI) and has been approved for use in combination antiretroviral therapy (cART) for ART experienced and naïve HIV-1 patients.[163-166] INI inhibit HIV-1 DNA transfer into host DNA; a pivotal step in forward HIV-1 transmission and disease progression. Dolutegravir is a second generation INI with greater binding to integrase compared to the first generation INI raltegravir and elvitegravir.[171, 257] This results in a higher genetic barrier to resistance with dolutegravir against wild-type and ART resistant HIV-1.[257, 258] Dolutegravir's efficacy and lack of selection for resistance has been demonstrated in four randomized clinical trials with a total of 2344 ART-naïve patients.[227, 228, 259, 260] Additionally, no major resistance was acquired in 715 ART experienced, INI naïve patients without raltegravir resistance at treatment initiation.[261] In INI experienced patients, responses declined in patients with codon 148 mutations in integrase.[262-264] These data combined indicate that dolutegravir is an effective addition to the antiretroviral drugs repertoire.

INI based cART are recommended first-line treatments in the latest guidelines combined with emtricitabine/tenofovir disoproxil-fumarate or lamivudine/abacavir. Although 4 single-tablet regimens (STR) are available, consisting of 3 antiretroviral drugs, all have their specific side-effects, drug-drug-interactions (DDI), contra-indications. Antiretroviral drug switches occur frequently.[176,265] Furthermore, the increased focus on cost-containment may result in the need for more cost-effective treatments.[186]

Reducing the drug burden in cART to dual or antiretroviral monotherapy could be a preferable option for a considerable number of patients, as long as suppression can be maintained. Monotherapy has only been studied with boosted protease inhibitors (PI). Although PI monotherapy can be an option to maintain suppression, it has not been widely adopted due to the pill-burden, pill-size, and increased viral load monitoring as patients might reintensify. Last, all PIs are combined with the CYP450-3A4 pharmacological boosters, ritonavir or cobicistat, posing patients at risk for DDI.

Dolutegravir is a small single tablet with a limited number of DDI. Given the high resistance barrier, dolutegravir may be an antiretroviral monotherapy option. No data from randomized trials on dolutegravir monotherapy are currently available. In this

case series we systematically analyzed 5 HIV-1 patients that switched from cART to dolutegravir maintenance monotherapy and describe the first experiences with this strategy.

METHODS

This is a prospective case series at the Erasmus University Medical Center, Rotterdam, the Netherlands. We selected HIV-1 infected patients, suppressed <50 copies/mL on cART for >1 year. Previous virological failure was not allowed. Some patients had high pill burdens due to comorbidities that needed medical therapy. Therefore, antiretroviral therapy with as few pills as possible was favoured. Four patients were on STR and preferred to remain on STR. HLAB57-01 positivity, unacceptable toxicity or DDI could be present as contra-indications to current or alternative combinations of recommended NRTI backbones (lamivudine/abacavir or emtricitabine/tenofovir disoproxil-fumarate) or third antiretroviral agents (PI, NNRTI or INI). The decision to start dolutegravir monotherapy was made after extensive counselling and done in accordance with good clinical practice and the Helsinki declaration. Patients had to provide consent. Additional medical ethical approval was not required because the patients were informed that dolutegravir monotherapy is off-label use due to contra-indications to other drugs. We predefined safety rules and patients would be switched back to their prior regimen upon a confirmed HIV-RNA >50 copies/mL. HIV-RNA was measured at week 0, 4, 8, 12, and every 6 weeks thereafter.

RESULTS

Five Caucasian HIV-1 infected males were included (Table 1). Four patients were >55 years of age. All were suppressed to HIV-RNA <50 copies/mL on NNRTI based cART. None had prior virological failure or documented resistance. Three patients (2, 4, 5) were always on efavirenz/emtricitabine/tenofovir disoproxil-fumarate, Patient 1 initiated raltegravir/emtricitabine/tenofovir disoproxil-fumarate due to chemotherapy and switched to rilpivirine/emtricitabine/tenofovir disoproxil-fumarate 1 month later. Patient 3 initiated indinavir/lamivudine/zidovudine in 1996 which was switched to nelfinavir/lamivudine/zidovudine in 1998, nevirapine/lamivudine/zidovudine in 1999, and nevirapine/lamivudine/tenofovir disoproxil-fumarate in 2006. All patients were

Table 1. Patient characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age	46	68	68	63	59
CD4 nadir (cells/mm^3)	170	230	320	120	60
Historical viral load (copies/mL)[1]	500	45000	82000	>100000	>100000
HIV diagnosis (year)	2013	1993	1993	2007	2007
Duration of viral load <50 copies/mL[2]	1.5 years	8 years	19 years	9 years	8 years
cART initiation year	2013	2007	1996	2007	2007
cART prior to switch	RPV/FTC/TDF	EFV/FTC/TDF	NVP/3TC/TDF	EFV/FTC/TDF	EFV/FTC/TDF
Prior INI	RAL	None	None	None	None
Clinical context	-HLAB57-01+ -Seminoma testis -Osteoporotic fractures -HCV and RPV induced hepatitis -Comedication[3]	-HLAB57-01+ -TDF related proximal tubular dysfunction -CVD with TIA -Comedication[3]	-Rheumatoid arthritis -Psoriasis -Diabetes -Steatohepatitis -Polyneuropathy -Comedication[3]	-Thromboangiitis obliterans with limb amputations -CKD stage 5, dialysis with residual diuresis -Comedication[3]	-CKD stage 3B -Hypertension -CVD: TIA, PCI -Comedication[3]
Latest viral load on DTG	<20 copies/mL	<20 copies/mL	<20 copies/mL	8150 copies/mL	<20 copies/mL
Viral blip on DTG monotherapy	None	None	None	120 copies/mL (week 12)	None
Time on DTG monotherapy at the latest viral load	48 weeks	48 weeks	48 weeks	30 weeks	30 weeks

[1] Highest viral load ever observed before start of cART.

[2] Duration of viral load <50 copies/mL, or <500 copies/mL prior to 2002, before the start of dolutegravir monotherapy.

[3] Relevant comedication. Patient 1: alendronic acid, vitamin d, testosterone. Patient 2: clopidogrel, atorvastatin, antacids. Patient 3: methotrexate, diclofenac, ezetimib, pravastatin, omeprazole, pregabaline, betamethasone, omeprazole, metformin. Patient 4: acetylsalicylic acid, nifidipine, bumetanide, labetalol, atorvastatin, pregabaline, esomeprazole, calcium carbonate, sevelamer, alfacalcidol. Patient 5: clopidogrel, acetylsalicylic acid, bisoprolol, losartan, hydrochloorthiazide, amlodipine, doxazosin, isosorbide mononitrate, pantoprazole, pravastatin.

Abbreviations: 3TC = lamivudine. CKD = chronic kidney disease. CVD= cardiovascular disease. DTG = dolutegravir. EFV = efavirenz. FTC = emtricitabine. HCV= hepatitis C virus. NVP = nevirapine. PCI = percutaneous coronary intervention. RPV = rilpivirine. TDF = tenofovir disoproxil fumarate. TIA = transient ischemic attack.

HIV-RNA suppressed within 6 months of cART initiation and had ≥2 HIV-RNA measurements yearly during follow up. Patients 3 and 5 were hepatitis-B virus (HBV) negative, patients 2 and 4 were adequately vaccinated, and patient 1 had cleared HBV.

Patients 1 and 2 were HLAB57-01 positive, and developed progressive tenofovir disoproxil-fumarate related proximal tubular dysfunction and multiple vertebral osteoporotic fractures on tenofovir disoproxil-fumarate. Patient 3 had a severely increased cardiovascular disease (CVD) risk, progressive steatohepatitis, and nephrotoxic medication. Patient 4 had hemodialysis with significant residual diuresis due chronic kidney disease (CKD) stage 5 caused by a vascular disease while on tenofovir disoproxil-fumarate. Patient 5 developed CKD stage 3B on tenofovir disoproxil-fumarate, and had secondary CVD prophylaxis. The comorbidities, contraindications and comedication prevented the use of many antiretrovirals in these patients.

The HIV-RNA remained <50 copies/mL at all time-points in patients 1, 2, 3 and 5 up to 48 weeks of follow-up after the switch to dolutegravir monotherapy. In patient 4, HIV-RNA remained <50 copies/mL at week 4 and 8, followed by 120 copies/mL at week 12. Week 13, 18, and 24 HIV-RNA were <50 copies/mL, but week 30 HIV-RNA was a confirmed 8150 copies/mL without acquired resistance. At week 24, the plasma dolutegravir trough concentration (C_{trough}) was 0.18 mg/L. Twenty-four hours later after dolutegravir administration following this plasma drug level, the C_{trough} remained 0.19 mg/L prior to haemodialysis. This patient had started taking calcium-carbonate three times daily as part of his dialysis therapy, which he took with dolutegravir that might explain his low plasma dolutegravir level. HIV-RNA resuppressed to 629 and 84 copies/mL at 4 and 12 weeks after switching to efavirenz/emtricitabine/tenofovir disoproxil-fumarate.

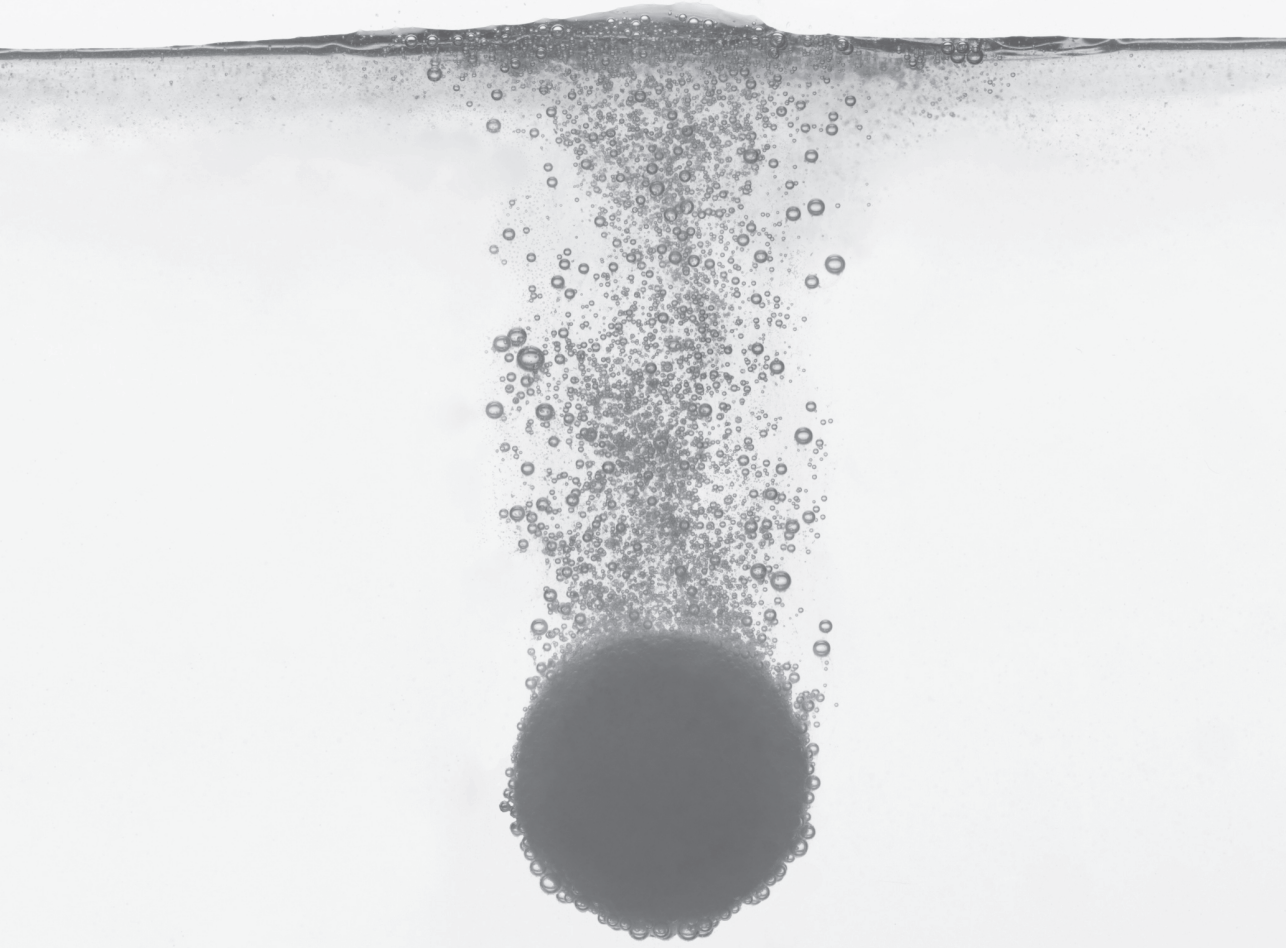
None of the patients experienced dolutegravir related side-effects. The alanine aminotransferase of patient 1 and 3 declined, patient 2 had resolution of proximal tubular dysfunction, and the urine protein/creatinine ratio declined from 758 to 323 mg/mmol in patient 4. The estimated creatinine clearance declined in patient 2 (-6 mL/min) and patient 5 (-13 mL/min).

DISCUSSION

This study provides the first insights into the potential clinical use of dolutegravir monotherapy as a treatment strategy, which may become a valid treatment option in a well selected HIV-1 infected patient population suppressed on cART, if future prospective studies confirm our initial encouraging short term results. Our patients all represented selected and unusual cases in which cART had become complicated for the reasons described. This case series is in no way proof of the long-term efficacy of dolutegravir monotherapy but rather illustrates the potential that dolutegravir may have in mono- or maybe duo-therapy. Similar to PI monotherapy, a pre-cART CD4 <200 cells/mm^3 and HIV-RNA >100.000 copies/mL might be unfavourable. Comedications, mineral supplements, and renal impairment with dialysis could also reduce dolutegravir exposure and might be more important with monotherapy.[266, 267] Future trials should evaluate these issues. We considered dolutegravir monotherapy to be a safe and a reasonable option in our patients because all had contra-indications to current or alternative regimens and none had ever failed prior cART. Therefore they all had NNRTI or PI based regimen options if dolutegravir monotherapy failed. We decided to use dolutegravir monotherapy instead of dolutegravir/lamivudine or dolutegravir/emtricitabine to ensure that patients would not develop resistance to lamivudine or emtricitabine upon failure. This would preserve the option to switch patients back to their original regimens. HIV-RNA was intensively monitored after the switch to assure that dolutegravir could be discontinued as soon as HIV-RNA would rebound.

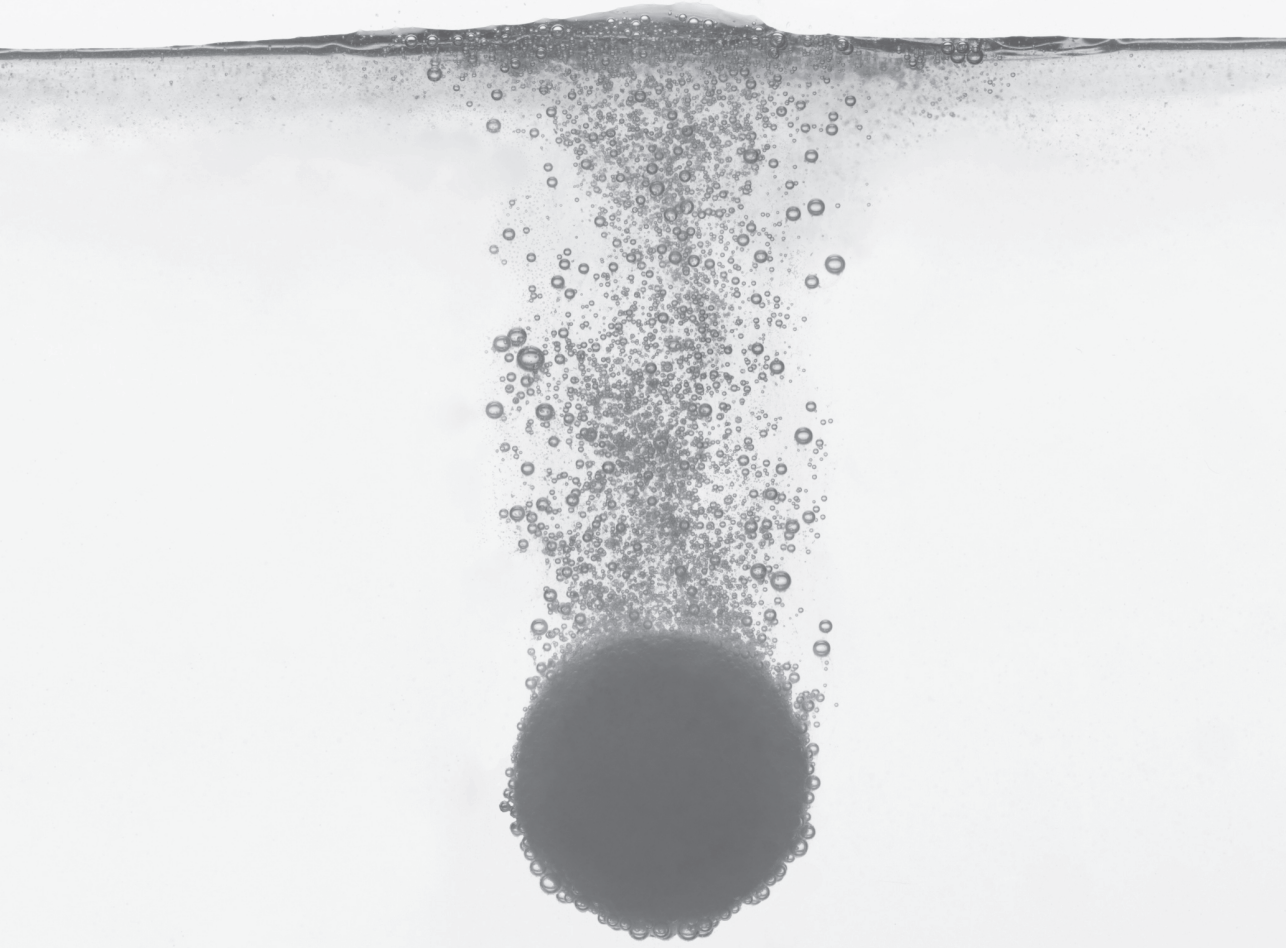
The potential advantages of dolutegravir monotherapy without the use of NRTI could be relevant for a considerable number of patients. Renal tenofovir disoproxil-fumarate toxicity or the potential increased risk on myocardial infarction with abacavir would be avoided. Unnecessary drug toxicity and CVD management are important in the ageing HIV-1 population. Second, drug costs would be substantially lower compared to recommended first-line cART regimens. Third, dolutegravir monotherapy might be a more favourable treatment option compared to PI monotherapy. PI monotherapy has been shown to be safe and non-inferior to cART in randomized clinical trials in patients with a pre-cART CD4 nadir >200 cells/mm^3 and a HIV-RNA <100.000 copies/mL.[268-271] However, PI are associated with more DDI than dolutegravir, significant side effects and dyslipidaemia in subsets of patients. The pill-size and daily number of ritonavir boosted PIs remains substantial compared to dolutegravir monotherapy.

PI monotherapy has not become part of HIV treatment guidelines. dolutegravir might have the potential to become a more acceptable maintenance monotherapy if future clinical trials confirm that viral rebound is not just delayed but also prevented by long-term dolutegravir monotherapy. Preliminary results on dolutegravir/lamivudine dual therapy were favourable in 20 ART naïve patients. Also, as yet 2 unpublished case series that were presented at the EACS conference 2015 and that also included heavily pretreated patients, showed virological failure with the occurence of INI resistance in 4 patients during dolutegravir monotherapy. All 4 had received raltegravir or elvitegravir containing cART in the past and 1 had previously failed INI containing cART.[272-274] We advocate that the first studies should focus on patients in which PI monotherapy was always non-inferior to cART. As such, a randomized trial is ongoing in the Netherlands and includes patients with a CD4 nadir >200 cells/mm^3 and a HIV-RNA <100.000 copies/mL to test the hypothesis that dolutegravir maintenance monotherapy is non-inferior to cART in selected HIV-1 patients.[275]



Part 3

Comorbidity during antiretroviral treatment



Chapter 7

Renal toxicity of concomitant exposure to tenofovir and inhibitors of tenofovir's renal efflux transporters in patients infected with HIV type 1.

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ABSTRACT

Introduction

Exposure to tenofovir disoproxil fumarate (TDF) may cause renal toxicity. Inhibitors of TDF's apical multidrug-resistance-associated protein efflux-transporters (MRPs) in the renal proximal tubule could enhance this unwanted effect.

Methods

We performed a cohort study involving patients with human immunodeficiency virus type 1 (HIV) infection. All patients had a suppressed viral load and were receiving TDF as a part of combination antiretroviral therapy. Data on mean cumulative defined daily doses (DDDs) of MRP inhibitors (NSAIDs, PDE5-i, salicylates, dipyridamole) were collected. The effects of MRP inhibitors on the estimated glomerular filtration rate (eGFR) and proximal tubular function were evaluated by generalized linear models, with adjustment for renal- and HIV-specific factors.

Results

A total of 721 HIV-infected patients were included (76.3% were male; median age 45 years; median CD4+ T-cell count 600 cells/mm^3). The median duration of TDF exposure was 54 months, and the total cumulative exposure duration was 3484 patient-years. Three hundred twenty-one patients had MRP inhibitor exposure, ranging from 0.02 to 120 mean DDDs/month. Exposure to MRP inhibitors was associated with an additional mean eGFR change of -1.4 mL/min (95% confidence interval [CI], -2.9 to .1 mL/min) over 12 months in patients with ≥1 year of continuous TDF exposure. Associations were observed between MRP inhibitor exposure and eGFR declines of >10 mL/min (odds ratio [OR], 1.38; 95% CI, .97 to 1.95), or >25% (OR, 2.14; 95% CI, 1.19 to 3.85) since initiation of TDF therapy. Overall, no clinically significant associations were found between MRP inhibitor exposure and abnormal protein, glucose, or phosphate handling in the proximal tubule or with the presence of ≥2 of these markers.

Conclusion

Concomitant incidental exposure to MRP inhibitors and TDF did not result in major additional TDF-related renal toxicity in HIV-infected patients.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is recommended as nucleoside reverse transcriptase inhibitor (NRTI) for treatment of human immunodeficiency virus type 1 (HIV) infection in combination antiretroviral therapy (cART).[163, 167] Prolonged TDF exposure in clinical practice has been associated with renal impairment, characterized by glomerular filtration rate (GFR) decline and proximal tubular dysfunction.[181] Use of boosted protease inhibitors (PIs) instead of nonnucleoside reverse transcriptase inhibitors in cART increases TDF's renal toxicity.[182]

The pharmacokinetic profile of the prodrug TDF includes conversion to tenofovir and excretion by glomerular filtration or proximal tubular secretion. Renal injury is probably related to intracellular tenofovir accumulation in proximal tubule cells, which results in mitochondrial DNA depletion and cytotoxicity.[276] The organic anion transporter 1 enables tenofovir influx at the basolateral side of tubular epithelial cells. Apical multidrug-resistance-associated protein 2 (MRP-2) and, predominantly, MRP-4 regulate tenofovir's active secretion in pre-urine. Mutations in the genes ABCC2/4 (encoding MRP-2/4) can impair tenofovir's transport,[277] and may contribute to renal impairment in vivo.[278-280] Nonsteroidal antiinflammatory drugs (NSAIDs), anticoagulants, and erectile dysfunction drugs can also inhibit tenofovir's primary efflux transporter MRP-4 (and often MRP-2) in vitro.[281-284] The capacity of these frequently prescribed drugs to inhibit MRP (as defined by the half maximal inhibitory concentration [IC_{50}]) is concentration dependent. Salicylates have the highest IC_{50}, whereas the IC_{50} values of others are considerably lower.

The potential interaction of MRP inhibitors with TDF has never been systematically studied in vivo, and whether concomitant exposure causes additional renal toxicity is unknown. The principle aim of this study was to evaluate whether the concurrent use of TDF and MRP inhibitors is associated with additional GFR decline or with proximal tubular dysfunction in HIV-infected patients receiving TDF-containing cART.

METHODS

Study Design

This was a cohort study, performed at the Erasmus University Medical Center, Rotterdam, the Netherlands, that involved adult HIV-infected patients receiving TDF-containing cART. Patients visited their HIV physician at least once per 6 months and were recruited between 1 February and 1 September 2014. Activities at visits included measurement of serum creatinine, phosphate, glucose, and HIV RNA levels and spot testing of urine with dipstick urinalysis to evaluate glycosuria and to quantify creatinine, total protein, albumin, and phosphate levels. All patients provided written informed consent. The study was approved by the institutional ethical board, conducted in accordance with good clinical practice, and registered (clinical trials registration NTR4618; available at: http://www.trialregister.nl).

Exposure to physician-prescribed and over-the-counter (OTC) NSAIDs (ie, ibuprofen, diclofenac, naproxen, celecoxib, etoricoxib, indomethacin, meloxicam, and aspirin), salicylates (for cardiovascular disease [CVD] prevention), dipyridamole, and phosphodiesterase-5 inhibitors [PDE5-i], i.e. sildenafil, tadalafil, and vardenafil) over 6 months was determined using structured patient interviews conducted by the researchers. Anthropometric, demographic, clinical characteristics and adherence data were collected. The exposure to potential nephrotoxic drugs (ie, acyclovir, trimethoprim-sulfamethoxazole, angiotensin-converting-enzyme inhibitors [ACEi], and angiotensin-receptor blockers [ARB]) was extracted from the patients' electronic medical files. Clinical data included cART history, HIV RNA levels, CD4+ T-cell counts, HIV transmission route, and TDF treatment duration. We measured serum creatinine levels (in μmol/L, divided by 88.4 equals mg/dL) at inclusion, at TDF initiation, and 12 months (±3 months) prior to study inclusion. Comorbidity data collected were history of hypertension (or a blood pressure of >150/100 mm Hg at study inclusion), history of diabetes (or a glucose level of >11.0 mmol/L at inclusion), and hepatitis C virus infection. Previous macrovascular complications, angina pectoris, or heart failure defined CVD. Chronic kidney disease (CKD) at inclusion was categorized on the basis of the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, as follows: (1) an eGFR of >60 mL/min and a ratio of urinary albumin level to creatinine level (ACR) of <3 mg/mmol (low risk); (2) either an eGFR of 45-59 mL/min without an ACR of >3 mg/mmol or an eGFR of >60 mL/min with an ACR of 3-30 mg/mmol (moderate risk); (3) an eGFR of 30-44 mL/min with an

ACR of <3 mg/mmol, an eGFR of 45-59 mL/min with an ACR 30-300 mg/mmol, or an eGFR of >60 mL/min with an ACR of >300 mg/mmol (high risk); and (4) all other combinations of eGFR and ACR values (very high risk).[285] The KDIGO guidelines on acute kidney injury (AKI) were used to evaluate whether patients would meet the criteria for possible AKI at study inclusion, compared with their kidney function 12 months earlier (assumed to represent the baseline eGFR).[286] The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) risk score was used to categorize patients with a low (<0 points), medium (0-4), or high (≥5) risk of CKD.[287] We calculated the mean monthly cumulative defined daily doses (DDDs) of OTC and physician-prescribed MRP inhibitors according to World Health Organization guidelines.[288] This value was used to categorize patients on the basis of increasing MRP exposure into 4 quartiles of comparable sizes. All GFRs were estimated from the serum creatinine level, according to the CKD Epidemiology Collaboration (CKD-EPI) formula. The CKD-EPI formula is recommended for HIV patients by the Infectious Diseases Society of America.[289, 290] The urine ACR, the ratio of protein level to creatinine level (PCR), the ratio of albumin level to protein level (APR), the fractional excretion of phosphate (FEPO), and the tubular maximum reabsorption of phosphate per liter of GFR (TmPO4/GFR) were calculated. Hypophosphatemia, increased FEPO, decreased TmPO4/GFR, glycosuria without hyperglycemia, and tubular proteinuria defined tubular dysfunction. The presence of ≥2 markers defined proximal tubulopathy.[290]

Hypophosphatemia was defined as a serum phosphate concentration of <0.8 mmol/L (mmol/L divided by 0.334 equals mg/dL). The FEPO was considered abnormal if >20% or, in hypophosphatemic patients, >10%. A TmPO4/GFR of <0.8 mmol/L was considered abnormal. If the tubular reabsorption of phosphate (TRP) was ≤0.86, TmPO4/GFR was calculated by multiplying the serum phosphate level by the TRP; and if the TRP was >0.86, TmPO4/GFR was calculated by multiplying the serum phosphate level by the result of the following equation: [0.3 × TRP]/[1-(0.8 × TRP)].[291] The ACR was categorized as normal (<3 mg/mmol), moderately increased (3-30 mg/mmol), or severely increased (if >30 mg/mmol). A PCR of <15 mg/mmol was considered normal. Tubular proteinuria was defined as a urine APR of <0.4, provided that the PCR was ≥20 mg/mmol.[290, 292]

Statistical Analysis

The primary outcome was the eGFR decline over 12 months. Secondary outcomes were an eGFR decrease of >10 mL/min, an eGFR decrease of >25%, and the overall eGFR decline since TDF initiation; the presence of proximal tubular dysfunction; and potential AKI at study inclusion. These outcomes were analyzed in patients with ≥12 months of continuous TDF exposure at the time of study inclusion. Comparisons were made between patients who had or had not been exposed to MRP inhibitors and between patients in the highest quartile of exposure and those without exposure. The evaluation of the following 2 subgroups was included in the protocol: an analysis of proximal tubule function in patients with <12 months of continuous TDF exposure at inclusion and an analysis of patients with NSAID exposure only. All outcomes were analyzed in patients with HIV suppression (defined as an HIV RNA load of <500 copies/mL), to minimize the influence of HIV replication on renal toxicity.[293]

Baseline data are reported as medians with interquartile ranges (IQRs) or as numbers of patients with percentages. The relationship of MRP inhibitor exposure with mean eGFR changes over time was assessed by independent t tests, and the continuous markers of proximal tubulopathy (ie, PCR, APR, and FEPO) and median MRP inhibitor exposure were evaluated with Wilcoxon rank sum tests. χ^2 tests were performed to assess associations between exposure groups and eGFR declines of >10 mL/min since TDF initiation, eGFR declines of >25% since TDF initiation, and markers of proximal tubulopathy.

Multivariable generalized linear models were constructed for an adjusted analysis of the effect of MRP inhibitor exposure on eGFR decline over 12 months and to calculate adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for an eGFR decline of >10 mL/min since TDF initiation, an eGFR decline of >25% since TDF initiation, and markers of proximal tubulopathy. The models were corrected for age, sex, ethnicity (African or other), HIV transmission route (male-male sex, injection drug use, and other), comorbidities, cART (PI and other), nephrotoxic medication, weight, CD4+ T-cell count, KDIGO CKD risk group, duration of TDF use, and baseline eGFR. A P value <.05 was considered statistically significant.

RESULTS

Baseline Characteristics

Of 893 eligible HIV-infected patients receiving TDF-containing cART, 731 (81.9%) consented to participation. The majority (n = 721) had a suppressed HIV RNA load (defined as <500 copies/mL) and contributed to 3484 patient-years of TDF exposure. Their characteristics at study inclusion are shown in Table 1.

A total of 627 patients had been continuously exposed to TDF for least 12 months at the time of inclusion. These participants were predominantly males (76.9%) with HIV transmission through male-male sexual contact (56.5%). The median age was 46 years. Patients were mostly of non-African origin (78.6%) and had a high CD4+ T-cell count (median, 620 cells/mm^3). Patients had received TDF-containing cART for a median of 62 months, contributing to 3434 patient-years of cumulative exposure. The median D:A:D CKD risk score at TDF initiation was -1. Most patients had an eGFR of >90 mL/min (median, 93 mL/min), without albuminuria (median ACR, 0.7 mg/mmol). The KDIGO CKD classification at inclusion was low or moderate in 99.4% of patients. Four males, aged 54-66 years, had high KDIGO CKD classifications. Two of these 4 patients did not have comorbidities and were not using nephrotoxic drugs, 1 had diabetes mellitus, and 1 had hypertension and a membranous glomerulopathy for which an ARB was used. Eighty-four patients (13.4%) had a PCR of >20 mg/mmol (median, 28.8 mg/mmol; IQR, 20.8-45.2 mg/mmol), including 2 patients with a PCR of >200 mg/mmol (361.7 and 1220.0 mg/mmol). A total of 64 of these 84 patients (76.2%) had an APR of <0.4 (median, 0.17; IQR, 0.08-0.26).

Two hundred eighty-six patients with a minimum of 12 months of TDF exposure had been exposed to MRP inhibitors. The mean monthly cumulative total MRP inhibitor exposure ranged from 0.02 to 120.0 DDDs/month (median, 1.4 DDDs/month; IQR, 0.3-5.7 DDDs/month). The range within the quartiles were 0.02-0.3 DDDs/month for quartile 1 (n = 73), 0.4-1.3 DDDs/month for quartile 2 (n = 69), 1.4-5.6 DDDs/month for quartile 3 (n = 73), and 6.2-120.0 DDDs/month for quartile 4 (n = 71). The median exposure to MRP inhibitors was 30.0 DDDs/month (IQR, 10.0-33.1 DDDs/month) in the highest quartile. Patients with any MRP inhibitor exposure had received TDF for a median of 55 months, and patients without exposure had received TDF for a median of 65 months (P = .016). Patients at highest exposure and patients

Table 1. Characteristics of suppressed HIV-1 infected patients on TDF containing cART.

	Overall (n=721)		With MRP inhibitor exposure (n=321)		Without MRP inhibitor exposure (n=400)	
	N	**(%)**	**N**	**(%)**	**N**	**(%)**
Male sex	550	(76.3)	255	(79.4)	295	(73.8)
Age (median, IQR)	45	(38 - 53)	46	(38 - 53)	45	(37 - 45)
Ethnicity						
Caucasian	434	(60.2)	218	(67.9)	216	(54.0)
African or African descent	161	(22.3)	51	(15.9)	110	(27.5)
Other	126	(17.5)	52	(16.2)	74	(18.5)
HIV-1 Transmission						
MSM	407	(56.4)	210	(65.4)	197	(49.3)
Heterosexual	254	(35.2)	82	(25.5)	172	(43.0)
IV drug use	20	(2.8)	11	(3.4)	9	(2.3)
Other	5	(0.7)	3	(0.9)	2	(0.4)
Unknown	35	(4.9)	15	(4.8)	20	(5.0)
CD4 count, cells/mm^3 (median, IQR)	600	(460 - 780)	600	(460 - 815)	610	(460 - 750)
TDF containing regimen						
NNRTI based	634	(87.9)	278	(86.7)	356	(89.0)
BPI based	83	(11.5)	42	(13.1)	41	(10.3)
Other	4	(0.6)	1	(0.2)	3	(0.7)
>1 year TDF exposure	627	(87.0)	286	(89.1)	341	(85.2)
Time on TDF, months (median, IQR)	54	(21 - 87)	49	(21 - 81)	(58)	(22 - 90)
Comorbidities						
Hypertension	85	(11.8)	33	(10.3)	52	(13.0)
Diabetes mellitus	32	(4.4)	11	(3.4)	21	(5.3)
Hepatitis C	28	(3.9)	17	(5.3)	11	(2.8)
Cardiovascular disease	51	(7.1)	32	(10.0)	19	(4.8)
Renal interacting co-medication						
Trimethoprim-sulfamethoxazole	9	(1.2)	3	(0.9)	6	(1.5)
ACEi or ARB	52	(7.2)	23	(7.2)	29	(7.3)
Acyclovir	16	(2.2)	9	(2.8)	7	(1.8)
D:A:D CKD risk score (median, IQR)	-1	(-2 - 1)	-1	(-2 - 1)	-1	(-2 - 1)
KDIGO CKD risk classification						
Low	581	(80.6)	255	(79.4)	326	(81.5)
Moderate	117	(16.2)	54	(16.8)	63	(15.8)
High	4	(0.6)	2	(0.6)	2	(0.5)
Insufficient information	19	(2.6)	10	(3.2)	9	(2.2)
eGFR, mL/min (median, IQR)	94	(82 - 106)	90	(78 - 103)	97	(84 - 109)

eGFR was calculated using the CKD-Epidemiology Collaboration formula.

Abbreviations: ART, combination antiretroviral therapy; BPI, boosted protease inhibitor; CKD, chronic kidney disease; D:A:D, data collection on adverse events of anti-HIV drugs; eGFR, estimated glomerular filtration rate; IQR, interquartile range; KDIGO, kidney disease: improving global outcomes; MRP, multidrug resistance protein; TDF, tenofovir disoproxil-fumarate.

without exposure had received TDF for a comparable median duration (69 vs 65 months; P = .794).

At least 1 NSAID was used by 202 patients (median, 0.5 DDDs/month; IQR, 0.2-2.0 DDDs/month). This exposure consisted predominantly of diclofenac (n = 51; median, 1.0 DDDs/month; IQR, 0.2-2.4 DDDs/month) or ibuprofen (n = 141; median, 0.4 DDDs/month; IQR, 0.2-1.0 DDDs/month). Thirty-eight patients used other NSAIDs (median, 2.5 DDDs/month; IQR, 0.3 to 17.5 DDDs/month). PDE5-i was used by 116 patients (94.0% received sildenafil; median, 2.0 DDDs/month; IQR, 0.7-4.0 DDDs/month). Twenty-eight patients used salicylates (71.4% received acetylsalicylic acid 80 mg) as CVD prophylaxis, including 3 patients who were also using dipyridamole. All patients using salicylates (30 DDDs/month) alone or in combination with dipyridamole (60 DDDs/month) were in the highest quartile. For the 4 patients with high KDIGO CKD classifications, only exposure to sildenafil (4.0 DDDs/month in 2) but not to NSAIDs or anticoagulants was observed.

Data from patients without complete quantitative urinalysis (n = 19) were not used for related interferential statistics. Twelve of these 19 patients had negative results of urine dipstick analyses of protein and glucose. The remaining 7 patients did not undergo urinalysis and had eGFRs of 86-126 mL/min.

eGFR Decline

Exposure to MRP inhibitors did not have a major effect on the eGFR decline in patients with at least 1 year of continuous TDF exposure at inclusion (Figure 1). Overall, eGFR changed by a mean of -1.5 mL/min (95% CI, -2.3 to -.8 mL/min) over the previous year. The kidney status in 6 patients would be classified as AKI stage 1 (≥26.5 μmol/L serum creatinine level increase), on the assumption that their creatinine level had remained stable from the time it was last measured until just prior to the observed increase in the level at inclusion. Their creatinine level increase ranged from 29 μmol/L to 54 μmol/L. Four of these 6 patients had used MRP inhibitors (0.03-45.0 DDDs/month). The mean decreases in eGFR were comparable between patients without exposure (change, -1.2 mL/min) and those with any MRP inhibitor exposure (change, -1.9 mL/min; P = .424) or those in the highest quartile of MRP inhibitor exposure (change, -1.1 mL/min; P = .919). The mean eGFR decline since TDF initiation was higher for patients with any exposure, compared with patients without exposure (change, -11.4 vs -8.6 mL/min; P = .008). Of all patients with any MRP

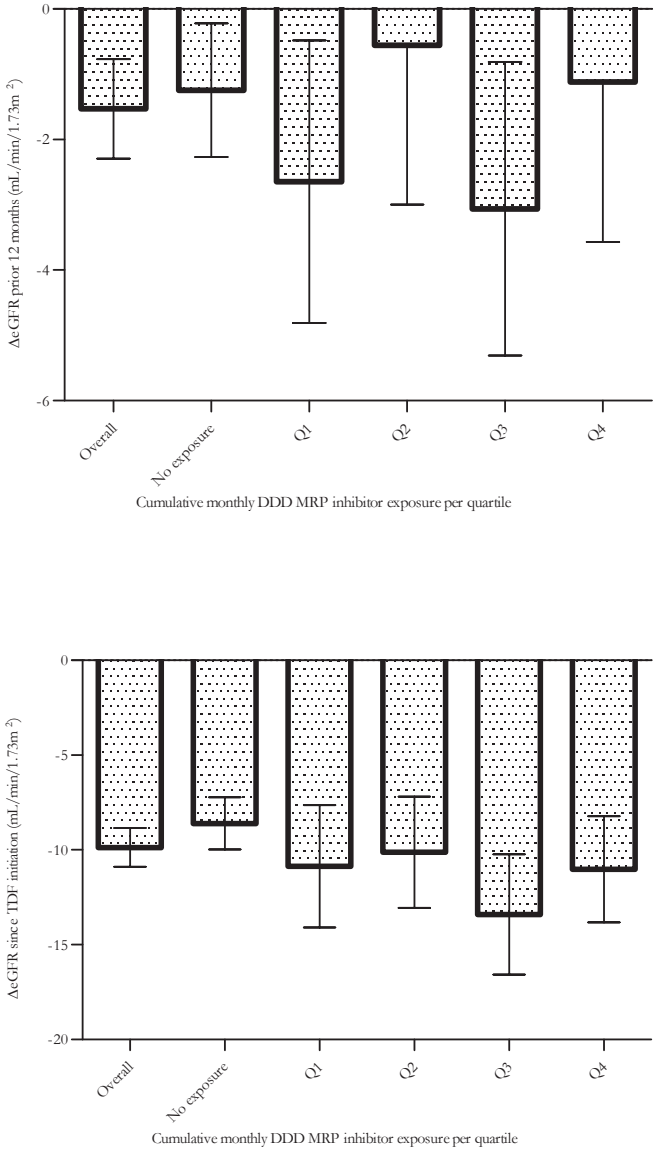


Figure 1. Bars represent the mean estimated glomerular filtration rate (eGFR) decline (with 95% confidence intervals) over 12 months and since initiation of tenofovir disoproxil fumarate (TDF) therapy. The eGFR decline for all patients and per category of cumulative monthly defined daily doses (DDDs) of multidrug resistance protein transporter (MRP) inhibitor exposure are shown. GFRs were estimated according to the Epidemiology Collaboration formula. Quartile ranges are 0.02–0.3 DDDs (quartile 1 [Q1]; n = 73), 0.4–1.3 DDDs (Q2; n = 69), 1.4–5.6 DDDs (Q3; n = 73), and 6.2–120.0 DDDs (Q4; n = 71).

inhibitor exposure, 49.7% had eGFR declines of >10 mL/min (compared with 42.8% without exposure), and 13.3% had a >25% decline in the eGFR since TDF initiation (compared with 8.2%). TDF treatment was discontinued owing to renal impairment in 17 of 66 patients with eGFR declines of >25%; the median MRP inhibitor exposure tended to be higher in these 17 patients (2.6 vs 0.03 DDDs/month; P = .055).

After multivariable adjustment, exposure to MRP inhibitors was not significantly associated with an additional eGFR decline over the previous 12 months (change, -1.4 mL/min; 95% CI, -2.9 to 0.1 mL/min; P = .067). Associations between MRP inhibitor exposure versus no exposure and eGFR declines of >10 mL/min (OR, 1.38; 95% CI, .97 to 1.95; P = .074) or >25% (OR, 2.14; 95% CI, 1.19 to 3.85; P = .011) since TDF initiation were found. Notably, these associations were predominantly driven by patients in quartile 3. For these patients, exposure to MRP inhibitors had an effect on eGFR decline (change, -2.3 mL/min; 95% CI, -4.6 to .0; P = .053) and increased the adjusted ORs for eGFR declines of >10 mL/min (2.06; 95% CI, 1.18 to 3.60; P = .011) and >25% (2.88; 95% CI, 1.29 to 6.42; P = .010) since TDF initiation.

Tubular Dysfunction

No major effects of MRP inhibitor exposure on the markers of proximal tubular dysfunction were observed in patients who had received TDF for at least 12 months at inclusion. These results are shown in Table 2. Overall, the median proteinuria level was 110 mg/L, and the median PCR was 9.5 mg/mmol. The median PCR was not different between patients without exposure to MRP inhibitors (9.9 mg/mmol) and patients with any (9.1 mg/mmol; P = .094) or the highest (9.0 mg/mmol; P = .710) exposure to MRP inhibitors. The patients without exposure and those with the highest exposure also had comparable median APRs (0.25 and 0.16, respectively; P = .362) and frequencies of an APR of <0.4 (72.9% and 78.6%, respectively; P = .671), both of which were assessed when the PCR was >20 mg/mmol. Multivariable adjusted models showed no increased OR yielded by tubular proteinuria for MRP inhibitor exposure overall (0.76; 95% CI, .37 to 1.56; P = .451) or for the highest quartile, compared with no exposure. Patients at highest exposure to MRP inhibitors had comparable rates of hypophosphatemia as compared to those without exposure. There were no significant differences in median FEPO (12.3% and 15.1%, respectively; P = .083) or frequencies of decreased TmPO4/GFR (P = .327). The proportion of patients with an abnormal FEPO was highest for patients in the third and fourth quartile of MRP inhibitor exposure. No increased multivariable adjusted OR associated with an abnormal FEPO

Table 2. Markers of proximal tubule toxicity in suppressed HIV-1 patients on TDF containing treatment (n=627) without and with exposure to inhibitors of renal multidrug resistance protein transporters.

	No exposure (n=341)		Quartile 1 (n=73)		Quartile 2 (n=69)		Quartile 3 (n=73)		Quartile 4 (n=71)	
Urine protein dipstick negative (No., %)	312	(91.5)	69	(94.5)	64	(92.8)	64	(87.7)	62	(87.3)
Proteinuria, mg/L (median, IQR)	110	(60-175)	100	(60-160)	110	(70-153)	115	(70-203)	140	(60-273)
Urine ACR, mg/mmol (median, IQR)	0.8	(0.4-1.8)	0.6	(0.3-1.9)	0.7	(0.4-2.2)	0.8	(0.4-1.5)	0.7	(0.4-2.8)
Urine PCR, mg/mmol (median, IQR)	9.9	(7.2-14.5)	8.2	(6.6-12.2)	9.6	(7.0-12.9)	9.9	(7.1-13.9)	9.0	(7.4-16.2)
Urine PCR >15 mg/mmol (No., %)	76	(22.3)	13	(17.8)	12	(17.4)	15	(20.5)	19	(26.8)
Urine APR (median, IQR)[1]	0.25	(0.14-0.49)	0.15	(0.06-0.42)	0.22	(0.07-0.52)	0.22	(0.09-0.32)	0.16	(0.11-0.37)
FEPO, % (median, IQR)	12.3	(8.0-17.3)	11.6	(7.8-16.8)	11.7	(7.4-17.2)	14.4	(9.6-21.4)	15.1	(10.2-20.0)
Hypophosphatemia (No., %)	76	(22.3)	9	(12.3)	13	(18.8)	14	(19.2)	16	(22.5)
Abnormal FEPO (No., %)	73	(21.4)	15	(20.5)	14	(20.3)	26	(35.6)	24	(33.8)
TmP/GFR <0.8 mmol/L (No., %)	123	(36.1)	21	(28.8)	19	(27.5)	29	(39.7)	30	(42.3)
Normoglycemic glycosuria (No., %)	9	(2.6%)	0	(0.0)	1	(1.5)	0	(0.0)	0	(0.0)

[1]Urine APR calculated in patients with PCR>20 mg/mmol.

Abbreviations: ACR, albumin-creatinin ratio; APR, albumin-protein ratio; FEPO, fractional excretion of phosphate; IQR, interquartile range; PCR, protein-creatinin ratio; TmP/GFR, Renal tubular maximum reabsorption of phosphate per liter of GFR.

was observed with overall MRP inhibitor exposure (OR, 1.24; 95% CI, .82 to 1.87; P = .303), although the OR associated with an abnormal FEPO was significantly increased (2.00; 95% CI, 1.13 to 3.87; P = .019) for patients in the third quartile. Glycosuria was rarely observed, occurring in 10 of 611 normoglycemic individuals, and no relationship with exposure to MRP inhibitors was noted (P = .211).

Thirty-nine patients had at least 2 markers of proximal tubulopathy. These included 21 patients (6.1%) without exposure and 9 patients (12.7%) at highest exposure to MRP inhibitors (P = .054). Multivariable analysis found no significant association between MRP inhibitor exposure overall (OR, 0.65, 95% CI, .26 to 1.61; P = .350) or per quartile and the presence of at least 2 markers of proximal tubulopathy. TDF was discontinued in 14 of 39 patients with at least 2 markers of proximal tubulopathy; median MRP inhibitor exposure duration was not significantly higher in these 14 patients (0.47 vs 0.0 DDDs/month; P = .239).

Subgroup Analysis

Ninety-four patients had <1 year of continuous TDF exposure at inclusion, including 35 patients with MRP inhibitor exposure. Their cumulative exposure ranged from 0.06 to 30.5 DDDs/month (median, 1.1 DDDs/month; IQR, 0.1-4.0 DDDs/month). Three of 94 patients had a PCR of >20 mg/mmol (median, 7.9 mg/mmol; IQR, 6.3-11.1 mg/mmol), including 1 patient with an APR of <0.4. Two patients had normoglycemic glycosuria, and a comparable proportion of patients with and without exposure (20.0% and 20.3%, respectively) had an abnormal FEPO. The presence of 2 markers of proximal tubulopathy was apparent in 2 patients.

The 202 patients with NSAID exposure were categorized into 4 quartiles. The median exposure per quartile was 0.1 DDDs/month (IQR, 0.06-0.1 DDDs/month) for quartile 1, 0.25 DDDs/month (IQR, 0.22-0.33 DDDs/month) for quartile 2, 0.83 DDDs/month (IQR, 0.6-1.7 DDDs/month) for quartile 3, and 5.0 DDDs/month (IQR, 2.6-16.3 DDDs/month) for quartile 4. Patients at highest exposure had a mean additional eGFR change of -2.3 mL/min (95% CI, -4.9 to .2; P = .076) in the previous year and an increased OR (2.81; 95% CI, 1.21 to 6.49; P = .016) associated with a >25% eGFR decline, compared with patients without exposure. No association of patients with the highest NSAID exposure and the presence of tubular proteinuria (P = .262) or abnormal FEPO (P = .675) were observed. Patients with the most exposure

to NSAIDs had an OR of 3.78 (95% CI, 1.00 to 14.31; P = .05) associated with the presence of at least 2 markers of proximal tubular dysfunction.

Diclofenac exposure in 51 patients was evaluated separately because diclofenac is the most potent MRP inhibitor and widely used. Median diclofenac exposure in those patients was 1.0 DDDs/month (range, 0.02-90.0 DDDs/month). Exposure to diclofenac was not associated with a significant eGFR decline in the previous year of TDF exposure (change, -2.2 mL/min; 95% CI, -4.9 to .5; P = .114), although it was associated with an increased OR (3.69; 95% CI, 1.63 to 8.36; P = .002) in association with a >25% eGFR decline since TDF initiation, compared with those without exposure. No significant associations between diclofenac exposure and tubular proteinuria (P = .574), abnormal FEPO (P = .548), or at least 2 markers of proximal tubular dysfunction (P = .743) were observed.

One additional sensitivity analysis was performed because of the unexpected large contribution of low-dose salicylates (associated with the highest IC_{50} in vitro) to the highest quartile of total MRP inhibitor exposure. This analysis showed that, when salicylates were not used to calculate cumulative total MRP inhibitor exposure, the patients in the highest quartile had an additional annual eGFR decline of -2.7 mL/min (95% CI, -5.1 to -.4; P = .024) after multivariable adjustment. No associations with markers of tubular dysfunction were observed in this analysis.

DISCUSSION

This study evaluated whether frequently used and often freely available drugs that are known to inhibit tubular MRP in vitro may increase TDF-related renal toxicity in vivo. Our results do not indicate major clinically relevant additional TDF-related renal tubular injury or additional eGFR decline in the previous year due to the concomitant exposure to MRP inhibitors and TDF. However, this conclusion can primarily be made for patients with a suppressed HIV load who received TDF-containing cART for >12 months and had incidental low-dose exposure to inhibitors of MRP. Although higher exposure to MRP inhibitors was not associated with proximal tubular dysfunction or eGFR decline over the previous 12 months, we observed consistent associations between eGFR declines since TDF initiation and higher exposure to MRP inhibitors. These observations were supported by the NSAID and diclofenac subgroup analyses.

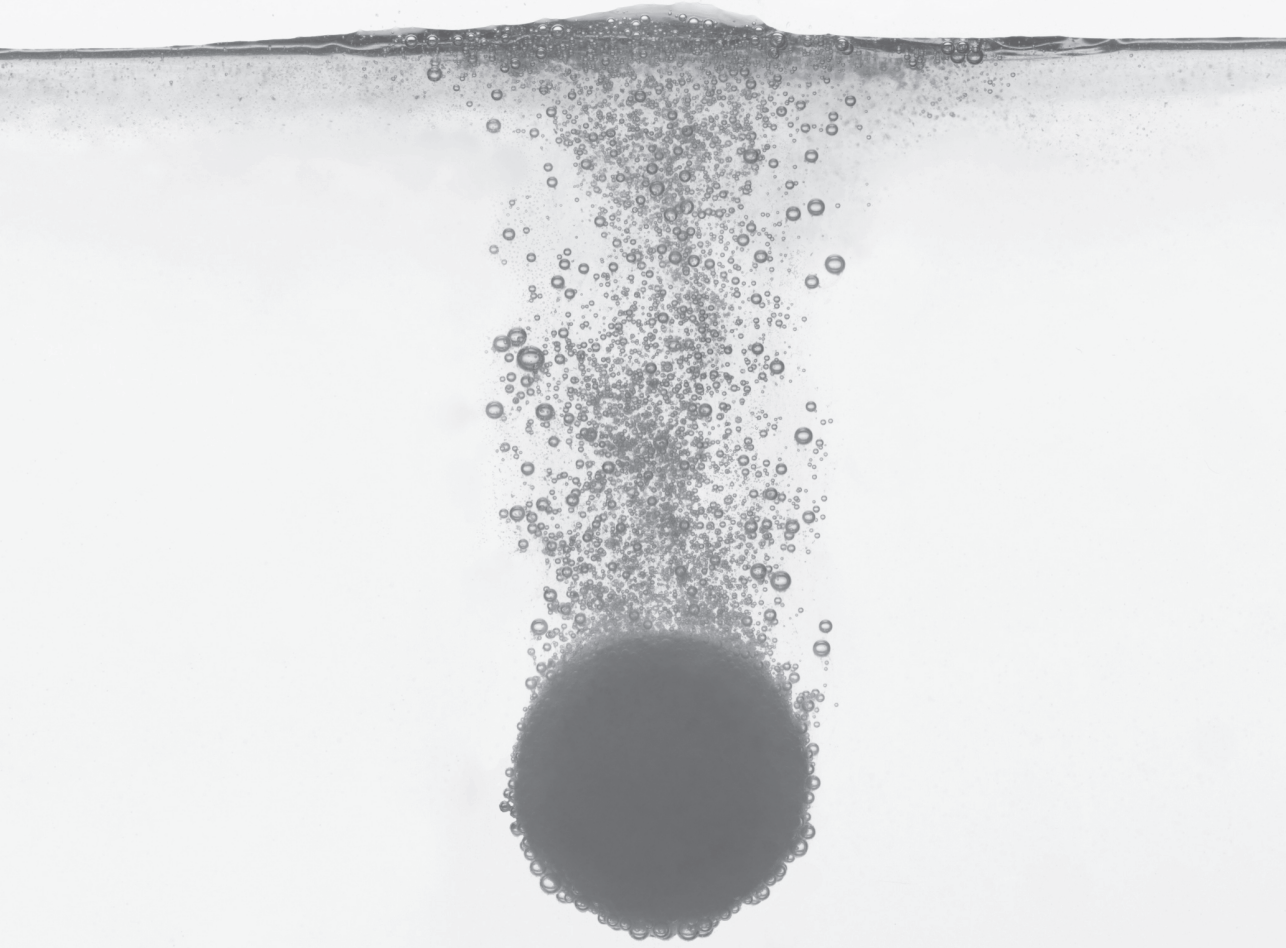
This indicates that, although MRP inhibitors do not promote TDF-related tubular nephrotoxicity, they can independently accelerate CKD progression by decreasing the eGFR in patients infected with HIV. Chronic, high-dose MRP inhibitor exposure in HIV-infected patients receiving TDF-containing regimens, especially diclofenac, warrants close eGFR monitoring.

This is the first study to evaluate the renal toxicity of prescribed and OTC MRP inhibitors in HIV-infected patients receiving TDF. Large cohorts have identified patient- and HIV-related predictors for eGFR declines during TDF-containing cART.[181, 182, 294] However, MRP inhibitors were not evaluated in these studies and may have been an important confounder. Only 1 small retrospective case series described a high frequency (14.6%) of renal injury following physician-prescribed diclofenac.[295] This study did not evaluate other MRP inhibitors, did not state the HIV RNA suppression rate, and did not correct for measured covariates, and it lacked a control group of individuals receiving TDF without diclofenac. This hinders the interpretation of this small study.

Importantly, our observed annual eGFR decline and tubular injury frequency were smaller than described in the studies mentioned above. It is possible that the much longer duration of TDF exposure in our cohort (62 months), compared with that in other cohorts (median, ≤12 months) and case series (38 months), is attributable to selection bias. Patients with obvious TDF-related renal toxicity probably discontinued TDF prior to the start of our study. These patients likely included those at highest risk for TDF-related renal toxicity, comprising patients with high D:A:D risk scores or unfavorable ABCC2/4 polymorphisms. The effects of TDF and MRP inhibitors are possibly increased in these populations.[287] Moreover, only a minority received cART with a PI backbone. Therefore, our reassuring results cannot be extrapolated to patients initiating TDF or those receiving short-term TDF-containing cART, especially regimens with a PI backbone. The relationship between MRP inhibitors and renal impairment in these populations can only be evaluated in randomized clinical trials or cohorts with adequate registration of prescribed and OTC medicines This study has limitations. The study was designed to compare patients in the highest quartile to those without MRP inhibitor exposure. Recall bias might have influenced calculated MRP inhibitor exposure. Also, patients in the highest quartile of MRP inhibitor exposure had an unexpected relatively large contribution of salicylate exposure. Low-dose salicylates have much lower potency for MRP inhibition than NSAIDs in vitro

and, possibly, in vivo.[282] The absent relationship of MRP inhibitor exposure in the highest quartile was especially surprising since some statistically significant effects of this factor on eGFR decline were observed for patients in the third quartile. Omitting low-dose salicylates from the calculation of total MRP inhibition showed that the highest quartile (not the third-highest quartile) was associated with additional eGFR decline. This may indicate that the inhibitory potency of low-dose salicylates is not of clinical relevance. The relatively small number of patients with high exposure to potent MRP inhibitors (such as diclofenac) still prevents firm conclusions. Also, the relationship between MRP inhibitor exposure and eGFR decline might also be explained by NSAID-related reduced glomerular blood flow, rather than TDF toxicity. Underlying medical conditions in HIV-infected patients warranting NSAID or PDE5-i exposure (eg, rheumatoid arthritis) may also result in renal injury promoting eGFR decline. Moreover, the relative effect of MRP inhibitors on intracellular TDF accumulation in vivo is probably not only a function of whether patients were sufficiently exposed to MRP inhibitors, but is also related to intracellular accumulation of MRP inhibitors (potentially altered by drugs or genetic variations).[296] Furthermore, the clinical significance of the small FEPO changes remains unclear. Common conditions (eg, hypovitaminosis D) and sample collection regardless of fasting state may have influenced the interpretation of tubular dysfunction. Impaired reabsorption of other solutes in the proximal tubulus (eg, low-molecular-weight proteins, uric acid, and bicarbonate) is not observed during routine care and could have influenced the interpretation of possible proximal tubular dysfunction. However, the presence of phosphaturia despite hypophosphatemia and normoglycemic glycosuria is particularly specific for proximal tubulopathy, were measured in this study, and used in routine care. Together, a separate evaluation of these factors by prospective collection of data on exposure to different MRP inhibitors in larger patient cohorts would provide more specific conclusions. The inadequacy of the data collection on potential exposure to OTC NSAIDs and other MRP inhibitors in current HIV cohorts, however, makes this evaluation impossible.

In conclusion, the renal effects of TDF will continue to be relevant, especially since more patients worldwide will initiate first-line cART, including TDF. Together, the results of this study do not provide evidence for major additional TDF-related renal toxicity due to the incidental concomitant exposure to frequently used drugs that inhibit MRP in a population of individuals with HIV suppression who are receiving long-term TDF-containing cART.



Chapter 8

Lipids and cardiovascular risk after switching HIV-1 patients on nevirapine and emtricitabine/ tenofovir-DF to rilpivirine/emtricitabine/ tenofovir-DF.

C. Rokx, A. Verbon, B.J.A. Rijnders.

AIDS Res Hum Retroviruses. 2015. Apr;31(4):363-7.

ABSTRACT

Introduction

Antiretroviral therapy-related dyslipidemia increases the risk of cardiovascular disease (CVD) and is less frequently observed with nevirapine. Whether substituting rilpivirine for nevirapine has dyslipidemic consequences and alters CVD risk is unknown. The aim of this prospective open-label clinical trial was to evaluate serum lipids, cardiovascular risks, and lipid treatment goals over 48 weeks after switching from nevirapine to rilpivirine.

Methods

Fifty HIV-1-suppressed patients on stable once-daily nevirapine plus emtricitabine/tenofovir-DF were switched to single-tablet rilpivirine/emtricitabine/tenofovir-DF. Lifestyle, weight, systolic blood pressure (SBP), ≥6 hours overnight fasting lipids, 10-year Framingham risk scores (FRS), and Adult Treatment Panel III (ATP-III) lipid goals were evaluated over 48 weeks.

Results

Patients were 82% males, were a median of 45 years of age, and were on nevirapine for a median of 66 months. Diets, exercise levels, body mass index, and smoking status did not change during follow-up. At week 24, significant changes ($p < 0.001$) were seen in mean [95% confidence interval (CI)] total cholesterol (-0.67 mmol/liter, CI: -0.50 to -0.83), low-density lipoprotein cholesterol (-0.36, CI: -0.21 to -0.51), and high-density lipoprotein cholesterol (-0.28, CI: -0.20 to -0.35). The total cholesterol/high-density lipoprotein cholesterol ratio increased 0.20 (CI: 0.02 to 0.37; $P = 0.029$). Triglycerides did not change and the SBP decreased 6 mmHg (CI: -1.7 to -10.3; $P = 0.007$). Week 48 lipid profiles and SBP were similar to week 24. The median FRS did not change during follow-up (-0.7%, $P = 0.119$). More patients achieved ATP-III low-density lipoprotein cholesterol (+14.9%; $P = 0.016$) and total cholesterol goals (+25.5%; $p < 0.001$).

Conclusion

The lipid profile changes after substituting rilpivirine for nevirapine did not significantly influence FRS, although SBP and the ATP-III low-density lipoprotein and total cholesterol goals improved.

INTRODUCTION

HIV-1-infected patients are at increased risk for cardiovascular disease (CVD) compared to the HIV-1-uninfected population.[297] This pro-atherogenic status is driven by HIV-1-induced inflammation and an increased prevalence of traditional CVD risk factors.[298, 299] Chronic untreated HIV-1 infection, smoking, diabetes, hypertension, and dyslipidemia are important modifiable cardiovascular risk factors.[300] Although essential for controlling HIV-1 replication, antiretroviral therapy (ART) has been associated with atherogenic lipid changes and increased CVD.[300, 301] ART-induced dyslipidemia varies with the drugs used. The use of nevirapine in the 2NN, ARTEN, and OCTANE trials,[302-304] and rilpivirine in the ECHO, THRIVE, and SPIRIT trials[235, 236, 250, 305] has been associated with more favorable lipid profiles compared to efavirenz and protease inhibitors. In these large randomized clinical studies, rilpivirine and nevirapine had different effects on serum lipids. Rilpivirine had neutral effects on low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C). The use of nevirapine resulted in a favorable HDL-C increase, although LDL-C and TC also increased.

Based on these results, nevirapine has been commonly used in HIV-1 patients at risk for CVD. Nonetheless, nevirapine is not recommended in first-line ART,[164] has significant toxicity,[234] and should not be used at higher CD4 counts. Nevirapine induces cytochrome P450 (CYP) 3A4, which causes drug-drug interactions and could lower the antilipid effects of statins.[306] In contrast to nevirapine, rilpivirine is recommended in first-line ART, is better tolerated, and does not significantly influence CYP3A4. Rilpivirine could therefore be preferred over nevirapine as a nonnucleoside reverse transcriptase inhibitor in antiretroviral regimens.

However, whether the substitution of rilpivirine for nevirapine has dyslipidemic consequences and alters the risk of CVD is unknown. This trial evaluates changes in serum lipids, cardiovascular risks, and lipid treatment goals over 48 weeks after switching from nevirapine to rilpivirine in HIV-1 patients.

METHODS

In this prospective, open-label clinical trial, HIV-1 patients were switched from once daily nevirapine plus emtricitabine/tenofovir-DF to once daily coformulated rilpivirine/emtricitabine/tenofovir-DF. Participants were HIV-1-infected patients, 18 years or older, using nevirapine plus emtricitabine/tenofovir-DF for over 9 months with plasma HIV-1 RNA levels <50 copies/ml for at least 6 months. Patients with historically documented virological failure, baseline resistance, an estimated glomerular filtration rate <60 ml/min, the inability to use rilpivirine with a meal of at least 500 kcal, pregnancy, or concomitant proton-pump inhibitor use were excluded. We collected demographical data and medical history at baseline. Patients visited the outpatient clinic and we assessed (changes in) diet, recommended exercise levels (30 min of moderate exercise minimal 5 days/week),[307] comedication, smoking status, self-reported adherence with pill counts, weight, body-mass index (BMI), and automatic blood pressure measurements at baseline, week 4, week 12, week 24, and week 48. Overnight fasting (≥6 hours) TC, LDL-C, HDL-C, triglycerides (TG), TC/HDL-C ratios, and glucoses were measured at all time points.

We evaluated the changes in TC, LDL-C, HDL-C, TG, TC/HDL-C ratio, glucose, blood pressure, and BMI during 48 weeks of follow-up. The 10-year risk on CVD was estimated by using the Framingham risk score (FRS) of the Copenhagen HIV Program.[308, 309] The Adult Treatment Panel III (ATP-III) criteria of the National Education Cholesterol Program were used to categorize TC, LDL-C, HDL-C, and TG.[310] In short, the FRS stratifies patients into low (<10.0%), intermediate (10.0% to 19.9%), or high (≥20.0%) risk groups on CVD. The ATP-III categories are CVD or FRS >20.0% (high risk), ≥2 risk factors on CVD with FRS <20.0% (intermediate risk), and <2 risk factors (low risk). Smoking, hypertension, unfavorable HDL-C, familial premature CVD, and age each counts as one risk factor The ATP-III LDL-C levels (mg/dl * 0.0259 equals mmol/liter) are categorized into <2.59 mmol/liter (optimal), 2.59 to 3.36 mmol/liter (near optimal), 3.37 to 4.13 mmol/liter (borderline), 4.14 to 4.91 mmol/liter (high), and ≥4.92 mmol/liter (very high). Depending on the ATP-III risk category, the LDL-C treatment goals are <2.59 mmol/liter for patients at high risk, <3.37 mmol/liter for patients at intermediate risk, <4.14 mmol/liter for patients at low risk, and <4.92 mmol/liter for patients without risk factors on CVD. TC is classified in ATP-III as desirable (<5.18 mmol/liter), borderline (5.18 to 6.19 mmol/

liter), and high (>6.19 mmol/liter). An HDL-C above 1.04 mmol/liter is favorable and TG below 3.89 mmol/liter is considered normal according to ATP-III.

The data are described as means with 95% confidence intervals (95% CI) or standard errors of mean, medians with interquartile ranges (IQR), or numbers with percentages, when appropriate. Paired t-tests were used to analyze the changes in serum lipids, glucose, BMI, and blood pressure between baseline and week 24 and between week 24 and week 48. The Wilcoxon signed-rank test was used to evaluate the change in median FRS. McNemar's test was used to compare changes in FRS categories and ATP-III treatment goals between baseline and week 24. A two-sided p value was prespecified at 0.05. Analyses were done with SPSS 21.0 and Graphpad Prism 5.0.

All included patients provided written informed consent after at least 7 days of consideration. The study was approved by the institutional ethics review board and was done in accordance with good clinical practice and the Helsinki Declaration. The trial was registered at www.trialregister.nl with clinical trial number NTR3368.

RESULTS

Table 1 shows the baseline demographic data, anthropometrics, and metabolic parameters of the 50 included participants. Patients were mostly white men having sex with men in the fourth decades of their lives. They used nevirapine for a median of 66 months and had a median CD4 count of 565 (IQR: 470-730) cells/mm^3. Nevirapine was used as first-line cART in 26 patients; 24 patients had switched to nevirapine from efavirenz (n = 14) or protease inhibitors (n = 10) because of side effects or for convenience reasons. Thirty patients (60%) were smokers or former smokers, nine (18%) were on antihypertensive treatments or had a history of hypertension, and one patient had well-controlled diabetes type-II. One patient had previous CVD. Two patients used statins for over 6 months prior to study entry. Their statin dosages remained unchanged on follow-up. Nine patients achieved the recommended exercise levels. No changes in diets, physical exercise levels, or smoking status were reported by the patients over 48 weeks and no additional lipid-lowering therapies were initiated. Three patients discontinued the study because of the side effects of rilpivirine or because of study noncompliance. Forty-seven patients (94%) completed the study and

Table 1. Baseline demographic and clinical characteristics of virologically suppressed HIV-1 infected patients switching from nevirapine to rilpivirine.

		RPV Switch Group (n=50)	
Age, years		45	(39 - 54)
Male sex		41	(82%)
HIV-1 Transmission			
MSM		31	(62%)
Heterosexual		18	(36%)
Intravenous drug use		1	(2%)
Region of Origin			
Netherlands		34	(68%)
Africa or African descent		11	(22%)
Asia		3	(6%)
Latin-America		2	(2%)
Smoking History			
Current		22	(44%)
Former		8	(16%)
Never		20	(40%)
Statins		2	(4%)
Time on NVP	(months)	66	(39 - 127)
CD4 count	(cells/mm^3)	565	(470 - 730)
Systolic BP	(mmHg)	130	(124 - 141)
Diastolic BP	(mmHg)	83	(78 - 89)
BMI	(kg/m^2)	25.1	(21.3 - 27.0)
TC	(mmol/L)	5.05	(4.58 - 5.50)
HDL-C	(mmol/L)	1.46	(1.17 - 1.77)
LDL-C	(mmol/L)	3.08	(2.56 - 3.87)
TG	(mmol/L)	1.05	(0.81 - 1.81)
TC/HDL-C	(ratio)	3.83	(2.45 - 4.50)
Glucose	(mmol/L)	5.00	(4.68 - 5.30)

Categorical variables are presented as No. (%) and continuous data are medians (IQR).
Abbreviations: BMI, body mass index; BP, blood pressure; HDL-C, high-density-lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density-lipoprotein cholesterol; MSM, men having sex with men; NVP, nevirapine; TC, total cholesterol; TG, triglycerides.

were included in the analysis. None of the 47 patients experienced virological failure during 48 weeks of follow-up.

The mean lipid changes from baseline to week 48 are shown in Figure 1. By week 24, the mean (95% CI) TC decrease was -0.67 (-0.50 to -0.83; p < 0.001). The HDL-C decreased -0.28 (-0.20 to -0.35; p < 0.001) and the LDL-C decreased -0.36 (-0.21 to -0.51; p < 0.001). The TC/HDL-C ratio increased significantly by +0.20 (0.02 to 0.37; P = 0.029 and TG decreased nonsignificantly by 0.14 (-0.29 to 0.01; P = 0.074). Glucoses were similar at baseline and week 24 (P = 0.786). The systolic blood pressure decreased a mean of -6.0 mmHg (95% CI: -1.7 to -10.3; P = 0.007). The diastolic blood pressure (-0.6 mmHg, 95% CI: -3.1 to 1.9; P = 0.613) and BMI (+0.2 kg/m^2, 95% CI: -0.1 to 0.5; P = 0.113) did not change over 24 weeks. Excluding the two patients on statins did not influence the changes in TC (-0.66), HDL-C (-0.28), LDL-C (-0.35), ratio (+0.21), or TG (-0.13) over 24 weeks. The changes in LDL-C or systolic blood pressure were not observed in the included African patients. Week 48 lipids, glucose, blood pressure, and BMI did not change significantly compared to week 24 (P = 0.228 or higher for all). The median FRS decreased nonsignificantly (P = 0.119) from 8.4% (IQR: 2.8 to 20.3) at baseline to 7.7% (IQR: 2.2 to 16.9) at

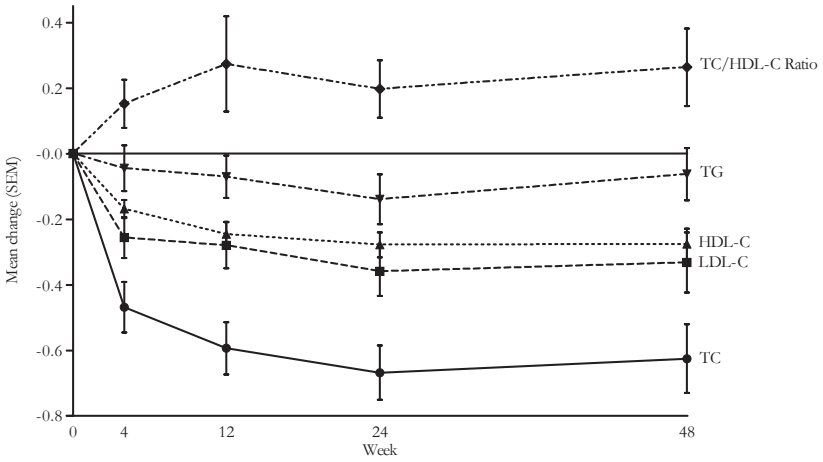


Figure 1. The mean changes (SEM) in fasting serum lipids (in mmol/L) and TC/HDL-C ratio over 48 weeks in HIV-1 infected patients after a nevirapine to rilpivirine switch. Abbreviations: HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; SEM, standard error of mean; TC, total cholesterol; TG, triglycerides.

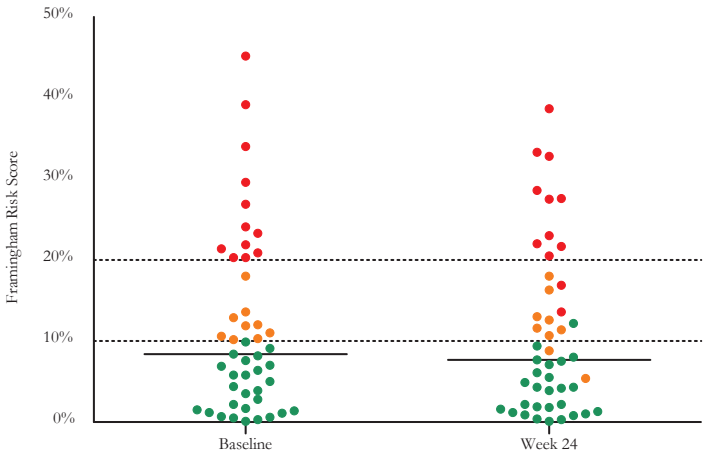


Figure 2. Framingham risk scores at baseline on nevirapine and at week 24 on rilpivirine. Patients have low (<10%, green), intermediate (10% to 20%, orange) or high (>20%, red) 10 year risk of cardiovascular disease. The solid lines are the median Framingham risk scores. The dots represent the individual patients and their colors at week 24 represent baseline Framingham risk score categories.

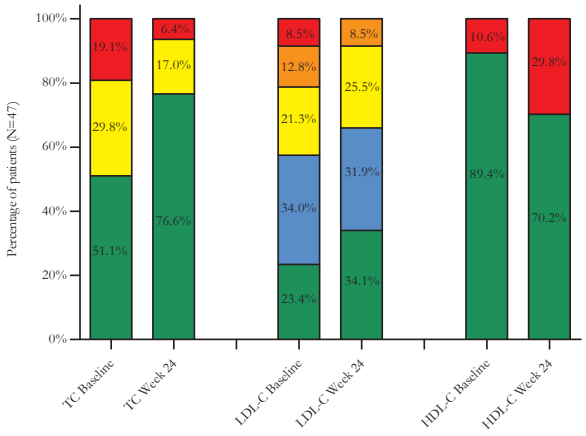


Figure 3. Distribution of Adult Treatment Panel-III recommended TC, LDL-C and HDL-C levels at baseline on nevirapine and at week 24 on rilpivirine. Treatment categories from optimal to unfavorable for TC are <5.18 (green), 5.18 to 6.19 (yellow) or >6.19 mmol/L (red). Categories for LDL-C are <2.59 (green), 2.59 to 3.36 (blue), 3.37 to 4.13 (yellow), 4.14 to 4.91 (orange) or ≥4.92 mmol/L (red) and categories for HDL-C are ≥1.04 (green) or <1.04 mmol/L (red). Abbreviations: HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TC, total cholesterol.

week 24 (Figure 2). The distribution of patients between the FRS categories did not change significantly between baseline and week 24 (P = 0.5); four patients decreased and one patient increased in FRS category from baseline to week 24. The patients' baseline and week 24 systolic blood pressures were used to calculate their FRS at these respective time points. Figure 3 shows ATP-III lipid treatment goals at baseline and week 24. At week 24, 76.6% (36/47) of patients had desirable TC levels according to ATP-III treatment goals compared to 51.1% (24/47) at baseline (difference: 25.5%; p < 0.001). For LDL-C, 22/47 (46.8%) patients did not achieve the desired LDL-C levels according to their ATP III risk categories at baseline. These 22 patients included all 12 high-risk patients with FRS >20%. According to ATP III guidelines, lifestyle or (non) medical LDL-C lowering interventions were indicated in these patients. By week 24 on rilpivirine, 15/47 patients (31.9%) did not fulfill LDL-C treatment goals (difference: 14.9%; P = 0.016). Two of the 12 patients with FRS > 20% at baseline achieved the desired LDL-C level according to their ATP-III risk category on rilpivirine at week 24. HDL-C levels were below 1.04 mmol/liter in 5/47 patients (10.6%) at baseline and 14/47 patients (29.8%) at week 24 (difference: 19.2%; P = 0.004). All patients had normal TG according to their ATP-III classification at baseline and week 24.

DISCUSSION

In this study we show that after substituting rilpivirine for nevirapine in virologically suppressed HIV-1-infected patients the lipid profile changes did not result in significant alterations of the FRS on 10-year CVD. The systolic blood pressure decreased and significantly more patients achieved TC and LDL-C treatment goals after switching ART to rilpivirine. The results indicate that in patients at risk for CVD or with ART-related dyslipidemia, rilpivirine may be a good alternative for nevirapine. Patients who have to replace nevirapine for toxicity, for drug-drug interactions, or because a single tablet regimen is desired may switch to rilpivirine without a negative impact on their cardiovascular risk profiles.

This is the first study to evaluate the potential dyslipidemic consequences and CVD risk alterations after switching from nevirapine to rilpivirine in HIV-1 patients. Nevirapine is commonly regarded as the least atherogenic antiretroviral drug based on results from previous clinical trials.[311] HIV-1 patients using nevirapine had a higher mean HDL-C compared to patients on efavirenz, boosted atazanavir, or boosted

lopinavir.[302-304] The association between nevirapine and higher HDL-C was also observed in our study. An increased production of apolipoprotein A1, HDL-C's major apolipoprotein, in patients using nevirapine could be a possible explanation.[302, 312]

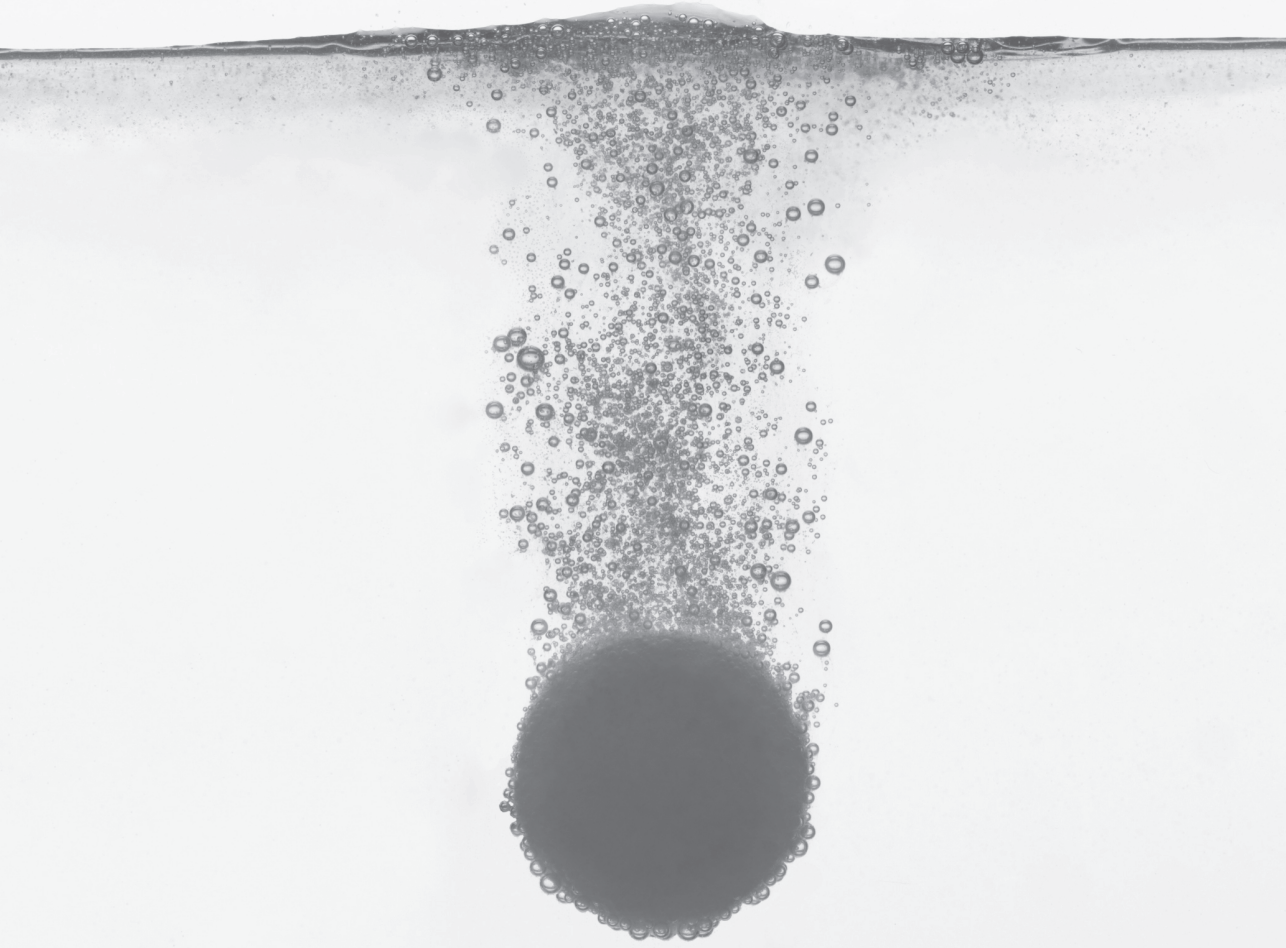
Whether a nevirapine-induced HDL-C increase results in antiatherogenic effects and prevents CVD has not yet been demonstrated. However, HIV-1 patients using nevirapine also had increases in LDL-C and TC levels. These LDL-C and TC alterations in patients using nevirapine were lower compared to efavirenz or boosted lopinavir but higher compared to boosted atazanavir. No LDL-C or TC increases were observed in patients using rilpivirine with emtricitabine/tenofovir-DF in contrast to patients using efavirenz,[235, 236, 305] and LDL-C and TC levels were lower in patients using rilpivirine compared to boosted protease inhibitors.[250] The use of rilpivirine was not associated with major changes in HDL-C in these trials. Although a lower HDL-C is a risk factor for CVD, the LDL-C remains the primary target of cholesterol-lowering therapy according to ATP-III. The results of our study indicate that the use of rilpivirine might aid in achieving LDL-C treatment goals. The LDL-C lowering effects of statins metabolized by CYP3A4 might be more pronounced by the use of rilpivirine instead of nevirapine, although this hypothesis needs additional confirmation.

This study has limitations. First, no control group of patients on nevirapine was included for comparison of lipid profiles and CVD risks as the participants were their own controls. This study's conclusions would be strengthened if the study outcomes were observed in patients randomly assigned to continue nevirapine or to initiate rilpivirine. A more detailed diet and exercise registration would aid the interpretation. Selection bias of motivated patients could have positively influenced the results. The generalizability of these results to less frequently monitored patients, or to patients from various ethnicities, remains to be elucidated.

The follow-up of 48 weeks was sufficient to observe the initial changes and plateauing of metabolic parameters but was too short to evaluate the effects on lipids and CVD risk alterations on morbidity and mortality. Furthermore, lipids and medical cardiovascular risk management should be considered in addition to maintaining a healthy lifestyle and smoking cessation. Finally, unexpected findings without direct plausible biological mechanisms, such as the blood pressure changes, should be interpreted with caution and confirmed in future studies. The Hawthorne effect (change in behavior due to the study participation) could explain the observed blood

pressure changes. However, an increased systolic blood pressure on nevirapine has been observed previously in the randomized OCTANE trial.[304] Despite the study limitations, the observed lipid and blood pressure changes were highly significant. It is therefore unlikely that larger studies would find completely different results, which would indicate that our observations occurred solely due to chance.

In conclusion, we did not observe relevant alterations in FRS as a consequence of changed fasting lipid profiles after substituting rilpivirine for nevirapine, although significantly more patients achieved LDL-C and TC ATP-III treatment goals and the systolic blood pressure improved on rilpivirine. Our study results indicate that rilpivirine could be used as an alternative for nevirapine as a treatment option for HIV-1 patients at risk for CVD.



Chapter 9

Peginterferon Alfa-2a for AIDS-Associated Kaposi Sarcoma: Experience With 10 Patients.

C. Rokx, M.E. van der Ende, A. Verbon, B.J.A. Rijnders.

Clin Infect Dis. 2013. Nov;57(10):1497-9.

ABSTRACT

In this observational cohort study, 10 patients with extensive or treatment-refractory AIDS-associated Kaposi sarcoma were treated with peginterferon alfa-2a. Tumor responses were observed in 9 patients with a median progression-free survival of 645 days. Peginterferon alfa-2a could be an effective therapy for extensive or treatment-resistant Kaposi sarcoma.

INTRODUCTION

Kaposi sarcoma (KS) is the most common AIDS-defining cancer.[183] Highly active antiretroviral therapy (HAART) is the first-line treatment for patients with limited AIDS-associated KS (AIDS-KS), resulting in tumor responses in most patients.[313, 314] As a result, HAART improved the prognosis of AIDS-KS and replaced radiotherapy and chemotherapy as first-line therapies. Nonetheless, initially limited KS can progress despite adequately suppressed human immunodeficiency virus (HIV) RNA or can already be extensive or rapidly progressive at the time of diagnosis, requiring additional therapy on top of HAART.[315, 316] Systemic chemotherapy, including liposomal anthracyclines or taxanes, may also be needed at the time of KS-associated severe immune reconstitution inflammatory syndrome (IRIS) after HAART initiation.[314, 317] Combining liposomal anthracyclines with HAART results in tumor responses in 80% of patients.[318] Patients with AIDS-KS who failed previous systemic chemotherapy are usually treated with paclitaxel, leading to overall tumor responses in approximately half of the patients for a median of 9 months.[319] Thus, adjuvant chemotherapy in combination with HAART has indisputably improved tumor responses of AIDS-KS. However, the treatment remains challenging due to relapses, disease progression, and treatment toxicity.[318, 319]

Interferon alfa was approved for the treatment of AIDS-KS and hypothesized to be effective against both HIV and human herpesvirus 8 as an antiviral and immunomodulatory agent. Responses were observed in 40% of patients, although significant side effects frequently occurred; nowadays, interferon alfa is rarely used.[320] The pegylated formulations of interferon alfa showed superior efficacy and less toxicity compared with non-pegylated interferon alfa in other viral disease.[321] Therefore, combining peginterferon alfa with HAART could be a promising therapy for advanced-stage AIDS-KS. We report on our experiences with peginterferon alfa-2a (peg-IFN; Pegasys®) therapy for advanced-stage AIDS-KS in 10 patients.

METHODS

We identified all AIDS-KS patients treated with peg-IFN until June 2013 at the Erasmus MC University Hospital, Rotterdam, the Netherlands. The medical charts of these patients were reviewed on sex, age, route of transmission, $CD4$ cell counts,

HIV RNA, and HAART. Data included pathology reports of KS lesions, KS staging, KS therapy, treatment responses, and toxicity. One hundred eighty micrograms of peg-IFN was subcutaneously administered once weekly except for patients 1 and 10, who received 135 μg. Treatment duration was not predefined and was based on the speed and completeness of the observed treatment response.

The criteria of the AIDS Clinical Trials Group were used for AIDS-KS staging.[322] Patients were staged on tumor extension (T), immunological status (I), and evidence of systemic symptoms of HIV disease (S) as 0 or 1, with 1 defining a poor prognosis. A validated prognostic index on disease progression and mortality was used to calculate prognostic scores.[317] Starting at 10 points, patients received -3 for having KS as the first AIDS-defining illness, -1 for each full 100 cells/μL CD4 count greater than zero at diagnosis of AIDS-KS, +2 for age >50 years, and +3 for S1 disease. A score <5 points represents a low risk, whereas a score >12 points represents a high risk of mortality.

The physician's documented clinical assessment and digital imaging were used to analyze treatment responses and progression-free survival (PFS). Responses were characterized as clinical complete response, partial response, stable disease, or progressive disease.[322] PFS was calculated from the initiation of peg-IFN to last documented treatment responses. Patients without progressive disease and patients who were lost to follow-up were censored at the date of last clinical assessment. Clinical and laboratory adverse events (AEs) were graded by using the Common Toxicity Criteria version 4.0.

RESULTS

Eight AIDS-KS patients were men who have sex with men and 2 were African females (Table 1). AIDS-KS was diagnosed between 1991 and 2012 and histologically confirmed in 9 patients. KS was the AIDS-defining illness in 6 patients (patients 2, 3, 4, 5, 6, 9). Patients were treated with peg-IFN between February 2005 and May 2013. All were on HAART at peg-IFN initiation, including 8 with HIV RNA <400 copies/mL for a median of 12.5 months (range, 4-47 months). Patient 1 had HIV RNA 20.000 copies/mL with triple nucleoside reverse transcriptase inhibitor therapy for an extensively resistant HIV type 1. This regimen was switched to boosted atazanavir, tenofovir, zidovudine, and lamivudine during peg-IFN resulting in HIV RNA <50 copies/mL.

Table 1. Characteristics of patient with AIDS-associated Kaposi's sarcoma and treatment responses on peginterferon alfa-2a.

No.	Age / Sex	HIV Diagnosis	KS Diagnosis	Previous KS chemotherapy	PS[1]	Stage[2]	Start PegIFN	Duration (months)	Response[2]	PFS (days)
1	44 / M	1987	09-1991	ABV, PLD, paclitaxel	9	T1 I0 S1	02-2005	9	CCR	699[3]
2	35 / M	2002	01-2006	PLD	7	T1 I0 S1	04-2010	12	CCR	1050
3	28 / M	2000	01-2007	PLD	10	T1 I1 S1	03-2008	5	CCR	590[3]
4	35 / M	2009	05-2009	None	10	T1 I1 S1	07-2010	6	CCR	902
5	41 / M	2010	12-2010	None	13	T1 I1 S1	02-2012	1	PD	0†
6	28 / M	2008	01-2012	None	4	T1 I0 S0	06-2012	3	CCR	343
7	50 / M	2002	08-2011	PLD, paclitaxel	9	T1 I0 S1	02-2012	4	SD	72
8	45 / M	2011	03-2012	PLD	12	T1 I1 S1	11-2012	5	PR	216
9	26 / F	2010	05-2010	None	11	T1 I0 S1	08-2010	5	CCR	936
10	21 / F	2003	08-2004	Paclitaxel	11	T1 I0 S1	12-2005	12	CCR	2661

[1] Prognostic score at diagnosis of AIDS-associated Kaposi's sarcoma.[317]

[2] Tumor staging at the start of peginterferon alfa-2a and evaluation of responses.[322]

[3] Patients that were lost to follow up. Time of sustained treatment response to last clinical assessment is calculated.

† Patient died 29 days after initiating peginterferon alfa-2a of due to progression of disseminated Kaposi's sarcoma and multicentric Castleman's disease.

Abbreviations: ABV: adriamycine, bleomycine, vinblastine; CCR: clinical complete response; KS: Kaposi's sarcoma; PD: progressive disease; PegIFN: peginterferon alfa-2a; PFS: progression free survival; PLD: pegylated liposomal doxorubicine; PR: partial response; PS: prognostic score; SD: stable disease.

Patient 7 was treated with peg-IFN twice, with HIV RNA 901 copies/mL at the start of the first course of peg-IFN. Although HIV-RNA declined to a nadir of 127 copies/mL, this patient eventually had virological failure 6 months after the start of peg-IFN. Extensive reverse transcriptase resistance led to a switch to boosted darunavir, tenofovir, emtricitabine, and raltegravir, resulting in HIV RNA 51 copies/mL at the second course of peg-IFN. All patients had T1 disease, including 7 with extensive cutaneous and visceral AIDS-KS. Patient 8 had oral and visceral AIDS-KS with regional lymph node involvement. Patient 6 and 9 had extensive cutaneous ulcerative and edematous AIDS-KS of the lower extremities. Systemic symptoms (S1) were apparent in 9 patients. Eight patients had intermediate prognostic mortality scores ≥5 and ≤12 points. Two patients (7 and 10) had progressive disease and 4 patients (1, 2, 3, and 8) had partial response or stable disease on previous chemotherapy. At the time of peg-IFN initiation, progressive disease was present and occurred within 6 months of the last chemotherapy. Four patients received peg-IFN without prior chemotherapy. In patient 4, peg-IFN was used for prominent facial KS lesions unresponsive to 1 year of photodynamic therapy and HAART, leading to severe psychological distress and social isolation. The previous use of cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone for multicentric Castleman disease in patient 5 led to renal and cardiac toxicity, which, together with the patient's critical situation, prevented the use of chemotherapy for AIDS-KS. Patients 6 and 9 received peg-IFN for severe IRIS after HAART initiation, which progressively affected both patients' mobility. Prior to peg-IFN, patient 1 was treated with the antiherpesvirus agent cidofovir and patient 3 and 10 with valganciclovir. Radiotherapy was used in patient 1 during the pre-HAART era and in patient 7 for progressive AIDS-KS after the first peg-IFN course.

Treatment responses were observed in 9 patients with a median PFS of 645 days, including 7 patients with clinical complete response and 1 patient with a partial response. The first course of peg-IFN in patient 7 resulted in stable disease until progression of tumorous edema, and pulmonary AIDS-KS led to discontinuation. Systemic chemotherapy was restarted with pulmonary radiotherapy without effect. A second course of peg-IFN resulted in response of cutaneous lesions, although the pulmonary lesions remained progressive. Patient 5 was critically ill and died within 29 days of respiratory insufficiency due to progressive AIDS-KS and multicentric Castleman disease. All 8 patients with clinical complete response or partial response have had their responses sustained until the most recent clinical assessment to date. Patients 1 and 3 were lost to follow-up, although treatment responses were sustained

until last assessments. All tumor responses were evaluated by clinical examination, and radiological imaging was only used in cases of suspected clinical nonresponse (patients 5 and 7).

AEs were reported in all patients. None exceeded grade 3 toxicity or led to peg-IFN discontinuation. Gastrointestinal complaints, fatigue, and skin reactions were the most common clinical AE, each occurring in 4 patients. Other clinical AEs were fever (1 patient), depressive symptoms (2 patients), musculoskeletal pain (2 patients), and alopecia (2 patients). Laboratory AEs included 5 patients with anemia, 5 patients with grade 1/2 leukopenia (including 1 with grade 2 neutropenia), 7 patients with thrombocytopenia, and 7 patients with grade 2/3 CD4 lymphopenia. Grade 3 CD4 lymphopenia was apparent in 4 patients but no new opportunistic infections were seen. Infectious AEs were seen in 3 patients (2 upper respiratory tract infections, 1 herpes zoster, and 1 cellulitis).

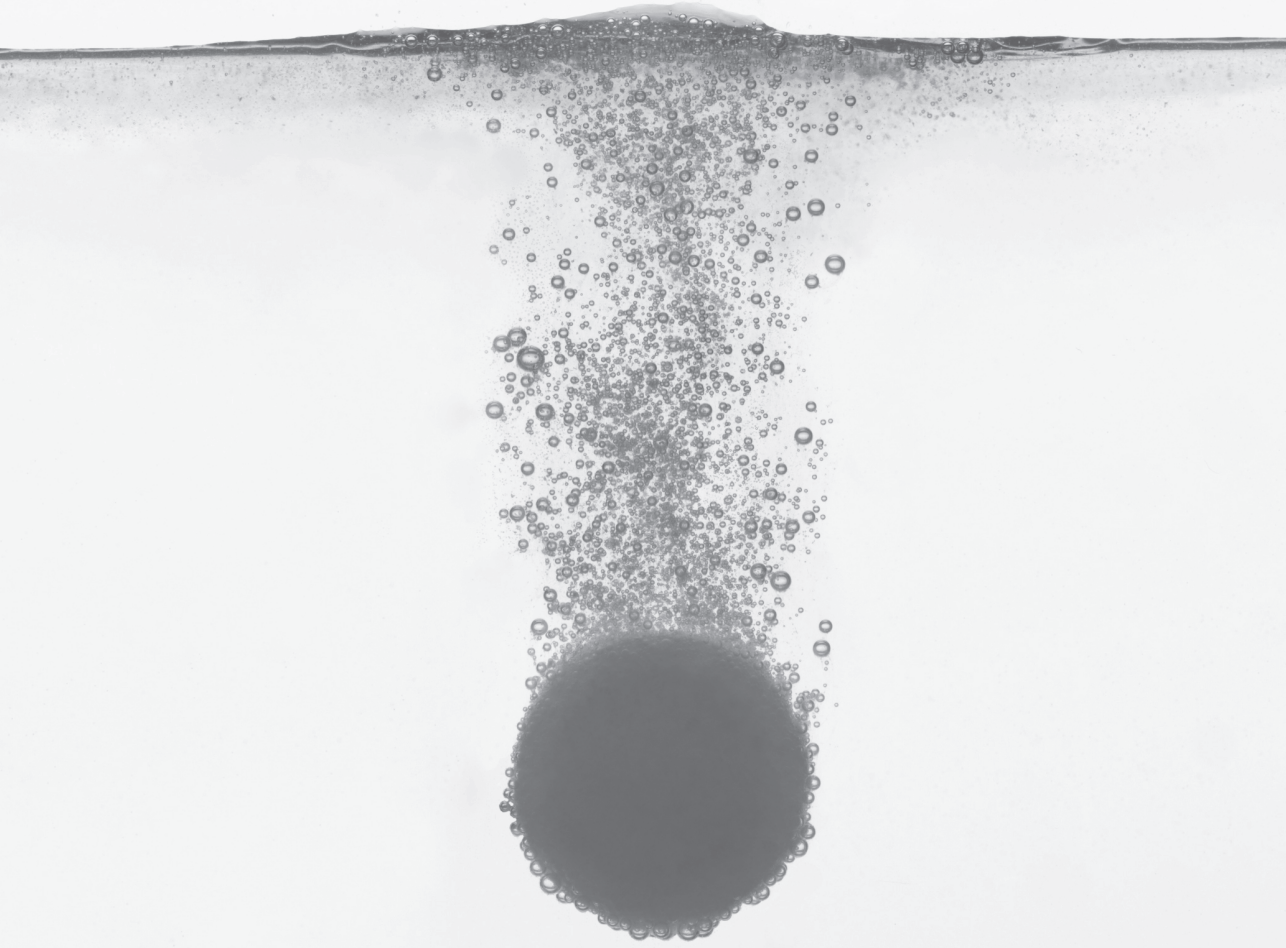
DISCUSSION

In this observational study, the administration of peg-IFN for AIDS-KS resulted in a partial or complete remission in 8 of 10 patients. Responses were durable until the end of follow-up with a median PFS of 2 years. AEs were observed in all, although none exceeded grade 3. Several patients, refractory to approved chemotherapeutic agents, benefited from peg-IFN as well.

This study is an uncontrolled observational cohort study without direct comparison with the current AIDS-KS standard of care. Therefore, definite conclusions cannot be drawn from these data. A selection bias could have occurred when the physicians selected the patients for peg-IFN therapy. Furthermore, the sample size is small and uncertainty on the true response incidence remains. However, our study provides a strong rationale for further investigation of peg-IFN in AIDS-KS. A possible study that could provide useful data is a randomized study on the role of peg-IFN to accelerate AIDS-KS regression after HAART initiation. This study should include standardized assessment of treatment responses, comparative toxicity evaluation, and quality-of-life assessment. Furthermore, future studies could identify subgroups of patients who would benefit most from peg-IFN and provide information on optimal timing and duration of peg-IFN. The findings of this study are nonetheless significant

for patients with advanced AIDS-KS. As tumor responses were observed in patients whose HIV RNA had already been successfully suppressed for a long time, we are convinced that a peg-IFN rather than a HAART effect was observed.

In conclusion, AIDS-KS is unique in its pathophysiologic association with 2 viruses. Combining peg-IFN with HAART could be an effective therapy in extensive or treatment-refractory AIDS-KS.



Chapter 10

Treatment of multicentric Castleman's disease in HIV-1 infected and uninfected patients: a systematic review.

C. Rokx, B.J.A. Rijnders, J.A.M. van Laar.

Neth J Med. 2015. Jun;73(5):202-10.

ABSTRACT

Introduction

Multicentric Castleman's disease (MCD) is frequently associated with human-herpesvirus (HHV)-8, especially in human immunodeficiency virus (HIV)-1 co-infections. The optimal treatment is unclear. This systematic review provides an overview of available evidence on chemotherapeutic and monoclonal antibody therapies directed against CD20, interleukin (IL)6 or IL6 receptor.

Methods

A systematic literature search of Embase, Medline, Web-of-Science, Scopus, PubMed publisher, Cochrane and Google Scholar was conducted for trials and cohort studies on MCD therapy. Baseline characteristics and reported endpoints were summarised and treatment efficacy was assessed by overall mortality rates.

Results

1817 studies were identified providing five trials and 14 cohort studies on 666 patients, including one randomised placebo-controlled trial. Ten studies reported on 450 HIV-1 positive patients. Most HIV-1 positive (99.7%), and 24.4% of HIV-1 negative patients were HHV-8 infected. Study populations and methods varied considerably. The use of rituximab was associated with better treatment responses and survival compared with chemotherapy without rituximab in HHV-8 associated, predominantly HIV-1 infected, MCD patients. Anti-IL6(receptor) antibodies might be promising second-line or salvage agents, at least in HIV-1 and HHV-8 negative patients. Kaposi sarcoma (re)activation with rituximab and MCD progression to aggressive lymphoma, or haemophagocytic lymphohistiocytosis were important complications

Conclusion

Optimal MCD treatment for HIV-1 and/or HHV-8 positive or negative patients remains unclear. The available evidence is of low quality due to study designs, treatment allocation bias, and publication bias. MCD patients remain at risk for developing lymphomas or haemophagocytic lymphohistiocytosis. Rituximab may have survival benefits for HHV-8 associated MCD, but it is related to Kaposi sarcoma exacerbations.

INTRODUCTION

Multicentric Castleman's disease (MCD) is a lymphoproliferative disorder affecting B-lymphocytes and plasma cells. An infection with the human herpesvirus (HHV)-8, especially in human immunodeficiency virus (HIV)-1 infected patients, has been frequently associated with MCD development.[323, 324] Three histological categories of MCD are identified: the plasma cell, hyaline vascular and mixed variants. The plasma cell variant is present in 80-90% of MCD cases. An HHV-8 infection results in the production of human and viral (HHV-8 DNA encoded) pro-inflammatory interleukin (IL)6 that induces plasma cell proliferation, and appears to be of importance in MCD pathogenesis.[325-327] MCD is diagnosed by histological evidence of affected tissues in patients with pro-inflammatory clinical symptoms.

Despite the ever-increasing number of patients with adequately controlled HIV-1 by combination antiretroviral therapy (cART), MCD incidence in HIV-1 patients is increasing.[184] The incidence in HIV-1 patients has been estimated at 2.3 per 10,000 patient-years in the pre-cART era prior to 1996, and 8.3 per 10,000 patient-years since 2000. In the general population, ten-year MCD prevalence is approximately 2.4 per million persons.[174, 184] The clinical course of MCD is seldom self-limiting and, if left untreated, associated with high mortality rates. However, the optimal treatment for MCD remains unclear.[314] MCD treatment strategies include chemotherapy, anti-CD20 antibodies (rituximab) and the use of antibodies directed against IL6 or the IL6 receptor (anti-IL6(R)). This systematic review aims to summarise available evidence of these MCD therapies and their potential complications.

METHODS

The primary purpose of this systematic review was to provide an overview of all conducted trials, prospective and retrospective cohort studies on chemotherapeutic or immunomodulatory (anti-CD20 and anti-IL6(R)) treatments of HHV8-associated and HHV8-unrelated MCD, both in HIV-positive and HIV-negative patients. The study conduct was in accordance with the PRISMA statement for systematic reviews.[328]

Search strategy

Studies were extracted from an extended search in Embase, Medline (OvidSP), Web-of-Science, Scopus, PubMed publisher, Cochrane Library and Google Scholar up to 16 December 2014. Results were limited to retrospective or prospective cohorts and clinical trials in humans from the English literature. The search was not restricted by age, HIV-1 status, or HHV-8 status. We searched the following medical subject heading terms in titles and abstracts: "Castleman" OR "Angiofollicular lymph node hyperplasia" AND "Chemotherapy" OR "Anti-CD20" OR "Rituximab" OR "Anti-IL6" OR "Tocilizumab" OR "Siltuximab". Duplicate findings were identified and removed. Initial screening of the titles and abstracts excluded animal studies, guidelines, identical publications and identified studies primarily on MCD. The titles and abstracts of the remaining studies were assessed on eligibility. We excluded studies that did not primarily report outcomes on the clinical effectiveness of chemotherapy, anti-CD20 or anti-IL6(R) for MCD, provided insufficient data on therapy outcomes or were available as conference abstracts only. We assessed the full text of studies on eligibility if the title and abstract were inconclusive. All eligible trials and cohort studies had to report on at least ten MCD patients on identical treatment consisting of chemotherapeutic, anti-CD20 or anti-IL6(R) therapies. Only studies with data on treatment outcomes and survival outcomes were included for the analysis. Case reports were excluded. We identified reports on identical patient series and included the most recent records. The final selected studies for analysis were cross-referenced for potential omitted relevant studies.

Data extraction

The following information was extracted from the studies: principle author, year of publication, study design, number of patients included, patient characteristics (age, gender), HIV-1 infection, HHV-8 status, cART, tissue histology, clinical course, therapy received, and treatment outcomes. The number of deaths and median or mean follow-up time were evaluated in all studies. If available, the reported survival rates were extracted for comparability reasons unless no survival rates were reported and the authors provided an alternative efficacy endpoint. We evaluated incidences of Kaposi sarcoma, lymphoma, and haemophagocytic lymphohistiocytosis (HLH) if reported. The results were reviewed on (pooled) descriptive characteristics and therapy outcomes. The levels of evidence and recommendations were graded according to the Oxford Centre for Evidence-Based Medicine levels of evidence.[329] No interferential

statistics were computed due to the heterogeneity of study designs and lack of uniform study endpoints.

RESULTS

Study and patient characteristics

Of 1817 studies identified by the search, 309 were eligible studies on MCD chemotherapy, anti-CD20 or anti-IL6(R) (Figure 1). These included 224 case reports, 47 case series on less than ten patients, and 13 reports on identical patient series. Nineteen studies, including five trials and 14 cohort studies, were included for analysis and provided data on 666 predominantly male (79.9%) patients with MCD. The level of evidence of all trials was grade 2B because of limited follow-up or absence of control groups. One cohort was grade 2B due to size, reported outcomes, follow-up duration and identification and correction of potential confounders.[330] All other cohorts were

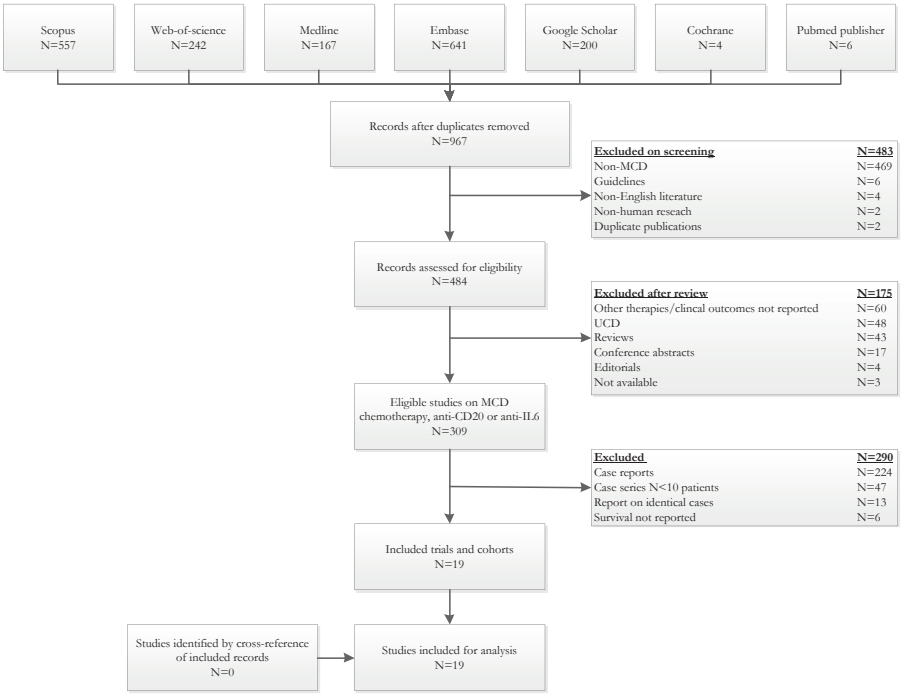


Figure 1. Search strategy and article selection.
Abbreviations: IL, interleukin; MCD, multicentric Castleman's disease; UCD, unicentric Castleman's disease.

low-quality studies of grade 4. Median age was 43 (range: 37-65) years. Data on gender and age were not available in one study.[331] HIV-1 was excluded by serology in all patients in five studies,[332-336] and in 13 of 21 patients in one study.[337] One trial did not report HIV-1 status.[338] Available HIV-1 test results were positive in 100% of patients in the remaining 11 studies, except in one retrospective cohort study (64% HIV-1 positive).[339] In total, 450 patients were HIV-1 positive and 216 were either HIV-1 negative or had an unknown HIV status. Apart from one study,[340] all studies were from the cART era and reported cART coverage in these studies was 65.4% (270/430 patients) at MCD diagnosis. The reported HIV-1 RNA suppression rate < 500 copies/ml was 40.8% and median CD4 cell count was 221 (range: 148-398) cells/mm^3.

MCD diagnosis was established by histological tissue examination in 98.5% of patients. Results on MCD variants were reported in 64 HIV-1 positive patients,[339-342] and 192 HIV-1 negative patients.[332, 334-338] Only the plasma cell and mixed variants were observed in 54.7% and 45.3% of HIV-1 patients respectively. MCD variants in HIV-1 negative patients were 49.5% plasma cell, 25.5% hyaline vascular, and 25.0% mixed variants. Splenectomies were performed in 39 patients and 29/39 were reported in studies conducted prior to 2008. Kaposi sarcoma foci were reported in four studies and apparent in 17 of 91 (18.7%) histologically examined MCD tissues.[340, 342-344] No histopathological evidence for lymphoma was found at MCD diagnosis although 13 studies either did not report results or excluded patients with evidence of lymphoma. The reported HHV-8 detection methods varied. Two studies did not report on HHV-8 status,[336, 345] two studies omitted the description of the detection methods,[338, 339] and six studies described multiple HHV-8 detection methods.[330, 331, 333, 341-343] Quantitative HHV-8 polymerase chain reaction (PCR) in plasma was used in 11 studies,[330, 333, 334, 337, 341-344, 346-348] including one trial that excluded HHV-8 infected patients.[334] ELISA or immunofluorescence antibody assays to latent nuclear antigens were used in three studies,[332, 342, 343] and six studies used HHV-8 PCR or immunohistochemistry on biopsy tissues.[330, 331, 333, 340, 341, 343] Excluding the studies that did not report or include HHV-8 patients,[334, 336, 345] HHV-8 test results were available for 416/525 patients (79.2%) and HHV-8 was demonstrated in 83.4% (347/416) of patients. These patients included 99.7% (326/327) of HIV-1 positive patients compared with 24.4% of HIV-1 negative patients with HHV-8.

MCD therapy and outcome

MCD treatments, survival and main therapy outcomes are shown in Table 1. Results are categorised according to HIV-1 status. Six cohort studies were predominantly on chemotherapy alone,[332, 335, 336, 340, 342, 347] nine studies were either on rituximab alone,[341, 343] or on rituximab/chemotherapy combined,[330, 333, 339, 344-346, 348] and four studies, including the only randomised placebo-controlled double-blind clinical trial on MCD therapy, were on anti-IL6(R).[332, 334, 337, 338] The cumulative number of patients treated by chemotherapy was 212, by rituximab this was 241 (including 163 patients on rituximab alone), and 130 were treated by anti-IL6(R). Eighty-three patients were treated by other or unreported therapies or received palliative care only. Of 212 patients on chemotherapy, 107 were treated by single cytostatics to control MCD recurrences including vinblastine, cyclophosphamide, chlorambucil, etoposide, doxorubicin and vincristine. Combination chemotherapy was used in 40 patients as first- or second-line regimens. One study did not specify the chemotherapies used in 65 patients.[330] Excluding prior corticosteroid exposure, rituximab was used as first-line therapy in 151 patients as single agent (n = 95) or in combination with chemotherapy (n = 56), predominantly etoposide (n = 36). As second-line therapy, rituximab monotherapy was used in 68 patients and rituximab/chemotherapy (predominantly liposomal doxorubicin) combined in 22 patients with (chemo)therapy dependent MCD. The majority of patients (105/130, 80.8%) on anti-IL6(R) therapy received prior systemic therapies.

The overall all-cause mortality rate was 137/666 patients (20.6%) at a median follow-up of 27 months. Mortality rates were 25.3% in HIV/HHV-8 positive and 10.6% in HIV-1 (and for the large majority HHV-8) negative patients. The causes of death were progressive MCD in 17.5% and 34.8% of HIV-1 positive patients and HIV-1 negative patients, respectively, infections (or AIDS in HIV-1 positive patients) in 12.3% and 13.0%, multi-organ failure in 4.4% and 4.5%, progression to lymphoma in 39.5% and 8.7%, and unreported or unknown in 21.9% and 39.0%. Kaposi sarcoma was the cause of death in 5/114 (4.4%) HIV-1/HHV-8 positive patients only. The mortality rates according to treatment modalities received during reported follow-up were 36.8% for chemotherapy alone, 10.1% for rituximab with or without chemotherapy, 7.7% for anti-IL6(R) and 30.1% for other therapies. Of note, one cohort study did not specify deaths according to therapy modalities and could not be used.[348] Furthermore, another cohort study only reported on deaths of MCD patients that developed non-Hodgkin's lymphoma.[331] Ten studies defined endpoints that showed broad variety

Table 1. Treatment outcomes reported in studies on MCD patients.

Reference	Study Design (n)	Primary Therapy (n)	HIV + (%)[a]	HHV8 + (%)[a]	Death (%)	FU (mo.)	Reported efficacy endpoint	Endpoint achieved (%)	Comments
HIV-1 status positive									
1996 Oksenhendler	Retrospective cohort (n=20)	Chemotherapy (16)[b] Other (4)	100	100	68.8 75.0	10	-	-	-
2002 Oksenhendler	Retrospective cohort (n=60)	Chemotherapy (60)	100	100	20.0	20	-	-	All patients received vinblastine or etoposide.
2004 Loi	Retrospective cohort (n=11)	Chemotherapy (11)	100	100	45.5	22	-	-	All patients received cyclophosphamide, chloorambucil or anthracyclines with steroids at unspecified disease stages
2005 Guilhot	Retrospective cohort (n=12)	Chemotherapy (11)[c] Rituximab (1)	100	100	36.4 100	41	-	-	-
2007 Bower	Non-randomized open-label single-arm phase II trial (n=21)	Rituximab (21)	100	100	4.8	12	Year 2 overall survival rate	95.0	Treatment naïve and patients with histological evidence of microlymphoma excluded.
2007 Gérard	Non-randomized open-label single-arm phase II trial (n=24)	Rituximab (24)	100	100	8.3	12	Year 1 overall survival rate	92.0	Second-line rituximab after MCD control by chemotherapy. Lymphoma and KS were excluded.
2011 Bower	Prospective cohort (n=61)	Rituximab (35) Rituximab +/etoposide (14) Other/NR (12)	100	100	8.2 - 33.3	50	Year 2 overall survival rate	94.0 - 42.0	Mortality and overall survival rate calculated on rituximab and rituximab+/etoposide treated patients. Patients on etoposide had poorer performance state.
2011 Hoffmann	Retrospective cohort (n=52)	Rituximab (10) Rituximab +/chemotherapy (4)[d] Chemotherapy (22)[d] Other/NR (14/2)	100	NR	10.0 - 45.5 50.0	27	Year 1 complete response	94.0 - 39.0 14.0	Mortality and complete response rate calculated on rituximab and rituximab+/chemotherapy patients.

Table 1. Treatment outcomes reported in studies on MCD patients. (continued)

Reference	Study Design (n)	Primary Therapy (n)	HIV + (%)[a]	HHV8 + (%)[a]	Death (%)	FU (mo.)	Reported efficacy endpoint	Endpoint achieved (%)	Comments
2011 Stebbing	Retrospective cohort (n=52)	Rituximab (28) Rituximab +/etoposide (14) Other (10)	100	100	36.5 - -	49	Year 2 overall relapse-free survival	89.0 - -	Mortality and overall relapse free survival rate calculated on whole cohort. Seven patients with early progressive disease not in survival analysis.
2012 Gérard	Retrospective cohort (n=113)	Rituximab (44) Rituximab +/chemotherapy (4)[e] Chemotherapy (65)[e]	100	100	16.7 - 43.1	50	Year 2 overall survival rate	93.2 - 67.9	Mortality and overall survival rate calculated on rituximab and rituximab+/chemotherapy treated patients. Cytostatic monotherapy prior to rituximab in all patients.
2012 Ramasamy	Retrospective cohort (n=11)	Rituximab +/thalidomide (11)	64.0	91.0	9.1	22	-	-	Three patients had response after second round of rituximab+/thalidomide.
2014 Uldrick	Prospective cohort (n=17)	Rituximab+liposomal doxorubicine (17)	100	100	17.6	58	Clinical complete response after 2 cycles	88.0	Fourteen patients were pretreated by chemotherapy, rituximab, or antivirals. Consolidation therapy in 15 patients.
HIV-1 status negative or unknown									
2005 Nishimoto	Non-randomized open-label single-arm phase II trial (n=28)	Tocilizumab (28)	0	7.1	3.6	15	Week 16 any MCD disease improvement	100	Treatment naive and experienced patients.
2012 Xu	Retrospective cohort (n=19)	Rituximab +/chemotherapy (1)[f] Chemotherapy (12)[f] Other/none (6)	0	0	0 25.0 16.7	32	-	-	All patients had MCD with renal involvement.
2013 Dossier	Retrospective cohort (n=18)	Rituximab +/etoposide (10) Rituximab +/chemotherapy (3)[g] Chemotherapy (5)[g]	0	100	0 0 60.0	18	-	-	Four patients were lost to FU. 2/3 deaths were due to PEL.

Table 1. Treatment outcomes reported in studies on MCD patients. (continued)

Reference	Study Design (n)	Primary Therapy (n)	HIV + (%)[a]	HHV8 + (%)[a]	Death (%)	FU (mo.)	Reported efficacy endpoint	Endpoint achieved (%)	Comments
2013 Kurzrock	Non-randomized open-label single-arm phase I trial (n=37)	Siltuximab (37)	NR	2.7	8.1	29	Day 36 clinical benefit response ≥1 component.	87.0	Treatment naive and experienced patients.
2013 Zhu	Retrospective cohort (n=10)	Chemotherapy (10)[h]	0	NR	20.0	34	-	-	-
2014 Kawabata	Retrospective cohort (n=21)	Tocilizumab (12) Other (9)	0	0	8.3 22.2	98	-	-	Tocilizumab used in patients with severe or refractory MCD.
2014 van Rhee	Randomized double-blind placebo-controlled trial (n=79)	Siltuximab (53) Placebo (26)	0	0	3.8 15.4	14	Week 18 durable tumor symptomatic response	34.0 0	Treatment naive and experienced patients. Randomization according to baseline steroid use. All had best supportive care.

[a] Percentage positive results of patients tested.

[b] Vinblastine (9), cyclophosphamide (3), adriamycin/bleomycin/vinblastin (4).

[c] Etoposide (4) +/methylprednisolone (1), vinblastine (3) +/methylprednisolone (1), cyclophosphamide/methylprednisolone (1) +/vinblastine (1).

[d] Doxorubicin (4) +/vinblastine (2) +/rituximab (1), etoposide (4) +/doxorubicin/bleomycin (3) +/vincristine (1), cyclophosphamide/hydroxydaunorubicin/vincristine/prednison (3) +/etoposide (1) +/antivirals (4) +/rituximab (3).

[e] Chemotherapeutics not specified.

[f] Cyclophosphamide/vincristine/prednisone (6) +/thalidomide (2) +/hydroxydaunorubicin/rituximab (1), cyclophosphamide/corticosteroid (2), vincristine/prednisone (2).

[g] Vinblastine/etoposide/bleomycine (3), cyclophosphamide/hydroxydaunorubicin/vincristine/prednisone (1) +/etoposide (1) +/rituximab (1), cyclophosphamide/rituximab (1) +/doxorubicin/etoposide/prednisone (1).

[h] Cyclophosphamide/vincristine/prednisone (4), cyclophosphamide/hydroxydaunorubicin/vincristine/prednisone (6).

Abbreviations: FU, follow-up; HIV, human immunodeficiency virus; HHV8, human herpesvirus type8; KS, Kaposi sarcoma; MCD, multicentric Castleman disease; mo., months; NR, not reported; PEL, primary effusion lymphoma.

in definitions. Overall, first- or second-line rituximab containing therapy was more able to sustain remission and increase survival than chemotherapy alone, at least in HHV-8 positive, and often HIV-1 positive, MCD patients. The reported proportions of patients who achieved the endpoints were at least 88.0% when rituximab was part of the treatment, and at the most 67.9% with chemotherapy alone. For anti-IL6(R) monotherapy, the majority of patients achieved improvement on at least one disease component although durable tumour and symptomatic responses remained around 40% in this highly pre-treated group of predominantly HIV-1 and HHV-8 negative MCD patients.

Kaposi sarcoma, HLH and lymphoma

Kaposi sarcoma, HLH and lymphoma were frequently diagnosed prior to MCD diagnosis or during follow-up. Excluding the three antiIL6(R) trials and four studies that did not report on Kaposi sarcoma,[335-337, 348] Kaposi sarcoma was apparent in 244 of 429 patients. Progression of Kaposi sarcoma during follow-up occurred in 55 (12.8%), predominantly HIV-1 positive (96.4%), MCD patients. The majority of Kaposi progressions (67.3%) were observed in studies of patients treated with rituximab. In the studies that specifically reported on lymphoma development during MCD follow-up (n = 416), the incidence was 15.1%. Three studies reported on HLH, which was diagnosed in 34.3% of 143 patients at MCD diagnosis or during follow-up.[330, 333, 342]

DISCUSSION

The current systematic review indicates that the use of rituximab appears to provide a survival benefit, both in HIV-1 positive and HIV-1 negative, HHV-8 associated MCD patients in first- or second-line therapy (Grade B recommendation). Anti-IL6(R) showed promising results in controlling disease activity, at least in HIV-1 and HHV-8 negative patients (Grade B recommendation).

The optimal treatment of HHV-8 positive and HHV-8 negative MCD patients remains unclear and largely based on low-quality evidence. Etoposide and liposomal doxorubicin have been used with favourable results in combination with rituximab in HHV-8 associated MCD (Grade C recommendation). The use of chemotherapy alone was generally associated with higher mortality rates than in combinations with rituximab. This indicates that it might be preferable not to use chemotherapy

without rituximab (Grade C recommendation). Despite treatment, the clinical course of MCD is frequently complicated by exacerbations of Kaposi sarcomas, lymphoma development, or HLH, and the mortality remains high.

Disease progression to (often HHV-8 related) lymphoma is frequently observed in MCD and appears to be partially prevented by including rituximab in MCD treatment. In MCD, HHV-8 infected B-lymphocytes are able to coalesce and form microscopic lymphoma, which may express the CD20 antigen.[327, 349, 350] Ongoing IL6 receptor activation might be involved in the lymphoproliferative differentiation of these B-cells. Rituximab's protective effect could be due to the resulting HHV-8 infected B-lymphocyte depletion, which decreases cytokine levels involved in further B-cell proliferation. Despite the effect of rituximab on lymphoma development, HLH and Kaposi sarcoma are prevalent concomitant clinical complications in MCD. Kaposi sarcoma seems to be related to the rituximab exposure, and almost exclusively in HIV-1 infected patients. HIV-1 and HHV-8 can both trigger HLH, which is associated with a high mortality rate.[351] The combination HHV-8 infection and IL6 overproduction in MCD could result in a dysfunctional cascade of cytokine overproduction with T-lymphocyte and macrophage activation causing HLH, especially in immunocompromised HIV-1 patients.[352-355] Furthermore, a possible relation has been observed between low B-lymphocyte counts and increased risk for Kaposi sarcoma development with increased expression of HHV-8 gene products in Kaposi sarcomas after rituximab therapy.[356-358] A marked decrease in Kaposi sarcoma flares was observed if rituximab was combined with single-agent chemotherapies, mainly etoposide. The clinical implications of these observations are unclear. In our opinion, HHV-8 positive MCD patients should be evaluated for clinical signs of Kaposi sarcoma or its presence in tissue biopsy prior to the initiation of rituximab. If Kaposi sarcomas are present, the concomitant administration of chemotherapeutics (etoposide, liposomal doxorubicin or paclitaxel) might be recommended. However, the possible benefit of adding chemotherapeutics to rituximab on Kaposi flares or survival needs to be further evaluated. Furthermore, the effects of HHV-8 suppression by antiviral agents on Kaposi sarcoma and HLH development in the context of rituximab therapy for MCD is yet unknown. Last, the usefulness of cytokine levels and HHV-8 viral load for the monitoring of treatment effect, disease activity or for predicting patients at risk for MCD relapse after clinical remission and development of subsequent lymphoma, Kaposi sarcoma or HLH warrants further evaluation.[359, 360]

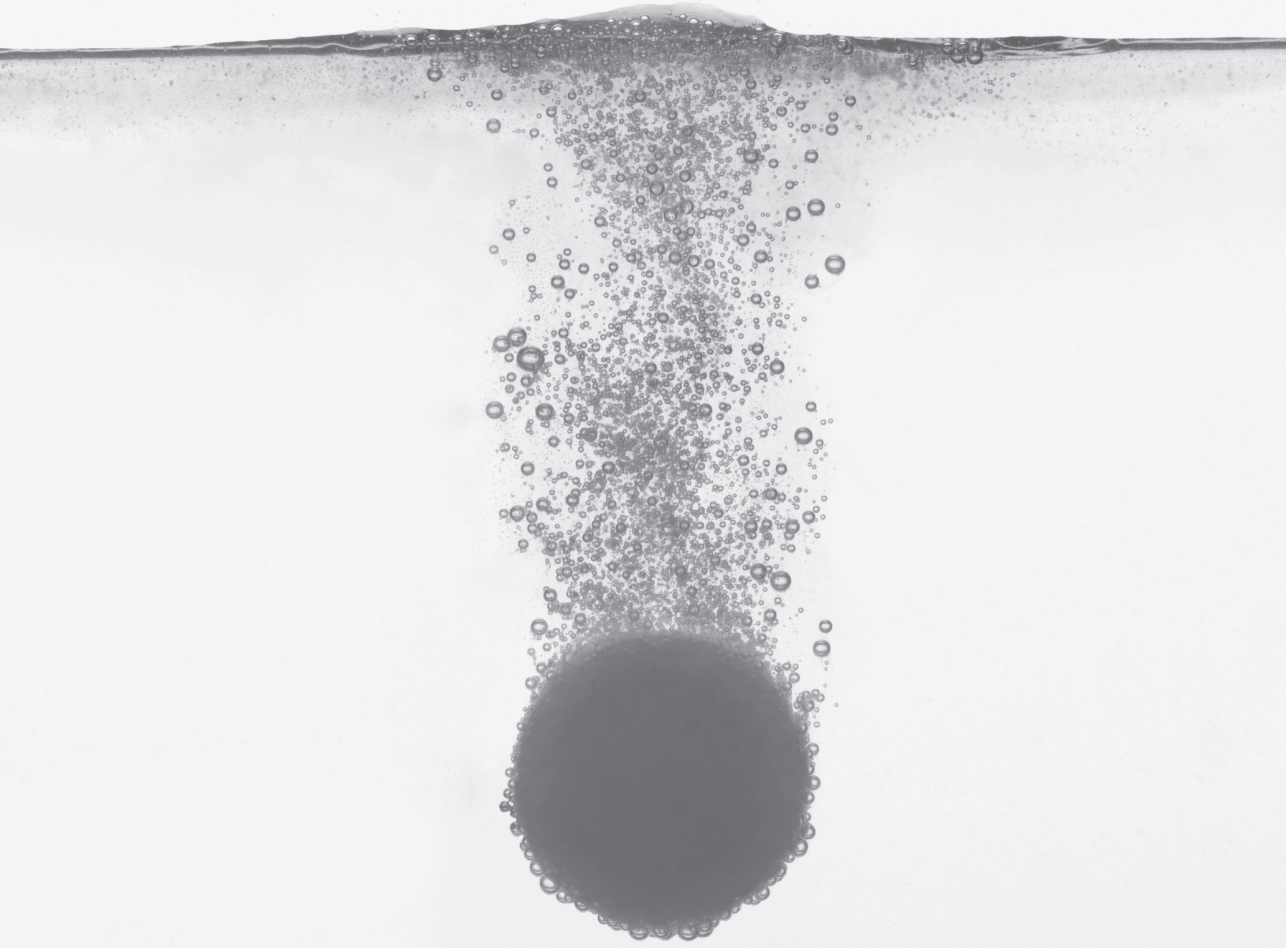
The monoclonal antibodies against IL6 and the IL6 receptor, siltuximab and tocilizumab, are not yet approved for the European market for the treatment of MCD. Siltuximab has a favourable opinion based on the benefit-to-risk balance and European market approval is recommended.[361] Tocilizumab has only been approved for the treatment of rheumatoid arthritis. Evidence is available from trials on the subgroup of MCD patients without HIV-1 or HHV-8 and the efficacy of these drugs in other patient groups remains to be elucidated. Issues on drug safety especially for the orphan medicine siltuximab are another important issue because of the limited experience with this drug. Additional trials on tocilizumab and siltuximab in MCD are ongoing.[362, 363]

The overall level of evidence of the studies was low and no definite conclusions can be drawn on the available evidence. All studies were obviously biased in several ways. Important confounders as HHV-8 status, HIV-1 infections, Karnofsky performance scores, detailed treatment information and the presence of Kaposi sarcoma or microlymphomas in tissue examinations were not uniformly investigated or reported. Unmeasured confounders have likely occurred during the covered time period, which make comparisons difficult. The large number of case reports and case series indicate publication bias, which cannot be evaluated due to the absence of registration databases for these studies. Selection bias of patients is a major limitation for interpreting the studies. The results of the cohort studies are predominantly influenced by possible treatment-allocation bias; the patients at highest risk for death received palliative care only or had aggressive and often fatal lymphomas for which chemotherapy was warranted. Therefore, the results should be interpreted cautiously. Lastly, an in-depth evaluation of treatment responses according to HHV-8 status might have been the preferred method from a pathophysiological point of view. The large amount of missing data regarding HHV-8 status, the heterogeneous use of variable HHV-8 detection methods, and other study limitations hindered this separation.

CONCLUSION

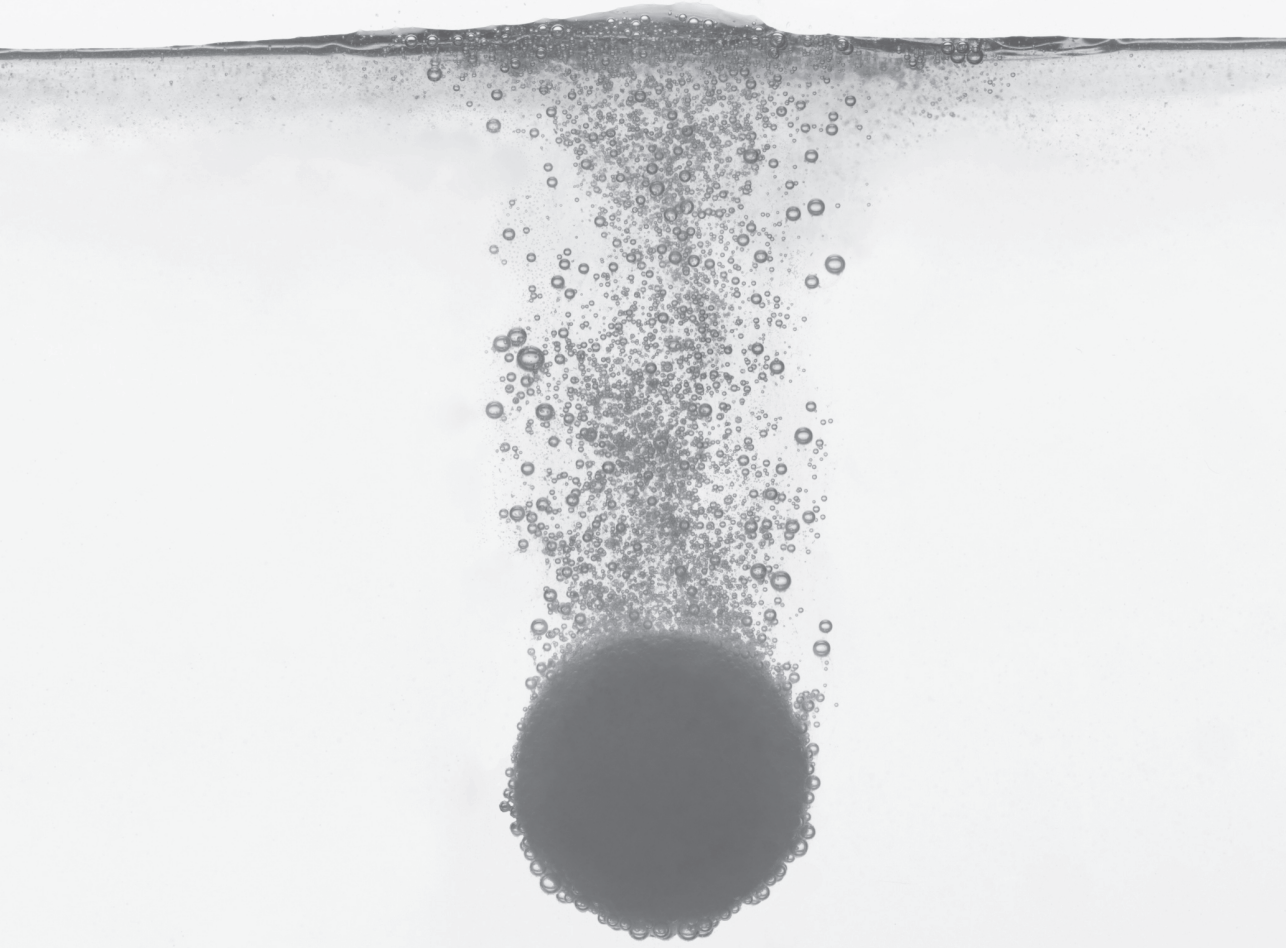
Based on the results of the present systematic review we cannot provide conclusive evidence-based treatment recommendations for optimal MCD therapy in HIV-1 infected or uninfected patients. Although the available evidence is of low quality, the use of rituximab appears to provide a survival benefit in HHV-8 associated MCD, and

anti-IL6(R) therapy might offer a treatment option after first-line treatment failure for HIV-1 negative patients without HHV-8 associated MCD.



Part 4

Perspectives



Chapter 11

Roundtable on the future management of HIV.

C. Rokx, D.D. Richman, M.M. Trutwin, G. Silvestri, J. van Lunzen, S. Khoo, M. Lichterfeld, M. Altfeld, C.F. Perno, P.W. Hunt, P. Mallon, J.K. Rockstroh, A.L. Pozniak, B. Clotet, C.A.B. Boucher.

J Virus Erad. 2015;1:e13-e22.

ABSTRACT

The Second European Round Table on the Future Management of HIV took place in Barcelona, 10-11 October 2014 and focused on the HIV-1 reservoir, strategies for HIV cure and primary HIV infection (PHI). Important issues in the HIV-1 reservoir research field are the validity of reservoir measurement techniques and the potential of new drugs to target latently infected cells. Current HIV-1 cure concepts are based on theoretical assumptions of biologically plausible mechanisms, supported by several clinical observations. Three main potential strategies are under investigation in order to achieve a sterilising cure or maintain HIV-1 remission: latency reversal resulting in antigen expression and viral cytolysis or immune targeted cell-death; immunological control of the reservoir; or replacement of the complete autologous haematopoietic and lymphoid stem-cell repertoire by transplantation. An interesting opportunity for restricting the size of the reservoir entails the early initiation of antiretroviral treatment (ART) during PHI. In terms of the reservoir, early treatment limits its size, alters its composition, and restricts the genetic variability of integrated proviral HIV-1 DNA. The challenges ahead involve the identification of patients undergoing seroconversion to HIV-1 and the prompt initiation of treatment. How the seemingly beneficial impact of early treatment will make cure more feasible, and whether the positive effects of the cure efforts outweigh the potentially negative impact of lifelong ART, are important aspects of future collaborative research prospects.

INTRODUCTION

The latent HIV-1 reservoir, new strategies for HIV-1 eradication and cure and the opportunity for early therapeutic intervention during primary HIV-1 infection (PHI) were the main topics at the Second Round Table on the Future Management of HIV (Barcelona, 10-11 October 2014). At present, the 'Berlin patient'[123] remains the only case of HIV-1 cure and the post-treatment viraemia controllers from the Visconti cohort[113] represent cases of apparent HIV-1 remission. In other instances, such as the 'Mississippi baby'[364, 365] and the 'Boston transplant patients'[366], viraemia relapse was delayed. These cases demonstrate both the feasibility and the difficulties in obtaining a sterilising cure or maintaining HIV-1 remission. None the less, these reports represent the foundation for ongoing research to characterise HIV-1 latency and efforts to substantially decrease viral reservoirs in order to achieve durable HIV-1 remission or a sterilising cure.

This year's round table brought together key investigators from specialties such as virology, immunology and pharmacology as well as clinicians and funders, to discuss the most recent scientific developments in HIV-1 cure and debate future research strategies. This review summarises the main advances in the research field and highlights the key challenges ahead to achieve an HIV-1 cure through future collaborative research activities.

OBSTACLES AND OPPORTUNITIES TO TARGET THE LATENT HIV RESERVOIR FOR ERADICATION STRATEGIES

Ongoing research activities have greatly expanded our knowledge on the latent HIV-1 reservoir. Douglas Richman (University of California, USA) provided an overview of the prospects in reservoir and eradication research. The two principal aims are: (1) the identification of cellular drug targets to safely activate HIV-1; and (2) the use of reliable reservoir measurements. HIV-1 latency is maintained by multiple restriction factors preventing the exposure of the viral long-terminal repeat (LTR) promotor region at nucleosome 1, blocking HIV transcription and subsequent mRNA formation. The best-studied latency-reversing agents (LRAs) in humans are inhibitors of histone deacetylases (HDACi). Histone deacetylases (HDACs) promote DNA chromatin folding and prevent transcriptional factors and polymerases from targeting the DNA

promotor regions. The targets include, but are not restricted to, integrated replication-competent but transcriptionally silent proviral HIV-1 DNA in latently infected cells. Of the four HDAC classes, HDAC subtypes 2 and 3 of class I are predominantly involved in maintaining HIV-1 latency.[367] The inhibition of HDAC disrupts chromatin architecture and exposes HIV-1 promotor regions for transcription. The maintenance of an HIV-1 repressive transcriptional environment is complex. The Merck corporation has screened over 2.9 million compounds to reverse latency, 66.5% of which affect unknown targets, 17.4% were farnesyltransferase inhibitors and 16.1% were HDACi.[368] Combining LRAs may have antagonistic, additive or synergistic effects *in vitro*, but whether these can be achieved *in vivo* remains unknown (Figure 1).

Validated measurements of the HIV-1 reservoir will be critical for interpreting the impact of candidate LRAs. Current methods are limited as they usually measure virus found only in blood and measurements have not yet been standardised. It is uncertain whether measures of virus in blood alone reflect other, more inaccessible and heterogeneous reservoirs. Additionally, while total HIV-1 DNA may reflect the size of the reservoir, it does not differentiate between integrated and unintegrated virus. Measurements of integrated (proviral) and 2-LTR circular HIV-1 DNA are the most sensitive methods for detecting presence of viral genome, but do not differentiate replication-competent from replication-incompetent integrated HIV-1 DNA.[369] Plasma single-copy and cell-associated HIV-1 RNA assays can provide confirmation of latency reversal, but do not discriminate between a situation where a few cells each produce high quantities of viral RNA or many cells a small amount. Cell-associated HIV-1 RNA measurements performed in limiting dilution assays might overcome this problem. Measuring the production of infectious virus using a limiting dilution viral outgrowth assay (VOA) is the current standard for quantifying the reactivation of viral replication-competent virus. However, the results of this labour-intensive, expensive and imprecise procedure are difficult to reproduce due to donor-cell dependency and the insensitivity of the assay at the detection limit. Therefore, VOAs probably miss some non-induced but replication-competent provirus. Most of the proviral populations in subjects initiating treatment during chronic infection contain defective viruses due to internal deletions, hypermutations and this indicates a potential for underestimation of the amount of intact and replication-competent provirus by VOA.[370] The size of the reservoir has been modelled to predict the time to rebound after treatment interruption,[371] and even with a continuous decline in the replication-

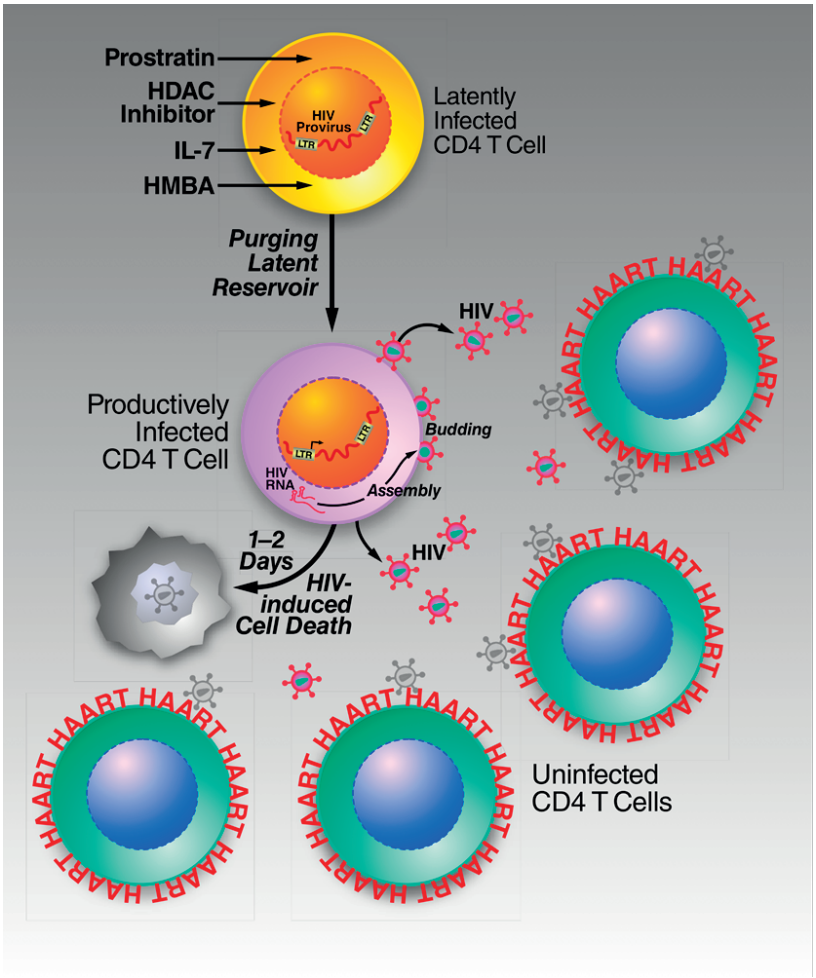


Figure 1. The use of histone deacetylases (HDAC) inhibitors alone or in combination with other latency reversing agents (IL7, prostratin, or hexamethylbisacetamide (HMBA)) to purge the persistent proviral HIV infection by activating latent HIV from CD4 T-cells. The use of antiretroviral treatment (HAART) is necessary to prevent HIV spread to uninfected CD4+ T-cells. Reproduced with permission from Richman et al. 2009 Science.

competent HIV-1 reservoir through the use of anti-latency drugs, predicting the time to plasma HIV-1 RNA rebound following treatment interruption remains stochastic.

ANIMAL MODELS FOR USE IN RESERVOIR STUDIES AND THE TESTING OF CURE CONCEPTS

Non-human primate (NHP) models are very useful tools for reservoir eradication research as host responses and simian immunodeficiency virus (SIV) replication profiles can be monitored under controlled conditions and allow use of intensive tissue analysis in pilot studies of new therapeutic interventions.[372] Their importance has recently been highlighted in a pivotal study of 20 rectally SIV-infected rhesus monkeys of Indian origin [in the absence of protective major histocompatibility complex (MHC) class I alleles *Mamu-A*01*, *Mamu-B*08* or *Mamu-B*17*].[109] These and previous results suggest a very early reservoir seeding prior to the detection of plasma viraemia that could not be prevented by the prompt initiation of suppressive ART.[373] Interestingly, proviral SIV DNA was absent in blood peripheral blood mononuclear cells (PBMCs) but detectable in lymph nodes and gut mucosa, and inversely correlated with local antiviral drug concentrations.

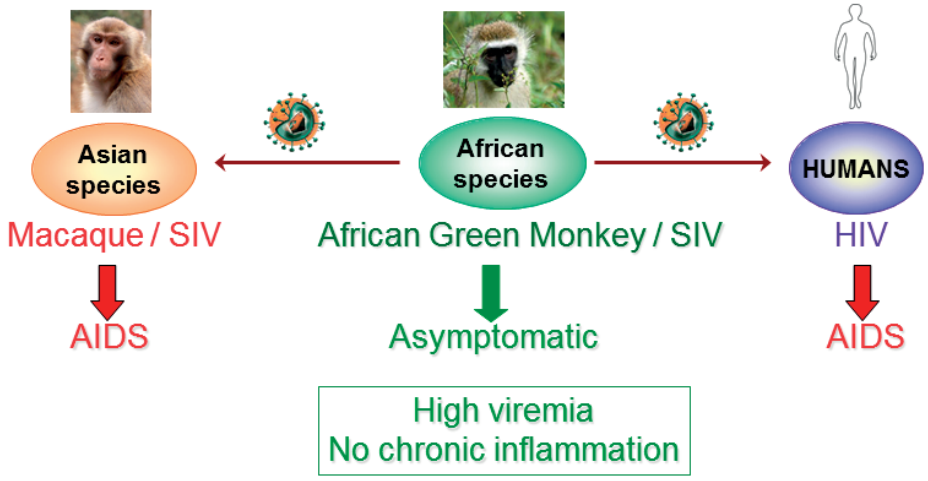


Figure 2. Primates with human or simian immunodeficiency virus (HIV/SIV) infections can be divided in natural hosts (for example the African Green Monkey) and non-natural hosts (humans, macaques). Natural hosts have the ability to tolerate high plasma viremia without aberrant chronic inflammation or progression to AIDS. Reproduced with permission.

Work by Michaela Müller-Trutwin's group (Institut Pasteur, Paris, France) has focused on SIV natural hosts and controllers in order to study the requirements for reservoir formation and inflammation control.[101] Natural NHP hosts do not have chronic inflammation, increased T cell activation, gastrointestinal T helper (Th) type 17 depletion or progression to AIDS. They also harbour reduced levels of SIV DNA in their secondary lymphoid organs and long-lived T lymphocytes despite high plasma viraemia (Figure 2). Research on regulation of inflammation control in SIV natural hosts centres on early innate immune responses such as natural killer (NK) cells, plasmacytoid dendritic cells (pDC) and myeloid dendritic cells in African green monkeys (AGM). These animals have pDCs with normal SIV-sensing capacity that produce a strong but rapidly controlled interferon (IFN) type I response during acute SIV infection. This downregulates IFN-stimulated genes and the activation of the adaptive immune.[69] How these and other immunological factors, in combination with possible epigenetic mechanisms, protect specific immune components and prevent chronic inflammation is the subject of ongoing research. Natural SIV controllers will be crucial to help decipher the development of immunological factors underlying the formation and control of viral reservoirs in tissues, particularly during early infection.

Guido Silvestri's group (Emory University, USA) uses NHP models to study immunological aspects of cure strategies. They have recently explored the feasibility of autologous haematopoietic stem cell transplantation (HSCT) following myelo-ablative total body irradiation in three SIV-infected rhesus macaques.[374] Despite successful HSCT under ART and undetectable PBMCs SIV DNA levels, rapid viraemia rebounds were observed in two of three animals following ART interruption. Necropsy of the third animal revealed SIV DNA in circulating CD4 T cells, spleen and LN. Thus, drastic haematopoietic reset alone is probably not sufficient to achieve a cure. Additional immunological approaches to HIV-1 cure that consider the complex differentiation pathways and activation stages of CD4+ T cells might be necessary (Figure 3). This group's ongoing research focuses on the immunological effects of three possible cure strategies in NHP models. The 'shock and kill' concept which assumes cytotoxic killing of latently infected cells after viral reactivation.[375] The importance of CD8+ mediated responses is stressed by the observation that their depletion in ART-treated SIVmac239-infected rhesus macaques resulted in viraemia rebound within days or weeks after depletion. Recovery of the CD8+ cells resulted in resuppression of viraemia.[376] Another experimental avenue involves preventing the activation of the

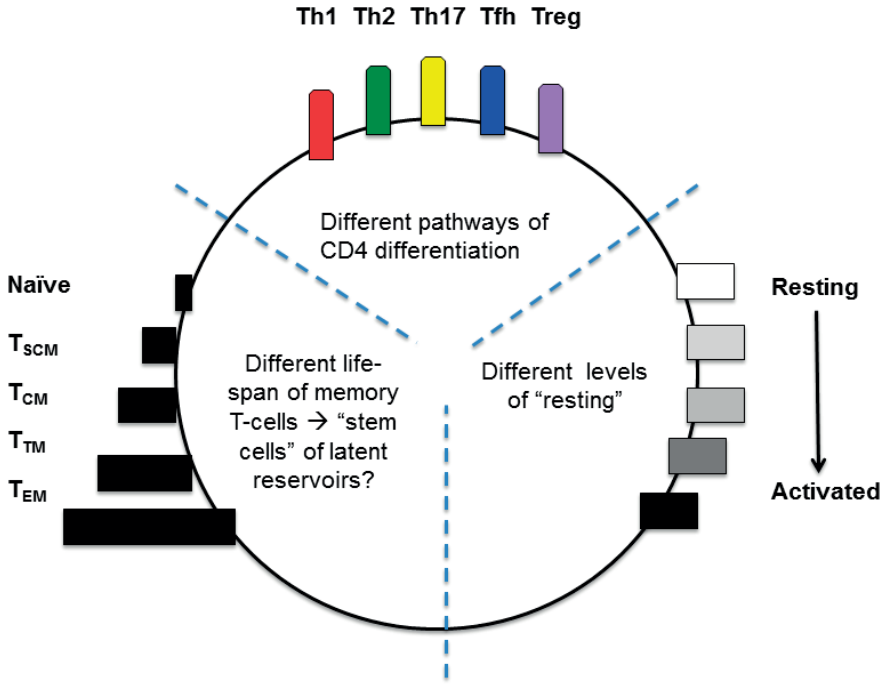


Figure 3. The complexity of the CD4+ T-cell considering its various subsets (Th1, Th2, Th17, Tfh, Treg), differentiations (naïve, Tscm, Tcm, Ttm, Tem), and levels of activation. Integrated proviral HIV-DNA may be present in all these CD4+ T-cells during a HIV infection which complicates the search for a sterilizing cure. Reproduced with permission.

latently infected memory T cell pool. Without viral production from these cells, the reseeding of the reservoir would be blocked and immune activation diminished. This hypothesis is supported by the fact that infusion of the immuno-regulatory cytokine interleukin (IL)-21 in eight SIVmac239-infected rhesus macaques on ART resulted in improved viraemic control over time and increased Th17 recovery compared to controls on ART alone. This improved Th17 recovery could limit gastrointestinal microbial translocation and its associated immune activation.

The persistence of a stable latent reservoir is facilitated by the homeostatic proliferation of central memory (T_{CM}) and stem-cell memory (T_{SCM}) T cell subsets.[104] T_{SCM} harbour high per-cell HIV-1 DNA levels, are archived during PHI and contribute progressively to the HIV-1 reservoir over time (from 1.0% during PHI to 24.0% in individuals on long-term ART). Testing using VOAs has confirmed the ability of T_{SCM} from individuals on long-term ART to produce replication-competent virions.[377]

The third type of cure concept involves producing a shift for long-lived memory T cells into shorter-lived transitional memory (T_{TM}) or effector memory (T_{EM}) T cells, thereby increasing the reservoir decay rate (i.e. 'push and vanish'). This may be achieved by combining proliferative-type cytokines (IL-7, IL-15) with co-inhibitors (PD1, LAG-3, TIGIT), by blocking T_{SCM} self-maintenance differentiation pathways or by exploiting the higher CCR5 levels on T_{EM} compared to T_{CM} with the use of maraviroc. This general concept was tested in SIV-infected sooty mangabeys, natural hosts, which have minimal SIV DNA in T_{SCM} and T_{CM}.[378, 379] However, 6 months of rapidly suppressive ART in two monkeys did not result in persistent viraemia control following ART interruption. Important caveats in this concept are the optimal identification of T_{CM} and T_{SCM} with replication-competent virus and the influence of the non-CD4 expressing reservoir (macrophages).

GENE THERAPY

One of the main challenges for obtaining a cure is the identification of HIV-specific, efficacious, safe and scalable interventions. Gene therapy could offer such a specific approach and Jan van Lunzen (University of Hamburg, Germany) provided an overview on the subject. The use of a limited number of gene-modified cells might be sufficient due to their beneficial effect on other cell types (bystander effect) or improved survival through natural selection *in vivo*. HIV-1 can be targeted by gene therapy at various stages, such as pre-integration, post-transcription or during viral assembly. Intervening at a pre-integrational stage could promote the preferential accumulation of gene-modified cells that do not carry HIV-1 DNA. The infusion of ex vivo expanded CD4+ T cells modified with the peptide M870/maC46, which inhibits gp41 fusion, was shown to be safe and resulted in temporary CD4+ T cell increases in a proof-of-concept study using multiple treatment-experienced patients with drug resistance.[380] *Ex vivo* CCR5 gene disruption upstream of the naturally occurring CCR5-Δ32 mutation by zinc finger nucleases (ZFN) has produced preferential expansion of infused autologous CCR5-modified CD4+ T cells (SB-728-T) in 12 ART-suppressed patients. However, treatment interruption resulted in viral rebound in all patients; one individual, heterozygous for CCR5-Δ32 deletion, re-suppressed before ART re-initiation.[381] Despite promising initial findings, the off-target effects of ZFN remain unknown.[382] The more-specific artificial restriction enzymes, TALENs (transcription

activator-like effector nucleases), were shown to induce a dose-dependent specific and efficient transient T cell receptor modification *in vitro* after mRNA-electroporation. T cells modified by TALENs showed reduced infectivity without a significant impact on other cell functions.[383] The selective CCR5 disruption might also promote a tropism shift towards CXCR4 usage, which has occurred in a CCR5-Δ32 homozygous stem-cell transplant recipient upon treatment interruption.[384] A combination of CCR5 gene disruption with M870/maC46 modification of T cells could theoretically prevent this phenomenon. Another approach includes the excision of integrated HIV-1 DNA by Tre-recombinases. These enzymes detect HIV-1 integrated LTRs and have been used successfully *in vitro*,[385] and in humanised mice with transplanted Tre-transduced human CD4+ T cells.[386] Future studies using this approach are planned.

In the absence of safe gene therapies to disrupt CCR5, the allogeneic stem-cell transplant of an HLA identical homozygous CCR5Δ32-deleted donor remains the only documented cure for HIV-1. Notably, the 'Berlin patient' is the only HIV-1 patient among seven others, who has survived a homozygous CCR5-Δ32 allogeneic stem-cell transplant. Apart from the stem-cell transplant-associated mortality, this therapy is restricted by the limited availability of adult donors with homozygous CCR5-Δ32 deletions and the necessity of HLA matching. Other stem-cell transplant-based approaches have failed to establish a cure. The use of wild type CCR5 stem-cell transplant in the two long-term (>20 years) HIV-1-infected Boston patients after reduced-intensity conditioning and the use of ART for 2-5 years post-transplantation, could not prevent viral rebound upon ART interruption. In these patients, no HIV-1 DNA was isolated from PBMCs and rectal tissue, VOA were negative and anti-HIV antibodies absent. These results indicate that new treatment combination approaches will be necessary to be tested in proof-of-concept studies. A multicentre collaborative project has therefore been initiated to investigate the potential for HIV-1 cure in patients requiring allogeneic stem-cell transplant for haematological disorders (EPISTEM), and is supported by the AmfAR Research Consortium on HIV Eradication (ARCHE).

PHARMACOKINETIC AND CLINICAL CHALLENGES OF ERADICATION STRATEGIES

Knowledge of the pharmacokinetic challenges required to purge the HIV-1 reservoir *in vivo* is essential for eradication research. Saye Khoo (University of Liverpool, UK) has stressed the need for research on ART and LRA penetration in HIV-1 sanctuary sites in order to: define target concentrations in light of the significant inter-patient variability of current methods; assess viral dynamic discrepancies between compartments; and track the evolution of drug resistance in separate compartments. Compartmental drug exposure depends not only on the ability of the drug to penetrate a compartment but also on the compartment-specific metabolic pathways and pharmacogenomics. The main tissue compartments discussed were the central nervous system (CNS), genital tract, breast milk, lungs and lymphoid tissues.

The effect of ART on the HIV-1 reservoir in the CNS remains incompletely understood. Although lower CNS penetration effectiveness (CPE) correlates with higher cerebrospinal fluid viral load and HIV-1 genetic diversity, the potential of ART with a high CPE score to improve cognitive function is disputed,[387] and its exposure in microglia and macrophages needs to be better defined. Targeting the CNS reservoir may further be limited by ART neurotoxicity or the selection of drug resistance due to plasma/CNS viral discordance as was found in participants with viral blips in PARTITION (Penetration of Antiretroviral Therapy into the Nervous System study).[388] Single nucleotide polymorphisms in enzymes involved in drug metabolism (e.g. CYP2B6 G516T for efavirenz) could also influence CNS drug exposure.[389] ART penetration into the genital tract is partially dependent on the variable expression of transporters, including P-glycoprotein, multidrug resistance protein (MRP)-2 and MRP-4, which are drug specific rather than class specific. However, results from the PARTNER study suggest that suppressed plasma HIV-1 RNA might be an adequate marker for HIV-1 RNA in the genital tract.[390]

Despite the importance of breast milk in viral transmission,[391] and its association with multiclass resistance in postpartum mother-to-child transmission, ART pharmacology in this compartment remains incompletely characterised. Treatment does not fully suppress cell-associated viral replication in CD4 T cells and macrophages in breast milk,[392, 393] and cell-to-cell HIV-1 transmission in the early postpartum period could be an important factor. HIV-1 transmission via breastfeeding can occur even when the

mother is seemingly aviraemic. In addition, HIV-1 resistance mutations not present in the mother have been transmitted to the child,[394] suggesting that the development of mutations might be driven by sub-therapeutic ART levels in breast-milk.

The relevance of the lungs in harbouring HIV-1 latently infected cells is unclear, although it has been shown that alveolar macrophages are preferentially infected compared to T cells and have irreversible impaired phagocytic function.[395] Macrophages and lymphoid tissue have shown variable drug levels with persistence of HIV-1 replication. Viral decay rates have been directly correlated with intracellular drug levels.[396, 397] Taken together, modern ART does not adequately control HIV-1 replication in all body compartments and improved formulation as well as new compounds with better penetration (more potent pro-drugs, for example TAF and cell-targeted nanoformulations) are necessary to promote reservoir eradication.

CLINICAL TRIALS

Several clinical trials have been conducted in humans in an attempt to purge latent HIV-1 from long-lived resting CD4+ T cells by LRAs, and were discussed by Mathias Lichterfeld (Harvard University, USA). Various drug classes (HDACi, protein kinase C activators, DNA methylation inhibitors) have been characterised or are already being tested *in vivo* as LRAs. HDACi are currently the most extensively studied LRAs. The anticonvulsant and HDACi, valproic acid, combined with intensified ART was the first compound shown to decrease the number of infected resting CD4+ T cells in four patients.[398] Studies with vorinostat, panobinostat and romidepsin have followed. A single orally administered dose of vorinostat was able to increase biomarkers of cellular acetylation and HIV-1 RNA expression in resting CD4+ T cells.[399] Repeated vorinostat exposure did not result in increased expression of cell-associated HIV-1 RNA.[400] Panobinostat is also administered orally and is active against HDAC subtypes 1, 2 and 3 with greater potency than vorinostat *in vitro*.[401] The administration of panobinostat, dosed three times weekly every second week for 8 weeks in a Phase I non-randomised trial in 15 aviraemic HIV-1 patients on ART showed an acceptable safety profile. This resulted in an increased level of cell-associated unspliced HIV-1 RNA and intermittent increases in plasma viraemia were observed in most patients.[402] Overall, no reduction in total and integrated HIV-1 DNA or in the VOA was observed. An interesting observation was that of nine patients who consented to

analytical treatment interruptions, three patients with sustained HIV-1 DNA reductions during panobinostat treatment had the longest time to viral rebound. This may result from an observed increase in magnitude and breadth of HIV-1 specific cytotoxic T lymphocyte (CTL) responses during panobinostat treatment. However, CTL function did not correlate with changes in HIV-1 DNA, nor did protective HLA class I alleles. Other explanations could be the increased expression of interferon-stimulated genes (preferentially in IL-28B CC carriers), or the effect on innate immunity such as NK cells. Romidepsin was able to reactivate HIV-1 *ex vivo*,[403] and is being tested as an intravenous drug in a randomised clinical trial (ACTG 5315). A non-randomised trial of several romidepsin treatments (day 0, 7, 14) in six patients has shown increases in cell-associated HIV-1 RNA and plasma viraemia after drug administration.[404] This compound was associated with a total of 36 drug-related adverse events during the 3 weeks of the study. Altogether, firm conclusions are difficult to draw from these preliminary and small human trials. These observations should be confirmed in larger groups of patients in order to exclude stochastic phenomena. It is important to stress that these current treatment strategies do not target HIV-1 alone. The potential of oncogene activation by the HDACi class stresses the need for pharmacovigilance programmes.

THE IMMUNOLOGICAL AND VIROLOGICAL IMPLICATIONS OF TREATING PRIMARY HIV-1 INFECTION

The evolving viro-immunological events and host dynamics in PHI are distinct from the later stage of the infection. PHI may represent a unique opportunity to interrupt immunopathogenesis, preserve vital host functions and prevent reservoir formation in long-lived and genetically diverse CD4+ memory T cells. Marcus Altfeld (Heinrich-Pette-Institut, Germany) presented data on early immunological responses to HIV-1 and treatment opportunities in PHI. Fiebig stages[60] divide PHI into windows of opportunity and encompass a 1-week eclipse phase post-infection; 4 weeks of progressively measurable disseminating virus with emerging antibody responses (Fiebig stages 1 to 4); and a potential point of no return in which a viraemia setpoint is reached by partial immunological control after the massive initial CD4+ T cell destruction (Fiebig stages 5 and 6).[405]

Early intervention may help limit immunological dysfunction with subsequent lower levels of T cell activation (including IP-10 and TNF-α), which predicts long-term clinical outcome for the individual.[406-408] Host factors, innate immunity, HIV-1 specific CTL responses and CD4+ T cells are all important factors in controlling viraemia during early PHI. Autologous virus-specific broadly neutralising antibodies (bNAbs) become increasingly important thereafter. NK cells, triggered by IFN-α, expand prior to the decline of peak plasma viraemia as the first cellular immune effector cells. These cells are able to recognise infected CD4+ T cells depending on the expression of activating and inhibitory NK immunoglobulin-like receptors.[73, 74] In a large international cohort of 615 patients with PHI (35.6% of whom were in Fiebig stage 4 or earlier) the presence of the HLA class 1 alleles B27 and B57 was associated with lower viral setpoints.[409] The immunodominance of HLA-B27- or B57-restricted CTL responses (both in magnitude and breadth) in PHI is associated with fewer symptoms, persists during chronic infection and can decrease the contribution of T_{CM} to the size of the reservoir.[80, 410-412] Apart from CTL, observations suggest that CD4+ T cells also demonstrate HIV-1-specific cytolytic responses during PHI.[82] In particular the emergence of Gag-specific granzyme A-positive CD4+ T cell responses was associated with better clinical outcomes.[83] Another subset of CD4+ T cells (Tfh) can provide immunological help to activate HIV-1-specific B cells, responsible for the production of neutralising antibodies; however, bNAbs are shown to develop after PHI in only a subset of patients. The preservation of CD4+ T cells and CXCR5+ PD-1+ CD4+ Tfh cells during PHI may be associated with better CTL function and higher levels of B cell activation and bNAb production.[93]

The initiation of ART during early PHI preserves immunological functions that could increase the probability of HIV-1 control in patients in the absence of therapy. The existence of HIV-1 post-treatment controllers following ART initiation in PHI was first reported around 2000.[413, 414] However, for the majority of patients who have initiated treatment during PHI, viral control has not been achieved on treatment interruption.[415] The reason for this limited durability of viraemic control and its rarity remains under investigation. The expression of protective HLA class I alleles (B57 in particular) has been linked to sustained control of viraemia post-treatment.[416] An important observation is that the majority of the 14 post-treatment HIV-1 controllers of the Visconti cohort did not carry these protective HLA class I alleles,[113] indicating a role for other viro-immunological factors in viral control as observed in these individuals.

The impact of HIV-1 on the pathogenesis and the impact of ART during PHI were described by Carlo Federico Perno (University of Rome, Italy). Despite advances in PHI diagnosis, intense viral replication and dissemination occur before current laboratory methods can detect the virus.[417] Several viral factors have a role in PHI dynamics and outcomes. Co-usage of the CXCR4 receptor in PHI might increase integrated HIV-1 DNA in naïve (CD45RA+ CCR7+) CD4+ T cells of patients in Fiebig stages 3/4. This was associated with rapid disease progression, despite a comparable amount of HIV-1 DNA in T_{CM} (CD45RA- CCR7+) and T_{EM} (CD45RA- CCR7-) in slow progressors.[418] Viral replication capacity (associated with CTL escape mutations such as T242N, or resistance mutations affecting viral fitness),[419] and *nef* function[420] were found to be decreased during PHI in HIV-1 controllers. During PHI, a maraviroc-sensitive CCR5 conformation of CD4+ T cells is more advantageous to the virus, with reduced glycosylation of the conserved envelope viral protein.[421] Both CTL pressure and early ART initiation might prevent the selection of the most replication-competent HIV-1 strains with the highest infectivity and fewest antibody targets. Gastrointestinal lymphoid tissue and blood in early Fiebig stages have been shown to contain more CCR5+ CD4+ T cells with lower levels of HIV-1 DNA and cell-associated HIV-1 RNA.[40, 422] Early ART initiation has been associated with reduced HIV-1 DNA and CD4+ T cell depletion. Some patients treated during Fiebig 1 reached HIV-1 DNA levels comparable to those of elite controllers (EC). Four perinatally infected children who initiated ART early (between 0.5 and 2.6 months of age) showed the absence of low level viraemia after a maximum of 18 years of follow up. HIV-1 seroreconversion was frequent, the amount of HIV-1 DNA in PBMCs decays over time, and no replication-competent virus was recovered from three of the four children.[423] Virological control prior to the age of 1 in perinatally infected infants correlated with a limited proviral reservoir size in PBMCs, a negative serostatus,[424] and improved clinical outcome.[425-427] Furthermore, ART during PHI prevented viral evolution in the gut lymphoid tissue,[428] and limited viral diversification of low-level viraemia, which suggests the presence of an early archived HIV-1 reservoir.[429] Early ART has been shown to benefit the CNS, with reduced levels of markers of inflammation (e.g. neopterin, white blood cells).[430] Together, a limited homogeneous viral reservoir and a more intact immune system following ART initiation during PHI rather than later during chronic infection may be an important prerequisite for successful future interventions in HIV-1 eradication. Additional interventions such as enhancing HIV-1-specific T cell immunity,[431] or neutralising antibody production may be necessary.

These issues highlight the need for additional hypothesis-driven studies on HIV-1 pathogenesis and the impact of early ART.

INFLAMMATION AND AGEING IN TREATED HIV-1 INFECTION

Peter Hunt (University of California, USA) and Paddy Mallon (University College Dublin, Ireland) discussed ongoing inflammation in the context of ageing HIV-1 patients (Figure 4). The predicted life expectancy of patients in the modern ART era has been increasing, particularly among those who have initiated ART at higher CD4 cell counts.[432] However, an overestimation may occur due to a survivor bias as the oldest patients in contemporary cohorts are most likely to have protective behavioural or genetic factors that have allowed them to survive advanced AIDS in the pre-ART era. A major challenge ahead will be the ageing HIV-1 population in sub-Saharan Africa. Many people in this group will have initiated ART at a more advanced disease stage.[433] HIV-1 is associated with increased rates of age-related morbidities, including cardiovascular disease (CVD), cancer, venous thromboembolism, type II diabetes, cognitive dysfunction and frailty, despite suppressive ART. Persistent inflammation has been increasingly recognised over the last few years as a possible contributor to

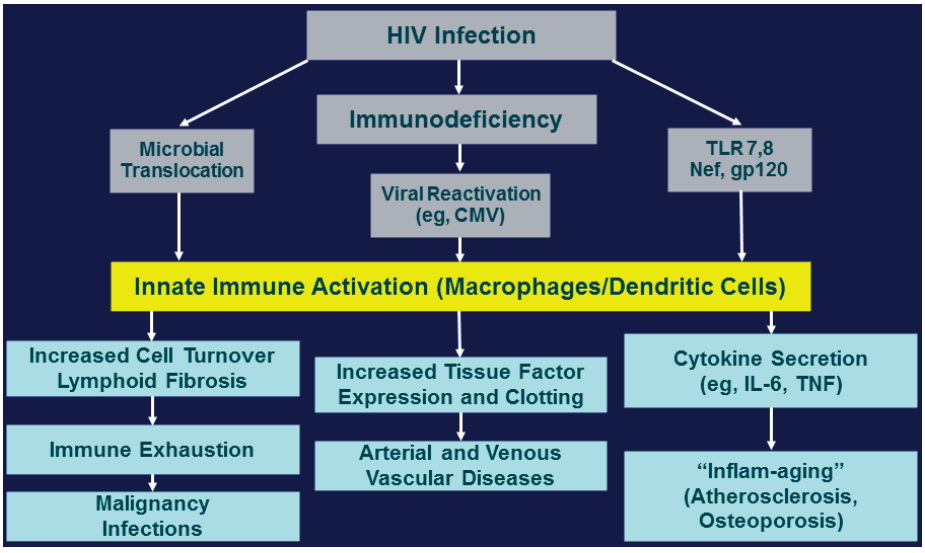


Figure 4. The relation between HIV infection and important factors driving ongoing inflammation, aging with HIV, and immunosenescence. Reproduced with permission from Appay et al.[98]

these risks.[434] Its importance in HIV-1 pathogenesis is suggested by NHP models in which clinical progression is more closely related to the ongoing massive aberrant immune activation than to viraemia levels.[70] Prolonged, effective ART is to some extent able to reduce immune activation as sustained virological suppression decreases levels of activated CD38+ HLA-DR+ CD8+ T cells, although not to normal levels.[435, 436] Observational studies have also found increased markers of inflammation (hsCRP, IL-6, D-dimer, cystatin-C) in treated HIV-1 patients compared with uninfected controls.[437] A nested case-control study of ART-suppressed participants in the SOCA cohort has shown that elevated markers of gut epithelial barrier dysfunction, innate immune activation and inflammation (IL-6, D-dimer, hsCRP, sCD14, i-FABP) all strongly predicted subsequent mortality.[98] Similarly, a pooled analysis of ART-suppressed control arms of the SMART, ESPRIT and SILCAAT studies has shown that a single IL-6 and D-dimer measurement continues to predict morbidity for the following decade.[438] This suggests the presence of an inflammatory setpoint that drives the long-term clinical risk of disease.

The mechanisms underlying persistent innate and adaptive immune activation are incompletely understood. Low-level viraemia arising from the stable reservoir while being treated with ART,[107] combined with microbial translocation resulting from the disrupted intestinal epithelial barrier and loss of mucosal immunity (CD4+ T cells and Th17 cells), as well as co-infections (e.g. CMV) are all suggested to contribute to persistent inflammation.[97] Some innate immune activation pathways may also exhibit feedback loops that perpetuate this inflammatory state.[439] For example, pro-inflammatory cytokines and lipopolysaccharide induce inoleamine 2,3-dioxygenase-1 (IDO-1) production in dendritic cells and macrophages, which in turn promotes tryptophan catabolism. These catabolites are neurotoxic (quinolinic acid), can impair T cell proliferation (kynurenine, picolinic acid) or cause Th17 depletion (3-hydroxy-anthralinic acid), which in turn causes lipopolysaccharide increases and results in a negative feedback loop of ongoing inflammation. Indeed, higher baseline kynurenine/tryptophan ratios have been associated with increased mortality in individuals starting ART.[440]

Several treatment strategies have been suggested to decrease inflammation in HIV-1 patients, ART-suppressed viraemia remaining a pivotal factor. Spontaneous HIV-1 controllers also display microbial translocation,[441] increased rates of monocyte activation (sCD163), atherosclerosis,[442] and lymphoid scarring.[443] Remarkably, EC

have even higher rates of CTL activation than ART-suppressed HIV patients. Treating EC with ART decreases their levels of immune activation, demonstrating that even very low levels of viral replication are sufficient to drive this phenomenon.[444] The initiation of ART within 6 months of HIV-1 seroconversion has led to modest decreases in CD4+ T cells (1.4%) and CTL activation (6.7%) compared with later ART initiation in the OPTIONS cohort.[445] Anti-inflammatory drugs (cyclosporine, inhibitors of toll-like receptor 4/pro-inflammatory cytokines) may reduce inflammation. Rosuvastatin was shown to decrease monocyte activation in the 48-week blinded placebo-controlled randomised SATURN-HIV trial.[446] A clinical endpoint study (REPRIEVE) with pitavastatin has started in 2015.[447] Restricting microbial translocation may be beneficial but recent studies with sevelamer,[448] mesalamine,[449] rifaximin,[450] and probiotics[451] have been unsuccessful in reducing microbial translocation or immune activation in HIV-1 patients. Whether treating co-infections reduces CD38+ HLA-DR+ CD8 activation and is clinically meaningful remains unclear; trials have observed both beneficial effects of valganciclovir in cytomegalovirus infections,[452] but also an absence of effect of valacyclovir in HSV-2 infections.[453] Finally, medical interventions could be irrelevant if the necessity of maintaining a healthy lifestyle (smoking cessation, exercise, diets) is overlooked.[454] As a conclusion, control of viraemia is not sufficient to prevent morbidity and inflammation. Strategies to reduce inflammation and prevent immune activation, or even produce immune responses that are beneficial without deleterious effects, should be further explored. The relationship between ongoing inflammation and premature immunosenescence is especially of concern for the ageing HIV-1 population. ART and associated CD4+ T cell recovery > 500 cells/mm^3 has led to mortality rates in suppressed HIV-1-positive individuals comparable to those who are uninfected.[455, 456] The current causes of mortality for HIV-1-positive individuals are non-AIDS related in the majority of cases. Non-AIDS-related morbidity is, however, increasing, especially in those over 50 years of age.[457] Three factors associated with accelerated ageing were highlighted by Paddy Mallon: immune dysfunction, bone disease and CVD. First, the T cell subsets are strikingly similar between populations of treated HIV-1-positive adults and HIV-1 uninfected elderly individuals.[458] The lower CD4/CD8 T cell ratio found in these populations has been correlated with a greater number of effector memory CD4+ and CD8+ T cells and fewer naïve CD4+ and CD8+ T cells,[459] and could predict non-AIDS-related morbidity.[173]

Secondly, bone mineral density (BMD) in HIV-1-positive patients is lower. Low BMD was much more prevalent in patients in the UPBEAT study, a prospective cohort of HIV-1-positive and -negative subjects from similar demographic backgrounds, regardless of the site of BMD measurement.[460] This lower BMD in HIV-1 patients seems multifactorial and higher levels of bone-turnover markers (osteocalcin, procollagen type 1 N-terminal, collagen type 1 cross-linked C-telopeptide) have been seen.[461] Not only HIV-1 infection itself, but also exposure to ART has been shown to reduce BMD, with most of the loss observed in the first year after ART initiation. Reductions in BMD have been observed with both first,[462] and second-line ART therapy in unsuppressed patients after first-line failure, with relatively greater BMD losses observed with the use of tenofovir disoproxil fumarate (DF).[463] In virologically suppressed patients, switching from tenofovir-DF to tenofovir-DF-sparing ART in the TROP and OsteoTDF studies,[464, 465] or vice versa in the SWAP and PREPARE studies.[466, 467] significant increases and decreases in BMD were seen, respectively. Calcium/25-OH vitamin D supplementation for mild-moderate vitamin D deficiency in patients initiating efavirenz/tenofovir-DF/emtricitabine prevented some BMD reduction over 48 weeks following treatment initiation.[468] Placebo-controlled randomised trials of bisphosphonates are ongoing.

Thirdly, CVD-related mortality is the predominant cause of death in virologically suppressed HIV-1 patients with good CD4 responses.[297] The pathogenesis of CVD in HIV-1 infection is complex with contributions from age, HIV-1 and lifestyle. However, there is 10% discrepancy between the predicted and actual CVD mortality rates in HIV-1-positive individuals,[469] and factors other than associations between CVD and exposure to abacavir or lopinavir, or dyslipidaemia, for example, are likely to be involved.[470, 471] One biologically plausible mechanism for the association between abacavir and CVD is that abacavir has been found to alter platelet function, as noted in a change in the soluble glycoprotein-6 level, which is involved in platelet activation.[472] Furthermore, patients with HIV-1 have increased rates of dyslipidaemia, characterised by low levels (<1.0 mmol/L) of the cardioprotective high-density lipoprotein cholesterol (HDL-C).[473] Whether ART-related dyslipidaemia,[311] and the use of interventions that increase levels of HDL-C influence clinical endpoints is unclear. Finally, HIV-1-related ongoing inflammation (particularly monocyte activation) affects CVD risk. Recent research has shown that platelet activation and endothelial dysfunction markers, but not the monocyte activation markers sCD163 or sCD14, were decreased after ART initiation.[474] The latter have been associated with changes

in carotid intima media thickness,[475] and non-calcified coronary plaques.[476, 477] Future challenges will be to study ART benefits in older patients, identify clinically useful markers of inflammation and identify key interventions in order to prevent HIV-1-related accelerated age-associated disease risks.[478]

IDENTIFICATION AND TREATMENT OF PATIENTS WITH PHI

PHI identification is currently restricted by both physician-(insufficient knowledge of either the clinical identification of the acute stage of the infection or lack of suitable tests to ascertain diagnosis) and patient-related factors (knowledge, shame or active denial of high risk). Jürgen Rockstroh (University of Bonn, Germany) emphasised the similarities about raising awareness for PHI and acute hepatitis C virus (HCV) infection and described the lessons learned in HCV. The acute HCV epidemic among HIV-1-infected MSM warranted a collaborative effort to halt onward transmission of the infection.[479] As a result, the European collaborative network (NEAT) introduced a joint definition for acute HCV in 2011.[480] As is the case for PHI, screening with antibody tests is not diagnostic of HCV infection shortly after high-risk transmission events and therefore HCV RNA tests should be used.[481] Acute HCV screening is cost-effective,[482] and early treatment is recommended as it benefits patients.[483] However, for prevention of the HCV epidemic, the unique opportunity for early intervention with new direct-acting antivirals (DAAs) has been missed. This is attributed to the high and non-reimbursed costs of DAAs (indicated for only advanced chronic HCV in Europe) and the increasing unwillingness of patients, or the lack of urgency for pegylated interferon- (peg-IFN) based treatment. Studies on peg-IFN-α-free DAA-based treatment strategies in acute HCV are planned. Several trials are ongoing to evaluate the efficacy of DAAs (boceprevir, telaprevir, sofosbuvir, ledipasvir) for 6, 8 or 12 weeks, with or without ribavirin or peg-IFN, and for various HCV genotypes. Despite the high sustained virological response rates of all-oral DAAs (> 95.0%), the previously mentioned factors combined with ineffective screening continues to fuel the MSM epidemic. Without changes in behaviour, the risk for reinfection in this particular patient group remains as high as 25.0% within 2 years of the first acute HCV episode.[484, 485]

In contrast to peg-IFN-based HCV treatment, current ART is well tolerated, reimbursed and patients are in general willing to receive treatment. Successful PHI

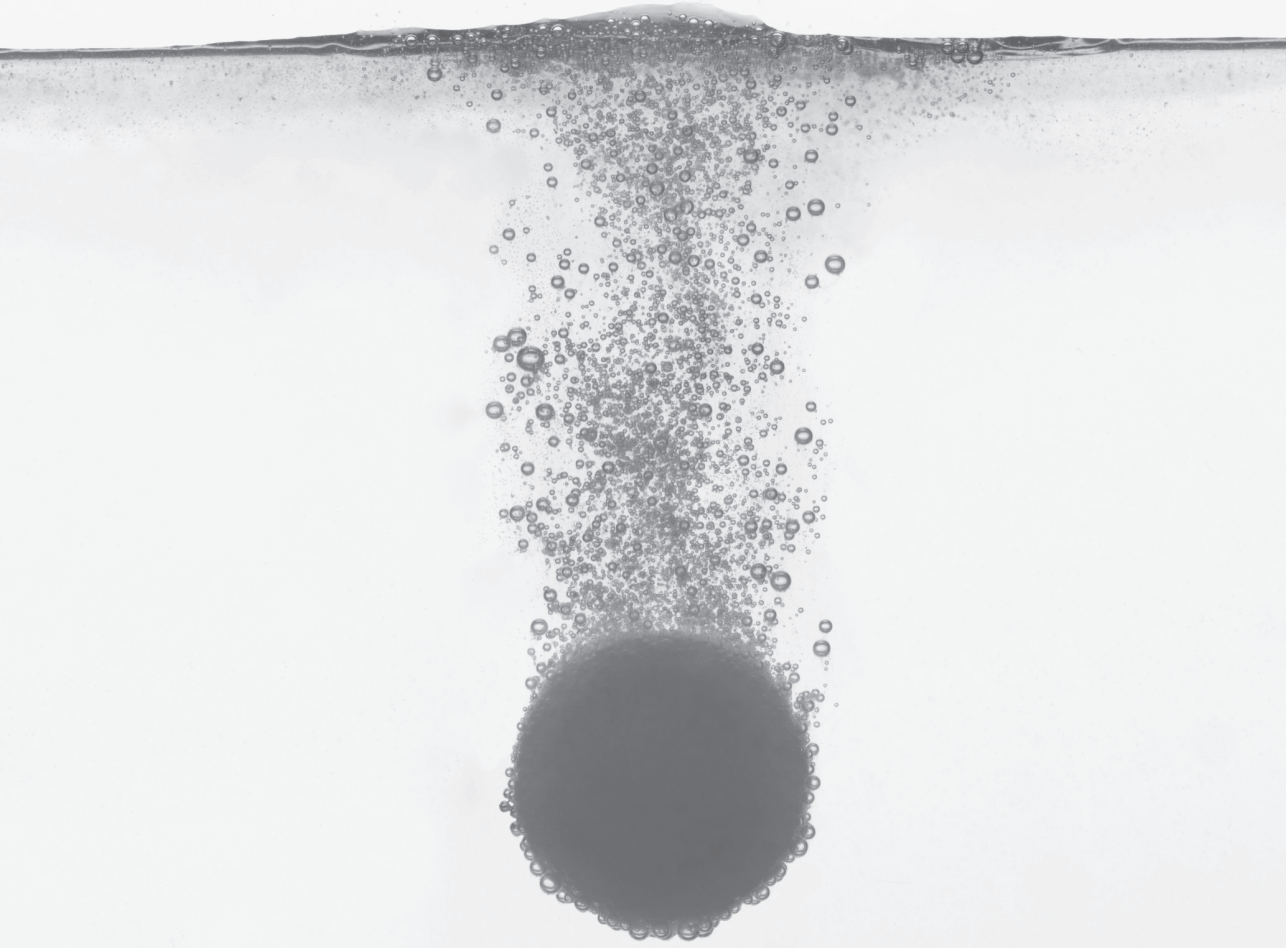
identification and prompt linkage to care are major goals and were highlighted by Anton Pozniak (Chelsea and Westminster Hospital, London). Although the symptoms of PHI are well described, they are not specific and the personal context (i.e. sexual preference, origin, substance abuse) is frequently unknown.[62] An important consideration in increasing PHI screening efforts and initiating early treatment is the prevention of amplified transmission during PHI. HIV-1 infectivity is highest during PHI,[64] and accounts for approximately half of all transmissions.[65] Patients who are unaware of their infection represent the minority of those who are infected but cause approximately half of all new HIV cases.[486] Relevant testing leading to a diagnosis of PHI is performed by physicians in relatively few patients despite the fact that most patients with PHI will already have had frequent voluntary antibody testing.[487] Furthermore, routine screening for HIV-1 in high-risk settings has been shown to be beneficial.[488] Together this argues for more active screening and supports targeting high-risk social environments (gay clubs, sex parties) together with sexual-contact tracing. Another important factor is the use of proper algorithms for the necessary tests to diagnose or exclude PHI. The window of opportunity is short and the utility of certain tests depends on the Fiebig stage.[489] The current fourth generation combined p24 antigen/HIV antibody ELISA can detect seroconversion approximately 2 weeks post-infection, which can be further reduced to 10 days by using RT-qPCR. A negative/indeterminate fourth generation ELISA in the setting of a recent high-risk event or clinical suspicion of PHI should be followed by RT-qPCR. A negative PCR in the eclipse phase warrants a repeated PCR after 1 week. Rapid point-of-care RT-qPCR tests can detect PHI when HIV-1 RNA typically exceeds 50,000 copies/mL.[490]

Although the above mentioned virological and immunological effects of early ART are of potential benefit to patients, no long-term trials are available to evaluate the benefits for an individual patient against the potential harm of lifelong early ART.[491] In trials conducted to date, PHI was defined as HIV-1 seroconversion within 6 months, which makes conclusions about outcomes difficult to generalise. The subgroup analysis of patients on ART within 12 weeks of seroconversion in the SPARTAC trial showed improved clinical outcome.[175] Overall, trials have shown that ART administration has led to improved short-term CD4+ T cell recovery, slowing the rate of CD4 T cell decline and may even lower viral setpoint after treatment discontinuation. Some trials have shown absent levels of HIV-1 DNA in CD4+ T cells with improved immune reconstitution in patients treated during Fiebig 1.[492] Very early ART may provide the opportunity for a successful treatment interruption in some patients, although

most do not achieve spontaneous control after treatment interruption. Furthermore, the factors predicting HIV-1 control post-treatment interruption remain unknown. No evidence exists to show that immunomodulators may improve immune responses during PHI and the optimal ART regimen to be initiated at this stage has yet to be determined. Importantly, none of the randomised trials have provided evidence that early ART has had an impact on AIDS, death or serious adverse events. Together, although ART during PHI decreases onward HIV-1 transmission and has probable biological benefits, definite clinical advantage at the individual level remains to be assessed. Nevertheless, screening efforts for PHI should continue to be improved and more therapeutic clinical trials, especially for patients in Fiebig 1 and 2 are needed.

CONCLUSION

A network of scientists, clinicians, funders and committed members of the HIV community continues to develop the HIV research field. An opportunity for collaborative European research lies ahead, which will increase the probability of producing breakthrough data on the HIV reservoir, cure strategies and PHI. The first steps towards an intensified collaboration have taken place and the commitment to fight for a common cause should benefit our joint research activities.



Chapter 12

Summarizing discussion

With the firm recommendation of immediate and lifelong treatment for all human immunodeficiency virus type 1 (HIV) infected patients, new questions have arisen. The central aim of this thesis is therefore to evaluate the safety and effectiveness of the drugs used in lifelong combination antiretroviral therapy (cART). This can contribute to the 'test and treat' and 'treatment as prevention' strategies. This thesis is divided in 4 parts. The studies in these 4 parts are designed to evaluate the efficacy of recommended first-line cART, the safety of antiretroviral treatment switch strategies, the care of comorbidities during cART and the perspectives in HIV management. In the last part of this thesis, we summarize and discuss the primary results of these studies, their clinical implications, and provide future prospects.

EFFICACY OF FIRST-LINE ANTIRETROVIRAL TREATMENT

The first part of this thesis concerns the lack of evidence regarding the relative efficacy of first-line lamivudine (3TC) or emtricitabine (FTC) combined with tenofovir disoproxil-fumarate (TDF) as nucleoside reverse transcriptase inhibitor (NRTI) backbone in non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) containing regimens. The efficacy of 3TC compared to FTC has been disputed by in vitro and in vivo experiments.[187, 205, 206] Well-powered randomized clinical trials (RCT) comparing 3TC and FTC directly without other variations in cART are unavailable. A difference in clinical efficacy would be alarming. The combinations of 3TC or FTC with TDF and either a NNRTI or a PI are the most frequently used regimens worldwide. Guidelines for HIV treatment consider 3TC and FTC to be clinically interchangeable components in first-line cART while this statement of equivalence has not been systematically studied. We have therefore investigated the

clinical efficacy of using either 3TC or FTC in combination with TDF as NRTI backbone.

Two cohort studies on 6322 cART naïve HIV patients provide evidence that the use of 3TC instead of FTC as part of a TDF containing NRTI backbone is associated with increased rates of virological failure with the NNRTI efavirenz (EFV) and nevirapine (NVP) (**Chapter 2**), but also that these compounds might be interchangeable when combined with PI (**Chapter 3**). Virological failure rates are increased with lower cluster of differentiation (CD)4+ T lymphocyte counts and higher viral loads at cART initiation. A promising finding is that once plasma HIV ribonucleic acid (RNA) is well suppressed, the virological failure rates do not differ. The selection of drug resistance mutations (DRM) is not different for 3TC and FTC with concomitant TDF/NNRTI use. DRM are rarely observed on TDF/PI based regimens.

These first clinical studies on treatment outcomes with FTC compared to 3TC in regimens of TDF containing NRTI backbones should have consequences. First, strong uncertainty regarding the clinical efficacy of widely used treatments can in particular be important for low and middle income countries. The absent routine HIV RNA monitoring could result in more AIDS related deaths. Second, increased failure rates may result in transmission of resistant HIV strains. Third, physicians should reject political or societal HIV cost containment incentives to favor the use of generic 3TC if this compound is associated with lower clinical effectiveness.[186] Last and importantly, worldwide recommended antiretroviral treatments by guidelines should preferably be based on proper evidence. Available phase III RCT that provide the basis of current recommendations for ART naïve patients have always compared FTC/TDF with 3TC coformulated with abacavir (ABC) or zidovudine (AZT) in PI, NNRTI or integrase inhibitor (INI) based regimens.[188-190, 197, 218, 220-222, 227, 228, 235, 236, 493-495] Notably, 3TC also appeared inferior when coformulated with ABC compared to FTC/TDF if HIV RNA was above 100.000 copies per milliliter (copies/mL). The statement of equivalence between 3TC and FTC in otherwise similar NRTI backbones is based on the absence of differences in 4 other RCT. The results from these 4 trials cannot be generalized to current cART recommendations on TDF containing NRTI backbones. The first RCT, FTC-302, is hampered by the use of the obsolete drug stavudine (d4T) instead of TDF and the administration of EFV or NVP in patients with low viral loads only.[210] The second open-label RCT, FTC-303, included already suppressed HIV infected patients, used first generation PI and d4T, and never included TDF.[213]

The third RCT, FTC-350, is a substudy of suppressed patients from FTC-303 who chose to be enrolled. The fourth RCT remains unpublished, is open label, included predominantly sub-Saharan women, and was underpowered to reject clinical meaningful differences.[212]

Our results are supported by subsequent smaller cohort studies. An European cohort study found increased failure rates with 3TC combined with TDF/EFV.[496] A Swiss cohort study showed higher virological failure rates with increased resistance rates on 3TC/TDF compared to FTC/TDF, although one third of the patients were ART experienced and those who had switched regimens for virological failure were excluded.[497] An Indian study found lower virological failure rates at lower CD4+ T lymphocyte counts for FTC/TDF/EFV compared to 3TC/TDF/EFV as single tablet regimens (STR).[498] Results of our study and others are unlikely to be explained by transmitted drug resistance as the rate of transmitted NRTI and NNRTI resistance has remained stable over time.[147, 499] Remarkably, 4 meta-analyses came to strikingly different conclusions regarding the clinical equality of 3TC and FTC although they included the same heterogeneic studies.[193, 199, 219, 500] This finding highlights the need for a robust well powered randomized study.

In conclusion, the current evidence to support the safe use of 3TC with TDF is insufficient. 3TC should not be administered in first-line TDF/NNRTI containing treatment regimens for ART naïve HIV patients if FTC is available. A switch to 3TC can be considered when the viral load is well suppressed.

ANTIRETROVIRAL TREATMENT SWITCH STRATEGIES

Antiretroviral drug switches are addressed in the second part of this thesis. The possibility to safely switch from one cART regimen to another is clinically relevant. Switching cART will sometimes be inevitable during lifelong therapy of ageing HIV infected patients. We evaluated three potential high-risk antiretroviral switches due to cytochrome P450 interactions (**Chapter 4**), acquired resistance on the same ART class (**Chapter 5**), and a novel monotherapy option (**Chapter 6**).

Cytochrome P450 3A4 (CYP3A4) is a member of the CYP450 family of enzymes responsible for metabolizing drugs including many chemotherapeutic and antiretroviral

medicines. The first generation NNRTI NVP and EFV induce CYP3A4. Substituting a CYP3A4 metabolized antiretroviral drug for a CYP3A4 inducing antiretroviral drug can be hypothesized to result in decreased drug exposure, selection of resistance and subsequent virological failure. We test this hypothesis in a study including suppressed HIV patients who switch their CYP3A4 inducer NVP to the CYP3A4 substrate rilpivirine (RPV) (**Chapter 4**).

A prospective open-label non-randomized controlled clinical trial is used to evaluate the efficacy, safety, and pharmacokinetics of substituting RPV/FTC/TDF for NVP/FTC/TDF in 50 suppressed switchers and 159 non-switchers. Virological suppression rates below 50 copies/mL remain high and comparable between the 2 groups during follow-up. The NVP levels are undetectable in all patients within 3 weeks and the RPV concentration is adequate 1 week after the switch. Substituting RPV for NVP results in ongoing virological suppression and does not have clinically relevant pharmacokinetic effects by CYP450 interactions.

The first generation NNRTI NVP is probably associated with inferior virological responses compared to other NNRTI.[501-503] NVP exposure results in more treatment discontinuations particularly due to toxicity.[234, 504, 505] The use of NVP is therefore no longer recommended in guidelines in high income countries. The results of our study show that patients can be safely switched to recommended regimens including RPV without the need for additional HIV RNA measurements, dosage increase or therapeutic drug monitoring due to concerns on ongoing CYP3A4 induction. The results can be extrapolated to other CYP3A4 metabolised first-line drugs including darunavir (DRV), elvitegravir (EVG) and dolutegravir (DTG) because RPV probably has a lower genetic barrier to resistance compared to these drugs.[506, 507] Our study results on sustained RPV efficacy have been confirmed by others studying switches from NVP, EFV and PI to RPV.[245, 247, 250, 508]

In conclusion, switching from first generation NNRTI to RPV should not be withold for concerns of CYP450 interactions and a subsequent increased risk of failure.

A STR is often preferred over multiple tablet regimens for convenience and optimal adherence. Resistance to NVP or EFV often results in cross-resistance to both drugs and selection of undetected minority resistant strains. Therefore, switching from one drug to another within the NNRTI drug class is generally not considered after a

previous treatment failure and selection of resistance mutations. An exception is the second generation NNRTI, etravirine. If this within-class switch is possible with RPV has not been studied before.

The results of our proof of concept study show that a within NNRTI class switch after previous failure with selection of an isolated K103N strain might be feasible when a HIV infected patient is first well suppressed on other regimens (**Chapter 5**). Three African women with an isolated K103N selected strain following first-line NNRTI failure switched to RPV/FTC/TDF and remain well suppressed without treatment emergent resistance during 48 weeks of follow up.

This is the first study to assess the clinical efficacy of RPV in acquired K103N containing strains. The activity of RPV is affected by the 181C mutation, but is not affected by most single mutations at positions 100, 103, 106, 179, 188, 190, 221, 230, and 236 in RT in vitro.[249] A previous study has provided the first evidence that RPV remains active in K103N containing transmitted HIV strains.[250] However, the efficacy of RPV in acquired resistance following failure on prior NNRTI is unknown. Theoretically, approximately 80% of selected strains after first generation NNRTI failure might still remain susceptible to RPV.[254] As K103N mutated strains are frequently selected following EFV failure, the potential for RPV as safe STR option in these single mutated strains could be considerable.[252]

A caveat can be that, in contrast to a transmitted K103N harboring strain, other DRM are selected following failure during treatment but not detected by standard population genotyping. Failure to successfully suppress the HIV infection with a suboptimal second-line therapy could induce the emergence of a more resistant virus. K103N might also facilitate the selection of K103N/E138K mutated strains.[253] Strains with other DRM might have been selected and archived in our patients that influence the long-term treatment success of RPV.[255, 256] The non-B subtype of our patients would not explain the results because all B and non-B subtypes have equal clinical responses to antiretroviral drugs, although acquired resistance on treatment varies between the subtypes.[509-511]

In conclusion, our results indicate that a substantial subgroup of HIV infected patients with an isolated K103N mutation, acquired on previous failing NNRTI regimens,

might benefit from RPV after virological suppression has been achieved on other second-line regimens.

The possibility to use a STR as second line therapy is an interesting option for patients and physicians. Patients with virological failure used to switch to multiple tablet PI based therapy. With RPV/FTC/TDF, EVG/cobicistat(c)/FTC/TDF and DTG/3TC/ABC, 3 STR are available that can be used after first generation NNRTI failure. As such, STR are becoming a valid treatment option for second-line HIV therapy in selected patients.

The STR DTG/3TC/ABC could in particular be preferred because of its efficacy and favourable DDI profile. However, the use of NRTI can still result in considerable toxicity or selection of NRTI resistance. The high genetic barrier of DTG makes DTG monotherapy an appealing option, if safe. We describe the first clinical experiences with DTG as antiretroviral monotherapy (**Chapter 6**).

In this study, 5 HIV infected patients on cART are switched to DTG monotherapy. All have contraindications to current and alternative combinations of antiretroviral drugs. The plasma viral loads remain well suppressed in 4 patients. However, one patient, with an unfavorable pre-cART viral load and CD4+ T lymphocyte count, experiences virological failure. This patient has low DTG plasma levels possibly due to hemodialysis and concomitantly used calcium tablets. The viral load of this patient resuppresses on cART. This study indicates that DTG monotherapy may be a maintenance option in selected HIV infected patients.

The robustness of DTG with 2 NRTI is shown in comparison to NNRTI, PI, and other INI based regimens, both in treatment naïve and treatment experienced patients. A remarkable finding is that no treatment emergent resistance is selected in ART naïve patients allocated to the DTG treatment arms during 96 weeks of follow up.[227, 228, 260, 512] The clinical responses to DTG are lower in ART experienced patients with previous virological failure and selection of resistance. Resistance is rarely acquired in 715 ART experienced and INI naïve HIV infected patients without baseline mutations to raltegravir (RAL) at treatment initiation.[261] Only the R263K and V151I, associated with low level resistance and significant reductions in viral fitness, are selected in patients without the 148 mutation at baseline. An interesting hypothesis is that additional mutations after R263K might not restore the replicative capacity

which could imply an evolutionary dead-end.[513, 514] Additional mutations in N155H/R263K mutated strains do not affect resistance or restore the replicative capacity in vitro.[515] However, clinical trials show that in pretreated INI experienced patients, the responses with DTG decline if codon 148 in integrase was mutated.[262-264]

The efficacy of antiretroviral monotherapy has been investigated with boosted PI and NRTI. The genetic barriers of NRTI and first-generation INI or NNRTI are insufficient for monotherapy. PI monotherapy (specifically ritonavir boosted DRV, atazanavir and lopinavir) is safe and non-inferior to cART in simplification RCT on well suppressed patients with a favorable CD4 nadir and viral load setpoint prior to cART initiation.[268-271] However, PI monotherapy is associated with DDI due to the pharmacological booster ritonavir, significant side effects, and the need of intensified viral load monitoring. The use of DTG monotherapy could mitigate toxicity, pill burden and DDI concerns. Furthermore, the omission of NRTI backbones including TDF and ABC decreases treatment toxicity.

In conclusion, our study provides the first insights in the clinical use of DTG as maintenance monotherapy. This strategy might be a valid treatment in selected HIV patients and preferable over PI monotherapy, provided that virological failure is not just delayed but also prevented by long term DTG monotherapy.

COMORBIDITIES DURING ANTIRETROVIRAL TREATMENT

Comorbidities that arise during cART form the third part of this thesis. Renal disorders, cardiovascular diseases (CVD) and malignancies are among the most prevalent comorbidities in HIV infected patients on cART. These comorbidities will become increasingly relevant for the ageing HIV population. In this thesis, the prevalence and novel aspects of TDF related nephrotoxicity (**Chapter 7**), CVD risk alterations by switching cART (**Chapter 8**), and the optimal treatment of the human herpesvirus type 8 (HHV8) related malignancies Kaposi sarcoma (KS) and multicentric Castleman's disease (MCD) that develop despite cART (**Chapter 9** and **Chapter 10**) are investigated.

Renal dysfunction

Renal dysfunction can develop due to HIV unrelated factors, an untreated HIV infection or as a result of cART. Untreated HIV patients have increased rates of glomerular disease compared to uninfected persons.[516] The most important glomerular diseases are HIV related associated nephropathy, HIV related immune complex disease and HIV related thrombotic microangiopathy.[517-519] Early diagnosis and timely cART initiation can prevent these renal disorders.

Antiretroviral therapy is also associated with acute and chronic kidney disorders.[181, 182, 287, 520] TDF related renal toxicity can result in increased glomerular filtration rate (GFR) decline, proximal tubular dysfunction, or both. The prevalence of tubular dysfunction is not clear and other as yet unidentified risk factors might enhance TDF related renal dysfunction.[521 522] In our study, we evaluate the prevalence of TDF related nephrotoxicity, and the effects of concomitant exposure to drugs that inhibit the primary renal tubular multidrug resistance protein transporter (MRP) of TDF as a novel explanatory factor of renal dysfunction (**Chapter 7**).

A total of 721 HIV infected patients on long-term TDF are included in a cohort study with quantification of their MRP inhibitor use. Exposure to MRP inhibitors is associated with increased progression to chronic kidney disease. No clinically significant relationships are found between exposure to MRP inhibitors and abnormal solute handling in the proximal tubule. Incidental exposure to MRP inhibitors in TDF treated HIV infected patients is unlikely to result in major additional TDF related renal toxicity.

Nonetheless, the results underline the importance to evaluate comedication, especially over-the-counter medication, in all HIV infected patients using TDF. The ability to restore kidney function following TDF discontinuation is unclear as are the factors influencing this recovery.[523, 524] The optimal method to identify patients at highest risk for developing tubular dysfunction remains unclear.[280, 525-528] Last, the best treatment strategies to mitigate TDF related renal toxicity are not well characterised. The use of ABC can be unwanted due to CVD risk concerns, and the efficacy of non NRTI treatment strategies might be suboptimal.[529] These strategies could be impacted by the introduction of tenofovir alafenamide fumarate (TAF).[530-532]

In conclusion, the renal effects of TDF will continue to be relevant with the worldwide expansion of cART. Concomitant medication should always be registered because major additional renal toxicity of ongoing exposure to potent MRP inhibitors and TDF cannot be excluded based on our study, and biomarkers for vulnerable patients are not readily available.

Cardiovascular diseases

Immune activation in HIV infected patients results in inflammation which is pro-atherogenic and increases the risk of CVD compared to HIV uninfected persons.[98, 297] Antiretroviral therapy lowers the inflammatory state.[173] Unfortunately, different antiretroviral drugs have different CVD risk profiles resulting in atherogenic lipid profiles or elevated blood pressure.[300, 301] A regimen with a more favorable lipid profile could be desirable in individuals at high risk for CVD.[297, 299] NVP has a favorable lipid profile compared to EFV or PI based regimens.[302-304] However, NVP has a lower virological efficacy. A within class switch from NVP to RPV could therefore be preferable. Dyslipidemia rates are low with RPV but a direct comparison with NVP is unavailable.[305] We have investigated the dyslipidemic alterations and CVD risk profiles after substituting RPV for NVP.

In a prospective open-label clinical trial, we evaluate the serum lipids, cardiovascular risk profiles, and lipid treatment goals over 48 weeks after switching NVP to RPV in 50 HIV infected patients (**Chapter 8**). We observe significantly altered lipid profiles and improved systolic blood pressure. More patients achieve internationally recognized total cholesterol and low-density lipoprotein goals on RPV although their high-density lipoprotein levels decline. These changes do not impact Framingham Risk Scores (FRS). These results argue against major risk profile changes following discontinuation of NVP and initiation of RPV.

Antiretroviral therapy will remain only one of the many factors in CVD risk management. Other CVD risk factors as hypertension, diabetes mellitus, and lifestyle including a sedentary life and smoking, are important modifiable risk factors. Strong evidence exists that dyslipidemia, in particular low high-density and high low-density lipoprotein cholesterols, inflammation and higher FRS impact CVD.[533-537] Large RCT on achieving cholesterol targets and prevention of cardiovascular events, such as conducted in HIV uninfected patients, are unavailable in HIV infected patients.[538] The only RCT on statin therapy in HIV has primarily focused on alleviation of im-

mune activation in those with already low cholesterol levels.[446] A study on the effect of statins in 6500 HIV infected patients on cART is ongoing.

CVD risk management is important in the ageing HIV population. Antiretroviral treatment strategies in this population must be accompanied by an integrated CVD risk analysis.[539] After lifestyle modifications have been shown to be insufficient, switching antiretroviral drugs to ameliorate the CVD risk prior to initiating additional drugs for CVD prevention is a valid treatment strategy. Apart from RPV, also INI have favorable lipid profiles in the phase III studies and may be used for this aim.

In conclusion, we do not observe relevant changes in CVD risk following NVP discontinuation and RPV initiation. Switching cART can be considered to improve the CVD risk.

Malignancies

The HHV8 related KS is the most common AIDS defining cancer prior to cART initiation.[183] The incidence of the another HHV8 related disease, MCD, remains unaffected despite the introduction of cART.[184] These HHV8 and HIV associated diseases can occur together but their malignant origins and viral gene expressions are different.[540] With KS, cART is often sufficient to cause remission. Additional therapy is necessary when KS develops or progresses despite cART. MCD always warrants additional therapy on top of cART. The current treatment for HIV infected patients who develop KS despite cART or MCD consists of chemotherapy. An immunomodulatory treatment could prevent the use of chemotherapy. We evaluate the efficacy of immunomodulatory treatments in patients with KS or MCD.

The efficacy of peginterferon is evaluated in an observational study of 10 patients with extensive or treatment-refractory AIDS-associated KS (**Chapter 9**). Tumor responses are observed in 9 patients with favorable progression free survival. A systematic review is conducted to evaluate survival in HIV patients with MCD (**Chapter 10**). The use of the monoclonal anti-CD20 antibody rituximab results in better treatment responses and survival compared to chemotherapy without rituximab. KS (re)activation with rituximab and MCD progression still occur despite treatment. Peginterferon for advanced KS or rituximab for MCD could be effective treatments in HIV infected patients.

Immunomodulatory treatment of virus related malignancies in HIV offers a novel clinical approach. Oncogenic development has been linked to impaired HHV8 cellular immunity.[541] HHV8 encodes homologs of human proteins involved in cell cycle regulation, proliferation, interferon (IFN) signaling tumor suppressor pathways and production of stimulatory cytokines.[542] The HLA class I restricted antigen presentation to cytotoxic T lymphocytes is also restricted by HHV8.[543] KS cells may proliferate in the setting of cytokines and the HIV tat protein in uncontrolled HIV.[544, 545] Type 1 IFN inhibits KS proliferation and downregulates KS stimulatory cytokines in humans.[546-548] Concomitant initiation of chemotherapy, or potentially also peginterferon, with cART in HIV patients with KS could have added value.[549] In this thesis, the feasibility to use peginterferon is shown both for extensive cutaneous disease to accelarate regression of invalidating lesions, and as second-line therapy when chemotherapy has failed.

Limited experience with IFN monotherapy in HIV associated MCD exists.[550-554] The initiation of antiretroviral therapy cannot stop MCD progression.[555, 556] Additional treatment for MCD is always necessary. The introduction of rituximab has resulted in improved outcomes in other B lymphocyte related malignancies.[557] Rituximab should also be the cornerstone in the treatment of MCD. A combination with a single chemotherapeutic agent can be considered if KS lesions are present or suspected in tissue biopsies. The use of interleukin 6 (receptor) antibodies should be further explored in HIV infected patients, especially when the CD4+ T lymphocyte count is below 50 cells/mm^3 and the use of rituximab increases mortality.[558]

A direct effect of antivirals against HHV8 related malignancies can also be expected. Limited trial experience is available with high dosed zidovudine/valganciclovir in MCD, and only anecdotal reports on the use of ganciclovir, cidofovir or foscarnet in MCD or KS exist.[559-562] Their definitive efficacy remains to be proven in KS and MCD. The use of valganciclovir alone in HHV8 infected patients decreases HHV8 viremia.[563] Novel strategies might include targeted therapy by exploiting the expression of virus associated metabolic pathways as tumor specific targets for radioisotope therapies.[564] Enhancing cytotoxic T lymphocytes, for example by programmed death-1 checkpoint inhibition, may restore antitumor effector function in HHV8 associated malignancies, similar to its efficacy shown in other cancers, lymphoma, and viral diseases.[565-568]

In conclusion, HHV8 associated malignancies remain relevant in the era of cART. Promising and effective immunomodulatory treatments are available. Rituximab should be the cornerstone for MCD treatment in HIV infected patients. Peginterferon for KS can be used in clinical practice despite not being formally evaluated in a RCT for this indication.

HIV: FROM PRESENT TO FUTURE

The worldwide dispersion of accessible and affordable treatment for HIV continues to demand an ongoing collaborative public effort. Those who are HIV infected and fortuitous to live in areas with access to treatment can effectively control their disease and have near normal life expectancies. The road towards present insights has been paved with clinical challenges. Four debates concerning HIV treatment have been settled by well-designed studies. The studies in this thesis aim to support the safe use of cART based on the outcomes of these 4 debates.

First, pre-exposition prophylaxis.[569-571] People know when they engage in risky sexual behavior. Condoms have been tried and are clearly insufficient to prevent HIV transmission alone. Sexual intercourse driven use of antiretroviral drugs protects sexual active persons from acquiring HIV, regardless of sexual preferences. People will not stop having sex and should not be disciplined with HIV for their incautious behavior; physicians are obliged to indiscriminately protect them. Second, cART should be used without interruption.[572] Interrupted or deferred treatment results in preventable deaths of HIV patients. The beneficial effects of current therapies outweigh toxicity and an uncontrolled virus is detrimental for survival. Third, prevent HIV using treatment as prevention.[129] cART prevents HIV transmission between serodiscordant couples. HIV patients should not only be treated for their own benefit, but also to protect their significant others. Physicians should not forget their responsibility to uninfected individuals at risk for HIV. Fourth, test and treat HIV upon a positive test.[174, 573] Deferred treatment decreases survival. Withholding cART for no good reason results in preventable HIV related morbidity. Practical concerns on adherence, costs and ART inconvenience should not intervene with therapy installment and should be dealt with.

These 4 strategies are the basis for current HIV treatment. The implementation of these aspects in the future management of HIV is discussed as the fourth topic in this thesis (**Chapter 11**). Improvement of the current and future antiretroviral strategies and reduction of comorbidities will remain important while strategies to evade the persistence of HIV will be further explored. The future research opportunities are likely to be found in 3 areas.

Best antiretroviral treatment practices

The optimal first-line therapy for different settings has not yet been established. Current registration RCT include young vital men from research rich countries with high CD4+ T lymphocyte counts. The findings cannot be indisputably generalized to women, elderly or AIDS patients. The efficacy of first-line cART, including 3TC and FTC, should preferably be shown by well-designed studies, in particular in populations that are currently underrepresented in studies. Additionally, a cost effectiveness analysis could aid in the distribution of scarce resources. Physicians should especially be involved in this process if policy makers prefer cheaper drugs despite a lower clinical efficacy. Novel drug classes, induction-maintenance strategies and drug formulations will have to prove their value in clinical practice. The paradigm of triple drug cART will further be questioned by studying dual- or monotherapy strategies. Future switch studies will elucidate the optimal population and drug dosage for monotherapy and the effect of resistance variants on safety. We have already initiated the DOMONO RCT on DTG monotherapy to test non-inferiority to cART in HIV infected patients.[275] HIV DNA and cell associated HIV RNA might be relevant measures to predict the safety of monotherapy in patients. Together, current and future treatment strategies will remain important research topics. On a global scale, 'test and treat' and 'treatment as prevention' strategies will have to be further implemented to decrease mortality and halt HIV transmission.

Reduction of comorbidity

Renal dysfunction, CVD and malignancies are discussed in this thesis, but also bone mineral density, metabolic, pulmonary and neurocognitive functions are important comorbidities in HIV infected patients. The most effective prevention and treatment for each of these comorbidities should be studied. Reducing immune activation should be a research focus. We will study the safety of cART on the risk of immune reconstitution. Intervening in microbial translocation is of interest. Antibodies to interleukin 6 could decrease the pro-inflammatory state associated with CVD. Comorbidities

during long-term cART exposure should be monitored. Other research topics are the effects of CVD prophylactic drugs on immune activation and clinical events, when to use them in HIV and the DDI of these drugs with cART. One aspect of CVD treatment, arterial and venous thromboembolic events, will be investigated in our PREDICT study. HIV specific CVD risk scores have to be evaluated prospectively and used in risk management. Strategies to predict and treat renal dysfunction in HIV patients on cART remain important. Research lines will include an assessment of the potency of TAF versus ABC to restore TDF induced renal dysfunction. The influence of MRP inhibitors on TDF related renal dysfunction should be further explored. In malignancies, preventive measures -smoking- and cancer screening in HIV are important. The effects of reducing HHV8 viremia, using interleukin 6 antibodies or combining cytostatics with rituximab on KS flares in MCD, the use of peginterferon with cART in KS, and the utility of cytokines and HHV8 load should be evaluated. Together, the management of comorbidities will remain a major challenge in HIV.

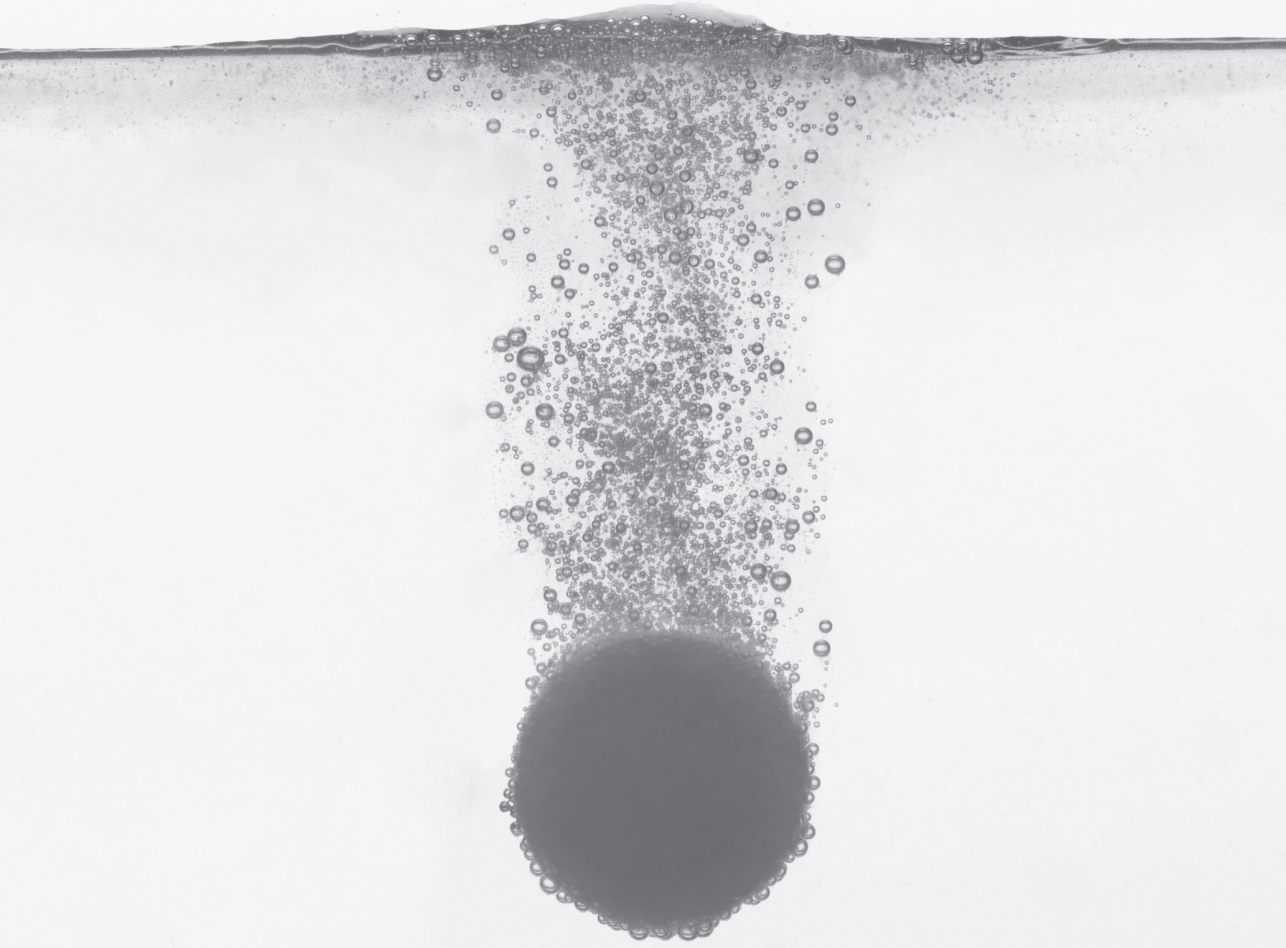
Clinical approach to HIV persistence

Antiretroviral therapy should be used unless a sterilizing or functional HIV cure is possible. Cure approaches are investigated by a research collaboration named the Erasmus MC HIV Eradication Group. One method is to purge latent HIV from its long-lived reservoir for targeted eradication: the shock and kill strategy. Latency reversing agents (LRA) and immunomodulatory approaches are explored using material of leucapherised HIV patients. These findings will be translated into studies to test their safety in clinical practice. Eradication trials with single LRA will be expanded to combinatorial approaches. A proof of principle study would be to combine histone deacetylase inhibitors and BAF complex inhibitors. Monoclonal antibodies or vaccines can be used to prime the immune system. Alternative ways may be the prevention of HIV reactivation, to promote reservoir differentiation to shorter-lived cells, or RNA interference. Gene editing by zinc-finger-nucleases, transcription-activator-like-effector-nucleases, or clustered regularly interspaced short palindromic repeats(CRISPR)-Cas is useful if off-target DNA effects are limited. The reservoir measurements will have to improve and reservoir biomarkers need to be identified. A collaborative NOVA study promotes diagnosing acute HIV and evaluates the effect of cART on the reservoir. These early treated patients with small reservoirs will be the preferential group for treatment interruption trials. Pilot interruption trials will need to predict responses based on proper biomarkers. A function of reservoir size and time to viral rebound could guide treatment interruption. The first trials will include

extensive sampling to detect residual reservoirs and long follow-up before HIV remission can be concluded. A cure does not necessarily restore previous damage; do cured patients have an increased risk on comorbidities just without HIV? Last, curing HIV does not protect one from acquiring HIV again. We have to think about the ethical and societal impact of the possibility to acquire HIV again after a cure.

CONCLUDING REMARKS

The focus of this thesis is the safety and effectiveness of the drugs used in lifelong cART. The initial survival of HIV infected patients is secured by the introduction of cART, the best moment of cART initiation is resolved, and the benefits of immediate and universal access to cART are clear. The main conclusions are: (1) that first-line cART should preferably not include 3TC if FTC is available, (2) that switch strategies with STR or monotherapy are relevant options in various clinical settings, (3) that TDF related renal dysfunction can be enhanced by concomitant medication, CVD risk profiles are stable on RPV after NVP cessation, immunomodulatory treatment of KS and MCD can be safe and effective, and (4) future clinical HIV management involves the treatment of acute HIV and cure strategies. Future research should further elucidate which first-line treatment is most effective, when switch strategies are safe, and how to optimally counteract drug toxicities and comorbidities until a definite answer to the persistence of HIV is formulated.



Chapter 13

Nederlandse samenvatting

Een infectie met het humaan immuundeficiëntie virus type 1 (HIV) leidt onbehandeld tot AIDS en de dood. HIV infecteert specifieke cellen van het immuunsysteem, voornamelijk de CD4 positieve T-lymfocyten. Deze cellen zijn cruciaal voor de goede werking van het immuunsysteem tegen infecties en kanker. HIV geïnfecteerde CD4 positieve T-lymfocyten worden vernietigd. Hierdoor vermindert de functie van het immuunsysteem progressief zolang HIV niet behandeld wordt. Als retrovirus bevat HIV het enzym reverse transcriptase. Dit zet het virale ribonucleïnezuur om in desoxyribonucleïnezuur (DNA). Dit virale DNA kan permanent in het menselijke DNA worden ingebouwd door het virale enzym integrase. Een klein deel van de geïnfecteerde cellen bevat latent virus dat niet repliceert. Dit wordt het virale reservoir genoemd.

HIV genezen is nog niet mogelijk: het reservoir is niet gevoelig voor de huidige therapie en blijft aanwezig. De medicamenteuze behandeling met antiretrovirale medicijnen is echter wel levensreddend. Succesvolle behandeling betekent een volledige onderdrukking van de replicatie van alle aanwezige virussen in het lichaam. Onvoldoende behandeling kan leiden tot therapiefalen met selectie van resistente virussen. De standaardbehandeling omvat combinaties van drie antiretrovirale medicijnen. De behandeling is gebaseerd op de principes 'test en behandel' en 'behandeling als preventie'. Het eerste principe houdt in dat de behandeling direct dient te worden gestart na het stellen van de diagnose HIV. Dit leidt tot een betere overleving van een HIV geïnfecteerde patiënt. Het tweede principe betekent dat een succesvolle behandeling van een HIV geïnfecteerde patiënt leidt tot bescherming van diens directe omgeving en daarmee de populatie waarin diezelfde patiënt zich bevindt. De behandeling dient levenslang te worden voortgezet. Bij het stoppen van de behandeling zal het virus vanuit het reservoir actief worden, met progressie van de ziekte tot gevolg. Wereldwijd zijn er bijna 40 miljoen mensen geïnfecteerd met HIV van wie het merendeel in

Afrika ten zuiden van de Sahara leeft. Slechts de helft van alle patiënten heeft in 2015 toegang tot levensreddende medicijnen.

Dit proefschrift beoogt zowel de effectiviteit en veiligheid van de antiretrovirale behandeling als de bijkomende comorbiditeit in HIV geïnfecteerde patiënten te onderzoeken. De achterliggende gedachte is dat de studies in dit proefschrift de verdere implementatie van optimale levenslange therapie volgens de bovenbeschreven principes voor alle HIV geïnfecteerde patiënten moet ondersteunen. Specifieker onderzoeken wij de effectiviteit van de eerstelijnstherapie, de veiligheid van behandelaanpassingen en de comorbiditeit gedurende de behandeling. Deze worden in dit proefschrift in drie aparte delen behandeld. In het vierde en laatste deel van dit proefschrift bespreken wij de implicaties van de studies en de uitdagingen voor de toekomst.

Twee belangrijke medicijnen in de eerstelijnstherapie van HIV zijn lamivudine en emtricitabine. Deze medicijnen worden als even effectief beschouwd door behandelrichtlijnen, zoals die van de Wereldgezondheidsorganisatie. Definitief bewijs voor deze aanname ontbreekt echter. Lamivudine en emtricitabine behoren tot de groep van nucleoside reverse transcriptase remmers. Vaak worden deze medicijnen gecombineerd met tenofovir disoproxil-fumaraat, ook een nucleoside reverse transcriptase remmer, en nog een derde medicijn. Dit derde medicijn is vaak een niet-nucleoside reverse transcriptase remmer, veelal efavirenz of nevirapine, of een protease remmer. Wereldwijd zijn deze combinaties van lamivudine of emtricitabine met tenofovir disoproxil-fumaraat in combinatie met nevirapine, efavirenz, of protease remmers de meest gebruikte behandelingen voor HIV. Basaal onderzoek toont echter aanwijzingen voor een verminderde effectiviteit van lamivudine. Adequaat opgezette klinische studies naar de effectiviteit van deze combinaties zijn er niet. In deel 1 van dit proefschrift onderzoeken wij of de effectiviteit van lamivudine en emtricitabine verschillend is wanneer deze worden gebruikt in combinatie met tenofovir disoproxil-fumaraat en nevirapine, efavirenz of een protease remmer in de behandeling van HIV geïnfecteerde patiënten.

In **hoofdstuk 2** en **hoofdstuk 3** vergelijken wij de effectiviteit van lamivudine en emtricitabine in twee klinische studies met in totaal 6322 HIV geïnfecteerde patiënten. Wanneer lamivudine in plaats van emtricitabine wordt gebruikt in combinatie met tenofovir disoproxil-fumaraat blijkt de kans op therapiefalen verhoogd. Dit is zowel het geval wanneer efavirenz als nevirapine als derde medicijn wordt gebruikt. De kans

op therapiefalen wordt ook verhoogd wanneer patiënten een gevorderde HIV infectie hebben voor de start van de behandeling. Lamivudine en emtricitabine blijken wel even effectief met een protease remmer of nadat de virale lading in het bloed eenmaal goed onderdrukt is. We concluderen daarom dat er onvoldoende bewijs is voor de ondersteuning van het gebruik van lamivudine met tenofovir disoproxil-fumaraat in HIV geïnfecteerde patiënten die starten met therapie. Een goed opgezette gerandomiseerde studie met voldoende patiënten dient eerst duidelijkheid te geven. Tot die tijd moet het gebruik van lamivudine bij voorkeur vermeden worden als emtricitabine beschikbaar is.

Bij het merendeel van de HIV geïnfecteerde patiënten zal de eerst gestarte behandeling ooit veranderen. Dit komt doordat patiënten levenslang behandeld worden en het succes van de behandeling zorgt voor een bijna normale levensverwachting. Ouderdom brengt gebreken waarvoor medicatie noodzakelijk kan zijn welke niet altijd te combineren is met de HIV medicatie. Daarnaast kan de HIV medicatie zelf comorbiditeit veroorzaken. Wisselingen in HIV medicatie zullen in toenemende mate voorkomen. Niet van alle medicatiewisselingen is bekend of deze veilig mogelijk zijn. In deel 2 van dit proefschrift onderzoeken wij drie mogelijk risicovolle medicatiewisselingen.

In **hoofdstuk 4** kijken wij of de effectiviteit van rilpivirine beïnvloed wordt door het direct daarvoor gestopte medicijn nevirapine. Nevirapine heeft een lange halfwaardetijd en versterkt de werking van een enzym dat rilpivirine afbreekt; het cytochroom P450 3A4. Hierdoor kunnen lagere rilpivirinespiegels ontstaan na een medicatiewissel. Dit kan leiden tot therapiefalen. In een klinische studie wisselen 50 patiënten met adequate virale onderdrukking hun nevirapine in voor rilpivirine. Deze groep wordt vergeleken met 159 patiënten die hun nevirapine continueren. De rilpivirine bloedspiegels zijn na één week adequaat. Na drie weken is nevirapine bij niemand meer aantoonbaar. De behandeling is in beide groepen even effectief. Hieruit concluderen wij dat nevirapine geen klinisch relevante invloed heeft op de effectiviteit van rilpivirine. Theoretische interacties rondom medicatiewisselingen staan behandelaanpassingen niet in de weg.

In **hoofdstuk 5** beschrijven wij onze studie naar de effectiviteit van rilpivirine in HIV geïnfecteerde patiënten met een geselecteerd K103N gemuteerd virus, verworven op efavirenz of nevirapine. Rilpivirine behoort tot dezelfde klasse geneesmiddelen als

efavirenz en nevirapine. Het gevaar kan zijn dat andere niet-gedetecteerde mutaties die wel tot resistentie tegen rilpivirine leiden ook geselecteerd zijn. Rilpivirine blijkt echter succesvol in 3 Afrikaanse vrouwen met een enkel K103N gemuteerd virus. Rilpivirine, en dus therapie met één pil per dag, kan dus geschikt zijn voor een grotere groep patiënten.

In **hoofdstuk 6** bestuderen wij de veiligheid van de integrase remmer dolutegravir als monotherapie. Monotherapie geeft minder bijwerkingen, minder comorbiditeit en minder problemen met andere medicijnen. Voorwaarde is dat één medicijn krachtig genoeg is om HIV volledig te onderdrukken. Dolutegravir monotherapie, gestart na een succesvolle reguliere behandeling, is succesvol in 4 van de 5 studiepatiënten. Monotherapie faalt echter in 1 patiënt. De combinatie van hemodialyse, een gevorderde HIV infectie en calciuminname kan het falen mogelijk verklaren. Dolutegravir monotherapie is mogelijk, maar waarschijnlijk alleen in een geselecteerde groep HIV geïnfecteerde patiënten.

Antiretrovirale behandeling is levensreddend maar kan ook schadelijke effecten hebben. Met name nierschade en hart- en vaatziektes komen als comorbiditeit vaak voor in de ouder wordende HIV populatie. Sommige HIV medicijnen kunnen de kans hierop verder vergroten. Daarnaast kunnen ondanks behandeling HIV gerelateerde kankers zich toch ontwikkelen. In deel 3 van dit proefschrift onderzoeken wij diverse aspecten van de aan HIV gerelateerde comorbiditeit. Hierbij focussen wij ons op de belangrijkste comorbiditeit in HIV, nierschade, hart- en vaatziektes, en twee kankers gerelateerd aan HIV: het Kaposi sarcoom en de ziekte van Castleman.

In **hoofdstuk 7** beschrijven wij de nierschade die kan optreden door tenofovir disoproxil-fumaraat. Dit middel wordt wereldwijd veel gebruikt. Nierschade is echter de belangrijkste beperking. Na omzetting tot de werkzame stof tenofovir in het bloed wordt dit middel door de nier verwijderd uit het lichaam. Hierbij wordt onder andere gebruik gemaakt van bepaalde kanalen in de tubulus van de nier. Het is onbekend of gelijktijdige blootstelling aan tenofovir en remmers van deze kanalen leidt tot meer nierschade. Voorbeelden van deze remmers zijn ibuprofen, diclofenac, sildenafil, en acetylsalicylzuur. In 721 HIV geïnfecteerde patiënten op tenofovir is deze blootstelling gemeten. Deze remmers blijken wel geassocieerd met een verhoogd risico op nierfunctieachteruitgang, maar niet met problemen in de niertubulus. Met deze studie kan

extra nierschade in HIV geïnfecteerde patiënten door blootstelling aan deze zonder recept verkrijgbare remmers en tenofovir disoproxil-fumaraat niet worden uitgesloten.

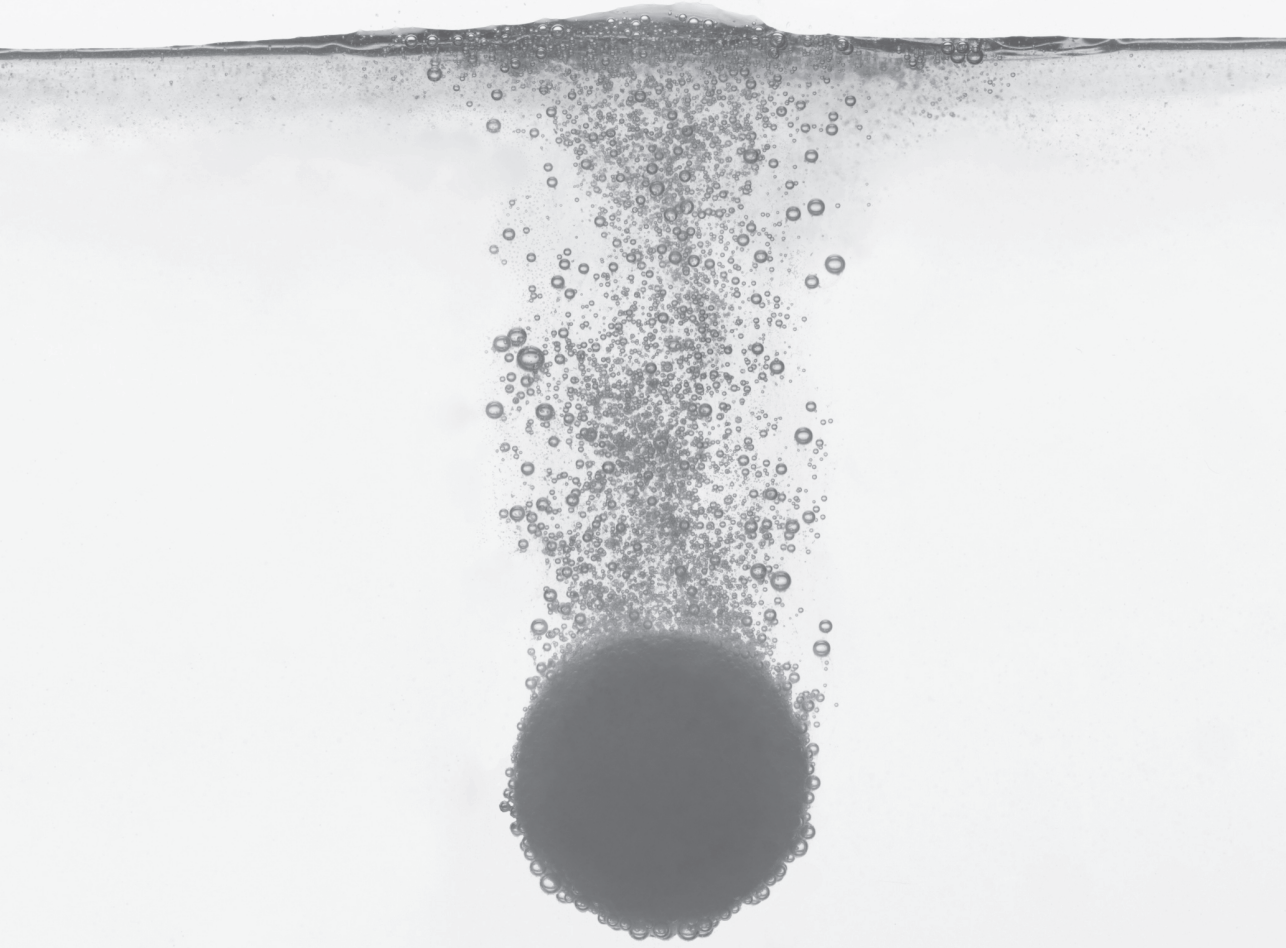
In **hoofdstuk 8** belichten wij het risico op hart- en vaatziektes na het wisselen van bepaalde HIV medicijnen. Het immuunsysteem van een HIV geïnfecteerde patiënt is geactiveerd doordat HIV niet te genezen is. Immuunactivatie verhoogt het risico op hart- en vaatziektes. Een ongunstig lipidenprofiel -dyslipidemie- is een belangrijke beïnvloedbare risicofactor in de preventie van hart- en vaatziektes. HIV medicatie kan dyslipidemie verergeren. Nevirapine geeft minder dyslipidemie, maar nevirapine is als HIV medicijn minder effectief. Het gebruik van andere HIV medicijnen kan daarom de voorkeur hebben. In een klinische studie wisselen 50 HIV geïnfecteerde patiënten nevirapine in voor rilpivirine. De lipidenprofielen veranderen significant. Dit leidt echter niet tot verandering van het berekende tienjaarsrisico op hart- en vaatziektes. Hieruit concluderen wij dat rilpivirine in plaats van nevirapine gebruikt kan worden zonder het risico op hart- en vaatziektes belangrijk te beïnvloeden. De invloed van de medicatie op dit risicoprofiel dient te worden meegewogen in de keuze voor een optimale behandeling. De HIV behandeling is dus een beïnvloedbare risicofactor voor hart- en vaatziektes.

In **hoofdstuk 9** en **hoofstuk 10** onderzoeken wij de behandeling van het Kaposi sarcoom en de multicentrische variant van de ziekte van Castleman in HIV. Deze kankersoorten zijn vaak geassocieerd met een humaan herpesvirus type 8 infectie en komen vaker voor bij HIV geïnfecteerde patiënten dan in de algemene bevolking. Beide kunnen ontstaan ondanks adequate behandeling van HIV. De behandeling bestaat dan uit chemotherapie. Effectieve immuuntherapieën kunnen het gebruik van chemotherapie voorkomen. Peginterferon blijkt in onze studie effectief in HIV geïnfecteerde patiënten met een gevorderd Kaposi sarcoom. De monoklonale antistof rituximab is effectiever dan chemotherapie als behandeling van patiënten met de ziekte van Castleman. Immuuntherapie van een gevorderd Kaposi sarcoom of de ziekte van Castleman lijken effectieve therapieën in HIV geïnfecteerde patiënten.

In het laatst deel van dit proefschrift bespreken wij de perspectieven van de HIV behandeling. Daarnaast geven we een samenvattende discussie van onze studies. In **hoofdstuk 11** belichten wij de toekomstige aanpak van HIV. Het actief opsporen van HIV infecties en direct behandelen leidt tot minder sterfte en verlaagt de transmissie van HIV. Daarnaast resulteert het starten van een behandeling tijdens een vroege HIV

infectie tot een beperking van het reservoir en immuunschade. Dit lijken gunstige voorwaarden voor toekomstige strategieën om HIV te genezen. De precieze aard en locatie van het virale reservoir en manieren om het reservoir te meten en te manipuleren zijn de uitdagingen van de toekomst. Uiteindelijk zal dit leiden tot de identificatie van patiënten bij wie de antiretrovirale behandeling succesvol gestopt zal kunnen worden.

De inzichten van dit proefschrift worden in **hoofstuk 12** besproken. De naar mijn mening belangrijkste conclusies zijn: (1) eerstelijnstherapie met tenofovir disoproxilfumaraat dient bij voorkeur geen lamivudine te bevatten wanneer emtricitabine beschikbaar is, (2) medicatieveranderingen naar één pil met een combinatie van medicijnen of monotherapie zijn mogelijk in diverse klinische situaties waarbij interacties, resistentie en comorbiditeit geen beperkende factoren hoeven te zijn, (3) nierschade bij tenofovir is geassocieerd met nieuwe factoren, het risico op hart- en vaatziektes wordt niet beïnvloed door het gebruik van rilpivirine in plaats van nevirapine, immuuntherapieën zijn effectief voor het Kaposi sarcoom en de ziekte van Castleman en (4) de toekomstige klinische aanpak van HIV omvat de behandeling van vroege HIV infecties en strategieën om HIV te genezen. Het overkoepelende doel van de studies in dit proefschrift is om bij te dragen aan een effectieve en veilige HIV behandeling voor alle HIV geïnfecteerde patiënten. De uitdaging ligt erin om te komen tot een verdere verbetering van deze behandeling en aanpak van comorbiditeit totdat we een definitieve oplossing vinden om HIV te genezen.



Chapter 14

References

1. Barre-Sinoussi F, Chermann JC, Rey F et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983; **220**: 868-71.
2. UNAIDS Global Statistics. http://www.unaids.org/en/resources/campaigns/2014/2014gapreport/factsheet.
3. Faria NR, Rambaut A, Suchard MA et al. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science* 2014; **346**: 56-61.
4. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med* 2011; **1**: a006841.
5. Gilbert MT, Rambaut A, Wlasiuk G et al. The emergence of HIV/AIDS in the Americas and beyond. *Proc Natl Acad Sci U S A* 2007; **104**: 18566-70.
6. Centers for Disease Control. Pneumocystis pneumonia--Los Angeles. *MMWR Morb Mortal Wkly Rep* 1981; **30**: 250-2.
7. Centers for Disease Control. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. *MMWR Morb Mortal Wkly Rep* 1981; **30**: 305-8.
8. UNAIDS Global Report 2015. http://www.unaids.org/sites/default/files/media_asset/AIDS_by_the_numbers_2015_en.pdf.
9. Muesing MA, Smith DH, Cabradilla CD et al. Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. *Nature* 1985; **313**: 450-8.
10. Stremlau M, Owens CM, Perron MJ et al. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 2004; **427**: 848-53.
11. Kohlstaedt LA, Wang J, Friedman JM et al. Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* 1992; **256**: 1783-90.
12. Sattentau QJ, Dalgleish AG, Weiss RA et al. Epitopes of the CD4 antigen and HIV infection. *Science* 1986; **234**: 1120-3.
13. Romani B, Engelbrecht S, Glashoff RH. Functions of Tat: the versatile protein of human immunodeficiency virus type 1. *J Gen Virol* 2010; **91**: 1-12.
14. Deng L, de la Fuente C, Fu P et al. Acetylation of HIV-1 Tat by CBP/P300 increases transcription of integrated HIV-1 genome and enhances binding to core histones. *Virology* 2000; **277**: 278-95.
15. Grewe B, Uberla K. The human immunodeficiency virus type 1 Rev protein: menage a trois during the early phase of the lentiviral replication cycle. *J Gen Virol* 2010; **91**: 1893-7.
16. Stoltzfus CM, Madsen JM. Role of viral splicing elements and cellular RNA binding proteins in regulation of HIV-1 alternative RNA splicing. *Curr HIV Res* 2006; **4**: 43-55.
17. Lama J, Mangasarian A, Trono D. Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef- and Vpu-inhibitable manner. *Curr Biol* 1999; **9**: 622-31.
18. Usami Y, Wu Y, Gottlinger HG. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature* 2015; **526**: 218-23.
19. Collins KL, Chen BK, Kalams SA et al. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* 1998; **391**: 397-401.
20. Zheng YH, Jeang KT, Tokunaga K. Host restriction factors in retroviral infection: promises in virus-host interaction. *Retrovirology* 2012; **9**: 112.
21. Kogan M, Rappaport J. HIV-1 accessory protein Vpr: relevance in the pathogenesis of HIV and potential for therapeutic intervention. *Retrovirology* 2011; **8**: 25.
22. Magadan JG, Bonifacino JS. Transmembrane domain determinants of CD4 Downregulation by HIV-1 Vpu. *J Virol* 2012; **86**: 757-72.
23. Gray RH, Wawer MJ, Brookmeyer R et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 2001; **357**: 1149-53.

24. Boily MC, Baggaley RF, Wang L et al. Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. *Lancet Infect Dis* 2009; **9**: 118-29.
25. Baggaley RF, White RG, Boily MC. HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. *Int J Epidemiol* 2010; **39**: 1048-63.
26. Keele BF, Giorgi EE, Salazar-Gonzalez JF et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* 2008; **105**: 7552-7.
27. de Witte L, Nabatov A, Pion M et al. Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat Med* 2007; **13**: 367-71.
28. de Jong MA, de Witte L, Oudhoff MJ et al. TNF-alpha and TLR agonists increase susceptibility to HIV-1 transmission by human Langerhans cells ex vivo. *J Clin Invest* 2008; **118**: 3440-52.
29. Kawamura T, Cohen SS, Borris DL et al. Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. *J Exp Med* 2000; **192**: 1491-500.
30. Feinberg H, Rowntree TJ, Tan SL et al. Common polymorphisms in human langerin change specificity for glycan ligands. *J Biol Chem* 2013; **288**: 36762-71.
31. Hussain LA, Lehner T. Comparative investigation of Langerhans' cells and potential receptors for HIV in oral, genitourinary and rectal epithelia. *Immunology* 1995; **85**: 475-84.
32. Geijtenbeek TB, Kwon DS, Torensma R et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* 2000; **100**: 587-97.
33. Deng H, Liu R, Ellmeier W et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996; **381**: 661-6.
34. Feng Y, Broder CC, Kennedy PE et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 1996; **272**: 872-7.
35. Weissenhorn W, Dessen A, Harrison SC et al. Atomic structure of the ectodomain from HIV-1 gp41. *Nature* 1997; **387**: 426-30.
36. Gallo SA, Puri A, Blumenthal R. HIV-1 gp41 six-helix bundle formation occurs rapidly after the engagement of gp120 by CXCR4 in the HIV-1 Env-mediated fusion process. *Biochemistry* 2001; **40**: 12231-6.
37. Dimitrov AS, Rawat SS, Jiang S et al. Role of the fusion peptide and membrane-proximal domain in HIV-1 envelope glycoprotein-mediated membrane fusion. *Biochemistry* 2003; **42**: 14150-8.
38. Rosen O, Sharon M, Quadt-Akabayov SR et al. Molecular switch for alternative conformations of the HIV-1 V3 region: implications for phenotype conversion. *Proc Natl Acad Sci U S A* 2006; **103**: 13950-5.
39. Long EM, Rainwater SM, Lavreys L et al. HIV type 1 variants transmitted to women in Kenya require the CCR5 coreceptor for entry, regardless of the genetic complexity of the infecting virus. *AIDS Res Hum Retroviruses* 2002; **18**: 567-76.
40. Ananworanich J, Schuetz A, Vandergeeten C et al. Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. *PLoS One* 2012; **7**: e33948.
41. Mattapallil JJ, Douek DC, Hill B et al. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* 2005; **434**: 1093-7.
42. Samson M, Libert F, Doranz BJ et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 1996; **382**: 722-5.
43. Martinson JJ, Chapman NH, Rees DC et al. Global distribution of the CCR5 gene 32-basepair deletion. *Nat Genet* 1997; **16**: 100-3.
44. Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* 1970; **226**: 1211-3.
45. Baltimore D. RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 1970; **226**: 1209-11.

46. Cohn LB, Silva IT, Oliveira TY et al. HIV-1 integration landscape during latent and active infection. *Cell* 2015; **160**: 420-32.
47. Mitchell RS, Beitzel BF, Schroder AR et al. Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. *PLoS Biol* 2004; **2**: E234.
48. Wagner TA, McLaughlin S, Garg K et al. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 2014; **345**: 570-3.
49. Maldarelli F, Wu X, Su L et al. HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 2014; **345**: 179-83.
50. Folks T, Powell DM, Lightfoote MM et al. Induction of HTLV-III/LAV from a nonvirus-producing T-cell line: implications for latency. *Science* 1986; **231**: 600-2.
51. Laguette N, Bregnard C, Benichou S et al. Human immunodeficiency virus (HIV) type-1, HIV-2 and simian immunodeficiency virus Nef proteins. *Mol Aspects Med* 2010; **31**: 418-33.
52. Perelson AS, Neumann AU, Markowitz M et al. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* 1996; **271**: 1582-6.
53. Ribeiro RM, Qin L, Chavez LL et al. Estimation of the initial viral growth rate and basic reproductive number during acute HIV-1 infection. *J Virol* 2010; **84**: 6096-102.
54. Bonhoeffer S, Funk GA, Gunthard HF et al. Glancing behind virus load variation in HIV-1 infection. *Trends Microbiol* 2003; **11**: 499-504.
55. Ho DD, Neumann AU, Perelson AS et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995; **373**: 123-6.
56. Chun TW, Carruth L, Finzi D et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997; **387**: 183-8.
57. Dimitrov DS, Willey RL, Sato H et al. Quantitation of human immunodeficiency virus type 1 infection kinetics. *J Virol* 1993; **67**: 2182-90.
58. Roberts JD, Bebenek K, Kunkel TA. The accuracy of reverse transcriptase from HIV-1. *Science* 1988; **242**: 1171-3.
59. Lauring AS, Andino R. Quasispecies theory and the behavior of RNA viruses. *PLoS Pathog* 2010; **6**: e1001005.
60. Fiebig EW, Wright DJ, Rawal BD et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003; **17**: 1871-9.
61. Mehandru S, Poles MA, Tenner-Racz K et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* 2004; **200**: 761-70.
62. Vanhems P, Hughes J, Collier AC et al. Comparison of clinical features, CD4 and CD8 responses among patients with acute HIV-1 infection from Geneva, Seattle and Sydney. *AIDS* 2000; **14**: 375-81.
63. Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. *N Engl J Med* 1998; **339**: 33-9.
64. Galvin SR, Cohen MS. The role of sexually transmitted diseases in HIV transmission. *Nat Rev Microbiol* 2004; **2**: 33-42.
65. Brenner BG, Roger M, Routy JP et al. High rates of forward transmission events after acute/early HIV-1 infection. *J Infect Dis* 2007; **195**: 951-9.
66. Doitsh G, Cavrois M, Lassen KG et al. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. *Cell* 2010; **143**: 789-801.
67. Doitsh G, Galloway NL, Geng X et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; **505**: 509-14.

68. Centers for Disease Control and Prevention: HIV Classification. http://www.cdc.gov/hiv/.
69. Jacquelin B, Petitjean G, Kunkel D et al. Innate immune responses and rapid control of inflammation in African green monkeys treated or not with interferon-alpha during primary SIVagm infection. *PLoS Pathog* 2014; **10**: e1004241.
70. Silvestri G, Sodora DL, Koup RA et al. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity* 2003; **18**: 441-52.
71. Brenchley JM, Silvestri G, Douek DC. Nonprogressive and progressive primate immunodeficiency lentivirus infections. *Immunity* 2010; **32**: 737-42.
72. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010; **140**: 805-20.
73. Alter G, Teigen N, Ahern R et al. Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. *J Infect Dis* 2007; **195**: 1452-60.
74. Alter G, Heckerman D, Schneidewind A et al. HIV-1 adaptation to NK-cell-mediated immune pressure. *Nature* 2011; **476**: 96-100.
75. Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. *Annu Rev Med* 2003; **54**: 535-51.
76. Dinter J, Duong E, Lai NY et al. Variable processing and cross-presentation of HIV by dendritic cells and macrophages shapes CTL immunodominance and immune escape. *PLoS Pathog* 2015; **11**: e1004725.
77. Fellay J, Shianna KV, Ge D et al. A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007; **317**: 944-7.
78. Goulder PJ, Phillips RE, Colbert RA et al. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat Med* 1997; **3**: 212-7.
79. Leslie AJ, Pfafferott KJ, Chetty P et al. HIV evolution: CTL escape mutation and reversion after transmission. *Nat Med* 2004; **10**: 282-9.
80. Altfeld M, Kalife ET, Qi Y et al. HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial CD8(+) T Cell Response against HIV-1. *PLoS Med* 2006; **3**: e403.
81. International HIV Controllers Study. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. *Science* 2010; **330**: 1551-7.
82. Klatt NR, Silvestri G. CD4+ T cells and HIV: A paradoxical Pas de Deux. *Sci Transl Med* 2012; **4**: 123ps4.
83. Soghoian DZ, Jessen H, Flanders M et al. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. *Sci Transl Med* 2012; **4**: 123ra25.
84. Douek DC, Brenchley JM, Betts MR et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 2002; **417**: 95-8.
85. Chevalier MF, Julg B, Pyo A et al. HIV-1-specific interleukin-21+ CD4+ T cell responses contribute to durable viral control through the modulation of HIV-specific CD8+ T cell function. *J Virol* 2011; **85**: 733-41.
86. Favre D, Lederer S, Kanwar B et al. Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. *PLoS Pathog* 2009; **5**: e1000295.
87. Perreau M, Savoye AL, De Crignis E et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J Exp Med* 2013; **210**: 143-56.
88. Scarlatti G, Tresoldi E, Bjorndal A et al. In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nat Med* 1997; **3**: 1259-65.
89. Weiss L, Donkova-Petrini V, Caccavelli L et al. Human immunodeficiency virus-driven expansion of CD4+CD25+ regulatory T cells, which suppress HIV-specific CD4 T-cell responses in HIV-infected patients. *Blood* 2004; **104**: 3249-56.

90. Brockman MA, Schneidewind A, Lahaie M et al. Escape and compensation from early HLA-B57-mediated cytotoxic T-lymphocyte pressure on human immunodeficiency virus type 1 Gag alter capsid interactions with cyclophilin A. *J Virol* 2007; **81**: 12608-18.
91. Brumme ZL, Brumme CJ, Heckerman D et al. Evidence of differential HLA class I-mediated viral evolution in functional and accessory/regulatory genes of HIV-1. *PLoS Pathog* 2007; **3**: e94.
92. Brumme ZL, Brumme CJ, Carlson J et al. Marked epitope- and allele-specific differences in rates of mutation in human immunodeficiency type 1 (HIV-1) Gag, Pol, and Nef cytotoxic T-lymphocyte epitopes in acute/early HIV-1 infection. *J Virol* 2008; **82**: 9216-27.
93. Cohen K, Altfeld M, Alter G et al. Early preservation of CXCR5+ PD-1+ helper T cells and B cell activation predict the breadth of neutralizing antibody responses in chronic HIV-1 infection. *J Virol* 2014; **88**: 13310-21.
94. Deeks SG, Schweighardt B, Wrin T et al. Neutralizing antibody responses against autologous and heterologous viruses in acute versus chronic human immunodeficiency virus (HIV) infection: evidence for a constraint on the ability of HIV to completely evade neutralizing antibody responses. *J Virol* 2006; **80**: 6155-64.
95. Mikell I, Sather DN, Kalams SA et al. Characteristics of the earliest cross-neutralizing antibody response to HIV-1. *PLoS Pathog* 2011; **7**: e1001251.
96. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J Pathol* 2008; **214**: 231-41.
97. Brenchley JM, Price DA, Schacker TW et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; **12**: 1365-71.
98. Hunt PW, Sinclair E, Rodriguez B et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. *J Infect Dis* 2014; **210**: 1228-38.
99. Streeck H, Brumme ZL, Anastario M et al. Antigen load and viral sequence diversification determine the functional profile of HIV-1-specific CD8+ T cells. *PLoS Med* 2008; **5**: e100.
100. Quigley M, Pereyra F, Nilsson B et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nat Med* 2010; **16**: 1147-51.
101. Paiardini M, Muller-Trutwin M. HIV-associated chronic immune activation. *Immunol Rev* 2013; **254**: 78-101.
102. Finzi D, Blankson J, Siliciano JD et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 1999; **5**: 512-7.
103. Finzi D, Hermankova M, Pierson T et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997; **278**: 1295-300.
104. Chomont N, El-Far M, Ancuta P et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 2009; **15**: 893-900.
105. Chun TW, Stuyver L, Mizell SB et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* 1997; **94**: 13193-7.
106. Churchill MJ, Deeks SG, Margolis DM et al. HIV reservoirs: what, where and how to target them. *Nat Rev Microbiol* 2016; **14**: 55-60.
107. Palmer S, Maldarelli F, Wiegand A et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci U S A* 2008; **105**: 3879-84.
108. Siliciano JD, Kajdas J, Finzi D et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med* 2003; **9**: 727-8.
109. Whitney JB, Hill AL, Sanisetty S et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 2014; **512**: 74-7.

110. Lorenzo-Redondo R, Fryer HR, Bedford T et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 2016; **530**: 51-6.
111. Rosenberg ES, Billingsley JM, Caliendo AM et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997; **278**: 1447-50.
112. Buchbinder SP, Katz MH, Hessol NA et al. Long-term HIV-1 infection without immunologic progression. *AIDS* 1994; **8**: 1123-8.
113. Saez-Cirion A, Bacchus C, Hocqueloux L et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog* 2013; **9**: e1003211.
114. Kosmrlj A, Read EL, Qi Y et al. Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. *Nature* 2010; **465**: 350-4.
115. Miura T, Brumme CJ, Brockman MA et al. HLA-associated viral mutations are common in human immunodeficiency virus type 1 elite controllers. *J Virol* 2009; **83**: 3407-12.
116. Elahi S, Dinges WL, Lejarcegui N et al. Protective HIV-specific CD8+ T cells evade Treg cell suppression. *Nat Med* 2011; **17**: 989-95.
117. Hersperger AR, Martin JN, Shin LY et al. Increased HIV-specific CD8+ T-cell cytotoxic potential in HIV elite controllers is associated with T-bet expression. *Blood* 2011; **117**: 3799-808.
118. Lambotte O, Ferrari G, Moog C et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS* 2009; **23**: 897-906.
119. Euler Z, van Gils MJ, Bunnik EM et al. Cross-reactive neutralizing humoral immunity does not protect from HIV type 1 disease progression. *J Infect Dis* 2010; **201**: 1045-53.
120. Scheid JF, Mouquet H, Feldhahn N et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature* 2009; **458**: 636-40.
121. Hessell AJ, Hangartner L, Hunter M et al. Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* 2007; **449**: 101-4.
122. Conway JM, Perelson AS. Post-treatment control of HIV infection. *Proc Natl Acad Sci U S A* 2015; **112**: 5467-72.
123. Hutter G, Nowak D, Mossner M et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 2009; **360**: 692-8.
124. Mocroft A, Vella S, Benfield TL et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. *Lancet* 1998; **352**: 1725-30.
125. Menendez-Arias L. Mechanisms of resistance to nucleoside analogue inhibitors of HIV-1 reverse transcriptase. *Virus Res* 2008; **134**: 124-46.
126. Tanser F, Barnighausen T, Grapsa E et al. High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. *Science* 2013; **339**: 966-71.
127. Nichols BE, Boucher CAB, van de Vijver DAMC. HIV testing and antiretroviral treatment strategies for prevention of HIV infection: impact on antiretroviral drug resistance. *J Intern Med* 2011; **270**: 532-49.
128. Granich RM, Gilks CF, Dye C et al. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. *Lancet* 2009; **373**: 48-57.
129. Cohen MS, Chen YQ, McCauley M et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; **365**: 493-505.
130. Maggiolo F, Callegaro A, Cologni G et al. Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. *J Acquir Immune Defic Syndr* 2012; **60**: 473-82.
131. Gandhi RT, Zheng L, Bosch RJ et al. The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. *PLoS Med* 2010; **7**.

132. Nettles RE, Kieffer TL, Kwon P et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. *Jama* 2005; **293**: 817-29.
133. Pasternak AO, Lukashov VV, Berkhout B. Cell-associated HIV RNA: a dynamic biomarker of viral persistence. *Retrovirology* 2013; **10**: 41.
134. Furtado MR, Callaway DS, Phair JP et al. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. *N Engl J Med* 1999; **340**: 1614-22.
135. Pasternak AO, de Bruin M, Jurriaans S et al. Modest nonadherence to antiretroviral therapy promotes residual HIV-1 replication in the absence of virological rebound in plasma. *J Infect Dis* 2012; **206**: 1443-52.
136. Doyle T, Smith C, Vitiello P et al. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. *Clin Infect Dis* 2012; **54**: 724-32.
137. Antiretroviral Therapy Cohort Collaboration. Impact of low-level viremia on clinical and virological outcomes in treated HIV-1-infected patients. *AIDS* 2015; **29**: 373-83.
138. Laprise C, de Pokomandy A, Baril JG et al. Virologic failure following persistent low-level viremia in a cohort of HIV-positive patients: results from 12 years of observation. *Clin Infect Dis* 2013; **57**: 1489-96.
139. Taiwo B, Gallien S, Aga E et al. Antiretroviral drug resistance in HIV-1-infected patients experiencing persistent low-level viremia during first-line therapy. *J Infect Dis* 2011; **204**: 515-20.
140. Ribeiro RM, Bonhoeffer S. Production of resistant HIV mutants during antiretroviral therapy. *Proc Natl Acad Sci U S A* 2000; **97**: 7681-6.
141. Bonhoeffer S, Nowak MA. Pre-existence and emergence of drug resistance in HIV-1 infection. *Proc Biol Sci* 1997; **264**: 631-7.
142. Gopalakrishnan S, Montazeri H, Menz S et al. Estimating HIV-1 fitness characteristics from cross-sectional genotype data. *PLoS Comput Biol* 2014; **10**: e1003886.
143. Tisdale M, Kemp SD, Parry NR et al. Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci U S A* 1993; **90**: 5653-6.
144. Paterson DL, Swindells S, Mohr J et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med* 2000; **133**: 21-30.
145. Maggiolo F, Ravasio L, Ripamonti D et al. Similar adherence rates favor different virologic outcomes for patients treated with nonnucleoside analogues or protease inhibitors. *Clin Infect Dis* 2005; **40**: 158-63.
146. Miller MD, Haddad M, Su C et al. Trends in HIV-1 reverse transcriptase resistance-associated mutations and antiretroviral prescription data from 2003-2010. *Antivir Ther* 2012; **17**: 993-9.
147. Hofstra LM, Sauvageot N, Albert J et al. Transmission of HIV Drug Resistance and the Predicted Effect on Current First-line Regimens in Europe. *Clin Infect Dis* 2015.
148. Boender TS, Hoenderboom BM, Sigaloff KC et al. Pretreatment HIV Drug Resistance Increases Regimen Switches in Sub-Saharan Africa. *Clin Infect Dis* 2015; **61**: 1749-58.
149. Pingen M, Sarrami-Forooshani R, Wensing AM et al. Diminished transmission of drug resistant HIV-1 variants with reduced replication capacity in a human transmission model. *Retrovirology* 2014; **11**: 113.
150. Brenner BG, Routy JP, Petrella M et al. Persistence and fitness of multidrug-resistant human immunodeficiency virus type 1 acquired in primary infection. *J Virol* 2002; **76**: 1753-61.
151. Hofstra LM, Nijhuis M, Pingen M et al. Evolution and viral characteristics of a long-term circulating resistant HIV-1 strain in a cluster of treatment-naive patients. *J Antimicrob Chemother* 2013; **68**: 1246-50.
152. Pingen M, Nijhuis M, de Bruijn JA et al. Evolutionary pathways of transmitted drug-resistant HIV-1. *J Antimicrob Chemother* 2011; **66**: 1467-80.

153. Pingen M, Wensing AM, Fransen K et al. Persistence of frequently transmitted drug-resistant HIV-1 variants can be explained by high viral replication capacity. *Retrovirology* 2014; **11**: 105.
154. Wittkop L, Gunthard HF, de Wolf F et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. *Lancet Infect Dis* 2011; **11**: 363-71.
155. Johnson JA, Li JF, Wei X et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. *PLoS Med* 2008; **5**: e158.
156. Metzner KJ, Giulieri SG, Knoepfel SA et al. Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naive and -adherent patients. *Clin Infect Dis* 2009; **48**: 239-47.
157. Concorde: MRC/ANRS randomised double-blind controlled trial of immediate and deferred zidovudine in symptom-free HIV infection. Concorde Coordinating Committee. *Lancet* 1994; **343**: 871-81.
158. Volberding PA, Lagakos SW, Koch MA et al. Zidovudine in asymptomatic human immunodeficiency virus infection. A controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. The AIDS Clinical Trials Group of the National Institute of Allergy and Infectious Diseases. *N Engl J Med* 1990; **322**: 941-9.
159. Hammer SM, Katzenstein DA, Hughes MD et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. AIDS Clinical Trials Group Study 175 Study Team. *N Engl J Med* 1996; **335**: 1081-90.
160. Delta: a randomised double-blind controlled trial comparing combinations of zidovudine plus didanosine or zalcitabine with zidovudine alone in HIV-infected individuals. Delta Coordinating Committee. *Lancet* 1996; **348**: 283-91.
161. Montaner JS, Reiss P, Cooper D et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. *Jama* 1998; **279**: 930-7.
162. Gulick RM, Mellors JW, Havlir D et al. Simultaneous vs sequential initiation of therapy with indinavir, zidovudine, and lamivudine for HIV-1 infection: 100-week follow-up. *Jama* 1998; **280**: 35-41.
163. European AIDS Clinical Society (EACS) HIV Treatment Guidelines. http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html.
164. Department of Health and Human Services (DHHS) HIV Treatment Guidelines. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.
165. Infectious Diseases Society of America (IDSA) HIV Treatment Guidelines. http://www.idsociety.org/Organism/#HIV/AIDS.
166. British HIV Association (BHIVA) HIV Treatment Guidelines. http://www.bhiva.org/guidelines.aspx.
167. World Health Organization (WHO) HIV Treatment Guidelines. http://www.who.int/hiv/pub/guidelines/en/.
168. HIV Drug Resistance Database. http://hivdb.stanford.edu/.
169. Larder BA, Bloor S, Kemp SD et al. A family of insertion mutations between codons 67 and 70 of human immunodeficiency virus type 1 reverse transcriptase confer multinucleoside analog resistance. *Antimicrob Agents Chemother* 1999; **43**: 1961-7.
170. Winters MA, Coolley KL, Cheng P et al. Genotypic, phenotypic, and modeling studies of a deletion in the beta3-beta4 region of the human immunodeficiency virus type 1 reverse transcriptase gene that is associated with resistance to nucleoside reverse transcriptase inhibitors. *J Virol* 2000; **74**: 10707-13.
171. Hightower KE, Wang R, Deanda F et al. Dolutegravir (S/GSK1349572) exhibits significantly slower dissociation than raltegravir and elvitegravir from wild-type and integrase inhibitor-resistant HIV-1 integrase-DNA complexes. *Antimicrob Agents Chemother* 2011; **55**: 4552-9.

172. Grijsen ML, Steingrover R, Wit FWNM et al. No Treatment versus 24 or 60 Weeks of Antiretroviral Treatment during Primary HIV Infection: The Randomized Primo-SHM Trial. *Plos Medicine* 2012; **9**.
173. Serrano-Villar S, Sainz T, Lee SA et al. HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality. *Plos Pathogens* 2014; **10**.
174. Insight START Study Group. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med* 2015; **373**: 795-807.
175. Investigators ST, Fidler S, Porter K et al. Short-course antiretroviral therapy in primary HIV infection. *N Engl J Med* 2013; **368**: 207-17.
176. Abgrall S, Ingle SM, May MT et al. Durability of first ART regimen and risk factors for modification, interruption or death in HIV-positive patients starting ART in Europe and North America 2002-2009. *AIDS* 2013; **27**: 803-13.
177. Smith CJ, Ryom L, Weber R et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* 2014; **384**: 241-8.
178. Schouten J, Wit FW, Stolte IG et al. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study. *Clin Infect Dis* 2014; **59**: 1787-97.
179. D. A. D. Study Group. Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med* 2007; **356**: 1723-35.
180. D. A. D. Study Group. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet* 2008; **371**: 1417-26.
181. Cooper RD, Wiebe N, Smith N et al. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010; **51**: 496-505.
182. Ryom L, Mocroft A, Kirk O et al. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis* 2013; **207**: 1359-69.
183. Clifford GM, Polesel J, Rickenbach M et al. Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. *J Natl Cancer Inst* 2005; **97**: 425-32.
184. Powles T, Stebbing J, Bazeos A et al. The role of immune suppression and HHV-8 in the increasing incidence of HIV-associated multicentric Castleman's disease. *Ann Oncol* 2009; **20**: 775-9.
185. Robinson D, Jr., Reynolds M, Casper C et al. Clinical epidemiology and treatment patterns of patients with multicentric Castleman disease: results from two US treatment centres. *Br J Haematol* 2014; **165**: 39-48.
186. Walensky RP, Sax PE, Nakamura YM et al. Economic savings versus health losses: the cost-effectiveness of generic antiretroviral therapy in the United States. *Ann Intern Med* 2013; **158**: 84-92.
187. Rousseau FS, Wakeford C, Mommeja-Marin H et al. Prospective randomized trial of emtricitabine versus lamivudine short-term monotherapy in human immunodeficiency virus-infected patients. *J Infect Dis* 2003; **188**: 1652-8.
188. Sax PE, Tierney C, Collier AC et al. Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. *N Engl J Med* 2009; **361**: 2230-40.
189. Post FA, Moyle GJ, Stellbrink HJ et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. *J Acquir Immune Defic Syndr* 2010; **55**: 49-57.

190. Gallant JE, DeJesus E, Arribas JR et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N Engl J Med* 2006; **354**: 251-60.
191. Pozniak AL, Gallant JE, DeJesus E et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naive patients: virologic, immunologic, and morphologic changes--a 96-week analysis. *J Acquir Immune Defic Syndr* 2006; **43**: 535-40.
192. Arribas JR, Pozniak AL, Gallant JE et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. *J Acquir Immune Defic Syndr* 2008; **47**: 74-8.
193. Tang MW, Kanki PJ, Shafer RW. A review of the virological efficacy of the 4 World Health Organization-recommended tenofovir-containing regimens for initial HIV therapy. *Clin Infect Dis* 2012; **54**: 862-75.
194. Maserati R, De Silvestri A, Uglietti A et al. Emerging mutations at virological failure of HAART combinations containing tenofovir and lamivudine or emtricitabine. *AIDS* 2010; **24**: 1013-8.
195. Marcelin AG, Charpentier C, Wirden M et al. Resistance profiles of emtricitabine and lamivudine in tenofovir-containing regimens. *J Antimicrob Chemother* 2012; **67**: 1475-8.
196. Svicher V, Alteri C, Artese A et al. Different evolution of genotypic resistance profiles to emtricitabine versus lamivudine in tenofovir-containing regimens. *J Acquir Immune Defic Syndr* 2010; **55**: 336-44.
197. Daar ES, Tierney C, Fischl MA et al. Atazanavir plus ritonavir or efavirenz as part of a 3-drug regimen for initial treatment of HIV-1. *Ann Intern Med* 2011; **154**: 445-56.
198. Campbell TB, Smeaton LM, Kumarasamy N et al. Efficacy and safety of three antiretroviral regimens for initial treatment of HIV-1: a randomized clinical trial in diverse multinational settings. *PLoS Med* 2012; **9**: e1001290.
199. Ford N, Shubber Z, Hill A et al. Comparative efficacy of Lamivudine and emtricitabine: a systematic review and meta-analysis of randomized trials. *PLoS One* 2013; **8**: e79981.
200. The Dutch HIV Monitoring Foundation (SHM). HIV monitoring report 2013. http://www.hiv-monitoring.nl/files/5913/8443/2799/SHM_MonitoringReport2013.pdf.
201. SHM Study protocol I13018: http://www.hiv-monitoring.nl/english/research/research-projects/.
202. The Dutch HIV Monitoring Foundation (SHM). Annual report 2012. http://www.hiv-monitoring.nl/index.php/download_file/view/405/371/.
203. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res* 2011; **46**: 399-424.
204. Supplementary data: http://cid.oxfordjournals.org/lookup/suppl/doi:10.1093/cid/ciu763/-/DC1.
205. Feng JY, Anderson KS. Mechanistic studies comparing the incorporation of (+) and (-) isomers of 3'TCTP by HIV-1 reverse transcriptase. *Biochemistry* 1999; **38**: 55-63.
206. Schinazi RF. Assessment of the relative potency of emtricitabine and lamivudine. *J Acquir Immune Defic Syndr* 2003; **34**: 243-5; author reply 5-6.
207. Moore KH, Barrett JE, Shaw S et al. The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. *AIDS* 1999; **13**: 2239-50.
208. Rey D, Hoen B, Chavanet P et al. High rate of early virological failure with the once-daily tenofovir/lamivudine/nevirapine combination in naive HIV-1-infected patients. *J Antimicrob Chemother* 2009; **63**: 380-8.
209. Towner W, Kerrigan H, LaRieve M, et al. Efficacy of a once daily (QD) regimen of nevirapine (NVP), lamivudine (3TC) and tenofovir (TDF) in treatment-naive HIV infected patients: a pilot study. In: Seventh International Congress on Drug Therapy in HIV Infection, Glasgow, Scotland, 14–18 November 2004. Abstract P49.

210. Sanne I, van der Horst C, Shaw A, et al. Two randomized controlled, equivalence trials of emtricitabine (FTC) to lamivudine (3TC). In: XIV International AIDS Conference, Barcelona, Spain, 7–12 July 2002. Abstract 4432.
211. Darin K, Scarsi K, Meloni S, et al. Clinical and virological outcomes of six first line regimens in a large ART program in Nigeria. In: XVIII International AIDS Conference, Vienna, Austria, 18–23 July 2010. Abstract THPE0117.
212. Mulenga L, Muwango A, Moyo C, et al. Efficacy of tenofovir disoproxil fumarate/emtricitabine and tenofovir disoproxil/lamivudine both in combination with efavirenz in antiretroviral-naive, HIV-1 infected Zambians. In: 7th IAS Conference on HIV Pathogenesis, Treatment and Prevention. Kuala Lumpur, Malaysia. 30 June–3 July 2013. Abstract TULBPE18.
213. Benson CA, van der Horst C, Lamarca A et al. A randomized study of emtricitabine and lamivudine in stably suppressed patients with HIV. *AIDS* 2004; **18**: 2269-76.
214. Sterne JA, May M, Costagliola D et al. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. *Lancet* 2009; **373**: 1352-63.
215. Rokx C, Fibriani A, van de Vijver DA et al. Increased virological failure in naive HIV-1-infected patients taking lamivudine compared with emtricitabine in combination with tenofovir and efavirenz or nevirapine in the Dutch nationwide ATHENA cohort. *Clin Infect Dis* 2015; **60**: 143-53.
216. Rokx C, Rijnders BJ. Evidence gathered from randomized clinical trials and observational studies on the equivalence of emtricitabine and Lamivudine. *Clin Infect Dis* 2015; **60**: 1732-3.
217. Ford N, Hill A, Vitoria M et al. Editorial commentary: Comparative efficacy of lamivudine and emtricitabine: comparing the results of randomized trials and cohorts. *Clin Infect Dis* 2015; **60**: 154-6.
218. Sax PE, Tierney C, Collier AC et al. Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results. *J Infect Dis* 2011; **204**: 1191-201.
219. Hill A, Sawyer W. Effects of nucleoside reverse transcriptase inhibitor backbone on the efficacy of first-line boosted highly active antiretroviral therapy based on protease inhibitors: meta-regression analysis of 12 clinical trials in 5168 patients. *HIV Med* 2009; **10**: 527-35.
220. Smith KY, Patel P, Fine D et al. Randomized, double-blind, placebo-matched, multicenter trial of abacavir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for initial HIV treatment. *AIDS* 2009; **23**: 1547-56.
221. Nishijima T, Takano M, Ishisaka M et al. Abacavir/lamivudine versus tenofovir/emtricitabine with atazanavir/ritonavir for treatment-naive Japanese patients with HIV-1 infection: a randomized multicenter trial. *Intern Med* 2013; **52**: 735-44.
222. Nishijima T, Komatsu H, Teruya K et al. Once-daily darunavir/ritonavir and abacavir/lamivudine versus tenofovir/emtricitabine for treatment-naive patients with a baseline viral load of more than 100 000 copies/ml. *AIDS* 2013; **27**: 839-42.
223. The Dutch HIV Monitoring Foundation (SHM). HIV monitoring report 2014. Page 4. http://www.hiv-monitoring.nl/files/8914/1527/1076/SHM_Monitoring_report_2014.pdf.
224. The Dutch HIV Monitoring Foundation (SHM). Annual report 2013. Page 24. http://www.hiv-monitoring.nl/files/2614/0117/8022/144142_HIV_JAARVERSLAG_2013_BW_GB.pdf.
225. Rubin DB. Multiple Imputation for Nonresponse in Surveys. New York, USA. John Wiley & Sons, 1987.
226. Supplementary data: http://onlinelibrary.wiley.com/doi/10.1111/hiv.12355/suppinfo
227. Raffi F, Rachlis A, Stellbrink HJ et al. Once-daily dolutegravir versus raltegravir in antiretroviral-naive adults with HIV-1 infection: 48 week results from the randomised, double-blind, non-inferiority SPRING-2 study. *Lancet* 2013; **381**: 735-43.

228. Clotet B, Feinberg J, van Lunzen J et al. Once-daily dolutegravir versus darunavir plus ritonavir in antiretroviral-naive adults with HIV-1 infection (FLAMINGO): 48 week results from the randomised open-label phase 3b study. *Lancet* 2014; **383**: 2222-31.
229. Martinez E, Arranz JA, Podzamczer D et al. A simplification trial switching from nucleoside reverse transcriptase inhibitors to once-daily fixed-dose abacavir/lamivudine or tenofovir/emtricitabine in HIV-1-infected patients with virological suppression. *J Acquir Immune Defic Syndr* 2009; **51**: 290-7.
230. Martin A, Bloch M, Amin J et al. Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-Lamivudine: a randomized, 96-week trial. *Clin Infect Dis* 2009; **49**: 1591-601.
231. Calza L, Manfredi R, Colangeli V et al. Efficacy and safety of atazanavir-ritonavir plus abacavir-lamivudine or tenofovir-emtricitabine in patients with hyperlipidaemia switched from a stable protease inhibitor-based regimen including one thymidine analogue. *AIDS Patient Care STDS* 2009; **23**: 691-7.
232. Martinez E, d'Albuquerque PM, Perez I et al. Abacavir/lamivudine versus tenofovir/emtricitabine in virologically suppressed patients switching from ritonavir-boosted protease inhibitors to raltegravir. *AIDS Res Hum Retroviruses* 2013; **29**: 235-41.
233. Campo R, DeJesus E, Bredeek UF et al. SWIFT: prospective 48-week study to evaluate efficacy and safety of switching to emtricitabine/tenofovir from lamivudine/abacavir in virologically suppressed HIV-1 infected patients on a boosted protease inhibitor containing antiretroviral regimen. *Clin Infect Dis* 2013; **56**: 1637-45.
234. Shubber Z, Calmy A, Andrieux-Meyer I et al. Adverse events associated with nevirapine and efavirenz-based first-line antiretroviral therapy: a systematic review and meta-analysis. *AIDS* 2013; **27**: 1403-12.
235. Molina JM, Cahn P, Grinsztejn B et al. Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial. *Lancet* 2011; **378**: 238-46.
236. Cohen CJ, Andrade-Villanueva J, Clotet B et al. Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial. *Lancet* 2011; **378**: 229-37.
237. Center For Drug Evaluation And Research. NDA 202-022/N-00 for TMC278 (rilpivirine) IR tablet, 25mg. Page 106. Trial R278474-C108.
http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202022Orig1s000ClinPharmR.pdf.
238. Crauwels H, Vingerhoets J, Ryan R et al. Pharmacokinetic parameters of once-daily rilpivirine following administration of efavirenz in healthy subjects. *Antivir Ther* 2012; **17**: 439-46.
239. Custodio JM, Yin X, Hepner M et al. Effect of food on rilpivirine/emtricitabine/tenofovir disoproxil fumarate, an antiretroviral single-tablet regimen for the treatment of HIV infection. *J Clin Pharmacol* 2014; **54**: 378-85.
240. Common Terminology Criteria for Adverse Events. Version 4.0, May 28, 2009. Published by US Department of Health and Human Services.
http://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf.
241. Burger D, Teulen M, Eerland J et al. The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma: a global proficiency testing program. *Ther Drug Monit* 2011; **33**: 239-43.
242. Holland DT, DiFrancesco R, Connor JD et al. Quality assurance program for pharmacokinetic assay of antiretrovirals: ACTG proficiency testing for pediatric and adult pharmacology support laboratories, 2003 to 2004: a requirement for therapeutic drug monitoring. *Ther Drug Monit* 2006; **28**: 367-74.
243. Center For Drug Evaluation And Research. NDA 202-022/N-00 for TMC278 (rilpivirine) IR tablet, 25mg. Page 346. Table 3: Population pharmacokinetic estimates of rilpivirine 25mg q.d. in antiretroviral treatment naive HIV-1 infected patients.

http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202022Orig1s000ClinPharmR.pdf.

244. Center For Drug Evaluation And Research. NDA 202-022/N-00 for TMC278 (rilpivirine) IR Tablet, 25mg. Page 363. Figure 12: median prediction of the likelihood of response as a function of TMC278 C0h. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202022Orig1s000ClinPharmR.pdf.
245. Mora-Peris B, Watson V, Vera JH et al. Rilpivirine exposure in plasma and sanctuary site compartments after switching from nevirapine-containing combined antiretroviral therapy. *J Antimicrob Chemother* 2014; **69**: 1642-7.
246. Crauwels HM, van Heeswijk RP, Buelens A et al. Impact of food and different meal types on the pharmacokinetics of rilpivirine. *J Clin Pharmacol* 2013; **53**: 834-40.
247. Mills AM, Cohen C, Dejesus E et al. Efficacy and safety 48 weeks after switching from efavirenz to rilpivirine using emtricitabine/tenofovir disoproxil fumarate-based single-tablet regimens. *HIV Clin Trials* 2013; **14**: 216-23.
248. Rokx C, Verbon A, Rijnders BJ. Successful switch to rilpivirine/tenofovir/emtricitabine in HIV-1-infected patients with an isolated K103N mutation acquired during prior nonnucleoside reverse transcriptase inhibitor therapy. *HIV Med* 2014; **15**: 611-4.
249. Azijn H, Tirry I, Vingerhoets J et al. TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. *Antimicrob Agents Chemother* 2010; **54**: 718-27.
250. Palella FJ, Jr., Fisher M, Tebas P et al. Simplification to rilpivirine/emtricitabine/tenofovir disoproxil fumarate from ritonavir-boosted protease inhibitor antiretroviral therapy in a randomized trial of HIV-1 RNA-suppressed participants. *AIDS* 2014; **28**: 335-44.
251. Arasteh K, Rieger A, Yeni P et al. Short-term randomized proof-of-principle trial of TMC278 in patients with HIV type-1 who have previously failed antiretroviral therapy. *Antivir Ther* 2009; **14**: 713-22.
252. Ngo-Giang-Huong N, Jourdain G, Amzal B et al. Resistance patterns selected by nevirapine vs. efavirenz in HIV-infected patients failing first-line antiretroviral treatment: a bayesian analysis. *PLoS One* 2011; **6**: e27427.
253. Asahchop EL, Wainberg MA, Oliveira M et al. Distinct resistance patterns to etravirine and rilpivirine in viruses containing nonnucleoside reverse transcriptase inhibitor mutations at baseline. *AIDS* 2013; **27**: 879-87.
254. Anta L, Llibre JM, Poveda E et al. Rilpivirine resistance mutations in HIV patients failing non-nucleoside reverse transcriptase inhibitor-based therapies. *AIDS* 2013; **27**: 81-5.
255. Gupta R, Hill A, Sawyer AW et al. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. *Clin Infect Dis* 2008; **47**: 712-22.
256. Santoro MM, Sabin C, Forbici F et al. Drug-resistance development differs between HIV-1-infected patients failing first-line antiretroviral therapy containing nonnucleoside reverse transcriptase inhibitors with and without thymidine analogues. *HIV Med* 2013; **14**: 571-7.
257. DeAnda F, Hightower KE, Nolte RT et al. Dolutegravir interactions with HIV-1 integrase-DNA: structural rationale for drug resistance and dissociation kinetics. *PLoS One* 2013; **8**: e77448.
258. Kobayashi M, Yoshinaga T, Seki T et al. In Vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. *Antimicrob Agents Chemother* 2011; **55**: 813-21.
259. Stellbrink HJ, Reynes J, Lazzarin A et al. Dolutegravir in antiretroviral-naive adults with HIV-1: 96-week results from a randomized dose-ranging study. *AIDS* 2013; **27**: 1771-8.
260. Walmsley SL, Antela A, Clumeck N et al. Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection. *N Engl J Med* 2013; **369**: 1807-18.

261. Cahn P, Pozniak AL, Mingrone H et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. *Lancet* 2013; **382**: 700-8.
262. Castagna A, Maggiolo F, Penco G et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. *J Infect Dis* 2014; **210**: 354-62.
263. Eron JJ, Clotet B, Durant J et al. Safety and efficacy of dolutegravir in treatment-experienced subjects with raltegravir-resistant HIV type 1 infection: 24-week results of the VIKING Study. *J Infect Dis* 2013; **207**: 740-8.
264. Akil B, Blick G, Hagins DP et al. Dolutegravir versus placebo in subjects harbouring HIV-1 with integrase inhibitor resistance associated substitutions: 48-week results from VIKING-4, a randomized study. *Antivir Ther* 2015; **20**: 343-8.
265. Cain LE, Phillips A, Lodi S et al. The effect of efavirenz versus nevirapine-containing regimens on immunologic, virologic and clinical outcomes in a prospective observational study. *AIDS* 2012; **26**: 1691-705.
266. Weller S, Borland J, Chen S et al. Pharmacokinetics of dolutegravir in HIV-seronegative subjects with severe renal impairment. *Eur J Clin Pharmacol* 2014; **70**: 29-35.
267. Song I, Borland J, Arya N et al. Pharmacokinetics of dolutegravir when administered with mineral supplements in healthy adult subjects. *J Clin Pharmacol* 2015; **55**: 490-6.
268. Pulido F, Arribas JR, Delgado R et al. Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and two nucleosides for maintenance therapy of HIV. *AIDS* 2008; **22**: F1-9.
269. Castagna A, Spagnuolo V, Galli L et al. Simplification to atazanavir/ritonavir monotherapy for HIV-1 treated individuals on virological suppression: 48-week efficacy and safety results. *AIDS* 2014; **28**: 2269-79.
270. Antinori A, Arribas J, Fehr J et al. The PROTEA trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV-1 RNA below 50 copies/mL. *J Int AIDS Soc* 2014; **17**: 19525.
271. Paton NI, Stohr W, Arenas-Pinto A et al. Protease inhibitor monotherapy for long-term management of HIV infection: a randomised, controlled, open-label, non-inferiority trial. *Lancet HIV* 2015; **2**: e417-26.
272. Cahn P, et al. Dolutegravir-Lamivudine as Initial Therapy in HIV-Infected, ARV Naive Patients: First Results of the PADDLE Trial. Presented at: European AIDS Conference (EACS) 22 October 2015, Barcelona, Spain.
273. Martinez E, et al. Dolutegravir Monotherapy in HIV-infected Patients with Sustained Viral Suppression: A 24-week Pilot Study. Presented at: European AIDS Conference (EACS) 22 October 2015, Barcelona, Spain.
274. Katlama C, et al. Dolutegravir Monotherapy in HIV-infected Patients with Suppressed HIV Viremia. Presented at: European AIDS Conference (EACS) 22 October 2015, Barcelona, Spain.
275. DOMONO study. Clinicaltrials.gov: NCT02401828.
276. Kohler JJ, Hosseini SH, Hoying-Brandt A et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Lab Invest* 2009; **89**: 513-9.
277. Kohler JJ, Hosseini SH, Green E et al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Lab Invest* 2011; **91**: 852-8.
278. Izzedine H, Hulot JS, Villard E et al. Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. *J Infect Dis* 2006; **194**: 1481-91.
279. Kiser JJ, Aquilante CL, Anderson PL et al. Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. *J Acquir Immune Defic Syndr* 2008; **47**: 298-303.

280. Rodriguez-Novoa S, Labarga P, Soriano V et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis* 2009; **48**: e108-16.
281. El-Sheikh AA, Masereeuw R, Russel FG. Mechanisms of renal anionic drug transport. *Eur J Pharmacol* 2008; **585**: 245-55.
282. El-Sheikh AA, van den Heuvel JJ, Koenderink JB et al. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2- and MRP4/ABCC4-mediated methotrexate transport. *J Pharmacol Exp Ther* 2007; **320**: 229-35.
283. Reid G, Wielinga P, Zelcer N et al. Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5. *Mol Pharmacol* 2003; **63**: 1094-103.
284. Reid G, Wielinga P, Zelcer N et al. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. *Proc Natl Acad Sci U S A* 2003; **100**: 9244-9.
285. Kidney Disease: Improving Global Outcomes (KDIGO) Chronic Kidney Disease Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013; 3:1–150.
286. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 2012; **120**: c179-84.
287. Mocroft A, Lundgren JD, Ross M et al. Development and validation of a risk score for chronic kidney disease in HIV infection using prospective cohort data from the D:A:D study. *PLoS Med* 2015; **12**: e1001809.
288. WHO Collaborating Centre for Drug Statistics Methodology. http://www.whocc.no/atc_ddd_index/.
289. Levey AS, Stevens LA, Schmid CH et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604-12.
290. Lucas GM, Ross MJ, Stock PG et al. Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2014; **59**: e96-138.
291. Payne RB. Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. *Ann Clin Biochem* 1998; **35 (Pt 2)**: 201-6.
292. Samarawickrama A, Cai M, Smith ER et al. Simultaneous measurement of urinary albumin and total protein may facilitate decision-making in HIV-infected patients with proteinuria. *HIV Med* 2012; **13**: 526-32.
293. Estrella M, Fine DM, Gallant JE et al. HIV type 1 RNA level as a clinical indicator of renal pathology in HIV-infected patients. *Clin Infect Dis* 2006; **43**: 377-80.
294. Mulenga L, Musonda P, Mwango A et al. Effect of baseline renal function on tenofovir-containing antiretroviral therapy outcomes in Zambia. *Clin Infect Dis* 2014; **58**: 1473-80.
295. Bickel M, Khaykin P, Stephan C et al. Acute kidney injury caused by tenofovir disoproxil fumarate and diclofenac co-administration. *HIV Med* 2013; **14**: 633-8.
296. Apiwattanakul N, Sekine T, Chairoungdua A et al. Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in Xenopus laevis oocytes. *Mol Pharmacol* 1999; **55**: 847-54.
297. Freiberg MS, Chang CC, Kuller LH et al. HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med* 2013; **173**: 614-22.
298. Hsue PY, Hunt PW, Wu Y et al. Association of abacavir and impaired endothelial function in treated and suppressed HIV-infected patients. *AIDS* 2009; **23**: 2021-7.

299. Triant VA, Lee H, Hadigan C et al. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* 2007; **92**: 2506-12.
300. Friis-Moller N, Weber R, Reiss P et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. *AIDS* 2003; **17**: 1179-93.
301. Fontas E, van Leth F, Sabin CA et al. Lipid profiles in HIV-infected patients receiving combination antiretroviral therapy: are different antiretroviral drugs associated with different lipid profiles? *J Infect Dis* 2004; **189**: 1056-74.
302. Podzamczer D, Andrade-Villanueva J, Clotet B et al. Lipid profiles for nevirapine vs. atazanavir/ritonavir, both combined with tenofovir disoproxil fumarate and emtricitabine over 48 weeks, in treatment-naive HIV-1-infected patients (the ARTEN study). *HIV Med* 2011; **12**: 374-82.
303. van Leth F, Phanuphak P, Stroes E et al. Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral-therapy-naive patients infected with HIV-1. *PLoS Med* 2004; **1**: e19.
304. Shaffer D, Hughes MD, Sawe F et al. Cardiovascular disease risk factors in HIV-infected women after initiation of lopinavir/ritonavir- and nevirapine-based antiretroviral therapy in Sub-Saharan Africa: A5208 (OCTANE). *J Acquir Immune Defic Syndr* 2014; **66**: 155-63.
305. Tebas P, Sension M, Arribas J et al. Lipid levels and changes in body fat distribution in treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 96 weeks in the ECHO and THRIVE trials. *Clin Infect Dis* 2014; **59**: 425-34.
306. Gerber JG, Rosenkranz SL, Fichtenbaum CJ et al. Effect of efavirenz on the pharmacokinetics of simvastatin, atorvastatin, and pravastatin: results of AIDS Clinical Trials Group 5108 Study. *J Acquir Immune Defic Syndr* 2005; **39**: 307-12.
307. Cardiovascular risk management guidelines (second revision). Article in Dutch. Huisarts Wet 2012;55(1):14-28. https://www.nhg.org/standaarden/volledig/cardiovasculair-risicomanagement.
308. Anderson KM, Odell PM, Wilson PW et al. Cardiovascular disease risk profiles. *Am Heart J* 1991; **121**: 293-8.
309. Copenhagen HIV Programme (CHIP). Framingham Risk Score Tools. http://www.chip.dk/TOOLS/Framingham/tabid/302/Default.aspx.
310. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). National Institutes of Health. http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3xsum.pdf.
311. Podzamczer D. Lipid metabolism and cardiovascular risk in HIV infection: new perspectives and the role of nevirapine. *AIDS Rev* 2013; **15**: 195-203.
312. Franssen R, Sankatsing RR, Hassink E et al. Nevirapine increases high-density lipoprotein cholesterol concentration by stimulation of apolipoprotein A-I production. *Arterioscler Thromb Vasc Biol* 2009; **29**: 1336-41.
313. Bower M, Fox P, Fife K et al. Highly active anti-retroviral therapy (HAART) prolongs time to treatment failure in Kaposi's sarcoma. *AIDS* 1999; **13**: 2105-11.
314. Bower M, Collins S, Cottrill C et al. British HIV Association guidelines for HIV-associated malignancies 2008. *HIV Med* 2008; **9**: 336-88.
315. Maurer T, Ponte M, Leslie K. HIV-associated Kaposi's sarcoma with a high CD4 count and a low viral load. *N Engl J Med* 2007; **357**: 1352-3.
316. Krown SE, Lee JY, Dittmer DP et al. More on HIV-associated Kaposi's sarcoma. *N Engl J Med* 2008; **358**: 535-6; author reply 6.
317. Stebbing J, Sanitt A, Nelson M et al. A prognostic index for AIDS-associated Kaposi's sarcoma in the era of highly active antiretroviral therapy. *Lancet* 2006; **367**: 1495-502.

318. Lichterfeld M, Qurishi N, Hoffmann C et al. Treatment of HIV-1-associated Kaposi's sarcoma with pegylated liposomal doxorubicin and HAART simultaneously induces effective tumor remission and CD4+ T cell recovery. *Infection* 2005; **33**: 140-7.
319. Tulpule A, Groopman J, Saville MW et al. Multicenter trial of low-dose paclitaxel in patients with advanced AIDS-related Kaposi sarcoma. *Cancer* 2002; **95**: 147-54.
320. Lane HC, Kovacs JA, Feinberg J et al. Anti-retroviral effects of interferon-alpha in AIDS-associated Kaposi's sarcoma. *Lancet* 1988; **2**: 1218-22.
321. Laguno M, Murillas J, Blanco JL et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV co-infected patients. *AIDS* 2004; **18**: F27-36.
322. Krown SE, Metroka C, Wernz JC. Kaposi's sarcoma in the acquired immune deficiency syndrome: a proposal for uniform evaluation, response, and staging criteria. AIDS Clinical Trials Group Oncology Committee. *J Clin Oncol* 1989; **7**: 1201-7.
323. Luppi M, Barozzi P, Maiorana A et al. Human herpesvirus-8 DNA sequences in human immunodeficiency virus-negative angioimmunoblastic lymphadenopathy and benign lymphadenopathy with giant germinal center hyperplasia and increased vascularity. *Blood* 1996; **87**: 3903-9.
324. Soulier J, Grollet L, Oksenhendler E et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood* 1995; **86**: 1276-80.
325. Osborne J, Moore PS, Chang Y. KSHV-encoded viral IL-6 activates multiple human IL-6 signaling pathways. *Hum Immunol* 1999; **60**: 921-7.
326. Aoki Y, Tosato G, Fonville TW et al. Serum viral interleukin-6 in AIDS-related multicentric Castleman disease. *Blood* 2001; **97**: 2526-7.
327. Du MQ, Liu H, Diss TC et al. Kaposi sarcoma-associated herpesvirus infects monotypic (IgM lambda) but polyclonal naive B cells in Castleman disease and associated lymphoproliferative disorders. *Blood* 2001; **97**: 2130-6.
328. Moher D, Liberati A, Tetzlaff J et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**: 264-9, W64.
329. Oxford Centre for Evidence-Based Medicint - Levels of Evidence (March 2009). http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/.
330. Gerard L, Michot JM, Burcheri S et al. Rituximab decreases the risk of lymphoma in patients with HIV-associated multicentric Castleman disease. *Blood* 2012; **119**: 2228-33.
331. Oksenhendler E, Boulanger E, Galicier L et al. High incidence of Kaposi sarcoma-associated herpesvirus-related non-Hodgkin lymphoma in patients with HIV infection and multicentric Castleman disease. *Blood* 2002; **99**: 2331-6.
332. Nishimoto N, Kanakura Y, Aozasa K et al. Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood* 2005; **106**: 2627-32.
333. Dossier A, Meignin V, Fieschi C et al. Human herpesvirus 8-related Castleman disease in the absence of HIV infection. *Clin Infect Dis* 2013; **56**: 833-42.
334. Van Rhee F, Wong RS, Munshi N et al. Siltuximab for multicentric Castleman's disease: A randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2014; **15**: 966-74.
335. Xu D, Lv J, Dong Y et al. Renal involvement in a large cohort of Chinese patients with Castleman disease. *Nephrol Dial Transplant* 2012; **27 Suppl 3**: iii119-25.
336. Zhu SH, Yu YH, Zhang Y et al. Clinical features and outcome of patients with HIV-negative multicentric Castleman's disease treated with combination chemotherapy: a report on 10 patients. *Med Oncol* 2013; **30**: 492.

337. Kawabata H, Kadowaki N, Nishikori M et al. Clinical features and treatment of multicentric castleman's disease : a retrospective study of 21 Japanese patients at a single institute. *J clin exp hematop* 2013; **53**: 69-77.
338. Kurzrock R, Voorhees PM, Casper C et al. A phase I, open-label study of siltuximab, an anti-IL-6 monoclonal antibody, in patients with B-cell non-hodgkin lymphoma, multiple myeloma, or castleman disease. *Clin Cancer Res* 2013; **19**: 3659-70.
339. Ramasamy K, Gandhi S, Tenant-Flowers M et al. Rituximab and thalidomide combination therapy for Castleman disease. *Br J Haematol* 2012; **158**: 421-3.
340. Oksenhendler E, Duarte M, Soulier J et al. Multicentric Castleman's disease in HIV infection: A clinical and pathological study of 20 patients. *AIDS* 1996; **10**: 61-7.
341. Bower M, Powles T, Williams S et al. Brief communication: Rituximab in HIV-associated multicentric Castleman disease. *Ann Intern Med* 2007; **147**: 836-9.
342. Guihot A, Couderc LJ, Agbalika F et al. Pulmonary manifestations of multicentric Castleman's disease in HIV infection: A clinical, biological and radiological study. *Eur Respir J* 2005; **26**: 118-25.
343. Gerard L, Berezne A, Galicier L et al. Prospective study of rituximab in chemotherapy-dependent human immunodeficiency virus-associated multicentric Castleman's disease: ANRS 117 CastlemaB trial. *J Clin Oncol* 2007; **25**: 3350-6.
344. Uldrick TS, Polizzotto MN, Aleman K et al. Rituximab plus liposomal doxorubicin in HIV-infected patients with KSHV-associated multicentric Castleman disease. *Blood* 2014; **124**: 3544-52.
345. Hoffmann C, Schmid H, Muller M et al. Improved outcome with rituximab in patients with HIV-associated multicentric Castleman disease. *Blood* 2011; **118**: 3499-503.
346. Bower M, Newsom-Davis T, Naresh K et al. Clinical features and outcome in HIV-associated multicentric Castleman's disease. *J Clin Oncol* 2011; **29**: 2481-6.
347. Loi S, Goldstein D, Clezy K et al. Castleman's disease and HIV infection in Australia. *HIV Med* 2004; **5**: 157-62.
348. Stebbing J, Adams C, Sanitt A et al. Plasma HHV8 DNA predicts relapse in individuals with HIV-associated multicentric Castleman disease. *Blood* 2011; **118**: 271-5.
349. Dargent JL, Lespagnard L, Sirtaine N et al. Plasmablastic microlymphoma occurring in human herpesvirus 8 (HHV-8)-positive multicentric Castleman's disease and featuring a follicular growth pattern. *APMIS* 2007; **115**: 869-74.
350. Dupin N, Diss TL, Kellam P et al. HHV-8 is associated with a plasmablastic variant of Castleman disease that is linked to HHV-8-positive plasmablastic lymphoma. *Blood* 2000; **95**: 1406-12.
351. Marsh RA, Vaughn G, Kim MO et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood* 2010; **116**: 5824-31.
352. Bower M, Veraitch O, Szydlo R et al. Cytokine changes during rituximab therapy in HIV-associated multicentric Castleman disease. *Blood* 2009; **113**: 4521-4.
353. Henter JI, Elinder G, Soder O et al. Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. *Blood* 1991; **78**: 2918-22.
354. Imashuku S, Hibi S, Fujiwara F et al. Hyper-interleukin (IL)-6-naemia in haemophagocytic lymphohistiocytosis. *Br J Haematol* 1996; **93**: 803-7.
355. Osugi Y, Hara J, Tagawa S et al. Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. *Blood* 1997; **89**: 4100-3.
356. Pantanowitz L, Fruh K, Marconi S et al. Pathology of rituximab-induced Kaposi sarcoma flare. *BMC Clin Pathol* 2008; **8**: 7.

357. Stebbing J, Gazzard B, Newsom-Davis T et al. Nadir B cell counts are significantly correlated with the risk of Kaposi's sarcoma. *Int J Cancer* 2004; **108**: 473-4.
358. Stratigos AJ, Malanos D, Touloumi G et al. Association of clinical progression in classic Kaposi's sarcoma with reduction of peripheral B lymphocytes and partial increase in serum immune activation markers. *Arch Dermatol* 2005; **141**: 1421-6.
359. Grandadam M, Dupin N, Calvez V et al. Exacerbations of clinical symptoms in human immunodeficiency virus type 1-infected patients with multicentric Castleman's disease are associated with a high increase in Kaposi's sarcoma herpesvirus DNA load in peripheral blood mononuclear cells. *J Infect Dis* 1997; **175**: 1198-201.
360. Oksenhendler E, Carcelain G, Aoki Y et al. High levels of human herpesvirus 8 viral load, human interleukin-6, interleukin-10, and C reactive protein correlate with exacerbation of multicentric castleman disease in HIV-infected patients. *Blood* 2000; **96**: 2069-73.
361. European Medicines Agency. Pending EC decisions. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003708/smops/Positive/human_smop_000658.jsp&mid=WC0b01ac058001d127.
362. Tocilizumab for KSHV-Associated Multicentric Castleman Disease. NCT01441063. Accessed on August 20, 2014. https://clinicaltrials.gov/ct2/show/NCT01441063?term=Castleman&rank=2.
363. A Study to Evaluate the Efficacy and Safety of CNTO328 Plus Best Supportive Care in Multicentric Castleman's Disease. NCT01024036. Accessed on August 20, 2014. https://clinicaltrials.gov/ct2/show/NCT01024036?term=Castleman&rank=3.
364. Persaud D, Gay H, Ziemniak C et al. Absence of detectable HIV-1 viremia after treatment cessation in an infant. *N Engl J Med* 2013; **369**: 1828-35.
365. Giacomet V, Trabattoni D, Zanchetta N et al. No cure of HIV infection in a child despite early treatment and apparent viral clearance. *Lancet* 2014; **384**: 1320.
366. Henrich TJ, Hu Z, Li JZ et al. Long-term reduction in peripheral blood HIV type 1 reservoirs following reduced-intensity conditioning allogeneic stem cell transplantation. *J Infect Dis* 2013; **207**: 1694-702.
367. Barton KM, Archin NM, Keedy KS et al. Selective HDAC inhibition for the disruption of latent HIV-1 infection. *PLoS One* 2014; **9**: e102684.
368. Hazuda D. ARV Drug Discovery: Integrase Inhibitors and Beyond. Bernard Fields Lecture. 20th Conference on Retroviruses and Opportunistic Infections (CROI) 2013. Atlanta, 3-6 March. Abstract 14. http://www.retroconference.org/2013b/Abstracts/48106.htm.
369. Eriksson S, Graf EH, Dahl V et al. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. *PLoS Pathog* 2013; **9**: e1003174.
370. Ho YC, Shan L, Hosmane NN et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 2013; **155**: 540-51.
371. Hill AL, Rosenbloom DI, Fu F et al. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc Natl Acad Sci U S A* 2014; **111**: 13475-80.
372. Saez-Cirion A, Jacquelin B, Barre-Sinoussi F et al. Immune responses during spontaneous control of HIV and AIDS: what is the hope for a cure? *Philos Trans R Soc Lond B Biol Sci* 2014; **369**: 20130436.
373. Bourry O, Mannioui A, Sellier P et al. Effect of a short-term HAART on SIV load in macaque tissues is dependent on time of initiation and antiviral diffusion. *Retrovirology* 2010; **7**: 78.
374. Mavigner M, Watkins B, Lawson B et al. Persistence of Virus Reservoirs in ART-Treated SHIV-Infected Rhesus Macaques after Autologous Hematopoietic Stem Cell Transplant. *PLoS Pathog* 2014; **10**: e1004406.
375. Deeks SG. HIV: Shock and kill. *Nature* 2012; **487**: 439-40.

376. Klatt NR, Shudo E, Ortiz AM et al. CD8+ lymphocytes control viral replication in SIVmac239-infected rhesus macaques without decreasing the lifespan of productively infected cells. *PLoS Pathog* 2010; **6**: e1000747.
377. Buzon MJ, Sun H, Li C et al. HIV-1 persistence in CD4+ T cells with stem cell-like properties. *Nat Med* 2014; **20**: 139-42.
378. Paiardini M, Cervasi B, Reyes-Aviles E et al. Low levels of SIV infection in sooty mangabey central memory CD(4)(+) T cells are associated with limited CCR5 expression. *Nat Med* 2011; **17**: 830-6.
379. Cartwright EK, McGary CS, Cervasi B et al. Divergent CD4+ T memory stem cell dynamics in pathogenic and nonpathogenic simian immunodeficiency virus infections. *J Immunol* 2014; **192**: 4666-73.
380. van Lunzen J, Glaunsinger T, Stahmer I et al. Transfer of autologous gene-modified T cells in HIV-infected patients with advanced immunodeficiency and drug-resistant virus. *Mol Ther* 2007; **15**: 1024-33.
381. Tebas P, Stein D, Tang WW et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med* 2014; **370**: 901-10.
382. Gaj T, Gersbach CA, Barbas CF, 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* 2013; **31**: 397-405.
383. Berdien B, Mock U, Atanackovic D et al. TALEN-mediated editing of endogenous T-cell receptors facilitates efficient reprogramming of T lymphocytes by lentiviral gene transfer. *Gene Ther* 2014; **21**: 539-48.
384. Kordelas L, Verheyen J, Beelen DW et al. Shift of HIV tropism in stem-cell transplantation with CCR5 Delta32 mutation. *N Engl J Med* 2014; **371**: 880-2.
385. Sarkar I, Hauber I, Hauber J et al. HIV-1 proviral DNA excision using an evolved recombinase. *Science* 2007; **316**: 1912-5.
386. Hauber I, Hofmann-Sieber H, Chemnitz J et al. Highly significant antiviral activity of HIV-1 LTR-specific tre-recombinase in humanized mice. *PLoS Pathog* 2013; **9**: e1003587.
387. Ellis RJ, Letendre S, Vaida F et al. Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder. *Clin Infect Dis* 2014; **58**: 1015-22.
388. Nightingale S, Geretti AM, Fisher M, Winston A, Nelson M, Taylor S, et al. HIV-1 RNA detection in cerebrospinal fluid in those on antiretroviral therapy experiencing incomplete viral suppression in plasma. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014, Boston, 3-6 March. Abstract 442. http://www.croiconference.org/sites/all/abstracts/442.pdf.
389. Sarfo FS, Zhang Y, Egan D et al. Pharmacogenetic associations with plasma efavirenz concentrations and clinical correlates in a retrospective cohort of Ghanaian HIV-infected patients. *J Antimicrob Chemother* 2014; **69**: 491-9.
390. Rodger A, Bruun T, Cambiano V, Vernazza O, Estrada V, van Lunzen J, et al. HIV transmission risk through condomless sex if HIV+ partner on suppressive ART: PARTNER study. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014, Boston, 3-6 March. Abstract 153LB. http://www.croiconference.org/sites/default/files/abstracts/153LB.pdf.
391. Kesho Bora Study Group. Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child transmission of HIV-1 (Kesho Bora study): a randomised controlled trial. *Lancet Infect Dis* 2011; **11**: 171-80.
392. Valea D, Tuaillon E, Al Tabaa Y et al. CD4+ T cells spontaneously producing human immunodeficiency virus type I in breast milk from women with or without antiretroviral drugs. *Retrovirology* 2011; **8**: 34.
393. Koulinska IN, Villamor E, Chaplin B et al. Transmission of cell-free and cell-associated HIV-1 through breast-feeding. *J Acquir Immune Defic Syndr* 2006; **41**: 93-9.

394. Zeh C, Weidle PJ, Nafisa L et al. HIV-1 drug resistance emergence among breastfeeding infants born to HIV-infected mothers during a single-arm trial of triple-antiretroviral prophylaxis for prevention of mother-to-child transmission: a secondary analysis. *PLoS Med* 2011; **8**: e1000430.
395. Jambo KC, Banda DH, Kankwatira AM et al. Small alveolar macrophages are infected preferentially by HIV and exhibit impaired phagocytic function. *Mucosal Immunol* 2014; **7**: 1116-26.
396. Fletcher CV, Staskus K, Wietgrefe SW et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc Natl Acad Sci U S A* 2014; **111**: 2307-12.
397. Gavegnano C, Detorio MA, Bassit L et al. Cellular pharmacology and potency of HIV-1 nucleoside analogs in primary human macrophages. *Antimicrob Agents Chemother* 2013; **57**: 1262-9.
398. Lehrman G, Hogue IB, Palmer S et al. Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. *Lancet* 2005; **366**: 549-55.
399. Archin NM, Liberty AL, Kashuba AD et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 2012; **487**: 482-5.
400. Archin NM, Bateson R, Tripathy MK et al. HIV-1 expression within resting CD4+ T cells after multiple doses of vorinostat. *J Infect Dis* 2014; **210**: 728-35.
401. Rasmussen TA, Schmeltz Sogaard O, Brinkmann C et al. Comparison of HDAC inhibitors in clinical development: effect on HIV production in latently infected cells and T-cell activation. *Hum Vaccin Immunother* 2013; **9**: 993-1001.
402. Rasmussen TA, Tolstrup M, Brinkmann CR et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *The Lancet HIV* 2014; **1**: e13-e21.
403. Wei DG, Chiang V, Fyne E et al. Histone deacetylase inhibitor romidepsin induces HIV expression in CD4 T cells from patients on suppressive antiretroviral therapy at concentrations achieved by clinical dosing. *PLoS Pathog* 2014; **10**: e1004071.
404. Sogaard OS, Graversen ME, Leth S et al. The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. *PLoS Pathog* 2015; **11**: e1005142.
405. McMichael AJ, Borrow P, Tomaras GD et al. The immune response during acute HIV-1 infection: clues for vaccine development. *Nat Rev Immunol* 2010; **10**: 11-23.
406. Pedersen C, Lindhardt BO, Jensen BL et al. Clinical course of primary HIV infection: consequences for subsequent course of infection. *BMJ* 1989; **299**: 154-7.
407. Liovat AS, Rey-Cuille MA, Lecuroux C et al. Acute plasma biomarkers of T cell activation set-point levels and of disease progression in HIV-1 infection. *PLoS One* 2012; **7**: e46143.
408. Vaidya SA, Korner C, Sirignano MN et al. Tumor Necrosis Factor alpha Is Associated With Viral Control and Early Disease Progression in Patients With HIV Type 1 Infection. *J Infect Dis* 2014; **210**: 1042-6.
409. Streeck H, Lu R, Beckwith N et al. Emergence of Individual HIV-Specific CD8 T Cell Responses during Primary HIV-1 Infection Can Determine Long-Term Disease Outcome. *J Virol* 2014; **88**: 12793-801.
410. Altfeld M, Addo MM, Rosenberg ES et al. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. *AIDS* 2003; **17**: 2581-91.
411. Descours B, Avettand-Fenoel V, Blanc C et al. Immune responses driven by protective human leukocyte antigen alleles from long-term nonprogressors are associated with low HIV reservoir in central memory CD4 T cells. *Clin Infect Dis* 2012; **54**: 1495-503.
412. Streeck H, Jolin JS, Qi Y et al. Human immunodeficiency virus type 1-specific CD8+ T-cell responses during primary infection are major determinants of the viral set point and loss of CD4+ T cells. *J Virol* 2009; **83**: 7641-8.
413. Lisziewicz J, Rosenberg E, Lieberman J et al. Control of HIV despite the discontinuation of antiretroviral therapy. *N Engl J Med* 1999; **340**: 1683-4.

414. Rosenberg ES, Altfeld M, Poon SH et al. Immune control of HIV-1 after early treatment of acute infection. *Nature* 2000; **407**: 523-6.
415. Kaufmann DE, Lichterfeld M, Altfeld M et al. Limited durability of viral control following treated acute HIV infection. *PLoS Med* 2004; **1**: e36.
416. Streeck H, Jessen H, Kuecherer C et al. Epidemiologically linked transmission of HIV-1 illustrates the impact of host genetics on virological outcome. *AIDS* 2009; **23**: 259-62.
417. Valcour V, Chalermchai T, Sailasuta N et al. Central nervous system viral invasion and inflammation during acute HIV infection. *J Infect Dis* 2012; **206**: 275-82.
418. Jiao Y, Song J, Sun X et al. Higher HIV DNA in CD4+ naive T-cells during acute HIV-1 infection in rapid progressors. *Viral Immunol* 2014; **27**: 316-8.
419. Miura T, Brumme ZL, Brockman MA et al. Impaired replication capacity of acute/early viruses in persons who become HIV controllers. *J Virol* 2010; **84**: 7581-91.
420. Kuang XT, Li X, Anmole G et al. Impaired Nef function is associated with early control of HIV-1 viremia. *J Virol* 2014; **88**: 10200-13.
421. Ping LH, Joseph SB, Anderson JA et al. Comparison of viral Env proteins from acute and chronic infections with subtype C human immunodeficiency virus type 1 identifies differences in glycosylation and CCR5 utilization and suggests a new strategy for immunogen design. *J Virol* 2013; **87**: 7218-33.
422. Yukl S, Liegler T, Abdel-Mohsen M, Sinclair E, Hatano H, Hecht F, et al. CD4 T-Cell Subset Composition of HIV Reservoirs in Gut, Lymph Node, and Blood During HAART. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 137. http://www.croiconference.org/sites/default/files/abstracts/137.pdf.
423. Luzuriaga K, Tabak B, Garber M et al. HIV Type 1 (HIV-1) Proviral Reservoirs Decay Continuously Under Sustained Virologic Control in HIV-1-Infected Children Who Received Early Treatment. *J Infect Dis* 2014.
424. Persaud D, Patel K, Karalius B et al. Influence of Age at Virologic Control on Peripheral Blood Human Immunodeficiency Virus Reservoir Size and Serostatus in Perinatally Infected Adolescents. *JAMA Pediatr* 2014.
425. Shiau S, Coovadia A, Technau KG, Strehlau R, Martens L, Pinillos F, et al. Greater Virologic Control in Infants Initiated On ART Before 6 Months of Age. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 924. http://www.croiconference.org/sites/default/files/abstracts/924_0.pdf.
426. Goetghebuer T, Haelterman E, Le Chenadec J et al. Effect of early antiretroviral therapy on the risk of AIDS/death in HIV-infected infants. *AIDS* 2009; **23**: 597-604.
427. Violari A, Cotton MF, Gibb DM et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med* 2008; **359**: 2233-44.
428. Evering TH, Mehandru S, Racz P et al. Absence of HIV-1 evolution in the gut-associated lymphoid tissue from patients on combination antiviral therapy initiated during primary infection. *PLoS Pathog* 2012; **8**: e1002506.
429. Kauffman RC, Villalobos A, Bowen JH et al. Residual viremia in an RT-SHIV rhesus macaque HAART model marked by the presence of a predominant plasma clone and a lack of viral evolution. *PLoS One* 2014; **9**: e88258.
430. Peterson J, Lee E, Fuchs D, Price RW, Spudich S. Early Antiretroviral Therapy Appears To Normalize Intrathecal Markers of Immune Activation. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston 3-6 March. Abstract 30. http://www.croiconference.org/sites/default/files/abstracts/30.pdf.

431. Lichterfeld M, Gandhi RT, Simmons RP et al. Induction of strong HIV-1-specific CD4+ T-cell responses using an HIV-1 gp120/NefTat vaccine adjuvanted with AS02A in antiretroviral-treated HIV-1-infected individuals. *J Acquir Immune Defic Syndr* 2012; **59**: 1-9.
432. Samji H, Cescon A, Hogg RS et al. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. *PLoS One* 2013; **8**: e81355.
433. Mills EJ, Barnighausen T, Negin J. HIV and aging--preparing for the challenges ahead. *N Engl J Med* 2012; **366**: 1270-3.
434. Deeks SG, Phillips AN. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. *BMJ* 2009; **338**: a3172.
435. Hunt PW, Martin JN, Sinclair E et al. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J Infect Dis* 2003; **187**: 1534-43.
436. Hunt PW, Landay AL, Sinclair E et al. A low T regulatory cell response may contribute to both viral control and generalized immune activation in HIV controllers. *PLoS One* 2011; **6**: e15924.
437. Neuhaus J, Jacobs DR, Jr., Baker JV et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis* 2010; **201**: 1788-95.
438. Grund B, Baker J, Deeks S, et al. Combined Effect of Interleukin-6 and D-dimer on the Risk of Serious Non-AIDS Conditions: Data from 3 Prospective Cohorts. Presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI) 2013. Atlanta, 3-6 march. Abstract 60. http://www.retroconference.org/2013b/Abstracts/47309.htm.
439. Favre D, Mold J, Hunt PW et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. *Sci Transl Med* 2010; **2**: 32ra6.
440. Byakwaga H, Boum Y, 2nd, Huang Y et al. The kynurenine pathway of tryptophan catabolism, CD4+ T-cell recovery, and mortality among HIV-infected Ugandans initiating antiretroviral therapy. *J Infect Dis* 2014; **210**: 383-91.
441. Hunt PW, Brenchley J, Sinclair E et al. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis* 2008; **197**: 126-33.
442. Pereyra F, Lo J, Triant VA et al. Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers. *AIDS* 2012; **26**: 2409-12.
443. Sanchez J, Hunt P, Jessurun J, et al. Persistent abnormalities of lymphoid structures in HIV viremic controllers. Presented at the 20th Conference on Retrovirusus and Opportunistic Infections (CROI) 2013. Atlanta, 3-6 March. Abstract 74. http://www.retroconference.org/2013/program/pocket-program.
444. Hatano H, Yukl S, Ferre A, et al. Prospective ART of Asymptomatic HIV+ Controllers. Presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI) 2013. Atlanta, 3-6 March. Abstract 75LB. http://retroconference.org/croi-2013-pocket-program.pdf.
445. Jain V, Hartogensis W, Bacchetti P et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. *J Infect Dis* 2013; **208**: 1202-11.
446. Longenecker CT, Hileman CO, Funderburg NT et al. Rosuvastatin preserves renal function and lowers cystatin C in HIV-infected subjects on antiretroviral therapy: the SATURN-HIV trial. *Clin Infect Dis* 2014; **59**: 1148-56.
447. REPRIEVE study. Clinicaltrials.gov: NCT02344290.
448. Sandler NG, Zhang X, Bosch RJ et al. Sevelamer Does Not Decrease Lipopolysaccharide or Soluble CD14 Levels But Decreases Soluble Tissue Factor, Low-Density Lipoprotein (LDL) Cholesterol, and Oxidized LDL Cholesterol Levels in Individuals With Untreated HIV Infection. *J Infect Dis* 2014.

449. Somsouk M, Dunham RM, Cohen M, et al. Mesalamine To Reduce Immune Activation During HIV Infection: A Randomized Controlled Trial. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 341. http://www.croiconference.org/sites/default/files/abstracts/341.pdf.
450. Tenorio AR, Chan ES, Bosch RJ et al. Rifaximin has a Marginal Impact on Microbial Translocation, T-cell Activation and Inflammation in HIV-Positive Immune Non-responders to Antiretroviral Therapy - ACTG A5286. *J Infect Dis* 2014.
451. Stiksrud B, Nowak P, Kvale D, et al. Decreased Levels of D-Dimer After Probiotic Supplementation in Patients Receiving ART. Presented at the Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 342. http://www.croiconference.org/sites/default/files/posters/342.pdf.
452. Hunt PW, Martin JN, Sinclair E et al. Valganciclovir reduces T cell activation in HIV-infected individuals with incomplete CD4+ T cell recovery on antiretroviral therapy. *J Infect Dis* 2011; **203**: 1474-83.
453. Yi TJ, Walmsley S, Szadkowski L et al. A randomized controlled pilot trial of valacyclovir for attenuating inflammation and immune activation in HIV/herpes simplex virus 2-coinfected adults on suppressive antiretroviral therapy. *Clin Infect Dis* 2013; **57**: 1331-8.
454. Longo V, Bonato M, Bossolasco S, et al. Brisk Walking Improves Inflammatory Markers in cART-Treated Patients. Presented at the Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 763. http://www.croiconference.org/sites/default/files/abstracts/763_0.pdf.
455. Rodger AJ, Lodwick R, Schechter M et al. Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. *AIDS* 2013; **27**: 973-9.
456. May MT, Gompels M, Delpech V et al. Impact on life expectancy of HIV-1 positive individuals of CD4+ cell count and viral load response to antiretroviral therapy. *AIDS* 2014; **28**: 1193-202.
457. Hasse B, Ledergerber B, Furrer H et al. Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin Infect Dis* 2011; **53**: 1130-9.
458. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. *Annu Rev Med* 2011; **62**: 141-55.
459. Tinago W, Coghlan E, Macken A et al. Clinical, immunological and treatment-related factors associated with normalised CD4+/CD8+ T-cell ratio: effect of naive and memory T-cell subsets. *PLoS One* 2014; **9**: e97011.
460. Cotter AG, Sabin CA, Simelane S et al. Relative contribution of HIV infection, demographics and body mass index to bone mineral density. *AIDS* 2014; **28**: 2051-60.
461. Cotter AG, Sabin CA, Simelane S, et al. Lower bone mineral density independently associated with both increased bone turnover and HIV-1 infection in HIV-positive subjects compared to HIV-negative subjects. Presented at the 7th IAS conference on HIV Pathogenesis, Treatment and Prevention 2013. Kuala Lumpur, 30 June-03 July..Abstract MOPE077. http://pag.ias2013.org/Abstracts.aspx?AID=2530.
462. McComsey GA, Kitch D, Daar ES et al. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. *J Infect Dis* 2011; **203**: 1791-801.
463. Martin A, Moore C, Mallon PW et al. Bone mineral density in HIV participants randomized to raltegravir and lopinavir/ritonavir compared with standard second line therapy. *AIDS* 2013; **27**: 2403-11.
464. Bloch M, Tong WW, Hoy J et al. Switch from tenofovir to raltegravir increases low bone mineral density and decreases markers of bone turnover over 48 weeks. *HIV Med* 2014; **15**: 373-80.

465. Negredo E, Domingo P, Perez-Alvarez N et al. Improvement in bone mineral density after switching from tenofovir to abacavir in HIV-1-infected patients with low bone mineral density: two-centre randomized pilot study (OsteoTDF study). *J Antimicrob Chemother* 2014.
466. Rasmussen TA, Jensen D, Tolstrup M et al. Comparison of bone and renal effects in HIV-infected adults switching to abacavir or tenofovir based therapy in a randomized trial. *PLoS One* 2012; **7**: e32445.
467. Cotter AG, Vrouenraets SM, Brady JJ et al. Impact of switching from zidovudine to tenofovir disoproxil fumarate on bone mineral density and markers of bone metabolism in virologically suppressed HIV-1 infected patients; a substudy of the PREPARE study. *J Clin Endocrinol Metab* 2013; **98**: 1659-66.
468. Overton ET, Chan ES, Brown TT, et al. High-dose vitamin D and calcium attenuates bone loss with ART initiation: results from ACTG A5280. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, March 3-6. Abstract 133. http://www.croiconference.org/sites/default/files/abstracts/133.pdf.
469. Law MG, Friis-Moller N, El-Sadr WM et al. The use of the Framingham equation to predict myocardial infarctions in HIV-infected patients: comparison with observed events in the D:A:D Study. *HIV Med* 2006; **7**: 218-30.
470. Worm SW, Sabin C, Weber R et al. Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. *J Infect Dis* 2010; **201**: 318-30.
471. Sabin C, Reiss P, Ryom L, et al. Is there continued evidence for an association between abacavir and myocardial infarction risk? Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 747LB. http://www.croiconference.org/sites/default/files/abstracts/747LB.pdf.
472. O'Halloran JA, Dunne E, Tinago W, et al. Effect of Switch From Abacavir To Tenofovir DF On Platelet Function Markers: A SWIFT Trial Substudy. Presented at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014. Boston, 3-6 March. Abstract 749LB. http://www.croiconference.org/sites/default/files/abstracts/749LB.pdf.
473. Cotter AG, Sabin CA, Simelane S, et al. Reduced high density lipoprotein cholesterol rather than elevated low density lipoprotein is the principal dyslipidaemia in HIV-positive subjects on contemporary antiretroviral therapy. Presented at the 14th European AIDS Conference (EACS) 2013. Brussels, 26-19 October. Abstract PE 11/28. http://www.eacsociety.org/Conference/PreviousConferences.aspx.
474. O'Halloran JA, Dunne E, et al. Effects of initiating antiretroviral therapy on markers of monocyte activation, endothelial dysfunction and platelet activation in HIV-1 infection. Antiviral Therapy 2014; 19 Suppl 2: A9.
475. Shaked I, Hanna DB, Gleissner C et al. Macrophage inflammatory markers are associated with subclinical carotid artery disease in women with human immunodeficiency virus or hepatitis C virus infection. *Arterioscler Thromb Vasc Biol* 2014; **34**: 1085-92.
476. Fitch KV, Srinivasa S, Abbara S et al. Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women. *J Infect Dis* 2013; **208**: 1737-46.
477. Longenecker CT, Jiang Y, Orringer CE et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. *AIDS* 2014; **28**: 969-77.
478. Shiels MS, Pfeiffer RM, Engels EA. Age at cancer diagnosis among persons with AIDS in the United States. *Ann Intern Med* 2010; **153**: 452-60.
479. Wandeler G, Gsponer T, Bregenzer A et al. Hepatitis C virus infections in the Swiss HIV Cohort Study: a rapidly evolving epidemic. *Clin Infect Dis* 2012; **55**: 1408-16.

480. European AIDS Treatment Network Acute Hepatitis C Infection Consensus Panel. Acute hepatitis C in HIV-infected individuals: recommendations from the European AIDS Treatment Network (NEAT) consensus conference. *AIDS* 2011; **25**: 399-409.
481. Thomson EC, Nastouli E, Main J et al. Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV. *AIDS* 2009; **23**: 89-93.
482. Linas BP, Wong AY, Schackman BR et al. Cost-effective screening for acute hepatitis C virus infection in HIV-infected men who have sex with men. *Clin Infect Dis* 2012; **55**: 279-90.
483. European Association for Study of Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420.
484. Martin TC, Martin NK, Hickman M et al. Hepatitis C virus reinfection incidence and treatment outcome among HIV-positive MSM. *AIDS* 2013; **27**: 2551-7.
485. Ingiliz P, Krznaric I, Stellbrink HJ et al. Multiple hepatitis C virus (HCV) reinfections in HIV-positive men who have sex with men: no influence of HCV genotype switch or interleukin-28B genotype on spontaneous clearance. *HIV Med* 2014; **15**: 355-61.
486. Marks G, Crepaz N, Janssen RS. Estimating sexual transmission of HIV from persons aware and unaware that they are infected with the virus in the USA. *AIDS* 2006; **20**: 1447-50.
487. Champenois K, Le Vu S, Cuzin L, et al. Factors associated with acute HIV infection diagnosis in MSM, ANRS-Opportunity study. 7th IAS Conference on HIV Pathogenesis, Prevention and Treatment 2013. Kuala Lumpur. Abstract MOAC0103. https://www.iasociety.org/Abstracts/A200748223.aspx.
488. Cohen MS, Gay CL, Busch MP et al. The detection of acute HIV infection. *J Infect Dis* 2010; **202 Suppl 2**: S270-7.
489. Cohen MS, Shaw GM, McMichael AJ et al. Acute HIV-1 Infection. *N Engl J Med* 2011; **364**: 1943-54.
490. Davis R, Dzoro S, Moyo S, et al. Optimizing a dried blood spot-based pooled RT-PCR technique for identification of acute HIV infections in Mochudi, Botswana. 19th IAS Conference on HIV Pathogenesis, Prevention and Treatment. Abstract no. TUPDB0203. http://www.iasociety.org/Abstracts/A200747137.aspx.
491. O'Brien M, Markowitz M. Should we treat acute HIV infection? *Curr HIV/AIDS Rep* 2012; **9**: 101-10.
492. Ananworanich J, Vandergeeten C, Chomchey C, et al. Early ART Intervention Restricts the Seeding of the HIV Reservoir in Long-lived Central Memory CD4 T Cells. Presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI) 2013, Atlanta, 3-6 March. Abstract 47. http://www.retroconference.org/2013b/Abstracts/46426.htm.
493. DeJesus E, Rockstroh JK, Henry K et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate versus ritonavir-boosted atazanavir plus co-formulated emtricitabine and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3, non-inferiority trial. *Lancet* 2012; **379**: 2429-38.
494. Sax PE, DeJesus E, Mills A et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3 trial, analysis of results after 48 weeks. *Lancet* 2012; **379**: 2439-48.
495. Lennox JL, DeJesus E, Lazzarin A et al. Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naive patients with HIV-1 infection: a multicentre, double-blind randomised controlled trial. *Lancet* 2009; **374**: 796-806.
496. Swartz JE, Vandekerckhove L, Ammerlaan H et al. Efficacy of tenofovir and efavirenz in combination with lamivudine or emtricitabine in antiretroviral-naive patients in Europe. *J Antimicrob Chemother* 2015; **70**: 1850-7.

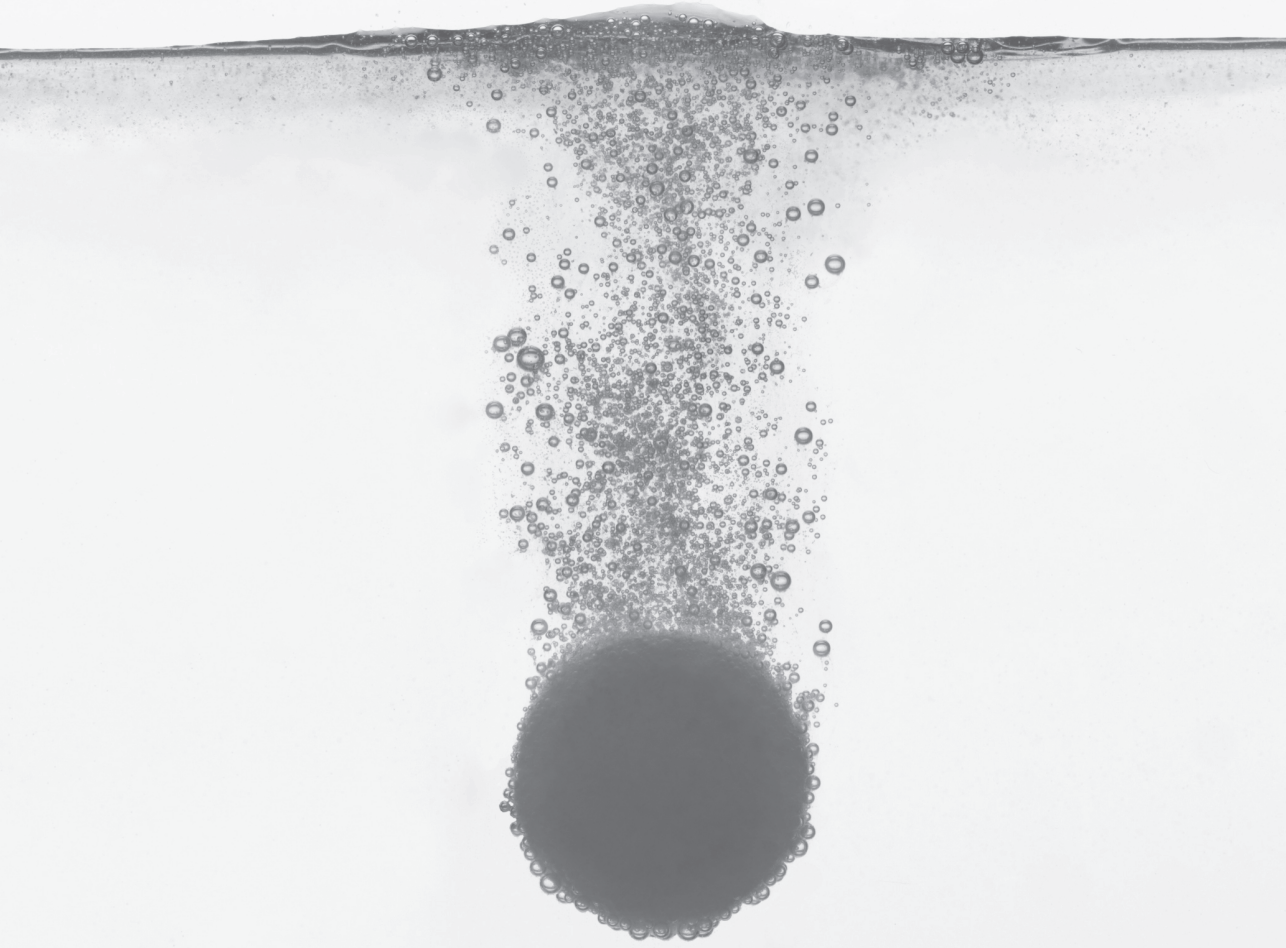
497. Yang WL, Kouyos RD, Scherrer AU et al. Assessing efficacy of different nucleos(t)ide backbones in NNRTI-containing regimens in the Swiss HIV Cohort Study. *J Antimicrob Chemother* 2015; **70**: 3323-31.
498. Dravid AN, Kulkami MM, Saraf CK, et al. Long Term Virologic Superiority of Emtricitabine over Lamivudine when Used in Fixed Dose Combination with Tenofovir/Efavirenz Among Patients with Low Baseline CD4 Counts in Resource Limited Settings. In: 15th European AIDS Conference. Barcelona, Spain. 21-24 October 2015. Abstract PE8/5.
499. Rhee SY, Blanco JL, Jordan MR et al. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. *PLoS Med* 2015; **12**: e1001810.
500. Teno-Res Study Group. Global epidemiology of drug resistance following failure of WHO recommended first line regimens for adult HIV-1 infection - a multi-centre retrospective cohort study. *Lancet Infect Dis* 2016; doi:10.106/pii: S1473-3099(15)00536-8.
501. Bonnet M, Bhatt N, Baudin E et al. Nevirapine versus efavirenz for patients co-infected with HIV and tuberculosis: a randomised non-inferiority trial. *Lancet Infect Dis* 2013; **13**: 303-12.
502. Pillay P, Ford N, Shubber Z et al. Outcomes for efavirenz versus nevirapine-containing regimens for treatment of HIV-1 infection: a systematic review and meta-analysis. *PLoS One* 2013; **8**: e68995.
503. Mbuagbaw LC, Irlam JH, Spaulding A et al. Efavirenz or nevirapine in three-drug combination therapy with two nucleoside-reverse transcriptase inhibitors for initial treatment of HIV infection in antiretroviral-naive individuals. *Cochrane Database Syst Rev* 2010: CD004246.
504. van Leth F, Phanuphak P, Ruxrungtham K et al. Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. *Lancet* 2004; **363**: 1253-63.
505. Kawalec P, Kryst J, Mikrut A et al. Nevirapine-based regimens in HIV-infected antiretroviral-naive patients: systematic review and meta-analysis of randomized controlled trials. *PLoS One* 2013; **8**: e76587.
506. Dailly E AC, Grégoire M, Reliquet V, Bouquié R, Billaud E, Hernando H, Bouchez S, Deslandes G, Hall N, Jolliet P, Raffi F. Influence of nevirapine administration on the pharmacokinetics of dolutegravir in patients infected with HIV-1. *J Antimicrob Chemother* 2015; **70**: 3307-10.
507. Pozniak A, Markowitz M, Mills A et al. Switching to coformulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus continuation of non-nucleoside reverse transcriptase inhibitor with emtricitabine and tenofovir in virologically suppressed adults with HIV (STRATEGY-NNRTI): 48 week results of a randomised, open-label, phase 3b non-inferiority trial. *Lancet Infect Dis* 2014; **14**: 590-9.
508. Allavena C, Dailly E, Reliquet V et al. Switching from tenofovir/emtricitabine and nevirapine to a tenofovir/emtricitabine/rilpivirine single-tablet regimen in virologically suppressed, HIV-1-infected subjects. *J Antimicrob Chemother* 2014; **69**: 2804-8.
509. Geretti AM, Harrison L, Green H et al. Effect of HIV-1 subtype on virologic and immunologic response to starting highly active antiretroviral therapy. *Clin Infect Dis* 2009; **48**: 1296-305.
510. Bhargava M, Cajas JM, Wainberg MA et al. Do HIV-1 non-B subtypes differentially impact resistance mutations and clinical disease progression in treated populations? Evidence from a systematic review. *J Int AIDS Soc* 2014; **17**: 18944.
511. Pant Pai N, Shivkumar S, Cajas JM. Does genetic diversity of HIV-1 non-B subtypes differentially impact disease progression in treatment-naive HIV-1-infected individuals? A systematic review of evidence: 1996-2010. *J Acquir Immune Defic Syndr* 2012; **59**: 382-8.
512. van Lunzen J, Maggiolo F, Arribas JR et al. Once daily dolutegravir (S/GSK1349572) in combination therapy in antiretroviral-naive adults with HIV: planned interim 48 week results from SPRING-1, a dose-ranging, randomised, phase 2b trial. *Lancet Infect Dis* 2012; **12**: 111-8.

513. Wares M, Mesplede T, Quashie PK et al. The M50I polymorphic substitution in association with the R263K mutation in HIV-1 subtype B integrase increases drug resistance but does not restore viral replicative fitness. *Retrovirology* 2014; **11**: 7.
514. Quashie PK, Mesplede T, Han YS et al. Characterization of the R263K mutation in HIV-1 integrase that confers low-level resistance to the second-generation integrase strand transfer inhibitor dolutegravir. *J Virol* 2012; **86**: 2696-705.
515. Anstett K, Fusco R, Cutillas V et al. Dolutegravir-Selected HIV-1 Containing the N155H and R263K Resistance Substitutions Does Not Acquire Additional Compensatory Mutations under Drug Pressure That Lead to Higher-Level Resistance and Increased Replicative Capacity. *J Virol* 2015; **89**: 10482-8.
516. Szczech LA, Grunfeld C, Scherzer R et al. Microalbuminuria in HIV infection. *AIDS* 2007; **21**: 1003-9.
517. Berliner AR, Fine DM, Lucas GM et al. Observations on a cohort of HIV-infected patients undergoing native renal biopsy. *Am J Nephrol* 2008; **28**: 478-86.
518. Haas M, Kaul S, Eustace JA. HIV-associated immune complex glomerulonephritis with "lupus-like" features: a clinicopathologic study of 14 cases. *Kidney Int* 2005; **67**: 1381-90.
519. Szczech LA, Gupta SK, Habash R et al. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney Int* 2004; **66**: 1145-52.
520. Ryom L, Mocroft A, Kirk O et al. Predictors of advanced chronic kidney disease and end-stage renal disease in HIV-positive persons. *AIDS* 2014; **28**: 187-99.
521. Reynes J, Cournil A, Peyriere H et al. Tubular and glomerular proteinuria in HIV-infected adults with estimated glomerular filtration rate >/= 60 ml/min per 1.73 m2. *AIDS* 2013; **27**: 1295-302.
522. Casado JL, Banon S, Santiuste C et al. Prevalence and significance of proximal renal tubular abnormalities in HIV-infected patients receiving tenofovir. *AIDS* 2016; **30**: 231-9.
523. Jose S, Hamzah L, Campbell LJ et al. Incomplete reversibility of estimated glomerular filtration rate decline following tenofovir disoproxil fumarate exposure. *J Infect Dis* 2014; **210**: 363-73.
524. Laprise C, Baril JG, Dufresne S et al. Association between tenofovir exposure and reduced kidney function in a cohort of HIV-positive patients: results from 10 years of follow-up. *Clin Infect Dis* 2013; **56**: 567-75.
525. Ezinga M, Wetzels JF, Bosch ME et al. Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. *Antivir Ther* 2014; **19**: 765-71.
526. Nishijima T, Komatsu H, Higasa K et al. Single nucleotide polymorphisms in ABCC2 associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: a pharmacogenetic study. *Clin Infect Dis* 2012; **55**: 1558-67.
527. Pushpakom SP, Liptrott NJ, Rodriguez-Novoa S et al. Genetic variants of ABCC10, a novel tenofovir transporter, are associated with kidney tubular dysfunction. *J Infect Dis* 2011; **204**: 145-53.
528. Labarga P, Barreiro P, Martin-Carbonero L et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS* 2009; **23**: 689-96.
529. Raffi F, Babiker AG, Richert L et al. Ritonavir-boosted darunavir combined with raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults infected with HIV-1: 96 week results from the NEAT001/ANRS143 randomised non-inferiority trial. *Lancet* 2014; **384**: 1942-51.
530. Mills A, Arribas JR, Andrade-Villanueva J et al. Switching from tenofovir disoproxil fumarate to tenofovir alafenamide in antiretroviral regimens for virologically suppressed adults with HIV-1 infection: a randomised, active-controlled, multicentre, open-label, phase 3, non-inferiority study. *Lancet Infect Dis* 2015.
531. Sax PE, Wohl D, Yin MT et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, non-inferiority trials. *Lancet* 2015; **385**: 2606-15.

532. Pozniak A, Arribas JR, Gathe J et al. Switching to tenofovir alafenamide, coformulated with elvitegravir, cobicistat, and emtricitabine, in HIV-infected patients with renal impairment: 48 week results from a single-arm, multi-center, open-label, Phase 3 study. *J Acquir Immune Defic Syndr* 2015.
533. Borges AH, O'Connor JL, Phillips AN et al. Factors Associated With Plasma IL-6 Levels During HIV Infection. *J Infect Dis* 2015; **212**: 585-95.
534. Ridker PM, Hennekens CH, Buring JE et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000; **342**: 836-43.
535. Ridker PM, Rifai N, Rose L et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002; **347**: 1557-65.
536. Barter P, Gotto AM, LaRosa JC et al. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007; **357**: 1301-10.
537. Petoumenos K, Reiss P, Ryom L et al. Increased risk of cardiovascular disease (CVD) with age in HIV-positive men: a comparison of the D:A:D CVD risk equation and general population CVD risk equations. *HIV Med* 2014; **15**: 595-603.
538. Ridker PM, Danielson E, Fonseca FA et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008; **359**: 2195-207.
539. Krikke M, Hoogeveen RC, Hoepelman A et al. Cardiovascular risk prediction in HIV-infected patients: comparing the Framingham, atherosclerotic cardiovascular disease risk score (ASCVD), Systematic Coronary Risk Evaluation for the Netherlands (SCORE-NL) and Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) risk prediction models. *HIV Med* 2015; doi:10.1111/hiv.12300.
540. Parravicini C, Chandran B, Corbellino M et al. Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. *Am J Pathol* 2000; **156**: 743-9.
541. Guihot A, Dupin N, Marcelin AG et al. Low T cell responses to human herpesvirus 8 in patients with AIDS-related and classic Kaposi sarcoma. *J Infect Dis* 2006; **194**: 1078-88.
542. Moore PS, Chang Y. Kaposi's sarcoma-associated herpesvirus-encoded oncogenes and oncogenesis. *J Natl Cancer Inst Monogr* 1998: 65-71.
543. Brander C, Suscovich T, Lee Y et al. Impaired CTL recognition of cells latently infected with Kaposi's sarcoma-associated herpes virus. *J Immunol* 2000; **165**: 2077-83.
544. Samaniego F, Markham PD, Gendelman R et al. Vascular endothelial growth factor and basic fibroblast growth factor present in Kaposi's sarcoma (KS) are induced by inflammatory cytokines and synergize to promote vascular permeability and KS lesion development. *Am J Pathol* 1998; **152**: 1433-43.
545. Fiorelli V, Barillari G, Toschi E et al. IFN-gamma induces endothelial cells to proliferate and to invade the extracellular matrix in response to the HIV-1 Tat protein: implications for AIDS-Kaposi's sarcoma pathogenesis. *J Immunol* 1999; **162**: 1165-70.
546. Tilg H, Mier JW, Vogel W et al. Induction of circulating IL-1 receptor antagonist by IFN treatment. *J Immunol* 1993; **150**: 4687-92.
547. Tilg H, Vogel W, Dinarello CA. Interferon-alpha induces circulating tumor necrosis factor receptor p55 in humans. *Blood* 1995; **85**: 433-5.
548. Koster R, Blatt LM, Streubert M et al. Consensus-interferon and platelet-derived growth factor adversely regulate proliferation and migration of Kaposi's sarcoma cells by control of c-myc expression. *Am J Pathol* 1996; **149**: 1871-85.
549. Mosam A, Shaik F, Uldrick TS et al. A randomized controlled trial of highly active antiretroviral therapy versus highly active antiretroviral therapy and chemotherapy in therapy-naive patients with HIV-associated Kaposi sarcoma in South Africa. *J Acquir Immune Defic Syndr* 2012; **60**: 150-7.

550. Pavlidis NA, Briassoulis E, Klouvas G et al. Is interferon-a an active agent in Castleman's disease? *Ann Oncol* 1992; **3**: 85-6.
551. Tamayo M, Gonzalez C, Majado MJ et al. Long-term complete remission after interferon treatment in a case of multicentric Castelman's disease. *Am J Hematol* 1995; **49**: 359-60.
552. Andres E, Maloisel F. Interferon-alpha as first-line therapy for treatment of multicentric Castleman's disease. *Ann Oncol* 2000; **11**: 1613-4.
553. Kumari P, Schechter GP, Saini N et al. Successful treatment of human immunodeficiency virus-related Castleman's disease with interferon-alpha. *Clin Infect Dis* 2000; **31**: 602-4.
554. Nord JA, Karter D. Low dose interferon-alpha therapy for HIV-associated multicentric Castleman's disease. *Int J STD AIDS* 2003; **14**: 61-2.
555. Dupin N, Krivine A, Calvez V et al. No effect of protease inhibitor on clinical and virological evolution of Castleman's disease in an HIV-1-infected patient. *AIDS* 1997; **11**: 1400-1.
556. Zietz C, Bogner JR, Goebel FD et al. An unusual cluster of cases of Castleman's disease during highly active antiretroviral therapy for AIDS. *N Engl J Med* 1999; **340**: 1923-4.
557. Sehn LH, Donaldson J, Chhanabhai M et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. *J Clin Oncol* 2005; **23**: 5027-33.
558. Kaplan LD, Lee JY, Ambinder RF et al. Rituximab does not improve clinical outcome in a randomized phase 3 trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin lymphoma: AIDS-Malignancies Consortium Trial 010. *Blood* 2005; **106**: 1538-43.
559. Uldrick TS, Polizzotto MN, Aleman K et al. High-dose zidovudine plus valganciclovir for Kaposi sarcoma herpesvirus-associated multicentric Castleman disease: a pilot study of virus-activated cytotoxic therapy. *Blood* 2011; **117**: 6977-86.
560. Casper C, Nichols WG, Huang ML et al. Remission of HHV-8 and HIV-associated multicentric Castleman disease with ganciclovir treatment. *Blood* 2004; **103**: 1632-4.
561. Berezne A, Agbalika F, Oksenhendler E. Failure of cidofovir in HIV-associated multicentric Castleman disease. *Blood* 2004; **103**: 4368-9; author reply 9.
562. Martin DF, Kuppermann BD, Wolitz RA et al. Oral ganciclovir for patients with cytomegalovirus retinitis treated with a ganciclovir implant. Roche Ganciclovir Study Group. *N Engl J Med* 1999; **340**: 1063-70.
563. Casper C, Krantz EM, Corey L et al. Valganciclovir for suppression of human herpesvirus-8 replication: a randomized, double-blind, placebo-controlled, crossover trial. *J Infect Dis* 2008; **198**: 23-30.
564. Fu DX, Tanhehco Y, Chen J et al. Bortezomib-induced enzyme-targeted radiation therapy in herpesvirus-associated tumors. *Nat Med* 2008; **14**: 1118-22.
565. Borghaei H, Paz-Ares L, Horn L et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* 2015; **373**: 1627-39.
566. Motzer RJ, Escudier B, McDermott DF et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med* 2015; **373**: 1803-13.
567. Ansell SM, Lesokhin AM, Borrello I et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 2015; **372**: 311-9.
568. Gardiner D, Lalezari J, Lawitz E et al. A randomized, double-blind, placebo-controlled assessment of BMS-936558, a fully human monoclonal antibody to programmed death-1 (PD-1), in patients with chronic hepatitis C virus infection. *PLoS One* 2013; **8**: e63818.
569. Molina JM, Capitant C, Spire B et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. *N Engl J Med* 2015; **373**: 2237-46.

570. Baeten JM, Donnell D, Ndase P et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med* 2012; **367**: 399-410.
571. Grant RM, Lama JR, Anderson PL et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med* 2010; **363**: 2587-99.
572. Strategies for Management of Antiretroviral Therapy Study Group (SMART). CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006; **355**: 2283-96.
573. TEMPRANO ANRS 12136 Study Group, Danel C, Moh R et al. A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. *N Engl J Med* 2015; **373**: 808-22.
574. Volberding PA, Deeks SG. Antiretroviral therapy and management of HIV infection. *Lancet* 2010; **376**: 808-22.



Chapter 15

Antiretroviral drug abbreviations

Publications

PhD portfolio

Curriculum vitae

Dankwoord

ANTIRETROVIRAL DRUG ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AZT	Azidothymidine
CBG	Cabotegravir
COBI	Cobicistat
d4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
DLV	Delavirdine
DOR	Doravirine
DRV	Darunavir
DTG	Dolutegravir
EFV	Efavirenz
ETR	Etravirine
EVG	Elvitegravir
FPV	Fosamprenavir
FTC	Emtricitabine
FTV	Fostemsavir
IDV	Indinavir
INI	Integrase inhibitor
LPV	Lopinavir
MVC	Maraviroc
NFV	Nelfinavir
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitor
RAL	Raltegravir
RPV	Rilpivirine
RTV	Ritonavir
SQV	Saquinavir
T-20	Enfuvirtide
TAF	Tenofovir alafenamide fumarate
TDF	Tenofovir disoproxil fumarate
TPV	Tipranavir
ZDV	Zidovudine

PUBLICATIONS

1. J. de Niet, R. Timman, **C. Rokx**, H.T.M. Jongejan, J. Passchier, E.L.T. van den Akker. Somatic complaints and social competence predict success in childhood overweight treatment. Int J Pediatr Obes. 2011 Jun;6(2-2):e472-9.

2. **C. Rokx**, M.E. van der Ende, A. Verbon, B.J.A. Rijnders. Peginterferon alfa-2a for AIDS-associated Kaposi sarcoma: experience with 10 patients. Clin Infect Dis. 2013 Nov;57(10):1497-9.

3. **C. Rokx**, R.M. Swart, A.A. van Houten, M.B. Leys, J.J. Duvekot, P.A. te Boekhorst. Thrombocytopenia during pregnancy. Ned Tijdschr Geneeskd. 2013;157(33):A6445.

4. **C. Rokx**, A. Verbon, B.J.A. Rijnders. Successful switch to rilpivirine/tenofovir/emtricitabine in HIV-1 infected patients with an isolated K103N mutation acquired during prior nonnucleoside reverse transcriptase inhibitor therapy. HIV Med. 2014 Nov;15(10):611-4.

5. **C. Rokx**, M. Blonk, A. Verbon, D. Burger, B.J.A. Rijnders. The efficacy, pharmacokinetics and safety of a nevirapine to rilpivirine switch in virologically suppressed HIV-1 infected patients. J Acquir Immune Defic Syndr. 2015 Jan 1;68(1):36-9.

6. **C. Rokx**, A. Fibriani, D.A.M.C. van de Vijver, A. Verbon, M. Schutten, L. Gras, B.J.A. Rijnders. Increased virological failure in naive HIV-1 infected patients taking lamivudine compared with emtricitabine in combination with tenofovir and efavirenz or nevirapine in the Dutch nationwide ATHENA cohort. Clin Infect Dis. 2015 Jan 1;60(1):143-53.

7. **C. Rokx**, A. Fibriani, D.A.M.C. van de Vijver, M. Schutten, L. Gras, B.J.A. Rijnders. More virological failure with lamivudine than emtricitabine in efavirenz and nevirapine regimens in the Dutch nationwide HIV cohort. J Int AIDS Soc. 2014 Nov 2;17(4 Suppl 3):19491.

8. **C. Rokx**, M. Blonk, A. Verbon, D. Burger, B.J.A. Rijnders. The efficacy, pharmacokinetics, safety and cardiovascular risks of switching nevirapine to rilpivirine in HIV-1 patients: the RPV switch study. J Int AIDS Soc. 2014 Nov 2:17(4 Suppl 3):19789.

9. **C. Rokx**, M. Blonk, A. Verbon, D. Burger, B.J.A. Rijnders. De effectiviteit en farmacokinetiek van het vervangen van nevirapine voor rilpivirine bij HIV-1 RNA onderdrukte patiënten. HIV bulletin. 2014;8(4):16-9.

10. **C. Rokx**, A. Verbon, B.J.A. Rijnders. Lipids and cardiovascular risk after switching HIV-1 patients on nevirapine and emtricitabine/tenofovir-DF to rilpivirine/emtricitabine/tenofovir-DF. AIDS Res Hum Retroviruses. 2015 Apr;31(4):363-7.

11. **C. Rokx**, B.J.A. Rijnders. How does weight influence tenofovir disoproxil-fumarate induced renal function decline? AIDS. 2015 Mar 13;29(5):643-5.

12. **C. Rokx**, B.J.A. Rijnders. Evidence gathered from randomized clinical trials and observational studies on the equivalence of emtricitabine and lamivudine. Clin Infect Dis. 2015 Jun;60(11):1732-3.

13. A. Duim, **C. Rokx**, E.C. van Gorp, B.J.A. Rijnders. Proximal tubular dysfunction in a HIV-1 patient with coadministered tenofovir disoproxil-fumarate and ibuprofen. AIDS. 2015 Mar 27;29(6):746-8.

14. **C. Rokx**, B.J.A. Rijnders, J.A. van Laar. Treatment of multicentric Castleman's disease in HIV-1 infected and uninfected patients: a systematic review. Neth J Med. 2015 Jun;73(5):202-10.

15. **C. Rokx**, D.D. Richman, M. Müller-Trutwin, G. Silvestri, J. van Lunzen, S. Khoo, M. Lichterfeld, M. Altfeld, C.F. Perno, P.W. Hunt, P. Mallon, J.K. Rockstroh, A.L. Pozniak, B. Clotet, C.A.B. Boucher. Second European Round Table on the Future Management of HIV 10-11 October 2014, Barcelona, Spain. J Virus Erad. 2015;1:211-220.

16. **C. Rokx**, H. Alshangi, A. Verbon, R. Zietse, E.J. Hoorn, B.J.A. Rijnders. Renal consequences in HIV-1 patients of concomitant exposure to tenofovir disoproxil-fumarate and inhibitors of tubular multidrug resistance protein transporters. J Infect Dis. 2016 Feb 15;213(4):561-8.

17. B.E. Nichols, H.M. Götz, E.C. van Gorp, A. Verbon, **C. Rokx**, C.A.B. Boucher, D.A.M.C. van de Vijver. Partner notification for reduction of HIV-1 transmission and related costs among men who have seks with men: a mathematical modeling study**.** PLoS One. 2015 Nov 10;10(11):e0142576.

18. T.C. Zondag, **C. Rokx**, K. van Lom, A.R. van den Berg, P. Sonneveld, W.A. Dik, G.J. van Doornum, K.H. Lam, J.A. van Laar. Cytokine and viral load kinetics in human herpesvirus 8-associated multicentric Castleman's disease complicated by hemophagocytic lymphohistiocytosis. Int J Hematol. 2015 Dec 21. Epub ahead of print.

19. **C. Rokx**, L. Gras, D.A.M.C. van de Vijver, A. Verbon, B.J.A. Rijnders. Virologic responses to lamivudine or emtricitabine when combined with tenofovir and a protease inhibitor in treatment naïve HIV-1-infected patients in the Dutch AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort. HIV Med. 2016 Feb 4. Epub ahead of print.

20. M. Stoszko, E. de Crignis, **C. Rokx**, M.M. Khalid, C. Lungu, R.J. Palstra, T.W. Kan, C.A.B. Boucher, A. Verbon, E.C. Dykhuizen, T. Mahmoudi. Small Molecule inhibitors of BAF; a promising family of compounds in HIV-1 latency reversal. EBiomedicine. 2016 Jan;3:108-121.

21. J.Gregson, M. Tang, N. Ndembi, R.L. Hamers, S.Y. Rhee, V.C. Marconi, L. Diero, K. Brooks, K. Theys, T.F. Rinke de Wit, M. Arruda, F. Garcia, S. Monge, H.F. Günthard, C.J. Hoffmann, P.J. Kanki, N. Kumarasamy, B. Kerschberger, O. Mor, C. Charpentier, E. Todesco, **C. Rokx**, L. Gras, E.K. Halvas, H. Sunpath, D. Di Carlo, A. Antinori, M. Andreoni, A. Latini, C. Mussini, A. Aghokeng, A. Sonnerborg, U. Neogi, W.J. Fessels, S. Agolory, C. Yang, J.L. Blanco, J.M. Juma, E. Smit, D. Schmidt, C. Watera, J. Asio, W. Kirungi, A. Tostevin, T. El-Hay, N. Clumeck, D. Goedhals, C. van Vuuren, P.A. Bester, C. Sabin, I. Mukui, M.M. Santoro, C.F. Perno, G. Hunt, L. Morris, R. Camacho, T. de Oliveira, D. Pillay,

E. Schulter, A. Murakami-Ogasawara, G. Reyes-Terán, K. Romero, S. Avila-Rios, S. Sirivichayakul, K. Ruxrungtham, S. Mekpresan, D. Dunn, P. Kaleebu, E. Raizes, R. Kantor, R.W. Shafer, R.K. Gupta. The TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. Lancet Infect Dis. 2016 Jan 28. Epub ahead of print.

22. **C. Rokx**, C.A.M. Schurink, C.A.B. Boucher, B.J.A. Rijnders. Dolutegravir as maintenance monotherapy: first experiences in HIV-1 patients. J Antimicrob Chemother. 2016 Feb 16. Epub ahead of print.

23. **C. Rokx**, J.A.M van Laar, B.J.A. Rijnders. Reply to: "Hemophagocytic Lymphohistiocytosis Associated With Bartonella Henselae Infection in an HIV-Infected Patient." Clinical Infectious Diseases. 2016. Epub ahead of print.

24. E. van Ander-Bode, S.K. Been, **C. Rokx**, M.E. van der Ende. Risk factors in an HIV-infected population for refraining from specialist care. AIDS Care. 2016. Epub ahead of print.

25. E. de Crignis, H. Rafati, E. Lemasters, L. van den Dries, **C. Rokx**, R. Gruters, E.C. van Gorp, A. Verbon, D. van den Heuvel, M.C. van Zelm, C.A.B. Boucher, T. Mahmoudi. Targeting the Wnt pathway for activation activates latent HIV and is synergistically enhanced upon concomitant inhibition of histone deacetylaction. Submitted.

26. **C. Rokx**, S. Vella, G. Pantaleo, Y. Lévy, C.A.B. Boucher. Unite forces to validate biomarkers in the quest for lasting HIV remission. AIDS. 2016. Epub ahead of print.

PHD PORTFOLIO

Candidate: Casper Rokx
Erasmus MC departments: Medical Microbiology & Infectious Diseases/Internal Medicine
Period: 2012-2016
Promotor: prof. dr. A. Verbon

Courses	Year
Research skills	
Statistics and SPSS. Erasmus MC MolMed Post Graduate School	2013
Biostatistics for clinicians. NIHES institute Rotterdam	2013
Good Clinical Practice GCP/BROK. Erasmus MC	2014
Scientific Integrity. Erasmus MC	2014
Workshops	
HIV Masterclass. Virology Education, Utrecht, the Netherlands	2013
Future management of HIV. Virology Education, Barcelona, Spain	2014
First HIV forum on INI. Virology Education, Barcelona, Spain	2015
Clinical courses	
Tropical Medicine course. Erasmus MC	2008
HIV course. Dutch Association of HIV-treating physicians	2010
Professional communication. Erasmus MC	2010
Video training on the job. NIGZ	2012
Fundamental Critical Care Support (FCCS) course	2012
Talent class for residents, Academy for Medical Specialist	2016

National and International Conferences

Oral Presentations

14th European AIDS Conference, Brussels, Belgium 2013

Increased risk of virological failure with lamivudine compared to emtricitabine in tenofovir and nevirapine containing antiretroviral therapy.

Dutch Association of HIV-treating Physicians Winter Congress, Amsterdam, the Netherlands 2014

Efficacy of FTC or 3TC with TDF in NVP or EFV containing regimens in ATHENA.

Dutch Association of HIV-treating Physicians Summer Congress, Utrecht, the Netherlands 2014

The efficacy, pharmacokinetics, safety of a nevirapine to rilpivirine switch.

HIV Drug Therapy Congress, Glasgow, Scotland 2014

More virological failure with lamivudine than emtricitabine in efavirenz and nevirapine regimens in the Dutch nationwide HIV cohort.

Science Days Internal Medicine, Erasmus MC, Antwerp, Belgium 2015

Renal consequences of concomitant exposure to tenofovir disoproxil-fumarate and inhibitors of tubular multidrug resistance protein-4 efflux transporters in HIV-1 patients.

Dutch Association of HIV-treating Physicians Winter Congress, Amsterdam, the Netherlands 2015

Renal consequences of concomitant exposure to tenofovir disoproxil-fumarate and inhibitors of tubular multidrug resistance protein-4 efflux transporters in HIV-1 patients.

Erasmus MC Science Day, Rotterdam, the Netherlands 2015

Renal consequences in HIV-1 patients of concomitant exposure to tenofovir disoproxil-fumarate and inhibitors of tubular multidrug resistance protein transporters.

1st HIV Forum Meeting, Virology Education, Barcelona, Spain 2015

Dolutegravir as maintenance monotherapy: first experiences in HIV-1 infected patients.

Post-CROI meeting, Virology Education, Utrecht, the Netherlands 2016

HIV pathogenesis and cure: where are we?

Poster Presentations

7th Netherlands Conference on HIV, Amsterdam, the Netherlands 2013

1. *Efficacy of FTC or 3TC with TDF in NVP containing regimens: single center experience.*
2. *Peginterferon alfa-2a for advanced AIDS-associated Kaposi sarcoma.*

HIV Drug Therapy Congress, Glasgow, Scotland. 2014

1. *More virological failure with lamivudine than emtricitabine in efavirenz and nevirapine regimens in the Dutch nationwide HIV cohort.*
2. *The efficacy, pharmacokinetics, safety, and cardiovascular risks of a nevirapine to rilpivirine switch.*

8th Netherlands Conference on HIV, Amsterdam, the Netherlands 2014

1. *Efficacy of FTC or 3TC with TDF in NVP or EFV containing regimens in ATHENA.*
2. *The efficacy, pharmacokinetics, safety and cardiovascular risks of a nevirapine to rilpivirine switch.*

22nd Conference on Retroviruses and Opportunistic Infections, Seattle, WA, USA 2015

Virological responses to lamivudine and emtricitabine in the nationwide ATHENA cohort.

15th European AIDS Conference, Barcelona, Spain 2015

Renal consequences in HIV-1 patients of concomitant exposure to tenofovir disoproxil-fumarate and inhibitors of tubular multidrug resistance protein transporters.

9th Netherlands Conference on HIV, Amsterdam, the Netherlands 2015

1. *Dolutegravir as maintenance monotherapy: first experiences in HIV-1 infected patients.*
2. *Renal consequences in HIV-1 patients of concomitant exposure to tenofovir disoproxil-fumarate and inhibitors of tubular multidrug resistance protein transporters.*

Awards

Martijn Verbrugge award for best scientific abstract. Dutch Association of HIV-Treating Physicians 2014

Young investigator award. HIV Drug Therapy Congress, Glasgow, Scotland 2014

Young investigator award. 22nd Conference on Retroviruses and Opportunistic Infections, Seattle, USA 2015

Young investigator and travel grant. 15th European AIDS Conference, Barcelona, Spain 2015

Teaching activities

Supervising reviews second year medical students
Eductional courses on HIV for medical students
Internal medicine subjects for interns
Infectious diseases courses for general practitioners

Supervising master theses

Hanin Alshangi, Elise Pieterman, Adacia T. Bourne. Medical students, Erasmus MC, Rotterdam, the Netherlands

1. Renal dysfunction on TDF.	2014
2. VTE in HIV.	2015
3. LRA and the HIV reservoir.	2016

Other activities

Coordinator of the Erasmus MC HIV Eradication Group	Ongoing
Chairman resident society Maasstad hospital	2011
Chairman resident society Erasmus MC	Ongoing

CURRICULUM VITAE

Casper Rokx was born on December 4th 1983 in Groningen, the Netherlands. In 2002 he graduated from the Marnix Gymnasium in Rotterdam and started his medical training at the Erasmus MC University Medical Center. His graduation research in 2008 focused on a pediatric clinical intervention study. He did a clinical internship at the Macha Mission Hospital in Macha, Zambia, after which he received his Medical Doctor degree in 2009. Subsequently, he started to work as a resident at the department of internal medicine of the Maasstad hospital in Rotterdam under the supervision of dr. M.A. van den Dorpel. He initiated his medical specialisation in internal medicine in 2010 under the supervision of prof. dr. J.L.C.M van Saase and dr. S.C.E. Klein Nagelvoort-Schuit. He concurrently started as a researcher in 2012 at the department of internal medicine under the supervision of prof. dr. A. Verbon and dr. B.J.A. Rijnders. He combined his research in HIV with his medical specialisation in internal medicine: this PhD thesis is the product of that period. In 2016 he started his specialisation as internist-infectiologist under the supervision of dr. J.L. Nouwen and dr. C.A.M Schurink. He will be an internist-infectiologist in 2018 and continue to do research in HIV.

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I'll be back before you can say Blueberry Pie.

HIV: Treatment and Comorbidity

Clinicians worldwide strive to improve HIV care for their patients. A clinical evaluation of treatment strategies is necessary to identify strategies that may jeopardize treatment effectiveness and patient safety. This thesis presents the results of clinical studies that assess these essential aspects of HIV care.