New insights into complications and treatment of HIV-1 infection

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New insights into complications and treatment of HIV-1 infection

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(met een samenvatting in het Nederlands)

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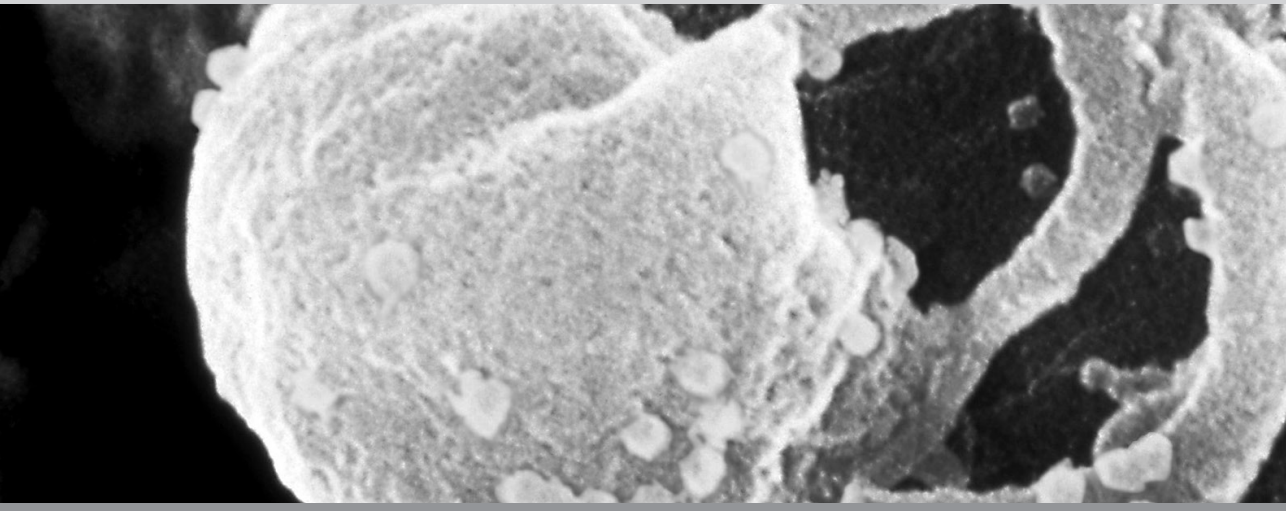
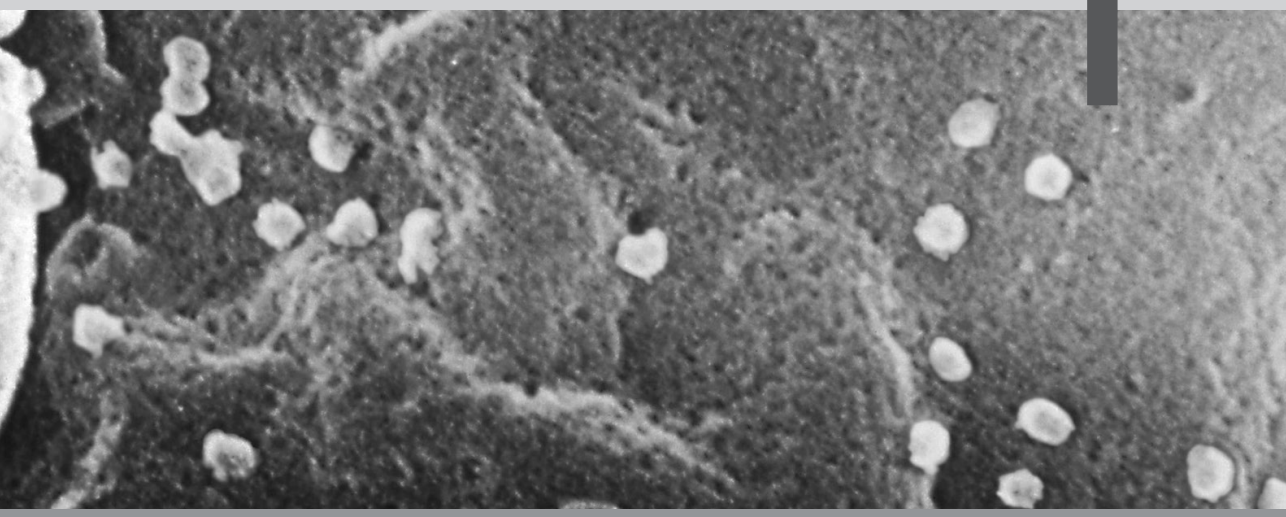
Voor mijn lief en de jongens

Aan mijn ouders

Contents



CHAPTER 1



General introduction and outline of the thesis

Worldwide 34 million people are estimated to be infected with the human immunodeficiency virus (HIV) and although much progress has been made in prevention and treatment of HIV-infection, this number continues to rise [1]. The introduction of combination antiretroviral therapy (cART) has decreased the mortality and morbidity of patients infected with HIV dramatically [2]. Although it has been reported that life expectancy in specific subgroups of HIV-infected patients is comparable with those having other chronic medical diseases (for example insulin treated diabetes) [3] and in some cohorts even to the general population [4, 5], cART does not seem to fully restore health in HIV-infected patients when compared to the general population [6-9]. It has been described that the incidence of non-AIDS defining diseases such as cardiovascular events and non-AIDS defining malignancies (non-ADM) [10, 11] is higher in HIV-infected patients as compared to the general population. In light of these observations, evidence is mounting that the aging process is occurring much faster in patients with HIV-infection compared to the general population, resulting in an increased incidence of comorbidities such as cardiovascular diseases, hypertension, diabetes mellitus, bone fractures and renal failure [12, 13]. This premature aging relates to the continuous presence of high levels of chronic inflammation and immune activation in HIV-infected patients [9].

It is now well accepted that the hallmark of HIV-infection – a gradual decline of $CD4^+$ T cells – is not merely caused by direct killing of these cells by infection with the HIV virus, but is the result of hyperactivation of the immune system in chronic HIV-infection, causing depletion of the naive T cell pool [14-18].

Although cART reduces immune activation, even in patients with an undetectable plasma HIV-RNA the level of activated T cells remains higher as compared to HIV-negative individuals [19]. Residual immune activation in cART treated patients is thought to be the result of a combination of factors, including continuous viral production/replication [20-23], bacterial translocation [24, 25] and HIV coinfections (e.g. CMV [26, 27]) .

Several studies have aimed to show the clinical implications of HIV-induced immune activation. Activated T cells have been found to be a strong independent predictor for clinical outcome, both in treated [28]

and untreated HIV-infection [16, 29]. In addition, it has been found that elevated levels of activated T cells are associated with a lower CD4 cell gain during cART [19, 28, 30]. Moreover, T cell activation and chronic inflammation has been found to be a contributing factor for the increased risk of HIV-infected patients for cardiovascular disease [31-33] as well. However, more research is needed to unravel the mechanism of residual immune activation and its clinical complications and to identify strategies to modulate immune activation.

The aim of this thesis was to study the clinical complications of HIV-infection and its treatment in the current era, and to investigate new therapeutic strategies.
First, in **chapter 2**, we review the mode of action and effectiveness of the CCR5-antagonist maraviroc (MVC), the only registered antiretroviral drug of the relatively new class of entry inhibitors and in this light discuss HIV coreceptor tropism, pharmacokinetics and side effects.
As mentioned above, the life expectancy of HIV-infected patients has improved enormously since the introduction of cART but the amount of non-AIDS related complications is increasing. In addition, a significant amount of patients still present themselves late in the course of their disease to clinical care (30-40%) [34, 35], sometimes resulting in major complications requiring critical care. In **chapter 3,** we investigate whether the long term outcome of HIV-infected patients that have been admitted to the intensive care unit (ICU) has improved as well. Since it has been found that non-AIDS defining complications are probably related to increased levels of immune activation and chronic inflammation, and that these levels are higher in patients with low $CD4^+$ T cell counts despite suppressive cART, we set out to investigate the risk for occurrence of long term clinical complications in this particular group of patients in **chapter 4.** The interest in the neurocognitive complications of HIV-infection has increased enormously during the past years, and the possible relation to ongoing viral replication/production in the central nervous system and the level of penetration of antiretroviral drugs is being studied increasingly. In **chapter 5**, we analyse the causes and consequences of HIV-replication in the central nervous system (a so-called 'HIV sanctuary site') in a patient with suppressed plasma HIV-RNA. The UMC Utrecht has relatively much experience with treat-

ment of HIV-infected patients with a MVC-containing regimen, which we describe in **chapter 6.** MVC is registered for treatment of patients harbouring a R5-trope virus population and a HIV coreceptor tropism assay is performed before start of therapy. In **chapter 7**, we analyze the virological effects of MVC therapy in a patient harbouring a virus population classified as dual/mixed-tropic according to the current cut-offs of the available tropism assays. HIV-patients with low $CD4^+$ T cell counts despite viral suppression are at increased risk for clinical complications [36-39], and therefore a need for immunomodulating therapies for this particular group of patients exists. In **chapter 8** we present the results of the "Maraviroc Immune Recovery Study (MIRS)", a randomised, multicenter study investigating the immunological effects of MVC intensification of cART in patients with a suboptimal immunological response on virological successful cART. In a substudy of the MIRS, we further investigated the immunological effects of MVC intensification, where we used heavy water (D_2O) labeling technique to estimate the life span of T cells. These results are presented in **chapter 9**. Finally, in **chapter 10**, the results of this thesis are summarized and discussed, and future perspectives are provided.

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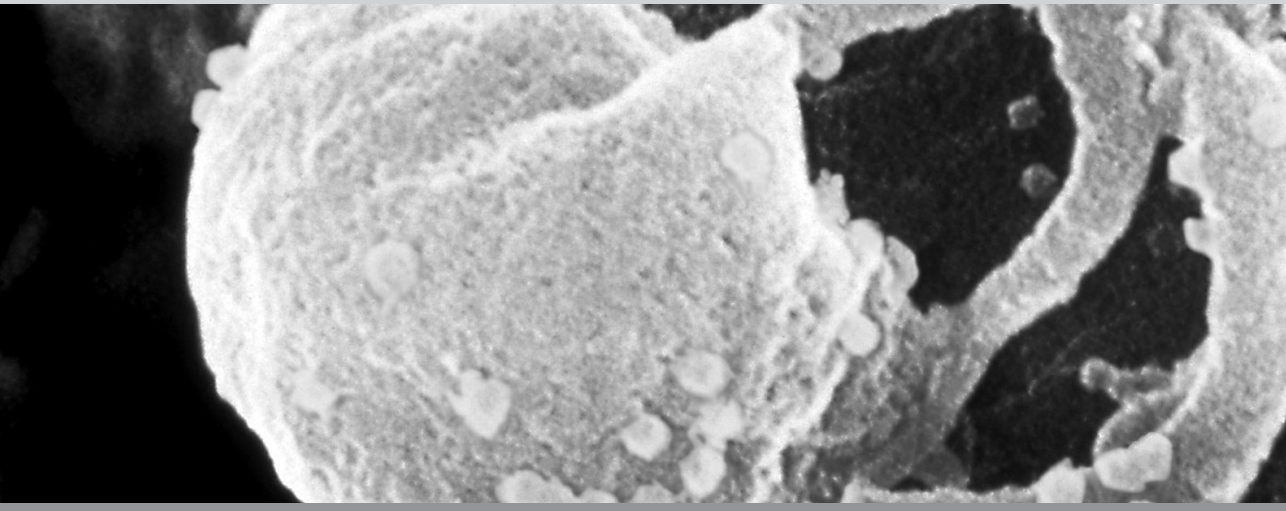
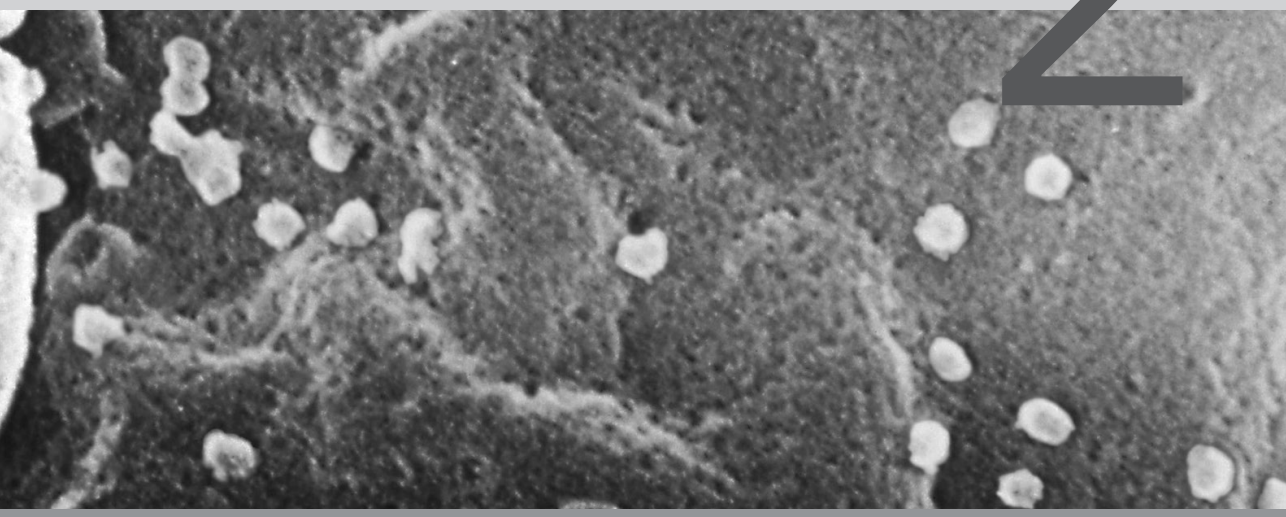


CHAPTER 2



The MOTIVATE trials: maraviroc therapy in antiretroviral treatment experienced HIV-1-infected patients

S.F.L. van Lelyveld, A.M.J. Wensing, A.I.M. Hoepelman

Expert Rev Anti Infect Ther. 2012 Nov;10(11):1241-7

Summary

Although the use of combination antiretroviral therapy (cART) has resulted in spectacular improvements in morbidity and mortality of HIV-1-infected patients, a need for the development of antiretroviral compounds with new mechanisms of action remains. Maraviroc (Celsentri®) is the only drug of the class of CCR5 antagonists registered for treatment for HIV-1-infected antiretroviral therapy experienced patients. Registration was based on the 'Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients (MOTIVATE) 1 and 2 studies', which compared the efficacy and tolerability of maraviroc in combination with optimized background therapy to placebo. The aim of this paper is to review the MOTIVATE studies, and to discuss issues related to maraviroc therapy in clinical practice such as assessment of HIV-1 coreceptor tropism.

Introduction

The introduction of combination antiretroviral therapy (cART) has resulted in spectacular improvements in morbidity and mortality of HIV-1-infected patients in the Western world [1]. As a result, life expectancy of HIV-1-infected patients in the Western world seems comparable to those having other chronic medical diseases (e.g. diabetes mellitus) [2] and in some cohorts even to the general population [3, 4]. However, evidence is accumulating that in patients with HIV-1-infection, despite viral suppression by cART, the process of aging is occurring much faster as compared to the general population resulting in a higher incidence of cardiovascular diseases and malignancies [5]. In addition, a large proportion of new diagnosed HIV-1-infected patients in Western-Europe present with advanced disease ($CD4^+$ T cell (CD4 cell) count of <200 cells/μL) [6]. Therefore, in general life expectancy of HIV-1-infected patients is still shorter compared to the general population [7] emphasising the need for more optimal treatment strategies.
Side effects of current cART and selection and transmission of drug resistance further enhance to the need for the development of antiretroviral compounds with new mechanisms of action [8-11].
Maraviroc (Celsentri®, Selzentry®) is the first drug of the class of CCR5 antagonists to be registered for treatment for HIV-1-infected antiretroviral therapy experienced patients, based on the 'Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients (MOTIVATE) 1 and 2 studies' [12, 13]. The aim of this paper is to review the MOTIVATE studies, and to discuss issues related to maraviroc therapy in clinical practice such as assessment of HIV-1 coreceptor tropism, tolerability and efficacy.

The MOTIVATE trials in antiretroviral-experienced HIV-1-infected patients

Background & rationale

Maraviroc is an antagonist for the chemokine (C-C motif) receptor 5 (CCR5 coreceptor), which is a G-protein-coupled transmembrane receptor able to bind CC-chemokines, such as CCL3 (MIP-1α), CCL4 (MIP-1β) en CCL5 (RANTES). CCR5 can be found on many different types of cells, such as CD4+ T cells, dendritic cells, monocytes and macrophages

[14]. The CCR5 receptor is suggested to be important in chemotaxis of macrophages and monocytes to sites of inflammation, and is involved in activation of T cells and cellular immunity. Besides its immunologic properties, the CCR5 receptor is, next to the chemokine (C-X-C motif) receptor 4 (CXCR4), a coreceptor for HIV-entry [15, 16].

The process of HIV entry initiates with binding of the HIV-1 envelop glycoprotein (gp) 120 to the cellular receptor protein CD4 on the target cell, resulting in conformational changes exposing the variable region of gp120 (V3 loop). The net charge of the V3 loop influences to which coreceptor the virus subsequently will bind.

CD4 and coreceptor binding leads to new conformational changes in gp120 enabling another envelop glycoprotein, gp41, to promote fusion between virion and cell [17]. 'Coreceptor tropism' refers to the ability of HIV-1 to enter $CD4^+$ cells using the CCR5 coreceptor ('R5-tropic virus'), the CXCR4 coreceptor ('X4-tropic virus'), or both coreceptors ('dual-tropic virus') [18]. Some patients are infected with a population of viruses with different coreceptor tropisms ('mixed-tropic virus'). The CCR5 coreceptor is of particular interest as a drug target, since persons homozygote for the CCR5-delta-32 allele repeatedly exposed to HIV-1 are at low risk to become infected. The CCR5-delta-32 allele contains an internal 32 base pair deletion, resulting in the inability to synthesize a functioning CCR5 protein [19]. The absence of the CCR5 receptor in these patients does not seem to result in significant clinical pathology, except for a possible a higher risk of symptomatic West Nile virus infection [20, 21].

The antiretroviral compound maraviroc is a small molecule, non-competitive allosteric inhibitor of the CCR5 coreceptor. Binding of maraviroc to CCR5 prevents interaction with the V3-loop of gp120, and thereby HIV entry and fusion.

The efficacy and safety of maraviroc was assessed in two randomized placebo-controlled phase 2a studies (A4001007 and A4001015) which included 63 patients infected with R5-tropic HIV-1. HIV-tropism was assessed by the original Trofile® assay (OTA; Monogram, CA, USA), a phenotypic tropism assay (see section titled 'Assessing HIV-1 coreceptor tropism' for details). Ten days maraviroc monotherapy with a dose of 100 mg BID or higher resulted in a maximal mean reduction of ≥1.6 $\log_{10}$ plasma HIV-RNA. Two participants experienced a tropism switch

(from R5-tropic virus to dual- or mixed tropic virus) during maraviroc monotherapy. In one patient R5-tropic virus rapidly regained dominance after maraviroc discontinuation, whereas in the other patient dual- or mixed tropic virus remained detectable at day 433. Maraviroc was tolerated well at all doses [22].
Based on these results the 'Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients (MOTIVATE) 1 and 2 studies' were initiated, which were designed to evaluate the efficacy and safety of maraviroc once daily or twice daily when added to an optimized background regimen (OBR) in antiretroviral experienced patients with R5-tropic HIV-1 [12, 13].

Design

The MOTIVATE 1 and 2 studies were parallel, randomized, double-blind, placebo-controlled, multinational phase 3 studies. MOTIVATE 1 was conducted in Canada and the United States, whereas the MOTIVATE 2 in Australia, Europe and the United States.
In short, patients aged 16 years or more, infected with R5-tropic HIV-1, with documented resistance to and/or at least 6 months of treatment experience with 3 different antiretroviral drug classes, with plasma HIV-1 RNA ≥5000 copies/mL, and on a stable antiretroviral regimen (or at least 4 weeks without cART) were randomized either to placebo or to once-daily or twice-daily maraviroc at a dose of 300 mg, all in combination with OBR. If necessary the maraviroc dose was adjusted to 150 mg once- or twice-daily, based on drug-drug interactions with other (antiretroviral) drugs. Off note, darunavir, raltegravir and etravirine were not yet approved at that time and therefore not allowed as OBR. The duration of the trial was 48 weeks, and continued with an open-label extension phase to 96 weeks. HIV-1 tropism was assessed by the original Trofile assay (OTA).

Data analysis

The primary endpoint was the mean change in $\log_{10}$ plasma HIV-RNA copies/mL between baseline and week 48, whereas secondary end points were plasma HIV-RNA levels <50 and <400 copies/mL, a decrease in plasma HIV-RNA 1.0 $\log_{10}$ or more from baseline, a change in CD4 cell count from baseline, and time to treatment failure. A safety analysis was performed as well. Results of the MOTIVATE-1 and -2 stud-

ies were pooled for an overall analysis, and treatment efficacy across various subgroups (such as baseline CD4 cell count strata, screening viral load strata, overall susceptibility score etc.) was analysed.

Results

Sixty-one percent of the 3244 patients that were screened were classified by OTA to be infected with R5-tropic HIV-1. Of these patients, 1075 were randomized, and 1049 patients received at least one dose of the study drug. Most patients in the studies were white (81-85%) and male (88-90%), the median CD4 cell counts were 167-171 cells/mm³ and median baseline HIV-1 RNA was 4.85-4.86 $\log_{10}$ copies/mL. Approximately half of the study participants (50-55%) had an OBR containing at least 2 active drugs according to a susceptibility score based on both genotype and phenotype, whereas the OBT of 41% of the study participants contained the fusion inhibitor enfuvirtide (categorized as "first use" in 59% of these patients). Baseline characteristics were similar between the study arms.

After 48 weeks, 143 (68%) study participants allocated to the placebo arm had discontinued treatment because of treatment failure, compared to 162 (39%) and 148 (35%) participants treated with maraviroc once or twice daily.

The main conclusion of the study was that study participants treated with maraviroc had a significant better virological and immunological response at week 48, as compared to placebo. The mean change in plasma HIV-1 RNA levels at week 48 was -0.79 $\log_{10}$ copies/mL in the placebo group compared to -1.68 and -1.84 $\log_{10}$ copies/mL in the maraviroc once or twice daily study arms ($p<0.001$); plasma HIV-RNA was undetectable (<50 copies/mL) in 179 (43%) and 194 (46%) participants treated with maraviroc once or twice daily, compared to 35 (17%) patients receiving placebo ($p<0.001$). Across all subgroups, the maraviroc arms were associated with a greater chance on complete virological response at week 48. A mean CD4 cell count increase of 124 cells/ µL in the maraviroc twice-daily arm, 116 cells/ µL in the once-daily arm, compared to 61 cells/ µL in the placebo arm ($p<0.001$) was observed.

Safety and tolerability

Overall a lower frequency of adverse events from any cause was found in the placebo arm as compared to the maraviroc arms (85% versus

91 and 92%), however no difference between the arms was found in treatment related adverse events. Diarrhoea, fatigue, fever, headache, nausea and upper respiratory tract infections were the most common adverse events. Differences in the rate of occurrence of fever (4% in placebo versus 2 and 6% in the maraviroc arms, overall p = 0.04) and headache (6% in the placebo versus 5 and 2% in the maraviroc arms, overall p = 0.03) were found. Of note, in this analysis no adjustment for study drug exposure was made, while the discontinuation rate was much higher in the placebo arm compared to the maraviroc arms (68% versus 39% and 35%), mostly because of lack of efficacy. No differences between the arms in the occurrence of serious adverse events were found.

Initially, concerns were present about potential hepatotoxic side effects of CCR5 antagonists, since the development of another CCR5 antagonist, aplaviroc (GlaxoSmithKline, London, UK) was discontinued because of severe hepatotoxicity [23]. In the MOTIVATE trials however, no significant differences in liver function test abnormalities between the study arms were found.

Virological failure and change in tropism

Retrospective analysis revealed a tropism switch from R5 tropism to dual- or mixed-tropism between screening and baseline, in 79 (8%) patients. One possible explanation for this observation might be that the virus population in these patients harbors a significant proportion of X4-virus, which is close to (but initially not above) the cut-off level for classification as a R5-tropic virus population. The observed switch in tropism between screening and baseline is most likey due to limited sensitivity of OTA, but it can not be excluded that progression to a slightly higher proportion of X4-virus between screening and baseline also occurred. As was to be expected, the rate of virological suppression in these patients was lower: 27 and 30% in the maraviroc arms, compared to 18% in the placebo arm. Analysis of 228 patients with a R5 tropism result at baseline and virological failure showed that 57% of patients receiving maraviroc had a D/M or X4 tropism result at time of treatment failure, compared to 6% of patients that received placebo. This suggests that D/M and X4 variants were selected for during CCR5 antagonist therapy. It has been shown that a tropism switch of HIV to X4 (“syncitium-inducing phenotype”) is predictive for an increased

rate of CD4 cell decline and progression to AIDS [24]. Therefore one of the concerns of CCR5 antagonist treatment was outgrow of X4- or dual-tropic minority variants with subsequent immunological deterioration. However in the MOTIVATE studies such deterioration was not observed, moreover the CD4 cell count increase was higher in the maraviroc arm as compared to the placebo arm. Moreover, in case of tropism switch during therapy, reversion to R5 tropism generally occurred if maraviroc was discontinued.

Reanalysis of the 48-week data using 454-sequencing for tropism assessment

The A4001029 trial was conducted to determine safety and efficacy of maraviroc in treatment experienced patients harbouring virus classified as D/M or X4-tropic by OTA [25], compared to placebo (both in combination with OBR). As was to be expected, at week 24 no significant decrease of plasma HIV-RNA was observed in the maraviroc arms compared to placebo. Initially a more prominent increase of the CD4 cell count was observed in the maraviroc twice daily arm (mean 27 cells/uL compared to placebo, p = 0.04), however at week 48 the difference was no longer significant

In a retrospective study the ultra deep 454-sequencing technique was applied to predict HIV coreceptor tropism on screening samples of participants of both the MOTIVATE and 1029 studies The ultra deep 454-sequencing technique is able to analyse all individual V3 sequences (sometimes thousands) from a single sample. Using this method twice as many patients treated with maraviroc were classified as non-R5 compared to OTA (n=240 versus 111). Overall, 454 sequencing matched the ability of OTA to predict the proportion of patients achieving <50 copies/mL [26].

Ninety-six week data

After 48-weeks, the MOTIVATE trial was extended with an open label phase to 96-weeks in which patients from both arms were offered therapy with maraviroc BID [27]. The vast majority of the patients in the maraviroc arms who had undetectable plasma HIV-RNA at week 48, continued to have undetectable plasma HIV-RNA at week 96 (81.4 and 86.7% in the once and twice daily arm). The median change in CD4 cell count at week 96 was comparable with week 48 (+89 and + 113

cells/μL). In the safety analysis, the frequencies of occurrence of adverse events were now adjusted for study drug exposure, and no difference was found between the maraviroc and placebo arms.

Experience with maraviroc containing CART in clinical practice

The available efficacy and tolerability data of maraviroc containing regimens from routine clinical practice are limited. Two retrospective cohort studies, from Spain and Germany, have been published [28, 29]. In the Spanish cohort 96.3% of patients (N = 46) had undetectable plasma HIV-RNA at week 48, with a mean CD4 cell count increase of 151 cells/μL. The OBR included a boosted protease inhibitor and raltegravir in the majority of patients (65.2%). In this cohort of therapy experienced patients with long standing HIV-infection (median 17 years) and high prevalence of hepatitis B and C coinfection (50%), maraviroc containing therapy was well tolerated. One patient developed an acute hepatitis possibly related to alcohol intake, however no definite cause could be established. Clinical events occurred in 8.7% of patients, including a non-Hodgkin lymphoma at week 36 in a patient with long standing disease and virological failure who experienced a CD4 cell gain >200 cells/μL after initiation of maraviroc containing therapy.

In the German cohort (N = 44) 78% of patients had undetectable plasma HIV-RN at month 6, whereas a median CD4 cell count increase of 124 cells/μL was observed. The major indication for maraviroc containing therapy in this heavily pre-treated cohort (median duration of cART 10.1 years; median number of previous drug combinations 9) was virological failure (56%). The median number of active drugs in the OBR was 2.5 (based on calculated genotypic sensitivity scores). Although the MVC containing regimens were generally well tolerated, liver enzyme abnormalities (< 3x upper limit of normal) developed in 6 patients. In 5 of these patients a casual relationship with maraviroc could not be excluded.

One prospective study from Italy has been published, where the efficacy and safety of the combination maraviroc, raltegravir and etravirine was evaluated in 28 antiretroviral therapy experienced patients[30]. All patients participated in the expanded access programs for these drugs (MK0518-023, raltegravir; A4001050, maraviroc; TMC125-C214, etravirine). At week 48, 93% of patients had a plasma HIV-RNA < 50 copies/mL, with a median CD4 cell count increase of 267 cells/μL. The regimens were well tolerated. Three serious adverse events occurred

(one anal cancer, one Hodgkin lymphoma and one recurrence of mycobacterial spondylodiscitis), and in one HCV-coinfected patient a transient increase in liver parameters was found due to alcohol abuse. No patients permanently discontinued maraviroc treatment.

Expert commentary

Assessing HIV-1 coreceptor tropism

Since maraviroc is registered for treatment of patients infected with R5-tropic HIV-1, viral coreceptor tropism has to be assessed before start of treatment with maraviroc [31]. Various tropism assays are available for use in clinical practice.

In the clinical trials, the phenotypic Trofile™ (Monogram, San Francisco, USA) assay was used for tropism assessment. In this assay, cell lines that express either the CCR5 or CXCR4 coreceptor are infected with a pseudovirus population containing a luciferase gene and envelope (including V3) genes of the patient derived viral population. When infection of one of these cell lines occurs, light will be emitted. This way tropism of the virus can be assessed [32]. Results of the Trofile are reported as R5 (CCR5 tropism), X4 (CXCR4 tropism) or D/M (dual or mixed tropism). When, due to technical reasons, no tropism results can be generated this will be reported as 'non-reportable'. The original Trofile assay (OTA), which was used in the MOTIVATE studies and the A4001029 trial, had limited sensitivity. It was able to detect X4 virus with 100% sensitivity if it made up at least 10% of the virus population (this was assessed using clonal mixtures) [18]. In 2008, the OTA has been replaced by an improved version, the 'enhanced sensitivity Trofile™ assay' (ESTA). It has been reported that the ESTA is able to detect X4 virus clones with 100% sensitivity when they comprise at least 0.3% of the virus population [33]. However, limited data are available on the sensitivity using clinical isolates. The ESTA can be performed both on viral RNA and pro-viral DNA. In Europe the ESTA is not readily available anymore.

Phenotypic tropism assays that are currently available in Europe are the classical MT-2 assay, the recombinant phenotypic Toulouse Tropism Test (Université Toulouse III Paul-Sabatier, Toulouse, France), and the Suisse developed PhenX-R (InPheno AG, Basel, Switzerland) which is a follow-up assay in a replicative format applied when the preceding XTrack^{c} genotypic assay ((InPheno AG) is not conclusive [34-36].

Genotypic tropism tests are based on amplification and sequence analysis of the V3 region of the virus population in a patient [18]. Population sequence analysis is the most frequently used technique; however these assays cannot detect minority variants less than 10-20% to the total virus population. Although the original Trofile is the only prospectively clinically validated tropism assay, population-based genotypic tropism prediction has been retrospectively clinically validated on stored plasma samples of the MOTIVATE studies, and in other cohorts [37, 38].
In order to predict the tropism of the virus, the obtained consensus sequence can be uploaded in a web based interpretation algorithm. Frequently used algoritms are the Position-Specific Scoring Matrix (PSSM) or the 'geno2pheno[coreceptor] algorithm' (http://www.geno2pheno.org/). In the geno2pheno algorithm, uploading the complete V3 sequence results into the generation of a quantifiable value, the 'false-positive rate' (FPR). The FPR reflects the chance that a R5 virus population is classified incorrectly as X4. By changing the cut-off value of the FPR one is able to define the sensitivity and specificity of the X4 prediction.
Genotypic tropism assays can be performed both on plasma HIV-RNA and on pro-viral DNA of PBMCs. The latter procedure can be used when plasma HIV-RNA in a patient is undetectable and a switch to maraviroc is warranted (for example, because of toxicity).
In these patients, it may also be possible to use the last sample with detectable plasma HIV-RNA for prediction of HIV coreceptor tropism. It has been shown that in patients with suppressed plasma HIV-RNA tropism switches seldom occur [39].
The European guidelines for HIV-1 tropism recommend to use the population-based genotypic tropism test, mostly because of practical and costs issues. These guidelines advise to use a cut-off FPR of 10% in most situations for both viral RNA and pro-viral DNA. This may be a rather conservative approach since the optimal cut-off (i.e. FPR) for R5-classification is not yet fully explored.
The DHHS guidelines recommend to use a phenotypic tropism assay when considering maraviroc treatment in a patient [40], because of the limited availability of genotypic tropism testing for routine care in the USA and the absence of prospective validation of genotypic tropism assays in clinical trials.

HIV-1 tropism and maraviroc therapy: not black and white

A small fraction X4-tropic virus in the total virus population of an HIV-1-infected patient can result into a D/M or X4 tropism classification, especially in case of the ESTA but also when using a genotypic tropism assay. It is currently unknown whether a small fraction X4-tropic virus (and what size of the fraction) has clinical relevance, especially since patients are generally treated with a combination of maraviroc and other active antiretroviral drugs.

In the reanalysis of the MOTIVATE and A4001029 studies with 454 genotypic tropism prediction (see above), it was found that the presence of 1-2% non-R5 HIV variants in the viral population impaired virological response (29% virological suppression at week 48 versus 65% when non-R5 was not detected) [26].

Of note, in a recent case study maraviroc was able to inhibit dual-tropic virus classified by ESTA [41]. These preliminary data points out that although viral variants are classified as X4 by a phenotypic tropism assay, they may preferably use the CCR5 coreceptor in vivo. Similar observation were observed in in vitro comparison studies by Svicher et al.[42], which suggests that maraviroc therapy in these patients might still be of benefit.

Prospective clinical research is necessary to explore the use of maraviroc in these situations, in order to prevent that maraviroc therapy is unnecessary withheld from these patients.

Resistance to maraviroc

Theoretically, virological failure on maraviroc therapy may occur via diverse mechanisms. Selection of a minority X4 variant that was already present in the virus population, resulting in a HIV coreceptor tropism switch; or development of new mutations that allow the virus to use the CXCR4 coreceptors (a change of HIV coreceptor tropism); alternatively, development of resistance is possible by selection of mutations that allow the virus to continue using the CCR5 coreceptors, despite blockade of these receptors by maraviroc. The latter two mechanisms especially concern mutations in the V3 region of the viral envelope protein gp120. The prevalence of these maraviroc resistance-related mutations in maraviroc naïve HIV-1-infected patients appears to be very low (<2%) [43]. Moreover, at present no convincing correlation has been found between individual mutations and maraviroc susceptibility

and no specified resistance pattern has been observed.
In clinical practice the first scenario (outgrowth of minority X4 population due to low selection pressure) is most likely to occur. Therefore, one has to be well informed about the tropism result and the possible presence of mutations resulting in resistance for other antiretroviral drugs in the regimen before starting maraviroc containing therapy.

Dosing and drug interactions
Based on in vitro testing of CCR5-tropic HIV-1 viruses, the geometric mean 90% inhibitory concentration (IC_{90}) of maraviroc against R5-tropic virus is 2.0 nmol/L [44, 45]. Based on week 24 efficacy data of the MOTIVATE studies and population pharmacokinetic analysis[13], the standard dosage of maraviroc is 300 mg twice daily. Maraviroc is a substrate for the cytochrome P_{450} 3A4 (CYP3A4) enzyme in the liver. Combination with other drugs that induce CYP3A4 (such as efavirenz) will result in a lower plasma maraviroc concentration, whereas combination with CYP3A4 inhibitors (such as protease inhibitors) will increase maraviroc plasma concentrations. Based on these findings, dose adjustment of maraviroc to 600 mg BID is necessary when maraviroc is combined with certain CYP3A4 inducing drugs, whereas the dose should be lowered to 150 mg BID when combined with drugs that inhibit CYP3A4[31]. Not only antiretroviral, but also other drugs are able to influence CYP3A4.
Before starting a maraviroc containing regimen, one should therefore specifically consider interactions with other (antiretroviral) drugs the patient is taking, and adjust the maraviroc dose accordingly. Since many patients are treated with protease inhibitors or efavirenz, this has to be done frequently in clinical practice.

Conclusion

The MOTIVATE studies as well as other observational cohort studies, demonstrated that a maraviroc containing regimen is effective in antiretroviral experienced patients infected with R5-tropic HIV-1 infection; moreover, side effects are mild [12, 28, 29]

Five-year view

There are some issues limiting the potential of maraviroc therapy in need to be solved during the next years. First, more long term data on maraviroc containing therapy from clinical trials and clinical practice are needed to establish a long term safety profile of maraviroc. Second, the logistic issues surrounding tropism testing have hampered use of the drug, especially in Europe. The introduction of the genotypic tropism test has now improved and simplified the logistics surrounding tropism testing significantly, making this less of an issue. The optimal cut-off FPR for R5-classification is not fully explored, with the risk that maraviroc treatment is unnecessary withheld in patients. Studies investigating and trying to solve these issues as the Modern Trial (A4001095) which explores the predictive value of different tropism assays are ongoing and should therefore continue during the next years. Third, maraviroc has to be dosed twice daily, in contrast to other new antiretroviral drugs for treatment experienced patients (such as the protease inhibitor darunavir) which can be taken once daily. Attempts to study alternative regimens with maraviroc once daily dosing in antiretroviral therapy naïve patients have been made such as the A4001078 trial, a phase IIb pilot trial which compared maraviroc plus atazanavir/ritonavir once daily versus tenofovir/emtricitabine plus atazanavir/ritonavir in ART naïve patients. Although this small study was not powered to detect differences between the arms, virological outcome at week 48 seemed in favor of the atazanavir/ritonavir arm, especially in patients with a baseline plasma HIV-RNA < 100,000 copies/mL (76.7% patients with plasma HIV-RNA <50 copies/mL in maraviroc arm versus 87.2% in atazanavir/ritonavir arm)[46]. The Modern trial will compare the efficacy of maraviroc with boosted darunavir once daily, as compared to emtricitabine/tenofovir with boosted darunavir, in antiretroviral treatment naïve patients (www.clinicaltrials.gov, NCT01345630). If once daily dosing appears to be safe, this would expand the therapeutic possibilities of maraviroc. The results are expected in 2013.

Key issues

- Sixty-one percent of the 3244 therapy experienced patients screened for the MOTIVATE trials were classified by OTA to be infected with R5-tropic HIV-1.
- At week 48, a larger decrease in plasma HIV-1 RNA was found in the maraviroc arms compared to placebo (-1.68 and -1.84 $\log_{10}$ copies/ mL in the maraviroc once and twice daily arms, compared to -0.79 in the placebo arm; $p<0.001$); the CD4 cell count increase was higher in the maraviroc arms compared to placebo (116 in the once-daily and 124 in the maraviroc twice-daily arm, compared to 61 cells/ µL in the placebo arm ($p<0.001$).
- No difference between maraviroc and placebo was found in treatment related and serious adverse events.
- These data, in combination with data from clinical practice, show that maraviroc is an effective and well tolerated therapy option for antiretroviral-experienced patients harboring R5-tropic virus populations.
- The European guidelines for HIV-1 coreceptor tropism testing advise to use a genotypic tropism assay before starting maraviroc containing therapy, whereas the DHHS guidelines recommend to use a phenotypic tropism assay.
- Current guidelines advises a relatively conservative 10% cut-off (i.e. FPR) for R5-classification using the geno2pheno algorithm, the optimal FPR is subject to further studies.
- Maraviroc is a substrate for the cytochrome P_{450} 3A4 (CYP3A4) enzyme in the liver; beware of interactions with other (antiretroviral) drugs.

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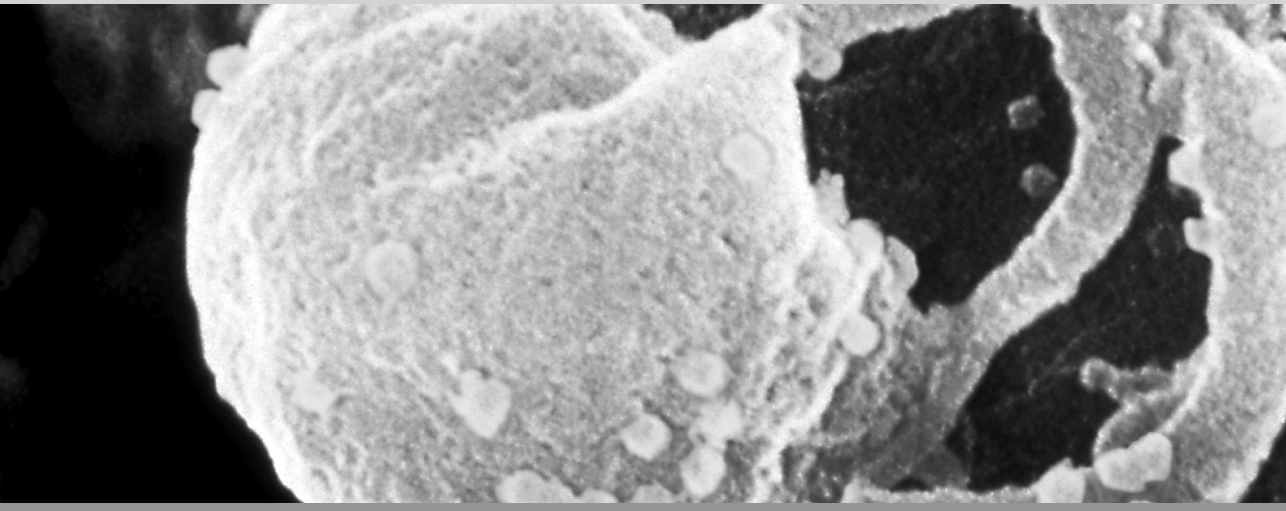
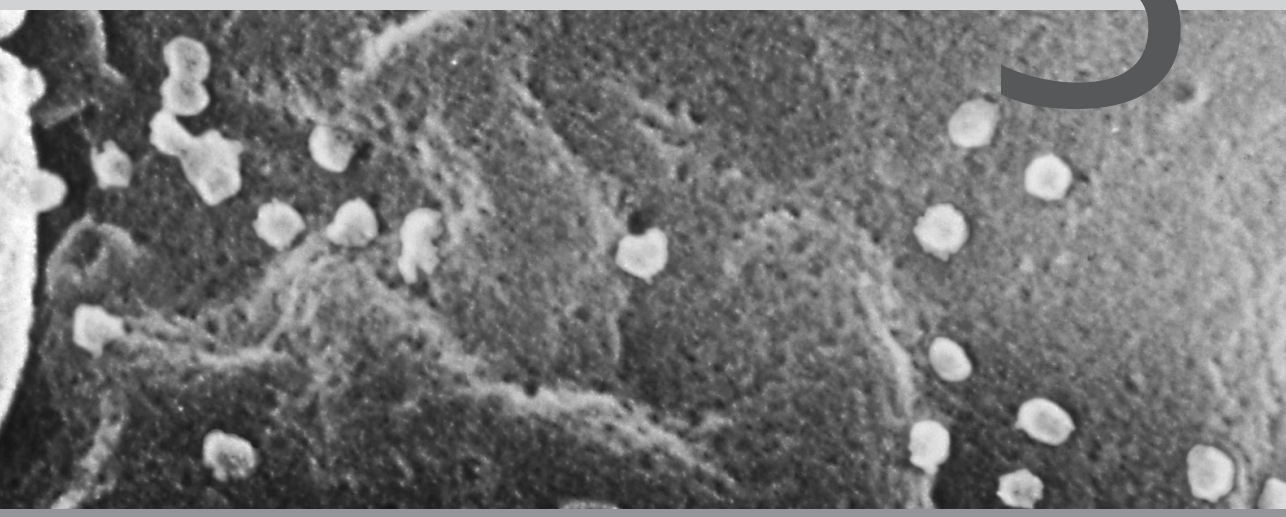


CHAPTER 3



Short- and long-term outcome of HIV-1-infected patients admitted to the intensive care unit

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Abstract

The purpose of this investigation was to analyse the impact of the availability of highly active antiretroviral therapy (HAART) on the long-term outcome of human immunodeficiency virus (HIV)-infected patients admitted to the intensive care unit (ICU). A retrospective cohort study of HIV-infected patients admitted to the ICU was undertaken. Outcomes in the pre-HAART era (1990–June 1996), early- (July 1996–2002), and recent-HAART (2003–2008) periods and total HAART era (July 1996–2008) were analysed and compared with those reported of the general population. A total of 127 ICU admissions were included. The 1-year mortality decreased from 71% in the pre-HAARTera to 50% in the recent-HAART period (p=0.06). The 5-year mortality decreased from 87% in the pre-HAART era to 59% in the early-HAART period (p=0.005). Independent predictors of 1-year mortality in the HAART era were age (odds ratio [OR]=1.16 [95% confidence interval [CI]=1.06–1.27]), APACHE II score>20 (6.04 [1.25–29.22]) and mechanical ventilation (40.01 [3.01–532.65]). The 5-year survival after hospitalisation was 80% and in the range of the reported survival of non-HIV-infected patients (83.7%). Predictors of 1-year mortality for HIV patients admitted to the ICU in the HAART era were all non-HIV-related. Short- and long-term outcome has improved since the introduction of HAART and is comparable to the outcome data in non-HIV-infected ICU patients.

Introduction

Worldwide, 33 million people are estimated to be infected with the human immunodeficiency virus (HIV), and this number continues to rise [1]. Complications of HIV-infection and its treatment may lead to intensive care admission. In the early years of the HIV pandemic, the admission of HIV-infected patients to the intensive care unit (ICU) was considered to be controversial due to the reported high short term mortality rates (60-81%) [2-4]. In the years thereafter, reported mortality rates improved but remained variable (24-81%) [5-7].

The introduction of highly active antiretroviral therapy (HAART) has dramatically improved the overall mortality and morbidity of patients infected with HIV [8]. As a result, the life expectancy of HIV-infected patients seems comparable to those having other chronic medical diseases (e.g. diabetes mellitus) [9] and in some cases even to the general population [10]. However, according to some analyses the life expectancy of HIV-infected patients still seems to be shorter than that of the general population [11]. The improvements in the prognosis have also had implications for critically ill HIV-infected patients. Since the beginning of the HAART era, the spectrum of illnesses of HIV-infected patients admitted to the ICU has changed and non-AIDS related ICU admissions are seen more often [7, 12]. The mortality rates of these patients admitted to the ICU during the HAART era have improved but remain highly variable. Recently reported ICU and hospital mortality is in the range 8.6 - 32% [13-15], although not all studies report an improvement of short term mortality rates in the HAART era [12, 16].

Data on the long term mortality of HIV-infected patients admitted to the ICU are scarce and originate from the early years of the HAART era, reporting a 2-year survival after ICU discharge of 70.8% [12], and a median overall survival of 324 days [17]. We set out to analyse both the short- and long-term mortality of HIV-positive patients admitted to the ICU in a large University Hospital before and after the introduction of HAART, and compared these with reported mortality rates of non-HIV-infected patients.

Methods

Subjects

A retrospective cohort study was conducted at the University Medical Center Utrecht (The Netherlands), a 1042-bed tertiary care center with a 32 bed ICU. The study population consisted of HIV-infected patients admitted to the ICU from 1990 to 2008. Patients were identified by a search of the hospital administration database. The inclusion criteria were as follows: age over 18 years and positive serology for HIV-1. Patients admitted for postoperative care or auto-intoxication were excluded. In case of more than one ICU admission per patient, data were collected for subsequent ICU admissions only if they occurred in the course of a separate hospitalisation.

The included patients were divided into cohorts representing the period before the introduction of HAART (1 July 1996 was used as the cut-off date for the availability of HAART), the pre-HAART era (1 December 1990 - 1 July 1996) and the HAART era (1 July 1996 - 31 December 2008). Because further improvement in the treatment of HIV-infected patients has been made during the HAART era itself, the HAART cohort was divided into an early-HAART period (1 July 1996 - 31 December 2002) and a recent-HAART period (1 January 2003 - 31 December 2008).

Data collection

Data were collected by the review of medical records. Variables included age, sex, nadir $CD4^+$ count, indication for ICU admission, use of HAART at ICU admission (HAART was defined as the use of least three different antiretroviral drugs) and need for mechanical ventilation during ICU stay. For the $CD4^+$ cell count and plasma HIV viral load (HIV-RNA [log10 copies/ml], if available) the most recent values at ICU admission (limited from six months prior to one week after ICU admission) were recorded. Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated for each ICU-admission[18]. For multivariate analysis, the APACHE II score was dichotomised into ≤ 20 and > 20. Where possible we calculated the Sepsis-related Organ Failure Assessment (SOFA) score [19] during admission. Medical charts were reviewed for indications for ICU admission; these were categorized as respiratory failure, sepsis, neurologic disease, circulatory shock or other indications. The categories for ICU admission were based on the criteria for organ failure used by our intensive care specialists. In our

hospital the following criteria derived from the SOFA score are used for organ failure assessment. Cardiovascular insufficiency: systolic bloodpressure < 90 mmHg, the need for >2000 ml fluid resuscitation to maintain a systolic blood pressure > 90 mmHg, vasoactive medication to maintain a systolic blood pressure > 90 mmHg or the mean arterial pressure (MAP) > 65 mmHg. Respiratory insufficiency: the need for invasive or non-invasive mechanical ventilation to maintain oxygen saturation >90% or paO2/FiO2 < 300 (either on mechanical ventilation or without mechanical ventilation). Renal failure: classification based on the RIFLE-criteria [20]. In short, a serum creatinine which is twice the normal creatinine of this patient before ICU admission and a urinary output < 0.5 ml/kg for at least 12 hours, or worse. Hepatic failure: bilirubin values twice the upper limit of normal (ULN). Cerebral failure: Glasgow coma scale ≤ 12. Coagulation: thrombocytes <150 x 10^9/L.
Admission due to pneumonia (all types) and to *Pneumocystis jirovecii* pneumoniae (PJP) was recorded. The final diagnosis at hospital discharge or death was listed and relation to AIDS was established using the 1993 Centers for Disease Control and Prevention (CDC) criteria [21]. Follow up of survival after hospital discharge occurred until 1 March 2009, by use of the hospital database, contacting the patients' general practitioner or the death registry of the community of Utrecht. Endpoints were ICU, hospital, 1-year and 5-year mortality and survival after hospital discharge. The HAART cohort was analyzed according to the use of HAART at the time of ICU admission, in order to be able to assess its effect on the short- and long-term mortality.

Statistical analysis
SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Continuous variables were compared using Student's *t*-test or the Mann-Whitney test, for categorical variables Chi-square or Fisher's exact test were used. Statistical significance was determined at $p < 0.05$. Survival after hospital discharge was analyzed by computing a Kaplan-Meier survival curve of the pre-HAART and HAART cohort, significance of difference in survival was determined using a log-rank test. Predictors of 1-year and 5-year mortality in the HAART era were analysed using logistic regression models. Variables were included in the multivariate analysis for $p < 0.1$ in the univariate analysis. For survival and long term mortality analysis, only the first ICU admission was included.

Results

Subjects

During the 18-year study period a total of 33,700 patients were admitted to the ICU, including 163 (0.5%) admissions of HIV-infected patients. After the exclusion of auto-intoxications (n = 18) and postoperative patients (n = 18), 127 ICU admissions of 117 HIV infected patients were analysed. Most patients (91.5%) were admitted to the ICU only once during the study period. The maximum number of separate hospitalisations, with an ICU admission, for a single patient was two. *Figure 1* shows a flow chart of the study cohort.

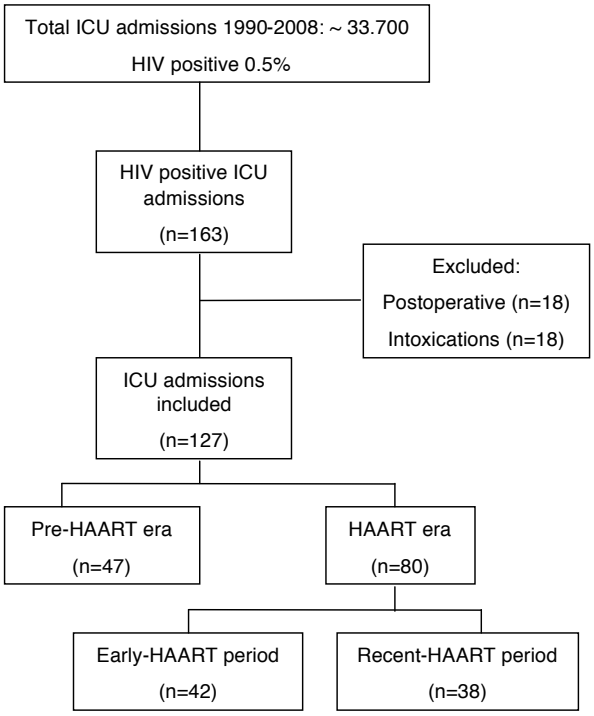

Figure 1. Flow chart of the included intensive care unit (ICU) admissions of human immunodeficiency virus (HIV)-infected patients, 1990–2008. n=number of patients; HAART=highly active retroviral therapy. Pre-HAART era: 1990–1996, HAART era: 1996–2008, early-HAART period: 1996–2003, recent-HAART period: 2003–2008.

Comparison of different time periods

The characteristics of the patients admitted to ICU during the pre-HAART era (n=47), early-HAART period (n=42) and recent-HAART period (n=38) are reported in *Table 1*. Respiratory failure was the main indication for admission on the ICU (58%), while sepsis, neurological diseases and circulatory shock accounted for respectively 12%, 11% and 16% of the ICU admission indications. Respiratory failure remained the main indication for ICU admission in the HAART era (57% versus 59% in the pre-HAART era). The use of mechanical ventilation increased during the HAART era (57% to 85%, p = 0.001). However, in the recent-HAART period we observed a non-significant decrease in the frequency of both pneumonia (29% compared to 49% in the pre-HAART era, p = 0.06) and PJP (5% compared to 26%, p = 0.08).

In approximately half of the patients (52%) the ICU diagnosis was AIDS-related. Although there was a trend for a higher CD4+ cell count at admission during the recent-HAART period (122 compared with 60 in the pre-HAART era, p=0.08), no difference in AIDS-related diagnosis was observed in the HAART era compared to the pre-HAART era (50% versus 55%). In 13% of patients, HIV-diagnosis was made during admission to ICU (no difference between the various time periods).

The length of ICU stay increased from 5 days in the pre-HAART era to 10 days in the recent-HAART period (p = 0.03). The median length of ICU stay was not significantly different between ICU survivors and non-survivors in the HAART era (6 versus 8 days, p = 0.19). However, in the pre-HAART era, the median duration of ICU stay was significantly longer in ICU non-survivors: 12 versus 3 days (p < 0.001).

We were able to calculate the SOFA score of 39 admissions in the HAART era (49%). We used the highest SOFA score and the initial score (first day of admission) for our analysis, since these are found to be predictive for ICU mortality [22]. There was no difference between the median highest SOFA score of patients admitted in the recent-HAART period compared with the early-HAART period (8.0 [range 32.0] and 7.0 [range 19.0], respectively; p = 0.11), while a trend for a higher initial SOFA score was found in the recent-HAART period (6.0 [range 13.0]) compared to the early-HAART period (3.5 [range 8.0], p = 0.06).

During the HAART era, a minority of all patients (36%) was treated with HAART on admission to the ICU. In addition, four patients started

Table 1. Characteristics of HIV positive patients admitted to ICU, 1990-2008.

Characteristics	All patients 1990-2008	Pre-HAART 1990-1996	Early-HAART 1996-2002
n	*127*	*47*	*42*
Age, years[a]	42 (22-73)	40 (22-68)	42 (28-63)
Male gender	104 (82)	37 (79)	35 (83)
Days since HIV diagnosis[b]	106 (0-6910)	500 (0-2263)	48 (0-4722)
HIV-diagnosis at ICU admission[c]	15 (13)	7 (16)	3 (8)
Use of HAART on admission	29 (23)	-	11 (26)
HAART use, days[a]	483 (6-2226)	-	1109 (20-2226)
≤ 6 months HAART use	16 (62)	-	3 (38)
CD4+ count, cells/μl[d]	73 (0-728)	60 (1-728)	31 (0-642)
< 200 CD4 cells/μl	77 (61)	23 (49)	30 (71)
≥ 200 CD4 cells/μl	25 (20)	6 (13)	6 (14)
CD4+ nadir count, cells/μl[e]	73 (0-728)	57 0-728)	30 (0-622)
HIV viral load, $\log_{10}$ copies/ml[f]	4.5 (1.5)	-	4.5 (1.4)
APACHE II score[a, g]	24 (6-41)	22 (6-39)	24 (10-41)
Use of ventilation	95 (75)	27 (57)	34 (81)
Invasive ventilation	88 (69)	27 (57)	29 (69)
Length of ventilation, days[h]	8 (1-58)	10 (1-58)	7 (1-37)
Pneumonia	62 (49)	23 (49)	28 (67)
PJP	30 (24)	12 (26)	16 (38)
ICU indication:			
Respiratory failure	74 (58)	27 (57)	29 (69)
Sepsis	15 (12)	3 (6)	7 (17)
Neurologic diseases	14 (11)	1 (2)	3 (7)
Circulatory shock	20 (16)	14 (30)	1 (2)
Other	4 (3)	2 (4)	2 (5)
AIDS-related ICU diagnosis	66 (52)	26 (55)	22 (52)
ICU stay, days	6 (1-60)	5 (1-58)	6 (1-46)
Hospital stay, days	20 (1-186)	20 (4-58)	19 1-108)

n = number of patients; HIV = Human Immunodeficiency Virus; ICU = Intensive Care Unit; ART = Antiretroviral Therapy; HAART = Highly Active Antiretroviral Therapy; APACHE II = Acute Physiology and Chronic Health Evaluation II; PJP = Pneumocystis jirovecii pneumoniae. Values are given as median (range) or number of ICU admissions (%), unless otherwise indicated.

Recent-HAART 2003-2008	HAART total 1996-2008	p-value[i]
38	*80*	
44 (26-73)	43 (26-73)	0.15
32 (84)	67 (84)	0.48
87 (0-6910)	71 (0-7303)	0.74
5 (15)	8 (11)	0.48
18 (47)	29 (36)	-
205 (6-1153)	483 (6-2226)	-
13 (72)	16 (62)	-
122 (0-578)	83 (0-642)	0.60
24 (63)	54 (68)	0.57
13 (34)	19 (24)	
101 (3-479)	80 (0-622)	0.38
4.5 (1.6)	4.5 (1.5)	-
26 (15-40)	25 (10-41)	0.08
34 (90)	68 (85)	0.001
32 (84)	61 (76)	0.08
10 (1-46)	8 (1-46)	0.80
11 (29)	39 (49)	0.98
2 (5)	18 (23)	0.65
18 (47)	47 (59)	0.89
5 (13)	12 (15)	0.17
10 (26)	13 (16)	0.02
5 (13)	6 (8)	0.001
0	2 (3)	0.63
18 (47)	40 (50)	0.56
10 (2-60)	7 (1-60)	0.17
36 (2-186)	22 (1-186)	0.15

[a]Mean (range); [b]Data were available for 122 ICU admissions; [c]First admissions were used for the calculation of percentages; [d]Data were available for 102 ICU admissions; [e]Data were available for 107 ICU admissions; [f]Mean $\log_{10}$ (standard deviation), data were available for 70 ICU admissions; [g]Data were available for 110 ICU admissions; [h]Data were available for 95 ICU admissions; [i]P-value of pre-HAART versus HAART cohort; - : not available

HAART during admission to the ICU. An increase in the use of HAART at ICU admission was noted during this period, increasing from 26% in the early- to 47% in the recent-HAART period (p=0.049). Patients on HAART at admission to the ICU had lower plasma HIV-RNA loads (3.4 versus 5.3 $\log_{10}$ copies/ml) and higher $CD4^+$ cell counts (106 versus 49 cells/µl). However, there was no significant difference in AIDS-related illnesses between these patients (45% versus 53%, p = 0.49).

Mortality

The overall ICU mortality was 37%, while the overall hospital mortality was 50% (*Table 2*). In the pre-HAART era, the ICU mortality was 47% and the hospital mortality was 60%, compared to 21% and 34% in the recent-HAART period (p = 0.01 and p = 0.02, respectively).

The overall 1-year mortality was 60%. There was a trend for decrease of 1-year mortality: 71% in the pre-HAART era compared to 53% in the HAART era (p=0.05). Due to the fact that some patients in our cohort were admitted to the ICU recently, 5-year mortality data from patients admitted in the HAART era was only available for 57 out of 72 patients. The 5-year mortality decreased from 87% in the pre-HAART era to 68 % in the overall HAART era (p = 0.03), being the lowest in the early-HAART period (59%, p=0.005).

Fifty-six patients survived hospitalization, the median overall survival after hospital discharge was 2430 days. Patients in the HAART era had a significantly better survival compared to the pre-HAART era (log-rank test p < 0.001 [*Figure 2*]). In the HAART era, 90% of patients were alive at 1 year and 80% at 2.5 and 5 years after hospital discharge.

Twenty-three patients survived their first admission to ICU, but died within one year. Fourteen patients died during further hospitalisation after discharge from the ICU. Ten of these patients (71%) died of conditions related to the reason for ICU admission, two (21%) causes of death were not related to ICU admission and one was unknown. Out of nine patients who died after hospitalisation but within one year, five deaths (56%) were unrelated to ICU admission, while two deaths were related (22%) and two (22%) were unknown.

No significant difference in short term mortality rates was found according to use of HAART on ICU admission. Observed ICU and hospital mortality were 35% and 55% in the HAART group compared to 29% and 39% in the group without HAART, respectively. However, higher

Table 2. Outcome of HIV-infected patients admitted to ICU, 1990-2008.

Outcome	All patients	Pre-HAART era	Early-HAART period	p-value [a]	Recent-HAART period	p-value[b]	HAART total	p-value[c]
ICU mortality	47 (37)	22 (47)	17 (41)	0.55	8 (21)	0.01	25 (31)	0.08
Hospital mortality	64 (50)	28 (60)	23 (55)	0.65	13 (34)	0.02	36 (45)	0.11
1-year mortality	68 (60)	32 (71)	21 (55)	0.13	15 (50)	0.06	36 (53)	0.05
5-year mortality	78 (76)	39 (87)	22 (59)	0.005	17 (85)	0.86	39 (68)	0.03

Total period of inclusion: 1990-2008; Pre-HAART era: 1990-June 1996; HAART era: July 1996-2008; early-HAART period: July 1996-2002; recent-HAART period: 2003-2008; HIV = Human Immunodeficiency Virus; ICU = Intensive Care Unit; HAART = Highly Active Antiretroviral Therapy. Values are given as number of patients (%).

P-values are given for comparisons between aearly-HAART period versus pre-HAART era, brecent-HAART period versus pre-HAART era and cHAART versus pre-HAART era.

For 1-year and 5-year mortality analysis only first admissions were used: 117 patients (pre-HAART 45, early-HAART 38 and recent-HAART 34). 113 out of 117 patients completed 1-year follow up (pre-HAART 45, early-HAART 38 and recent-HAART 30), while 102 patients completed 5-years of follow up (pre-HAART 45, early-HAART 37 and recent-HAART 20).

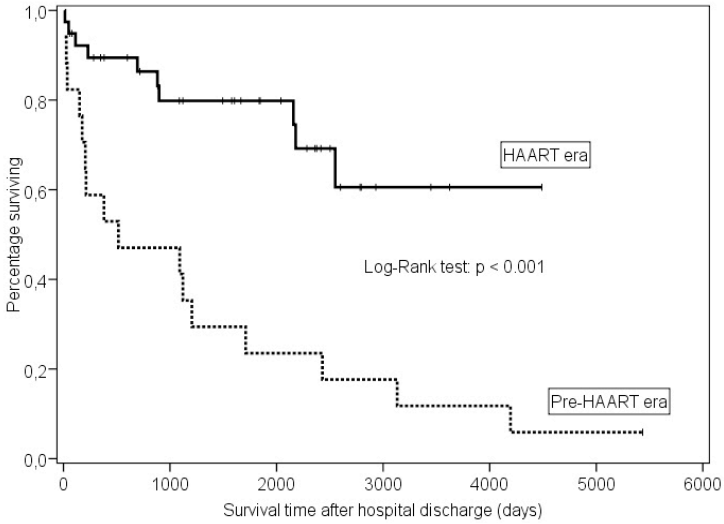

Figure 2. Kaplan–Meier survival curve for all HIV-infected patients admitted to an ICU and surviving until hospital discharge, stratified by time period. Solid line: HAART-era (1996–2008), dotted line: pre-HAART era (1990–1996). Survival after hospital discharge is significantly better in the HAART era compared to the pre-HAART era, using a log-rank test ($p<0.001$).

mortality after one year was observed in the group using HAART (70% versus 44%, p = 0.05), as was the case for 5-year mortality (85% versus 60%, p = 0.048). Out of four patients who started HAART during ICU admission, one died during ICU stay and another died during further hospitalization. No patients died from conditions related to immune reconstitution inflammatory syndrome (IRIS).

We found a trend for improvement of the ICU and hospital mortality for patients admitted with pneumonia (all types) in the HAART era (31% and 41% compared to 52% and 65% in the pre-HAART era; p = 0.10 and 0.07, respectively). Moreover, the 1- and 5-year mortality significantly improved during HAART era (38% and 44% compared to 70% and 80%; p = 0.02 and 0.001, respectively).

Thirty cases of PJP were recorded. When comparing the pre-HAART and HAART era, we found no difference in the ICU mortality (42% versus 33%, p = 0.71). The 1-year and 5-year mortality was decreased in the HAART era (38% and 47%) compared to the pre-HAART era (58% and 75%, respectively). However this was not statistically significant (p = 0.27 and 0.24, respectively).

Predictors of the 1-year mortality

Predictors of the 1-year mortality in the HAART era according to univariate analysis were older age, APACHE II score > 20 and the use of mechanical ventilation (*Table 3*). After multivariate logistic regression analysis, older age (odds ratio [OR] 1.16, 95% Confidence Interval [CI] =1.06-1.27), APACHE II score > 20 (OR 6.04, CI 1.25-29.22) and mechanical ventilation (OR 40.01, CI 3.01-532.65) remained independent predictors for the 1-year mortality. Neither HIV-related factors, nor the use of HAART on ICU admission were independent predictors for the 1-year mortality (OR 1.12, CI 0.35-3.56). The same independent predictors were found for the 5-year mortality in the HAART era: older age (OR 1.17, CI 1.03-1.33), APACHE II score > 20 (OR 5.86, CI 1.02-33.63)

Table 3. Univariate and multivariate predictors of 1-year mortality of HIV-infected patients admitted to ICU in the HAART era.

Variables	Univariate OR (95% CI)	p-value	Multivariate OR (95% CI)	p-value
Age	1.09 (1.03 - 1.16)	0.003	1.16 (1.06 - 1.27)	0.01
Male gender	0.93 (0.25 - 3.38)	0.91	-	-
Use of HAART on admission	2.86 (0.99 - 8.29)	0.05	1.12 (0.35-3.56)	0.78
CD4+ cell count, cells/µl[a]	1.00 (1.00 - 1.00)	0.72	-	-
CD4 < 200 cells/µl	1.00	-	-	-
CD4 ≥ 200 cells/µl	0.40 (0.07-2.29)	0.30	-	-
HIV viral load, $\log_{10}$ copies/ml[b]	0.90 (0.64 - 1.27)	0.56	-	-
APACHE II score > 20[c]	6.4 (1.61 - 25.52)	0.009	6.04 (1.25 - 29.22)	0.03
Mechanical ventilation	6.65 (1.32 - 33.65)	0.02	40.01 (3.01 - 532.65)	0.005
HIV related ICU diagnosis	0.56 (0.21 - 1.46)	0.23	-	-
PJP	0.44 (0.14 - 1.39)	0.16	-	-
ICU admission indication:				
Respiratory failure	1.00	-	-	-
Sepsis	0.95 (0.06-16.31)	0.97	-	-
Neurologic diseases	1.75 (0.08-36.29)	0.72	-	-
Circulatory shock	1.40 (0.07-28.12)	0.83	-	-
CD4+ cell nadir:				
<200 cells/µl	1.00	-		
≥200 cells/µl	0.40 (0.07-2.27)	0.30		

HIV = human immunodeficiency virus; ICU = intensive care unit; HAART = highly active antiretroviral therapy; APACHE II = Acute Physiology and Chronic Health Evaluation II; PJP = Pneumocystis jirovecii pneumonia; OR = odds ratio; 95% CI = 95% confidence interval; - = not applicable (statistically insignificant data were not included). 1-year mortality data was available of 68 patients in the HAART era, 36 patients died within 1-year after admission to the ICU. For multivariate analysis 67 patients were included (of which 35 patients died), due to one missing APACHE II score.

[a]data available for 61 patients; [b]data available for 57 patients; [c]data available for 67 patients.

and the use of mechanical ventilation (OR 210.40, CI 5.71-7753.51). PJP and the use of HAART on ICU admission were included in the model as well, but were not significant predictors for the 5-year mortality.

Discussion

In this study, we present recent data of both short and long term mortality of HIV-infected patients admitted to an ICU. Both short and long term mortality, as well as overall survival after hospital discharge has improved significantly in the HAART era.

The frequency of admission of HIV-infected patients to the ICU in our hospital increased during the study period (47 in the pre-HAART era, 80 in the HAART era). This might either be explained by a possible tendency to withhold ICU care to HIV-patients in the earlier days of the epidemic, or by the increase of the HIV epidemic. As in other studies [7, 14] respiratory failure remains the most common reason for ICU admission in our study (59% in the HAART era), while pneumonia remains an important ICU admission diagnosis (49% in the pre- HAART and HAART eras). The use of mechanical ventilation increased (85% in the HAART era compared to 57% in the pre-HAART era, p=0.001), while the percentage of patients admitted with respiratory failure remained the same. This might suggest that intensivists nowadays are more inclined to use this intervention in HIV-infected patients. The increase in duration of ICU admission (10 days in the recent-HAART period versus 5 days in the pre-HAART period, p = 0.03) is in line with this observation.

In contrast with other studies of HIV-patients admitted to the ICU [7, 12] and reports that the mortality of HIV-infected patients in general (due to non-AIDS related diseases) increases [23], the relative frequency of AIDS-related ICU diagnosis remained the same during the whole study period (50% in the HAART era versus 55% in the pre-HAART era, p = 0.56). This observation might be explained by the HIV-related patient characteristics in the current study. First, the median $CD4^+$ cell count on ICU admission remained low during the whole study period (the highest being 122 cells/μl in the recent-HAART period) indicating that this population remains at high risk for AIDS-related diseases. In addition, the percentage of patients on HAART at ICU admission was low (36% in the recent HAART era). These characteristics indicate that the

population admitted to the ICU in our study is not representative of the general HIV-infected patient population.

The improvement in short term mortality rates in the present study was most pronounced in the recent-HAART period. The ICU mortality of 21% and hospital mortality of 34% during this period are in line with recently published data of other cohorts [12-15], as is the 1-year mortality rate of 53% in the HAART era [12, 24]. More importantly, the ICU mortality in our cohort is in the same range as that reported in a non-HIV infected general ICU population (9.9 - 20.6%) [25, 26].

Data on long term mortality rates after ICU admission are very scarce. Besides a trend for the improvement of the 1-year mortality in the HAART era (53% compared to 71% in the pre-HAART era, p=0.05), we report a significant improvement in 5-year mortality in the HAART era (68% versus 87% in the pre-HAART era) of HIV-infected patients admitted to the ICU in our hospital. Despite this improvement the 5-year mortality rate is still higher than that reported in the HIV-negative ICU population (40.1 - 47%) [25, 26]. However, since a significant proportion of patients in our cohort was admitted recently and did not yet reach 5 years of follow up, a further decrease of the 5-year mortality rate might occur within the next couple of years. Moreover, we found that the long term survival of patients in the HAART era who survived hospitalisation (80% at 5 years) was in the range of the reported survival after hospital discharge of non-HIV infected patients (83.7%) [27], although the median age was lower in our cohort (42 versus 61 years). The long term mortality rates of patients admitted for PJP specifically seemed to improve in the HAART era as well (5-year mortality of 47% compared to 75% in the pre-HAART era), although this was not statistically significant (p = 0.24). In general, however, the long term mortality of patients admitted with a pneumonia (all types) during the HAART era decreased to 44% (compared to 87% in the pre-HAART era, p = 0.001). The improvements in the long-term mortality rates are probably the result of better treatment options for HIV-infection (HAART).

As was reported elsewhere as well [15] independent predictors for the 1-year and 5-year mortality in the HAART era were all non-HIV related: older age, APACHE II score > 20 and the use of mechanical ventilation. This suggests that other (non-AIDS related) co-morbidities might also be responsible for long term mortality, which is in line with the obser-

vations mentioned above that the mortality rates of HIV-infected patients admitted to the ICU seem close to that of the general ICU population. However, in the current retrospective study a systemic record of co-morbidities was not always available, so their potential effect on the long-term mortality rates in our study remains unknown.

The actual use of HAART at the time of ICU admission was not associated with a better outcome in our study. Moreover, we found higher 1-year and 5-year mortality rates in patients using HAART at ICU admission. We speculate that admission to the ICU while on HAART is an indication of an unsuccessful HAART treatment (underlined by the fact that the median $CD4^+$ cell count was only 106 cells/μL in this group of patients), resulting in fewer long term treatment options (for example, due to resistance to antiretroviral drugs). A previous study did not find differences in the long term mortality, based on the use of HAART at time of the ICU admission, either [17].

This study has limitations. First, this study was of retrospective nature. This could have resulted in uncontrolled confounders, such as differences in ICU admission policies (although the guidelines of our institution did not change during the study period) and treatment modalities, which might have influenced the outcome. Second, this study was conducted at a single institution and this limits the generalisability of our results. However, since we analysed data from both the pre-HAART and HAART era in our hospital, the improvements in survival since the introduction of HAART seem valid.

Third, larger studies with more endpoints might generate more statistical power to detect differences. Finally, no matched cohort of non-HIV patients admitted to the ICU for the comparison of mortality rates was available. We, therefore, had to use the mortality data of other cohorts reported in the literature in order to make a comparison.

Nowadays HIV-infected patients admitted to the ICU in the HAART era have a better outcome. Although the attitude of intensivists towards the admission of HIV-infected patients to an ICU seems to have changed, the recently published results of a multicenter survey performed in Italy suggest there is still room for improvement [28]. The objective of this study was to assess the policy of Italian intensivists towards critically ill HIV-infected patients in the HAART era. Only 30.4% of the ICU physicians considered the admission of HIV-infected patients

to be ‘always appropriate’. Apparently still more information and education is necessary to change the attitude towards the admission of HIV-infected patients to an ICU. In this cohort, the short term mortality and survival after hospital discharge are in the range of those reported of non-HIV infected patients [25-27], suggesting that the attitude towards admitting HIV-infected patients should be the same as in HIV-negative patients.

In conclusion, both the short- and -long term outcome of HIV-infected patients admitted to the ICU has improved during the HAART era. The results of this study emphasise that HIV infection is not a criterion to withhold intensive care treatment, since the outcomes seem almost in the range of those reported of the general population and predictors of long term mortality are all non-HIV related.

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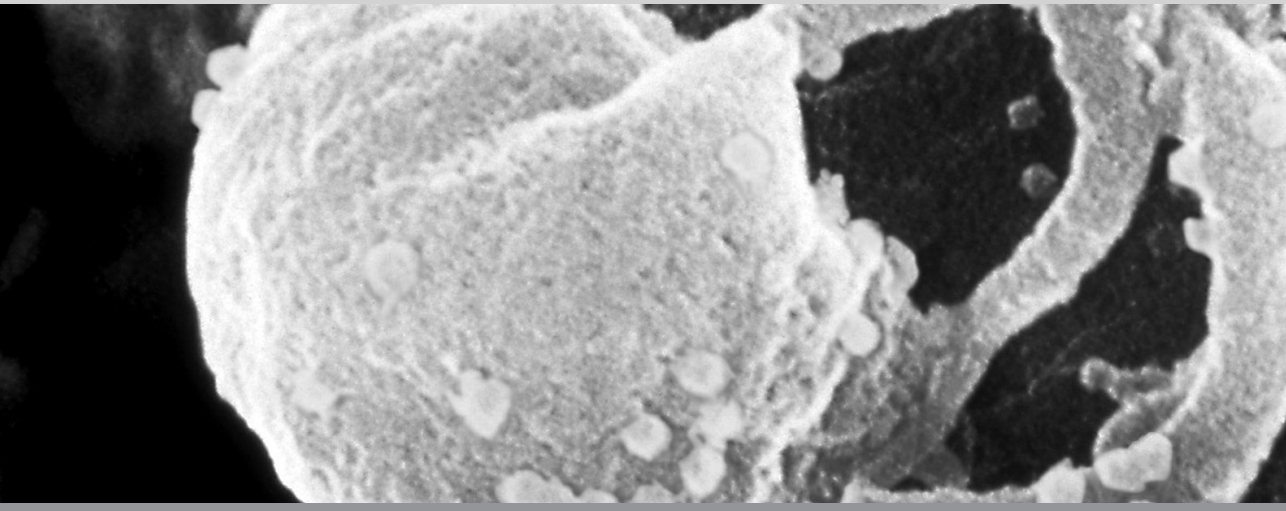
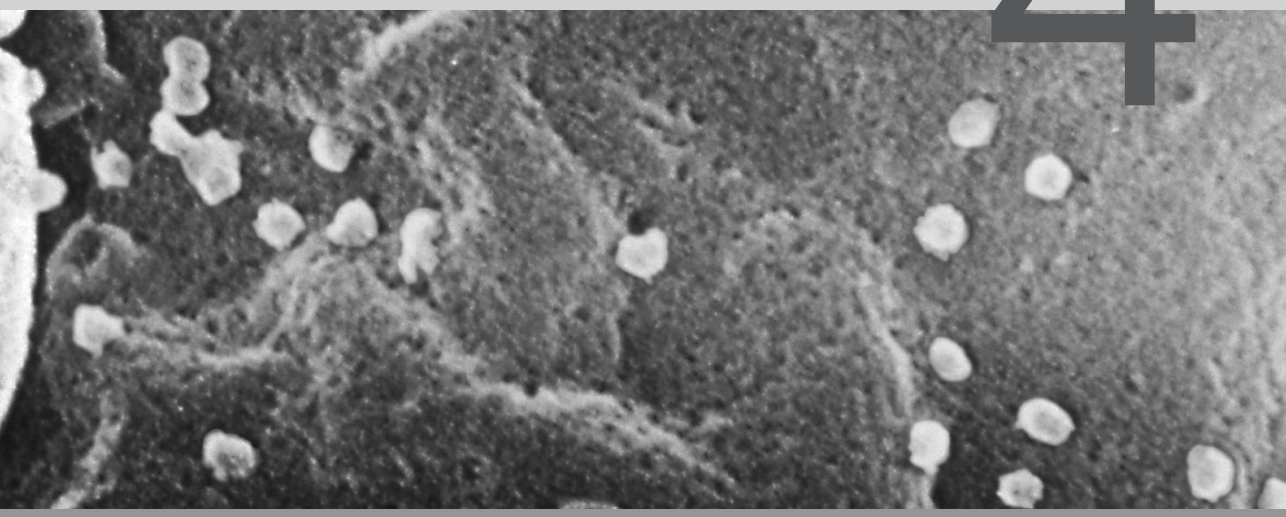


CHAPTER 4



Long term complications in patients with poor immunological recovery despite virological successful HAART in Dutch ATHENA cohort

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Abstract

Objective: We investigated the risk of AIDS and serious non-AIDS defining diseases according to the degree of immunological recovery after 2 years of virological successful antiretroviral therapy (HAART).

Design: Retrospective observational cohort study including HIV-infected patients treated with HAART resulting in viral suppression (<500 copies/ml).

Methods: Patients were grouped according to their CD4 cell count after two years of HAART: CD4 cell count <200 (A), 200-350 (B), 351-500 (C) or >500 cells/µl (D). Analysis was done to assess predictors for poor immunological recovery and the occurrence of a composite endpoint (death, AIDS, malignancies, liver cirrhosis and cardiovascular events), non-AIDS defining diseases (non-ADD), cardiovascular events (CVE) and non-AIDS defining malignancies (non-ADM).

Results: 3068 patients were included. Older age, lower CD4 cell nadir, and lower plasma HIV-RNA at start of HAART were independent predictors for a poor immunological recovery. The composite endpoint, non-ADD and CVE were observed most frequently in group A (overall log rank, p<0.0001, p=0.002 and p=0.01). In adjusted analyses, age was a strong independent predictor for all endpoints. Compared to group A, patients in group D had a lower risk for the composite endpoint (hazard ratio (HR) 0.54 (95% CI 0.33-0.87); patients in group B had a lower risk for CVE (HR 0.34 (95% CI 0.14-0.86).

Conclusions: Poor immunological recovery despite virological successful HAART is associated with a higher risk for overall morbidity and mortality and cardiovascular events in particular. This study underlines the importance of starting HAART at higher CD4 cell counts, particularly in older patients.

Introduction

The introduction of highly active antiretroviral therapy (HAART) has improved the mortality and morbidity of patients infected with HIV-1 dramatically [1]. As a result, life expectancy in specific subgroups of HIV-infected patients is comparable with those having other chronic medical diseases (for example insulin treated diabetes) [2] and in some cohorts even approaches this of the general population [3, 4]. Still, the incidence of non-AIDS defining diseases such as cardiovascular events and non-AIDS defining malignancies (non-ADM) [5, 6] is higher in HIV-infected patients compared to the general population.

Generally, treatment of HIV-infection with HAART suppresses viral replication, leading to recovery of $CD4^+$ T-cells (CD4 cells). Unfortunately, in 9-29% of patients treatment with HAART fails to achieve an adequate CD4 cell count despite virological suppression [7-12], known as 'immunological non response'.

Several studies show a poorer long term clinical outcome in terms of death, AIDS and non-AIDS defining diseases, in patients with an inadequate immunological response on HAART [7, 8, 10, 13]. However, these studies are limited due to sample size and/or do not have non-AIDS defining diseases such as cardiovascular events or non-ADM as solitary endpoints.

Therefore, we set out to assess predictors and long term clinical outcome – in particular the occurrence of cardiovascular events and non-ADM- of patients with a poor immunological recovery (low CD4 cell count) despite two years of virological successful HAART in the Dutch ATHENA cohort.

Methods

Design

Patients were included from the observational AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort, which follows HIV-positive patients who are registered in one of the 25 designated treatment centres in the Netherlands. Clinical data of patients who have decided not to opt out is anonymously recorded in a central database that is maintained by the Stichting HIV Monitoring [14] The ATHENA database includes information on patient demographics, immunological and vi-

rological parameters, detailed treatment data, data on adverse events and AIDS-defining and selected non-AIDS-defining clinical events. Data on malignancies have been collected since 1998, on cardiovascular events since 2000, and on liver disease since 2002. Data on cardiovascular events and non-AIDS defining malignancies are collected according to the Data Collection on Adverse events of Anti-HIV Drugs (D:A:D) study protocol (http://www.cphiv.dk). Cardiovascular risk factors such as family history for cardiovascular disease and smoking (having a history of smoking, no amount specified), as well as alcohol abuse (>28 glasses per week [men], >21 glasses per week [women]) are recorded.
Inclusion criteria for this study were: naive for antiretroviral therapy at the start of HAART (in or after 1998) at a minimal age of 16 years, at least two CD4 cell count measurements available between 1 and 2 years after starting HAART of which one between 1.5 and 2 years, observed suppression of viral load to <500 plasma HIV-RNA copies/ml within 9 months after starting HAART and plasma HIV-RNA viral load of <500 copies between 1.5 and 2 years after starting HAART. Exclusion criteria were: therapy interruptions longer than 2 weeks during the first 2 years after starting HAART, pregnancy 10 months prior to start HAART until end of follow-up, the combined use of tenofovir and didanosine and the use of immunosuppressive co medication during the first 2 years after starting HAART.
Baseline was defined as two years after starting HAART. Patients were categorized into 4 groups based on their last two CD4 cell counts: group A with CD4 cell count <200 cells/µl (both measurements below 200 cells/µl), group B with CD4 cell count between 200-350 cells/µl (one or both measurements above 200 cells/µl and below or equal to 350 cells/µl), group C with CD4 cell count between 351-500 cells/µl (one or both measurements above 350 cells/µl and both below or equal to 500 cells/µl) and group D with CD4 cell count >500 CD4 cells/µl (both measurements above 500 cells/µl). Hepatitis C infection at baseline was defined as positive result on a quantitative or qualitative HCV-RNA test or, if not available, positive hepatitis C virus (HCV) serology at baseline. Hepatitis B infection was defined as positive result on a hepatitis B surface antigen (HBsAg) test. Subjects were censored at their data of death or at the date of the last available plasma HIV-RNA measurement before March 1, 2009.

Outcomes

The endpoints of this study were: 1) a composite endpoint (cardiovascular events, all malignancies (except basal cell carcinoma), liver cirrhosis, AIDS or death); 2) non-AIDS defining diseases (non-ADM, liver cirrhosis and cardiovascular events); 3) cardiovascular events; and 4), non-ADM. Cardiovascular event was defined as a coronary bypass, coronary stenting and/or angioplasty, fatal and non-fatal myocardial infarction and cerebrovascular attack (CVA). Non-ADM was defined as any malignancy except Kaposi sarcoma, cervix carcinoma, non-Hodgkin lymphoma and basal cell carcinoma of the skin. Liver cirrhosis diagnosis was based on either histology or radiological reports.

Statistical methods

A logistic regression model was used to analyse the association between patient, clinical and HAART-regimen characteristics and poor immunological recovery (<200 CD4 cells/μL despite 2 years of virological successful HAART, group A). Independent variables included age, gender, smoking, HCV co-infection, alcohol abuse, time on protease inhibitor (PI)-based regimen, plasma HIV-RNA at start HAART, HIV transmission category (homosexual/heterosexual, IV-drug use), region of origin (Western Europe/North America, sub-Saharan Africa, Caribbean/Latin America) and nadir CD4 cell count before the start of HAART (nadir CD4).

Differences in time to endpoints after baseline during follow up between patient groups were analysed using Kaplan-Meier survival estimates and unadjusted and adjusted Cox proportional hazards models. Time to the composite endpoints was defined as the time between baseline and first occurrence of any of the individual endpoints.

Results

Baseline characteristics and degree of immunological recovery

The characteristics of the 3,068 selected patients are shown in *Table 1*, according to the groups of CD4 cell count recovery after 2 years of HAART.

The majority of the study population was men (83.4%) of western European or North American (65.0%) origin with a median age of 41 years. Most patients had acquired HIV through homosexual contact (57.5%). Total duration of follow up after baseline was 10,956 patient years.

Table 1. Baseline characteristics of the study population.

Variable	CD4 cell count (cells/mm³)				
	<200	*200-350*	*351-500*	*>500*	*Total*
Number	199 (6.5)	645 (21.0)	1413 (46.1)	811 (26.4)	3068 (100.0)
Male gender	176 (88.4)	544 (84.3)	1162 (82.2)	677 (83.5)	2559 (83.4)
Age (years)	45 (38-54)	42 (36-49)	40 (35-47)	39 (34-46)	41 (35-48)
Baseline CD4 (cells/μL)[a]	144 (110-163)	255 (215-280)	424 (360-490)	680 (610-1005)	360 (255-510)
CD4 nadir (cells/μL)	30 (10-70)	80 (25-140)	180 (96-238)	280 (200-371)	170 (70-250)
HIV-RNA at start HAART (log_{10} copies/ml)	5.2 (4.8-5.6)	5.0 (4.7-5.4)	5.0 (4.6-5.4)	5.0 (4.6-5.5)	5.0 (4.6-5.4)
HCV co-infection[b]	14 (7.0)	37 (5.8)	66 (4.7)	25 (3.1)	142 (4.7)
HBV co-infection[c]	15 (7.5)	41 (6.4)	103 (7.3)	35 (4.3)	194 (6.3)
Plasma HIV-RNA					
<50 copies/ml	173 (86.9)	551 (85.4)	1241 (87.8)	698 (86.1)	2663 (86.8)
<500 copies/ml	26 (13.1)	94 (14.6)	172 (12.2)	113 (13.9)	405 (13.2)
PI use during first two years after starting HAART					
No[d]	71 (35.5)	280 (43.4)	675 (47.8)	328 (40.4)	1354 (44.1)
0-12 months	44 ((22.1)	95 (14.7)	230 (16.3)	130 (16.0)	499 (16.3)
13-24 months	84 (42.2)	270 (41.9)	508 (36.0)	353 (43.5)	1215 (39.6)
Transmission risk group					
MSM	101 (50.8)	317 (49.1)	821 (58.1)	525 (64.7)	1764 (57.5)
IDU	7 (3.5)	17 (2.6)	31 (2.2)	10 (1.2)	65 (2.1)
Heterosexual	66 (33.2)	247 (38.3)	461 (32.6)	238 (29.4)	1012 (33.0)
Region of origin					
Western Europe/North America	138 (69.4)	379 (58.8)	902 (63.8)	574 (70.8)	1993 (65.0)
Sub-Saharan Africa	30 (15.0)	129 (20.0)	234 (16.6)	101 (12.5)	494 (16.1)
Caribbean/Latin America	20 (10.0)	73 (11.3)	166 (11.7)	83 (10.2)	342 (11.1)
Risk factors					
Smoking[e]	104 (71.7)	310 (71.1)	654 (68.5)	461 (76.8)	1529 (71.6)
No data	54 (27.1)	209 (32.0)	458 (32.4)	211 (26.0)	932 (30.4)
Family history of CVE	14 (14.6)	30 (11.3)	46 (7.6)	37 (9.4)	127 (9.3)
No data	103 (51.8)	380 (58.9)	806 (57.0)	419 (51.7)	1708 (55.7)
Alcohol abuse	18 (9.1)	46 (7.1)	101(7.2)	66 (8.1)	231 (7.5)
CVE before baseline	2 (1.0)	13 (2.0)	21 (1.5)	7 (0.9)	43 (1.4)

Data are given as number (percentage) or median (interquartile range). Abbreviations: CVE, cardiovascular events; HAART, highly active antiretroviral therapy; IQR, interquartile range; IDU, intravenous drug use; PI, protease inhibitor; HCV, hepatitis C virus; MSM, men who have sex with man.[a]Average of last 2 CD4+ cell measurements before inclusion, [b]HCV data available for 3029 patients, [c]HBV data available for 3060 patients, [d]0 months PI use (24 months of mainly Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) based HAART), [e]Percentage of known data,.

Based on the last two CD4 cell counts between 1-2 years after starting HAART, 199 (6.5%) patients were categorized into group A, 645 (21.0%) in group B, 1413 (46.0%) in group C and 811 (26.4%) in group D. At that time 86.8% of patients had a plasma viral load of < 50 copies/ml, while 13.2% had a viral load below 500 copies/ml. The median number of plasma HIV-RNA measurements per patient between start of HAART and baseline was 8 [interquartile range (IQR) 7-10].

Predictors for poor immunological recovery

Out of 733 patients with a nadir CD4 of 0-50 cells/μL, 139 (19.0%) patients had a poor immunological recovery (group A), compared to 30 (8.6%) out of 351 patients with a nadir of 51-100 and 28 (3.5%) out of 808 patients with a nadir of 101-200.

Older age was an independent predictor for a poor immunological recovery (*Table 2*), whereas higher nadir CD4 (nadir CD4 of 101-200 compared to 0-50 cells/μL) and a higher plasma HIV-RNA at start of HAART (>5.0 log copies/ml compared to <4.0 log copies/ml) was associated with a better immunological outcome [adjusted odds ratio (aOR) 0.13 (95% confidence interval (95% CI) 0.08-0.20) and aOR 0.50 (95% CI 0.27-0.91), respectively]. The length of use of different drug classes during the first 2 years after starting HAART was not independently associated with poor immunological recovery [13-24 months PI use compared to 0 months PI use (24 months of mainly Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) based HAART) , aOR 0.79 (95% CI 0.55-1.13)]. Results were similar when PI use was divided into boosted and unboosted PI use (data not shown).

CD4 cell count restoration after baseline

The CD4 cell count restoration after baseline was significantly different between the various groups (*Figure 1*). Five years after baseline (7 years after initiation of HAART) the median CD4 cell count was 320 (interquartile range (IQR) 240-410) for group A, 410 (IQR 323-510) for group B, 580 (IQR 450-710) for group C and 788 (IQR 630-1000) for group D (overall p <0.0001).

Table 2. Predictors for incomplete immune recovery (<200 CD4 cells/μL after 2 years of virological suppressive HAART).

Variable	Unadjusted OR (CI)	P	Adjusted OR (CI)	P
Age[a]	1.22 (1.14-1.30)	<0.0001	1.19 (1.10-1.29)	<0.0001
Female Gender	0.64 (0.41-1.00)	0.05	0..61 (0.37-0.99)	0.046
HCV co infection	1.27 (0.72-2.24)	0.42	1.59 (0..73-3.47)	0.24
PI-based regimen during first 2 years of HAART				
No (ref.)	1.00	-	1.00	-
0-12 months	1.75 (1.18-2.58)	0.005	1.26 (0.82-1.94)	0.30
13-24 months	1.34 (0.97-1.86)	0.08	0.79 (0.55-1.13)	0.19
Plasma HIV-RNA at start HAART				
<4.0 log copies/ml	1.00 (ref.)	-	1.00 (ref.)	-
4.0-5.0 log copies/ml	0.72 (0.40-1.28)	0.26	0.63 (0.34-1.20)	0.16
>5.0 log copies/ml	1.10 (0.64-1.89)	0.74	0.50 (0.27-0.91)	0.02
Transmission category				
MSM/heterosexual	1.00 (ref.)	-	1.00 (ref.)	-
IDU	1.89 (0.85-4.20)	0.12	1.73 (0.58-5.15)	0.32
Region of origin				
Western Europe/North America	1.00 (ref.)	-	1.00 (ref.)	-
Sub-saharan Africa	0.87 (0.58-1.31)	0.50	1.15 (0.71-1.87)	0.57
Caraibbean/latin America	0.84 (0.52-1.35)	0.46	0.84 (0.50-1.42)	0.51
Nadir CD4 cell count				
0-50 cells/uL	1.00 (ref.)	-	1.00 (ref.)	-
51-100 cells/uL	0.40 (0.26-0.61)	<0.0001	0.35 (0.23-0.54)	<0.0001
101-200 cells/uL	0.15 (0.10-0.23)	<0.0001	0.13 (0.08-0.20)	<0.0001

Abbreviations: CI, 95% confidence interval; CVE, cardiovascular events; HCV, hepatitis c virus; HAART, highly active antiretroviral therapy; IDU, intravenous drug use; MSM, men who have sex with man; OR, odds ratio; PI, protease inhibitor. aestimated OR per 5 years increase in age.

Morbidity and mortality in relation to degree of immunological recovery

During follow-up 213 patients reached the composite endpoint. Most events were observed in the group with poorest immunological recovery; the Kaplan-Meier (KM) estimate of the percentage of patients in group A with an event within 5 years after baseline was 17.2%, compared to 10.7% (B), 10.4% (C) and 7.3% (D), overall log rank, p <0.0001

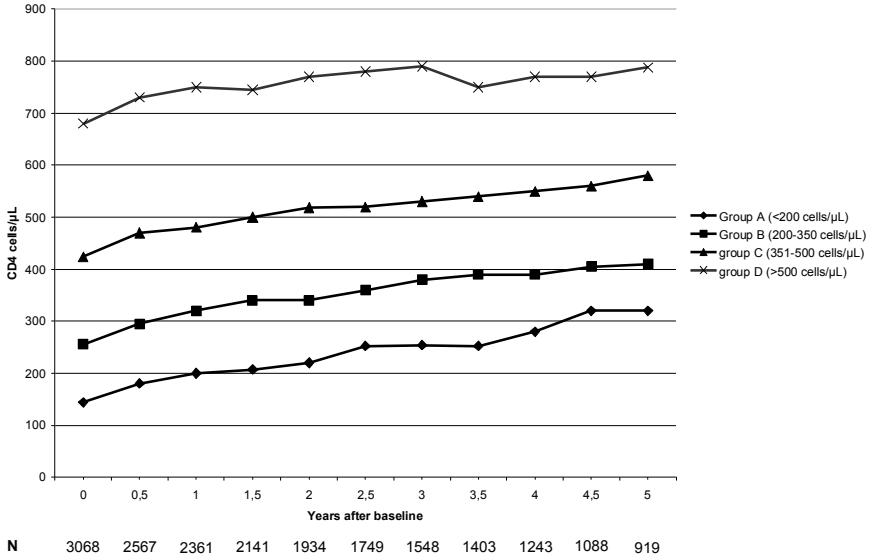

Figure 1. Median CD4 cell count during follow up, according to level of immunological recovery at baseline. CD4 cell count <200 cells/µL (group A), 200-350 cells/µl (group B), 351-500 cells/µl (group C) and >500 CD4 cells/µl (group D). N = total number of CD4 cell measurements available for this analysis at different time points.

(*Figure 2a*). In unadjusted Cox proportional hazard models a significant lower risk for an event was found in those patients with higher CD4 cell counts after 2 years of HAART (*Table 3*). After adjustment for age and other baseline confounders the hazard ratio (HR) of an event attenuated. Differences in the hazard of an event between groups D and A remained significant and the differences between B-C and A were borderline significant (overall p=0.09). Older age, smoking, acquisition of HIV-infection by intravenous drug use (IDU), and the occurrence of an non-AIDS defining event before baseline (cardiovascular event, non-AIDS defining malignancy or liver cirrhosis) were independently associated with the occurrence of an event.

112 patients reached the composite endpoint of non-AIDS defining diseases. Most events occurred in group A; the KM estimate for an event within 5 years was 10.9% for group A, compared to 5.6% (B), 4.9% (C) and 4.4% (D), overall log rank, p=0.002 (*Figure 2b*).

In unadjusted models, the risk for an event was significantly higher in group A compared to all other groups [table3, HR group D compared with A 0.34 (95% CI 0.19-0.63)]. Adjusted for age and other confound-

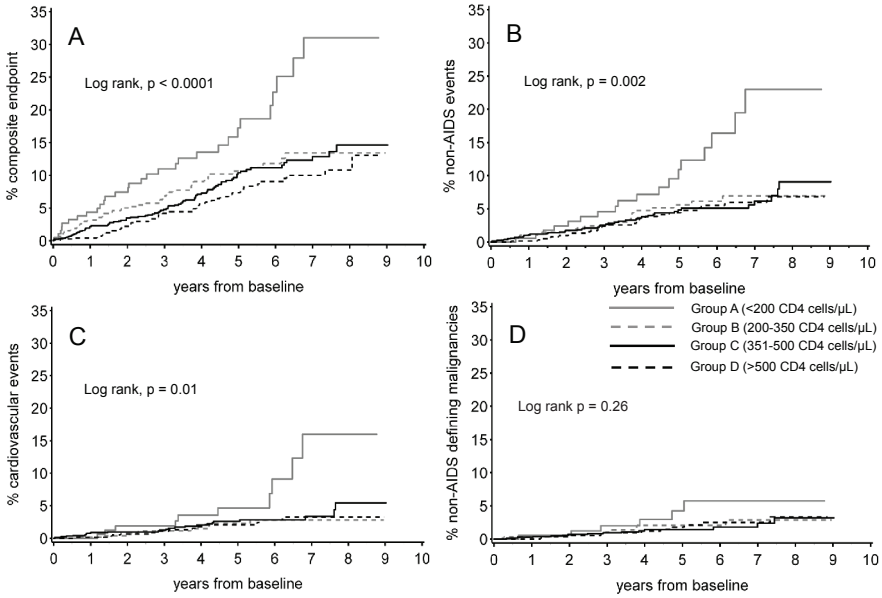

Figure 2. Kaplan-Meier curves of the occurrence of the various endpoints, according to the level of immunological recovery at baseline.
CD4 cell count <200 cells/µL (group A), 200-350 cells/µl (group B), 351-500 cells/µl (group C) and >500 CD4 cells/µl (group D). Composite endpoint (cardiovascular events, malignancies, liver cirrhosis, AIDS or death; A), non-AIDS defining diseases (cardiovascular events, non-AIDS defining malignancies and liver cirrhosis; B), cardiovascular events (C) and non-AIDS defining malignancies (D) during follow up. Overall log rank, p <0.0001 for the combined endpoint, p=0.002 for non-AIDS defining diseases and p=0.01 for cardiovascular events. For non-AIDS defining malignancies overall p=0.26, whereas p=0.05 for group C and 0.10 for group D compared to group A.

ers (IDU, gender, smoking, alcohol abuse, region of origin, occurrence of a non-AIDS defining disease before baseline and HCV co-infection), the hazard ratios comparing groups B-D with A remained well below one but the 95% confidence interval included 1 [HR group D compared with A, 0.58 (95% CI 0.31-1.07)]. Older age, IDU and a non-AIDS defining disease diagnosis before baseline were independent predictors for the occurrence of a new non-AIDS defining disease.
During follow up 57 patients experienced a cardiovascular event (CVE), consisting of 22 myocardial infarctions (6 fatal), 14 CVA (1 fatal), 20 coronary stenting and/or angioplasty procedures and 1 coronary bypass procedure. The KM estimate for a CVE (*Figure 2c*) within 5 years was significantly higher in group A (4.7%) compared to the other groups (2.2%

Table 3. Cox proportional hazards analysis of the occurrence of the composite endpoint, non-AIDS defining diseases, cardiovascular events and non-AIDS defining malignancies.

Variable	Composite endpoint				Non-AIDS defining diseases			
	HR (CI)	*p*	*aHR (CI)*	*p*	*HR (CI)*	*p*	*aHR (CI)*	*p*
Age[a]	1.06 (1.05-1.07)	<0.0001	1.05 (1.04-1.07)	<0.0001	1.09 (1.07-1.11)	<0.0001	1.08 (1.05-1.10)	<0.0001
Smoking	2.03 (1.34-3.07)	0.0008	1.60 (1.04-2.45)	0.03	2.21 (1.25-3.90)	0.006	1.47 (0.82-2.63)	0.19
Alcohol abuse	2.02 (1.39-2.94)	0.0002	1.38 (0.93-2.05)	0.11	2.14 (1.29-3.55)	0.003	1.47 (0.87-2.48)	0.15
Event before baseline	4.35 (2.72-6.98)	<0.0001	2.59 (1.58-4.27)	0.002	7.07 (4.10-12.19)	<0.0001	3.52 (1.96-6.33)	<0.0001
IDU	4.07 (2.47-6.69)	<0.0001	2.34 (1.16-4.71)	0.02	3.96 (2.00-7.85)	<0.0001	4.53 (1.64-12.54)	0.004
HCV at baseline	3.17 (2.07-4.85)	<0.0001	1.70 (0.93-3.10)	0.08	2.12 (1.07-4.20)	0.03	0.81 (0.30-2.21)	0.67
Groups of immunologic recovery (CD4 cells/µL)				0.09[b]				0.23[b]
Group A (<200)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
Group B (200-350)	0.53 (0.34-0.84)	0.006	0.66 (0.42-1.05)	0.08	0.42 (0.23- 0.79)	0.006	0.54 (0.29-1.01)	0.05
Group C (351-500)	0.46 (0.30-0.70)	0.0002	0.66 (0.43-1.01)	0.06	0.40 (0.23- 0.68)	0.0009	0.63 (0.36-1.08)	0.11
Group D (>500)	0.35 (0.22-0.55)	<0.0001	0.54 (0.33-0.87)	0.01	0.34 (0.19-0.63)	0.0005	0.58 (0.31-1.07)	0.08
	Cardiovascular events				**Non-AIDS defining malignancies**			
	HR (CI)	*p*	*aHR (CI)*	*p*	*HR (CI)*	*p*	*aHR (CI)*	*p*
Age[a]	1.11 (1.09-1.14)	<0.0001	1.10 (1.07-1.13)	<0.0001	1.07 (1.03-1.10)	<0.0001	1.06 (1.03-1.09)	<0.0001
Alcohol abuse	-	-	-	-	3.52 (1.73-7.19)	0.0005	2.76 (1.35-5.75)	0.006
Event before baseline	11.53 (5.46-24.36)	<0.0001	4.27 (1.83-9.94)	0.0008	-	-	-	-
Groups of immunologic recovery (CD4 cells/µL)				0.14[b]				0.75[b]
Group A (<200)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
Group B (200-350)	0.30 (0.12-0.74)	0.009	0.34 (0.14-0.86)	0.02	0.50 (0.18-1.42)	0.19	0.64 (0.23-1.81)	0.40
Group C (351-500)	0.41 (0.20-0.85)	0.02	0.62 (0.29-1.31)	0.21	0.41 (0.16-1.05)	0.06	0.60 (0.23-1.56)	0.30
Group D (>500)	0.31 (0.13-0.70)	0.005	0.52 (0.22-1.20)	0.12	0.44 (0.16-1.19)	0.10	0.61 (0.22-1.66)	0.33

Unadjusted (HR) and adjusted hazard ratios (aHR) are given for the risk of the occurrence of the composite endpoint, non-AIDS defining diseases, cardiovascular events and non-AIDS defining malignancies. In the model for the composite endpoint the variables gender and region of origin were also included; in the model for non-AIDS defining diseases the variables gender, smoking, alcohol abuse and region of origin; in the model for cardiovascular events the variables gender, family history of cardiovascular event and smoking; and in the model for non-AIDS malignancies the variable smoking was included as well (hazard ratios not shown in table). The variable 'event before baseline' is defined as the occurrence of a clinical event before baseline (depending on the model either the prior occurrence of a non-AIDS malignancy, cardiovascular events or liver cirrhosis for the composite endpoint and the endpoint of non-AIDS defining diseases, or the prior occurrence of a cardiovascular event). Abbreviations: CI, 95% confidence interval; IDU, acquisition of HIV-infection by intravenous drug use; HCV, hepatitis C virus co-infection; HBV, hepatitis B virus co-infection. [a]HR are given per 1 year increase in age; [b]overall p-value.

(B), 2.6% (C) and 2.0% (D)); overall log rank, p=0.01). In unadjusted models the risk for a CVE was significantly higher in group A compared to all other groups [*Table 3*; HR group D compared with A 0.31 (95% CI 0.13-0.70)]. The risk for CVE attenuated slightly in adjusted models, the HR comparing group B with A changed from 0.30 (95% CI 0.12-0.74) in unadjusted analysis to 0.34 (95% CI 0.14-0.85) in adjusted analyses. Other independent predictors for a CVE were older age and the occurrence of a CVE before baseline. The variables gender, family history of cardiovascular event and smoking were included in the model as well.
41 patients developed a non AIDS defining malignancy (non-ADM) during follow up. Recorded were 6 anal malignancies, 5 Hodgkin lymphomas, 4 breast cancers, 5 lung cancers, 2 prostate cancers, 2 pharyngeal malignancies and 17 malignancies of other or unknown type. The KM estimate for a non-ADM event within 5 years was highest in group A (4.3%, compared to 2.1% (B), 1.4% (C) and 1.8% (D), overall log rank, p=0.26 (*Figure 2d*). Differences in the occurrence of non-ADM between groups in unadjusted analyses were borderline not significant (compared to group A, p=0.05 for group C and p=0.10 for group D). In adjusted models (for age, alcohol abuse, smoking), low CD4 cell counts were not significantly associated with the occurrence of a non-ADM (*Table 3*). Age and alcohol abuse were independently associated with the occurrence of non-ADM.

Discussion

The principle finding of this study is that antiretroviral-naïve patients with a poor immunological recovery despite virological successful HAART are at higher risk for morbidity and mortality, and for cardiovascular complications in particular. Older age, low nadir CD4 cell count and low plasma HIV-RNA at the start of HAART were factors associated with a poor immunological recovery.
A significant proportion of patients in our study (27.5%) failed to reach a CD4 cell count of 350 cells/μL after 2 years of virological successful HAART. Moreover, even after 7 years of HAART the median CD4 cell count (320 (IQR) 240-410) in this group remained well below 500 cells/uL. We found that lower CD4 cell nadir before the start of HAART and older age were predictors for poor immunological recovery at 2 years, in line with previous reports [7-10, 15]. Moreover we found that a high

plasma viral load (>5.0 $\log_{10}$ copies/ml) at the start of HAART was associated with a better immunological outcome, as was reported in other studies [10, 16-18]. The reasons for this correlation have not yet been clearly elucidated. Reduced thymic function, increased immune activation leading to lymphoid tissue fibrosis and T-cell apoptosis, and human genetic polymorphisms are other factors reported to be involved in the mechanism of a poor immunological response [19-23]. We did not find and association between immunological recovery after 2 years of HAART and the use of protease inhibitors or HCV co-infection, in contrast to other studies [7, 8, 16]. Concerning the latter, this might be related to the low overall prevalence of HCV co- infection in our study (4.7%), whereas investigators who found a relation between HCV co-infection and immunological recovery reported a much higher prevalence (33.0%) [16].

In our study, patients with low CD4 cell counts after two years of virological successful HAART more often experienced fatal and non-fatal diseases (combined endpoint of death, AIDS defining diseases, malignancies, cardiovascular disease and liver cirrhosis). Besides the degree of immunological recovery, known risk factors such as older age, smoking, alcohol abuse, and IDU acquisition of HIV-infection were independent predictors. The higher risk for these diseases in these patients can be party explained by their average older age in our study. However, even after adjusting for age there was a significant association between a poor immunological recovery and an increased risk for events.

We analyzed the occurrence of non-AIDS defining diseases as a combined endpoint, and likewise found more events in the groups with lower degrees of immunological recovery. In models adjusted for age and other confounders we found a trend towards lower risk for non-AIDS defining disease in patient with better immunological recovery after two years of virological successful HAART. Studies have shown that low CD4 cell counts are not only related to the incidence of AIDS defining diseases, but are also associated with non-AIDS defining diseases [6, 24-27]. Associations between most recent CD4 cell count and the occurrence of cardiovascular disease [27-29], liver disease [30] and non AIDS-related malignancies [26] have been reported. Whereas other studies have reported data on mortality or combined endpoints of

AIDS and non-AIDS defining diseases in patients with a poor immunological response on HAART [7, 8, 10, 13], we are the first to report data on non-AIDS defining diseases as a solitary endpoint in these patients. Patients with a poor immunological recovery after two years of HAART were at a higher risk for cardiovascular events, for a large part because of their older age. In age-adjusted analyses (*Table 3*) we found that differences between the group with poorest immunological recovery (A) and groups B-D in the risk for a cardiovascular event remained large. However this difference was significant for group B only, while a trend was found for the other groups. Larger studies are necessary to confirm the finding of an increased risk for cardiovascular events in patients with low CD4 cell counts despite virological successful HAART. Chronic HIV-infection has now been recognized as a risk factor for cardiovascular disease [31]. Several studies report a relationship between low CD4 cell counts and occurrence of cardiovascular events; Triant et al. reported low CD4 cell count as an independent predictor for myocardial infarction [28], whereas data of the HIV Outpatient Study (HOPS) showed a relationship between latest CD4 cell count and cardiovascular disease [29]. Other studies found a relationship between the degree of immune activation, chronic inflammation and vascular dysfunction [32]. Moreover, the level of immune activation and chronic inflammation is reported to be higher in patients with a poor immunological response on HAART [33], despite viral suppression. Therefore a high level of immune activation in these patients could play a role in the development of cardiovascular complications.

In univariate analysis, we found a trend for the occurrence of more non-ADM in the group with the lowest degree of immunological recovery. It has been suggested that (duration of exposure to) immunodeficiency is related to the occurrence of non-ADM, since several studies have shown that both nadir CD4 cell count and current CD4 cell count are associated with a higher frequency of non-ADM [6, 26]. However, these studies have included both antiretroviral treated and untreated patients in their analysis, whereas in our study patients were included only if treatment with HAART resulted in virological success. In adjusted analysis we did not find an association between CD4 cell count after 2 years of HAART and the occurrence of non-ADM. Due to the low number of endpoints (41 non-ADM), the power of this study to answer this question might be limited.

The findings of the present study emphasize the importance of starting HAART at higher CD4 cell counts, in line with evidence that initiation of HAART at higher CD4 cell counts reduces mortality [34]. Moreover, since 31-39% of new diagnosed HIV-infected patients in Western-Europe present with a CD4 cell count of <200 cells/uL [35], these data suggest that more aggressive screening policies may need to be implemented.

Strengths of this study include the use of a well defined cohort followed prospectively where data is recorded in a standardized manner and the large number of patients included in this study. However, there are limitations as well. In some patients information on traditional risk factors was missing and data on conditions related to cardiovascular diseases such as blood lipids, hypertension and renal function were not included in this study. Furthermore, probably an even larger sample size might be necessary to confirm whether the association between persistent low CD4 cell counts on HAART and long term clinical complications (especially for non-AIDS defining diseases) is independent of older age. Finally, occurrence of CVE and NADM might be related to duration of HIV infection as HIV-associated biomarkers such as CD4 cell count nadir and markers of inflammation have been associated with an elevated risk of cardiovascular events [36]. We did not control for duration of (known) HIV-infection or nadir CD4 cell count. As nadir CD4 cell count is strongly correlated with the degree of immunological recovery after two years of HAART, a larger sample size would be necessary to disentangle these two effects as well.

In conclusion, our findings clearly stress the importance of achieving an adequate restoration of the immune system, to minimise the risk of serious diseases such as cardiovascular events. Our data suggest that starting HAART at higher CD4 cell counts, especially in older aged patients, may be beneficial. Further investigations are necessary to identify new immune-modulating therapeutic options for patients in whom HAART fails to achieve an adequate restoration of the immune system.

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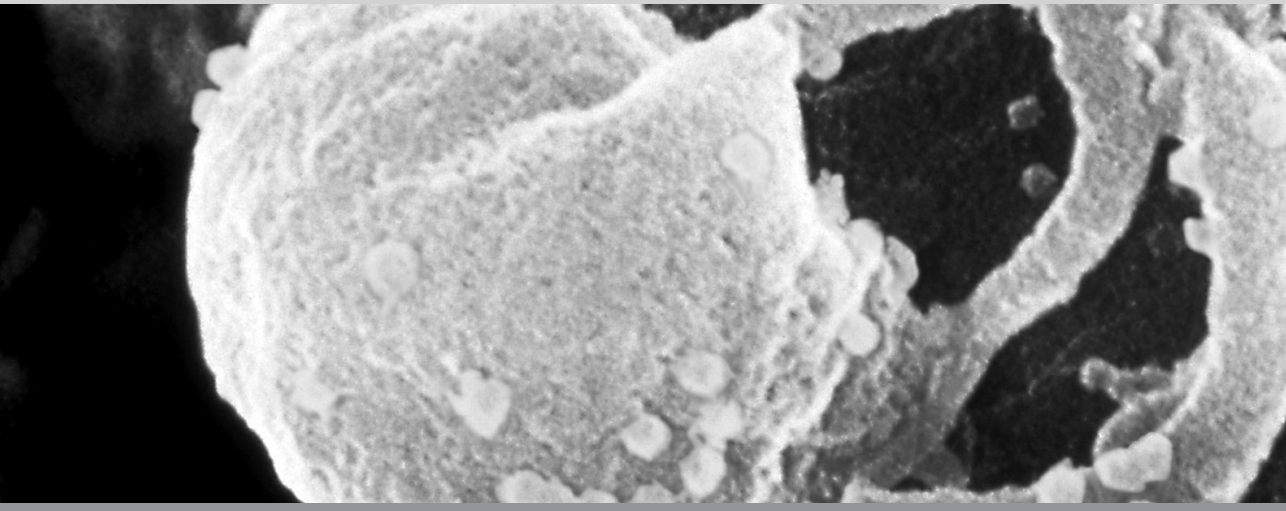
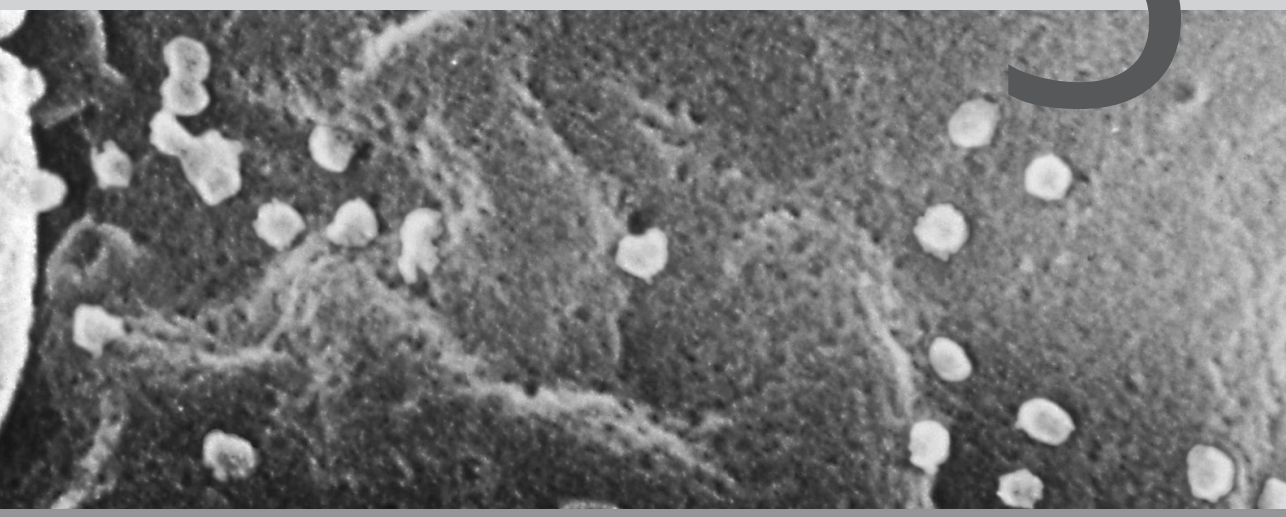


CHAPTER 5



Therapy failure following selection of enfuvirtide resistant HIV-1 virus in cerebrospinal fluid

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Abstract

We report the selection of enfuvirtide resistant HIV-1 virus in cerebrospinal fluid resulting in subsequent loss of viral suppression in the plasma. This case report stresses the potential danger of low level penetration of entry inhibitors into the central nervous system.

Introduction

Currently, 2 antiretroviral agents interfering with human immunodeficiency virus (HIV)–1 entry, enfuvirtide and maraviroc, are available for treatment of patients infected with multi-drug resistant HIV-1. Limited data are available regarding the level of penetration of these drugs into the central nervous system (CNS), a potential sanctuary site of HIV [1]. We describe selection of enfuvirtide resistant HIV-1 in cerebrospinal fluid (CSF) of a patient with undetectable plasma HIV-RNA, ultimately resulting in therapy failure.

Case report

In June 2007, a 50-year-old Caucasian male presented with sensory disturbances and unsteadiness of the lower extremities leading to an uncoordinated gait. He had been diagnosed with HIV-1 in 1994 for which he was initially treated with zidovudine monotherapy. During the years thereafter, several treatment changes were made because of virological failure and toxicity, with subsequent selection of a multidrug resistant HIV. The adverse effects subsided after each change in his antiretroviral regimen.

In 2005, the $CD4^+$ cell count was 38/mm^3 and plasma HIV-RNA was >750.000 copies/mL. Genotypic resistance analysis (sample p1, *Figure 1*a) identified multiple resistance related mutations in reverse transcriptase (M41L, E44D, D67N, L74V, A98G, K101H, V188I, Y188L, M184V, L210W, T215Y, K219E, H221Y) and protease (L10I, I13V, L33F, I54V, L63P, A71V, V77I, V82S, I84V, L90M), but no enfuvirtide related mutations [2]. Tropism testing (The Original Trofile Assay; OTA, Monogram Biosciences) reported presence of CCR5-tropic virus only.

Salvage therapy was subsequently started containing maraviroc, enfuvirtide, tenofovir, zidovudine and lamivudine (*Figure 1*a). On the basis of a resistance interpretation algorithm [3], maraviroc and enfuvirtide were the only fully active drugs in this regimen.

Immunologic recovery was rapidly achieved; the $CD4^+$ cell count increased to 579 cells/mm^3. However virological response was slow; after an initial response of >3-log decrease, prolonged low level replication was observed before the HIV-RNA was suppressed (<50 copies/ml, *Figure 1*a).

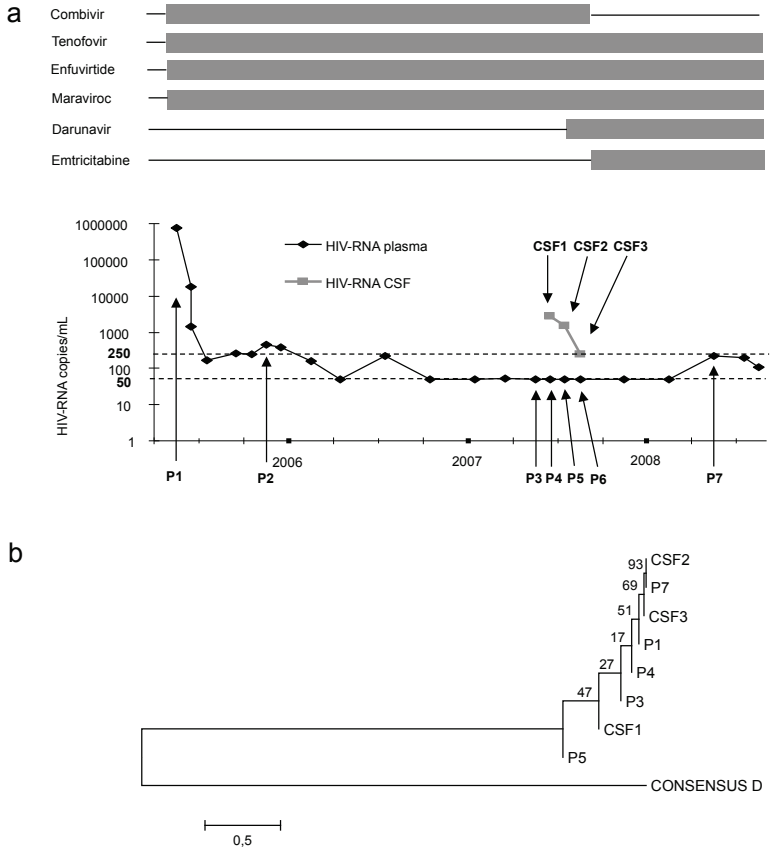

Figure 1. A, Plasma and cerebrospinal fluid (CSF) human immunodeficiency virus (HIV) RNA levels after the start of salvage regimen (bars). B, Phylogenetic tree of HIV-V3 sequences in CSF and plasma samples. P1–P7 correspond to the different plasma samples, and CSF1–CSF3 correspond to the different CSF samples. P2 and P6 could not be included in the phylogenetic tree because of insufficient material. Dosages were as follows: lamivudine (150 mg) plus zidovudine (300 mg) twice per day, tenofovir (245 mg twice per day), enfuvirtide (90 mg twice per day), maraviroc (300 mg twice per day, changed to 150 mg twice per day when darunavir was started), darunavir (600 mg twice per day; boosted by ritonavir), and emtricitabine (200 mg once per day).

Soon after initiation of the salvage regimen sensory disturbances of the feet occurred, followed by progressive gait problems 20 months later.

Neurological examination revealed a spastic gait, exaggerated knee reflexes, absent ankle reflexes and plantar reflexes according to

Babinsky. Sense of pain, temperature, touch, pressure, vibration and joint position were impaired in the lower extremities.
Laboratory examinations reported macrocytic anemia (hemoglobin 8.1 mmol/L, MCV 115 fL), which was compatible with the use of zidovudine and/or slight folic acid (5.8 nmol/L) and vitamin B12 (110 pmol/L) deficiency. Thoracic and lumbar spine magnetic resonance imaging (MRI) showed no abnormalities. Electromyography was undisturbed; however, tibial nerve somatosensory evoked potentials showed prolonged central conduction times, compatible with myelopathy. A CSF specimen revealed an elevated leucocyte count (48 leucocytes/mm^3), a slightly increased protein level (0.58 g/L) and a normal glucose level (3.9 mmol/L). Infection with *Treponema pallidum, Borrelia burgdorferi,* human T lymphotropic virus type 1, herpes simplex virus, and varicella zoster virus was excluded. On the basis of these results, subacute combined degeneration of the spinal cord due to vitamin B12-deficiency was recognized as the most likely cause for the patient`s neurological symptoms, and therapy with folic acid and vitamin B12 was initiated.
However, although HIV-RNA was undetectable in the concurrent plasma sample, analysis of the CSF demonstrated an HIV-RNA load of 2780 copies/mL (sample CSF1, *Figure 1*a).
Therefore active viral replication in the CNS as possible cause of his symptoms could not be excluded either.
To differentiate between active viral replication in CSF and viral release by infected cells only, the protease inhibitor (darunavir/r) which just had become available, was added. Within four weeks after this intervention, the CSF HIV-RNA load became undetectable (<250 copies/ml), indicating suppression of active viral replication (sample CSF3, *Figure 1*a). Despite suppression of HIV-RNA in CSF and correction of serum vitamin B12, the patient's neurological symptoms did not significantly improve. To eliminate potential neurotoxic drug effects, zidovudine was discontinued and lamivudine was switched to emtricitabine. Subsequently, his sensory disturbances disappeared slowly, although his gait problems persisted. In 2008, ten months after addition of darunavir/r to the patient's regimen, the plasma HIV-RNA level rebounded to 270 copies/mL (sample p7, *Figure 1*a).
We questioned whether viral replication in CSF was related to therapy failure and set out in-depth investigations.

Methods

The patient participates in the AIDS Therapy Evaluation Project, Netherlands (ATHENA) observational cohort, approved by the local and national institutional review board.

Resistance analysis
Longitudinal plasma and CSF samples were analysed (*Figure 1*a). Population genotypic analysis was performed (protease, RT and env gp41 and gp120-V3) [4,5]. Gp41 was amplified and sequenced (BigDye Terminator Cycle Sequencing kit) using the forward primer FRgp41F (GCWGGAAGCACATGGGCGC) and the reverse primer FRgp41R (TGTARTACCCTKCCTAACTCT). Large volume (4.3 ml) input was used to perform ultrasensitive analysis on plasma samples with HIV-RNA below precise quantification (<50 copies/mL) that still gave a positive HIV-RNA signal.

V3-phylogenetic analysis
HIV-V3 sequences were aligned to a reference sequence from the Los Alamos database (http://www.hiv-web.lanl.gov) using Clustal-software (http://megasoftware.net). Neighbour-joining phylogenetic trees were constructed from 377 nucleotides using Megav4.1 (Beta) software (http://megasoftware.net) and the maximum composite likelihood model and gamma distributed rates (gamma parameter 0.5). Bootstrap analysis was performed using the same methods (1000 replicates). A bootstrap value >70% was interpreted as support for clustering.

Drug level analysis
Drug levels were measured by liquid chromatography tandem mass spectrometry. Total enfuvirtide and maraviroc levels were measured in CSF and plasma 3 and 1.5 hours after drug intake, total darunavir level was measured in CSF and plasma 2.5 and 3.75 hours after drug intake.

Results

Genotypic analysis of CSF-derived HIV-RNA did not reveal changes at resistance-related positions in protease and reverse transcriptase, compared with the baseline plasma sample (samples, CSF1-2 and p1, *Figure 1*a) [2]. In addition, CSF-sequence analysis of the env-gp120 V3-loop showed no differences compared to baseline, suggesting no change in HIV-1 tropism.

Interestingly, analysis of the heptad repeat regions of env-gp41 revealed the enfuvirtide-related V38A mutation in the CSF (sample CSF2, *Figure 1*a), whereas ultrasensitive resistance analysis in the concurrent plasma sample (HIV-RNA level <50 copies/mL [~25 copies/mL]) did not show selection of enfuvirtide resistance related mutations (sample P6, *Figure 1*a). In addition, retrospective analysis of a sample obtained during the period of low-level replication after initiation of the regimen (HIV-RNA level, 439 copies/mL) did not reveal enfuvirtide mutations (sample P2, *Figure 1A*).

Subsequently, drug levels were measured to relate the differential selection of antiviral resistance in the 2 compartments to differences in drug pressure.

Total enfuvirtide concentrations of 3.74 and 0.055 µg/mL were measured in plasma and CSF, respectively. On the basis of plasma protein binding of 92%, the enfuvirtide concentration in CSF appeared to be 5.5 fold lower than the estimated free plasma concentration of 0.29 µg/mL [6]. The total plasma concentration of maraviroc was 0.146 µg/mL, corresponding to a free plasma concentration of 0,04 µg/mL (76% plasma protein binding [7]), while only traces of maraviroc below the limit of quantification (39ng/mL) could be detected in the CSF. The plasma level of darunavir was 3.3 µg/mL, corresponding to ~ 0.17 µg/mL of free darunavir (95% protein binding [8]). The darunavir concentration in CSF was estimated to be 0.019 µg/mL, which is 9 fold lower than the calculated free plasma concentration and is in line with recently published results [9].

At time of therapy failure 11 months later (sample p7, figure1a), genotypic analysis detected, for the first time, the V38A mutation in plasma. Phylogenetic analysis demonstrated close relationship (bootstrap value, 93%) of this rebound plasma population with the viral population in the CSF-sample (samples P7 and CSF2-3).

Discussion

In this case report, we describe selection of enfuvirtide resistant HIV in CSF during salvage therapy while HIV-RNA in the plasma was suppressed below the limit of quantification. Subsequent addition of boosted darunavir to the antiretroviral regimen resulted in suppression of HIV-RNA levels in the CSF. Although the CSF concentration of

darunavir was low, it was still in the range of the 50% inhibitory concentration of darunavir–susceptible virus (0.012–0.055 mg/mL) [9]. Investigations into CSF penetration of another boosted protease inhibitor also suggest that reduction of CSF HIV-RNA levels can be observed if CSF drug levels exceed the 50% inhibitory concentration for wild-type virus [10].

Currently, only limited data regarding the penetration of entry inhibitors into the CNS have been published. In this patient, we observed poor CSF penetration of maraviroc, in line with data of animal studies [11]. To our knowledge, these are the first data published on CSF maraviroc concentrations in humans. Recently, negligible penetration of enfuvirtide in CSF and presence of enfuvirtide-resistant HIV in CSF was reported in a patient with a transient increase in the CSF HIV-RNA level (while HIV-RNA was detectable in plasma) [12]. We demonstrate that viral replication resulting in selection of enfuvirtide resistant HIV-1 in the CSF can precede selection of enfuvirtide resistant virus in the plasma.

One could argue that enfuvirtide resistance was already selected in the plasma during the initial slow decay after start of salvage therapy. In our opinion, this scenario is unlikely, because we did not detect the V38A mutation in plasma at this point in time. Furthermore, at time of detection of the V38A mutation in CSF, no enfuvirtide resistance was observed in the concurrent plasma sample with an undetectable viral load using ultrasensitive analysis.

Therefore, the most likely explanation is that low CSF enfuvirtide concentrations (in the absence of adequate maraviroc levels) enabled viral replication in the CSF leading to subsequent selection of enfuvirtide drug-resistance. Enfuvirtide-resistant HIV eventually appeared in blood, possibly via migration of replication competent cells or altered blood-brain barrier permeability, resulting in therapy failure. This suggests that treatment with enfuvirtide should be combined with at least one active antiretroviral drug with adequate CNS penetration.

To our knowledge, this is the first case describing therapy failure after initial selection of resistant HIV-1 in a sanctuary site, illustrating the risk of low-level penetration of antiretroviral drugs in the CNS.

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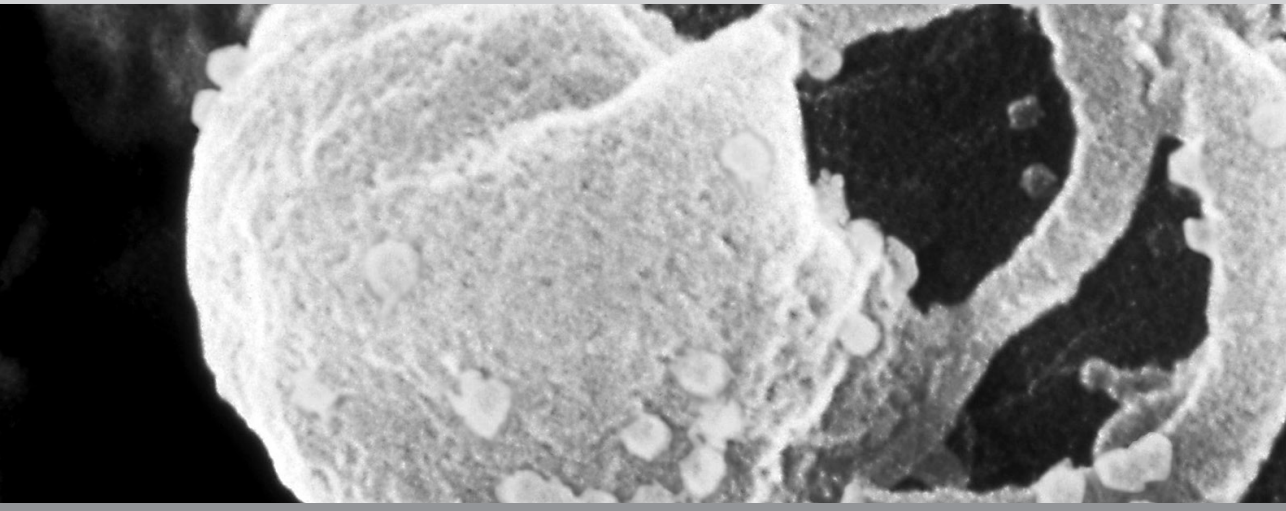
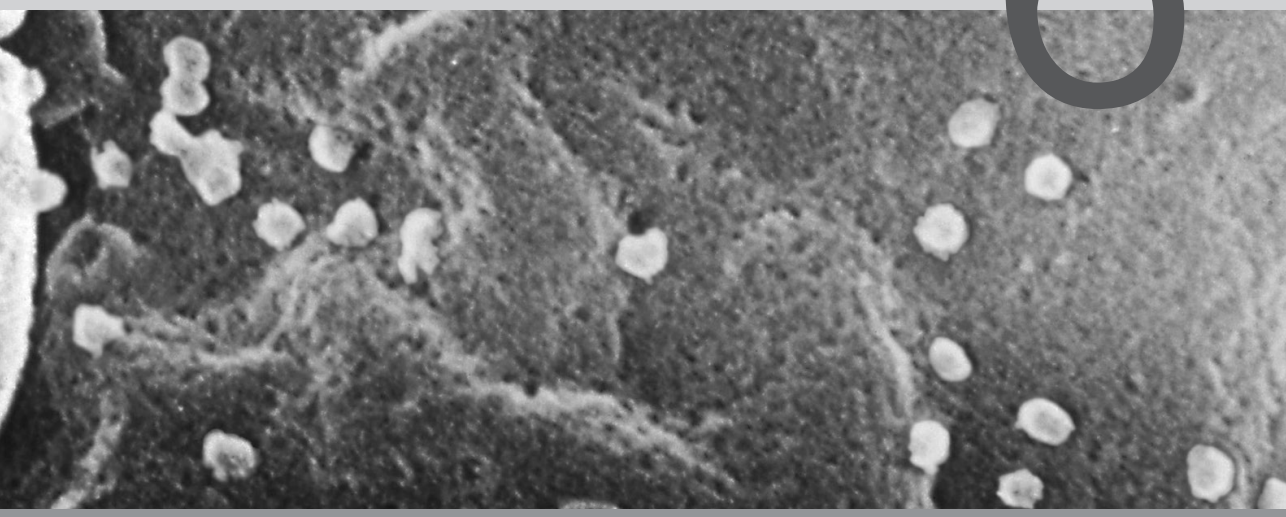


CHAPTER 6



Clinical outcome of maraviroc containing therapy in HIV-1-infected patients

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Submitted

Abstract

Background: Limited data are available about use of maraviroc (MVC) in clinical settings. We analyzed the clinical outcome of patients treated with MVC in the UMC Utrecht, and compared the predictive value of different tropism assays with respect to virological and immunological outcome.

Methods: HIV-1-infected patients treated with MVC were included in this cohort study. Baseline viral tropism was assessed and compared by phenotypic (Trofile, MT-2) and genotypic (Tropgene-V3) assays. Backbone activity was measured using a genotypic sensitivity Score (GSS) based on the Stanford HIVdb algorithm. Virologic (log_{10} copies plasma HIV-RNA/ml) and immunological (CD4 cells/μL) response was evaluated. Liver and kidney adverse events (grade III&IV) were recorded.

Results: 62 predominantly extensive pre-treated patients started MVC. Mean GSS of the backbone was 2.0 (1.9-2.2). One or more tropism assays were performed on samples of 52 patients (83.9%). Thirty (81.1%) samples were classified as R5 by Trofile, 26 (89.7%) by Tropgene-V3 and 18 (81.8%) by MT-2. In samples of 23 patients two or three different tropism assays were performed, with an observed concordance of 86.7-94.1%. Median baseline plasma HIV-RNA was log 3.0 [1.7-4.9], while median baseline CD4 cell count was 271 (96-438). Plasma HIV-RNA was undetectable (<50 copies/ml) in 82.1, 85.0 and 68.8%, of patients after 12, 24 and 36 months; median CD4 cell increase was 194 (IQR 104-283), 249 (IQR 164-412.5) and 296 (IQR 135-444) after 12, 24 and 36 months. When the virus population was classified as R5 according to Trofile, in 92.3% of patients plasma HIV-RNA was undetectable at month 24, compared to 90.0 and 100.0% according to Tropgene-V3 and MT-2. Three patients stopped MVC treatment because of suspected side-effects. Five patients died during follow-up. No adverse events seemed MVC related.

Conclusion: In this heavily pre-treated cohort, treatment with MVC was tolerated well and resulted in a good immunological and virological response. The results generated by the different tropism assays correlated well with each other and clinical outcome.

Introduction

Maraviroc (Celsentri®, Selzentry®) is the first drug of the class of CCR5 antagonists to be registered for treatment for HIV-1-infected antiretroviral therapy naive (USA only) and experienced patients, based on the 'Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients (MOTIVATE) 1 and 2 studies' [1, 2]. Maraviroc (MVC) is an antagonist for the chemokine (C-C motif) receptor 5 (CCR5 co-receptor), which is a G-protein-coupled transmembrane receptor able to bind CC-chemokines, such as CCL3 (MIP-1α), CCL4 (MIP-1β) en CCL5 (RANTES) [3]. The CCR5 receptor is, next to the chemokine (C-X-C motif) receptor 4 (CXCR4), a co-receptor for HIV-entry [4, 5]. The process of HIV entry initiates with binding of the HIV-1 envelop glycoprotein (gp) 120 to the cellular receptor protein CD4 on the target cell, resulting in conformational changes exposing the variable region of gp120 (V3 loop). The net charge of the V3 loop influences to which co-receptor the virus subsequently will bind. CD4 and co-receptor binding leads to a subsequent conformational change in gp120 enabling the envelope glycoprotein gp41 to protrude the cellular membrane and promote virion and target cell fusion [6]. 'Co-receptor tropism' refers to the ability of HIV-1 to enter $CD4^+$ cells using the CCR5 co-receptor ('R5-tropic virus'), the CXCR4 co-receptor ('X4-tropic virus'), or both co-receptors ('dual-tropic virus') [7]. Some patients are infected with a population of viruses with different co-receptor tropisms ('mixed-tropic virus').

The antiretroviral compound MVC is a small molecule, non-competitive allosteric inhibitor of the CCR5 co-receptor. Binding of MVC to CCR5 prevents interaction with the V3-loop of gp120, and thereby HIV entry and fusion. Since MVC is registered for treatment of patients infected with R5-tropic HIV-1, viral co-receptor tropism has to be assessed before start of treatment with MVC [8]. Genotypic tests predict viral co-receptor tropism based on the sequence coding for the V3 region of the viral envelope by means of interpretation algorithms (R5 or X4 prediction) [7].

Although MVC has been registered since 2008 (Europe), the quantity of available data from clinical practice regarding efficacy, tolerability and the predictive value of different tropism assays is limited. The aim of this study is to analyse the clinical outcome of treatment of a heterogeneous group of HIV-infected patients with a MVC containing regimen

in clinical practice, and to compare the use of different tropism assays for clinical purposes in our hospital.

Methods

The University Medical Center Utrecht (UMCU) is one of the Dutch HIV treatment centers. All patients participate in the AIDS Therapy Evaluation in the Netherlands (ATHENA) observational cohort, which has been approved by local and national institutional review boards. As of 31 December 2011, 19.531 patients were registered in this database. The UMCU is the fourth largest HIV-treatment center in The Netherlands.

Study population

We conducted a retrospective cohort study in the UMCU, and included patients with an HIV-1 diagnosis, aged >18 years and who had taken at least one dose of MVC. Patients were followed until discontinuation of MVC, death or May 1, 2012.

Data collection

Collected data included information about demographics, date of HIV diagnosis, antiretroviral medication use, clinical diagnoses, laboratory data, and patient outcomes. The most recent $CD4^+$ T (CD4) cell counts and plasma HIV-RNA measurements before the start of MVC were recorded as baseline values. The indications for MVC use were recorded; these were categorized as failure of previous ART, intolerance to other antiretroviral drugs, MVC intensification therapy (either in order to increase CD4 cell count in patients with suppressed plasma HIV-RNA or to further suppress plasma HIV-RNA in patients with low level viremia) or 'other' indications. Therapy failure was defined as a viral load (VL) > 50 copies/ml in two consecutive HIV-RNA measurements during ART and/or as selection of resistance related mutations by the previous regimens based on the IAS mutation list [9]. The optimized backbone regimen (OBR) that was used in combination with MVC was recorded, as were changes in OBR during MVC therapy. In case MVC was stopped, the date and indication for stopping were captured.

Immunological and virological response was assessed at time points with a three monthly interval from baseline. CD4 cell count and plasma HIV-RNA measurements within the range of one month before and one month after these time points were included. Virologic response

was evaluated as the proportion of patients with an HIV-RNA plasma level below a threshold of 50 copies/ml and 400 copies/ml, whereas the immunologic outcome was determined as the median change in CD4 cell count (cells/μL) compared to baseline.
For evaluating tolerability of a MVC containing regimen, laboratory abnormalities were graded according to the Division of Microbiology and Infectious Diseases (DMID)-defined grades of toxicity (DMID Adult Toxicity Table May 2001, National Institute of Allergy and Infectious Diseases, National Institutes of Health). If ALT values were 1.25-2.5 times above upper limit of normal reference (ULN) it was graded as 'mild' toxicity, a value of >2.5-5.0 times ULN was graded as 'moderate', >5.0-10.0 times ULN as 'severe' and a value of more than 10.0 times ULN was graded as 'very severe' toxicity. For creatinin these cut-off levels were >1.0-1.5, >1.5-3.0, >3.0-6.0 and >6.0 times ULN.

Tropism testing

Plasma samples were sent to Monogram Biosciences (San Francisco, USA) for phenotypic tropism testing. Initially, the Original Trofile™ (OTA) assay was used for tropism assessment. In this assay, cell lines that express either the CCR5 or CXCR4 co-receptor are infected with a pseudovirus population containing a luciferase gene and envelope (including V3) genes of the patient derived viral population. When infection of one of these cell lines occurs, light will be emitted [10]. Results of the OTA are reported as R5 (CCR5 tropism), X4 (CXCR4 tropism) or D/M (dual or mixed tropism). When, due to technical reasons, no tropism results can be generated this will be reported as 'non-reportable'. In 2008, the OTA has been replaced by an improved version, the 'enhanced sensitivity Trofile™ assay' (ESTA). It has been reported that the ESTA is able to detect X4 virus clones with 100% sensitivity when they comprise at least 0.3% of the virus population [11].
The MT-2 assay is a phenotypic in-house tropism assay using an MT-2 cell line expressing the CXCR4 coreceptor. MT-2 cells were maintained in culture medium [CM; RPMI1640 with L-glutamine (BioWhittaker), 10% fetal bovine serum (FBS; Biochrom AG) and 10 mg/L gentamicin (Gibco)]. Patient-derived PBMCs (1×10^6) prepared by Ficoll-Paque density gradient centrifugation were co-cultured in triplicate with 1×10^6 CXCR4+CCR5- MT-2 cells in CM [12]. Viral cultures were maintained for 3 weeks and monitored for syncytium formation. A viral population

is reported to be 'syncytium-inducing' (X4-, dual-, or mixed-tropic), or 'non-syncytium-inducing' (R5-tropic).
For genotypic prediction of HIV-1 coreceptor tropism ('Tropgene-V3' assay), viral RNA was isolated from 200–1000 mL of plasma/serum as described previously [13] or viral DNA was isolated from isolated 5×10^6 PBMCs. The V3 region of the envelope was amplified as previously reported [14]. In brief; V3 region was amplified with primers 6206V3F 5'-AGAGCAGAAGACAGTGGCAATGAGAGTGA-3', 7785R 5'-AGTGCTTCCTGCTGCTCCYAAGAACCC-'3 (Titan One Tube RT-PCR kit, Roche). The nested-PCR was performed with primers 6658F 5'-TGGGATCAAAGCCTAAAGCCATGTG-'3, 7371R 5'-GAAAATTCCCCTCCACAATT-'3 (Expand High-Fidelity PCR-System, Roche). Sequenced using primers 6957F 5'-GTACAATGTACACATGGAAT-'3 and 7371R or V3-4 5'-ACAGTACAATGTACACATGGAATTA-3' and V3-3 5'-AATTCCCCTCCACAATTAAAASTGTG-3' (Big dye Terminator Cycle seq kit v3,1, Applied Biosystems). Viral co-receptor tropism was predicted using 'geno2pheno [coreceptor] algoritm' [http://www.geno2pheno.org/; R5 prediction, >10%; and X4, ≤10% false positive rate (FPR)]. When patients experienced virological failure on MVC containing regimen the viral population at rebound was assed phenotypically with an MT-2 assay and tropism was predicted by genotypic tropism testing.

Cumulative Genotypic Sentitivity Score of optimized backbone regimen

The Genotypic Sensitivity Score (GSS) of the virus population to antiretroviral drugs was calculated for each patient using HIV sequences obtained on different time points before start of the MVC containing regimen. The sequences were uploaded in the Genotypic Resistance Interpretation Algorithm of the Stanford University HIV drug resistance database (http://hivdb.stanford.edu/pages/ algs/HIVdb.html). Antiretroviral drugs were scored as 1 when the virus was 'susceptible' or 'potential resistant', 0.5 when the virus was 'low' or 'intermediate' resistant and 0 in case of 'high resistance'. The cumulative GSS was the sum of the separate susceptibility scores of the different antiretroviral drugs in the OBR.

Statistical analysis

Continuous variables were compared using Student's t-test or the Mann-Whitney test, for categorical variables Chi-square or Fisher's exact test were used. Statistical significance was determined at $p < 0.05$.

Statistical significance was determined at $p<0.05$. All analyses were performed with SAS software (version 9.2, SAS institute Inc.).

Results

Baseline characteristics

Sixty-two patients were included, their baseline characteristics are depicted in *Table 1.* In 40 patients the plasma HIV-RNA was detectable (≥ 50 copies/ml) at the start of MVC, whereas in 22 patients MVC was started when plasma HIV-RNA was undetectable. Thirty-four (54.8%) patients started MVC because of virological failure of the previous regimen, 15 (24.2%) patients switched to a MVC containing regimen because of toxicity problems and in 12 (19.4%) patients cART was intensified with MVC (with the aim to increase CD4 count or to suppress low level viremia).

The median age of the included patients was 48.8 years (interquartile range (IQR) 46.1-51.5), 75.8% were male, and the majority acquired HIV through homosexual contact (53.2%) and originated from Western Europe (77.4%). Although the included patients in general had long-standing HIV-infection and were heavily pre-treated (median 14.2 (IQR 8.9-16.2) years since HIV diagnosis, median 11.5 (IQR 8.5-12.7) years since start cART), an optimized backbone regimen with a median cumulative GSS of 2.0 could nevertheless be constructed. Only 4 patients had a cumulative GSS ≤1.

Tropism

In 52 patients (83.9%) a tropism assay of any kind was performed (*Figure 1a*). An OTA/ESTA was performed in 37 patients (59.7%) whereas an MT-2 assay in 22 patients (35.5%), resulting in an R5 tropism classification in 30 (81.1%) and 18 (81.8%) patients, respectively (*Figure 1c*). The Tropgene-V3 assay was performed in 29 patients (46.8%), resulting in a mean FPR of 50.6 (95% CI 39.7-61.5) and R5 prediction in 26 (89.7%) patients. Genotypic tropism prediction was based on V3-sequences obtained from viral DNA in 8 (27.6%) of patients. In 14 patients all three tropism assays were performed, resulting in a concordance of 85.7% (12/14). In 23 (37.1%) patients at least two different tropism assays were performed (*Figure 1b*). Comparison of two different tropism assays resulted in a concordance of 86.7% (13/15) for OTA/ESTA compared to Tropgene-V3, 94.1% (16/17) for MT-2 assay compared to OTA/

Table 1 Baseline characteristics

	Total population *(n = 62)*	**Patients with VL ≥50 copies/ml** *(n = 40)*	**Patients with VL <50 copies/ml** *(n = 22)*	**p-value**
Age (years)[a]	48.8 (46.1-51.5)	46.3 (42.9-49.6)	53.5 (49.1-57.8)	0.02
Male sex	47 (75.8%)	26 (65.0%)	21 (95.5%)	0.01
Time since HIV diagnosis (years)[1]	14.2 (8.9-16.2)	14.2 (8.9-16.0)	14.2 (9.8-16.5)	0.72
Duration of ART (years)[2]	12.9 (8.1-14.6)	12.1 (5.4-14.3)	13.2 (9.3-14.9)	0.24
Duration of cART (years)[3]	11.5 (8.5-12.7)	11.4 (7.7-11.8)	12.5 (8.5-13.9)	0.10
Previous CDC-C diagnosis	33 (53.2%)	21 (52.5%)	12 (54.6%)	0.88
GSS [a 4]	2.04 (1.87-2.21)	1.96 (1.75-2.17)	2.21 (1.92-2.49)	0.18
CD4 nadir	56.5 (18.5-159.5)	42 (12-158)	65 (37-190)	0.23
$CD4^+$ cell count (cells/mm^3)	271 (96.0-438)	156 (48.5-360)	363.5 (271.0-760.0)	0.001
$CD4^+$ cell count < 200 cells/mm^3	24 (38.7%)	22 (55.0%)	2 (9.1%)	0.0004
HIV-RNA (log_{10} copies/mL)	3.0 (1.7-4.9)	4.7 (3.3-5.3)	<1.7	<0.0001
ALT (U/L)[5]	25.5 (19.0-41.5)	25.5 (19.0-39.0)	25.5 (18-43)	0.94
Creatinine (µmol/l)[6]	87.0 (74.0-103.0)	79.0 (71.0-96.0)	99.5 (79-138)	0.07
Region of origin:				
Western Europe	48 (77.4%)	27 (67.5%)	21 (95.5%)	0.01
Sub-Saharan Africa	8 (12.9%)	7 (17.5%)	1 (4.6%)	0.24
North/Latin America, Caribbean	4 (6.5%)	4 (10.0%)	0 (-)	0.29

Table 1 continued

	Total population (n = 62)	Patients with VL ≥50 copies/ml (n = 40)	Patients with VL <50 copies/ml (n = 22)	p-value
Transmission route:				
Homosexual	33 (53.2%)	17 (42.5%)	16 (72.7%)	0.03
Heterosexual	14 (22.6%)	11 (27.5%)	3 (13.6%)	0.34
IV drug use	2 (3.2%)	0 (-)	2 (9.1%)	0.11
Indication for MVC use:				
Virological failure	34 (54.8%)	34 (85.0%)	0 (-)	<0.0001
Intolerance to previous ARV(s)	15 (24.2%)	2 (5.0%)	13 (59.1%)	<0.0001
Intensification	12 (19.4%)	3 (7.5%)	9 (40.9%)	0.001
Other	1 (1.6%)	1 (1.6%)	0 (-)	-

Values expressed as median [interquartile range] or number of cases (%), unless otherwise indicated. a Mean [95% confidence interval]. Abbreviations: n = number of patients, VL = viral load, ART = antiretroviral therapy, cART= combination antiretroviral therapy, MVC= maraviroc, ALT = alanine aminotransferase, IV = intravenous. [1]Data available for 59 patients, [2]for 60 patients, [3]for 58 patients and [4]for 55 patients, [5]for 60 patients and [6]for 61 patients.

Table 2. HIV-subtypes, plasma HIV-RNA and results of the various tropism assays.

Patient	Subtype	Baseline RNA	Trofile		
			Type	*plasma RNA*	*Result*
1	B	<50	ESTA	3,6	R5
2	B	detectable			
3	B	detectable	OTA	3,7	R5
4	C	detectable	ESTA	6,5	R5
5	B	detectable	ESTA	5,5	DM
6	unknown	detectable			
7	A1	<50			
8	B	<50			
9	B	detectable	OTA	5,7	R5
10	B	detectable			
11	J	detectable	ESTA	4,7	R5
12	B	detectable			
13	B	<50			
14	B	detectable	ESTA	5,0	DM
15	B	<50			
16	unknown	<50			
17	B	detectable	OTA	5,6	R5
18	B	detectable	ESTA	4,7	R5
19	B	<50	ESTA	6,1	
20	D	detectable	ESTA	5,0	DM
21	CRF02_AG	detectable	ESTA	4,5	
22	unknown	detectable			
23	B	<50	ESTA	4,9	R5
24	unknown	<50	OTA	4,9	R5
25	CRF02_AG	detectable	OTA	5,3	R5
26	B	detectable	ESTA	3,6	R5
27	B	detectable	ESTA	2,9	R5
28	A1	detectable	OTA	5,7	R5
29	B	detectable	ESTA	5,3	R5
30	B	<50	ESTA	3,7	R5
31	unknown	<50			
32	B	<50			
33	B	<50			
34	B	detectable	ESTA	2,9	DM
35	CRF03_AB	<50			
36	B	detectable	OTA	5,0	DM
37	B	detectable	OTA	5,8	R5
38	B	detectable	ESTA	5,1	R5
39	C	<50			
40	B	detectable	OTA	3,7	R5
41	unknown	<50			

Patient	MT-2		Tropgene-V3			
	plasma RNA	*Result*	*plasma RNA*	*V3-sequence*	*FPR*	*Result*
1						
2			6,3	RNA	90,9	R5
3						
4						
5	5,6	NSI	5,6	RNA	41,4	R5
6						
7			1,7	DNA	74,0	R5
8			1,7			
9	5,7	NSI	5,7	RNA	58,6	R5
10			5,7	RNA	22,0	R5
11	4,7	NSI	4,7	RNA	82,3	R5
12	4,0	NSI	4,0	RNA	9,0	X4
13						
14	5,0	SI				
15						
16						
17	5,7	NSI	5,6	RNA	41,6	R5
18	4,7	NSI	4,7	RNA	79,5	R5
19	6,1	NSI	4,1	RNA	38,0	R5
20						
21	3,6	NSI	5,8	DNA	69,1	R5
22						
23						
24						
25	5,3	NSI	5,3	RNA	58,6	R5
26						
27						
28	5,7	NSI	5,7	RNA	96,5	R5
29	5,3	NSI	5,3	RNA	28,8	R5
30						
31						
32			1,7	DNA	1,7	X4
33						
34						
35						
36	4,7	SI	4,7	RNA	10,5	R5
37						
38	5,1	NSI	5,1	RNA	73,3	R5
39			1,7	DNA	74,4	R5
40						
41			1,7	DNA	71,7	R5

Table 2 continued

Patient	Subtype	Baseline RNA	Trofile		
			Type	*plasma RNA*	*Result*
42	B	<50	ESTA	5,9	R5
43	B	detectable	ESTA	5,9	R5
44	B	detectable	OTA	5,1	
45	B	detectable	OTA	5,3	R5
46	B	detectable	OTA	4,3	DM
47	B	detectable	ESTA	4,7	R5
48	B	<50	ESTA	3,9	R5
49	B	<50			
50	B	detectable	OTA	4,3	R5
51	B	detectable			
52	B	detectable	OTA	3,7	R5
53	B	detectable	ESTA	4,1	R5
54	unknown	<50			
55	B	detectable	ESTA	3,0	R5
56	CRF03_AB	<50			
57	C	detectable	OTA	5,9	R5
58	B	detectable	ESTA	4,1	DM
59	C	<50			
60	B	detectable	ESTA	3,3	R5
61	C	detectable	ESTA	4,9	
62	B	detectable	OTA	6,5	R5

Abbreviations: OTA = original Trofile assay; ESTA = Enhanced Sensitivity Trofile Assay; R5 = CCR5-tropic; DM = dual/mixed-tropic; X4 = CXCR4-tropic; NSI = non-syncytium inducing;

Patient	MT-2		Tropgene-V3			
	plasma RNA	*Result*	*plasma RNA*	*V3-sequence*	*FPR*	*Result*
42						
43	5,9	NSI	4,5	RNA	36,5	R5
44	5,1	NSI	5,0	RNA	53,8	R5
45	5,3	NSI	5,3	RNA	42,5	R5
46	4,3	SI				
47						
48						
49			1,7	DNA	33,9	R5
50						
51						
52	3,7	NSI	3,7	RNA	27,2	R5
53	4,1	NSI				
54						
55			3,0	RNA	87,8	R5
56			1,7	DNA	23,6	R5
57						
58	4,9	SI	4,1	RNA	4,8	X4
59			1,7	DNA	38,8	R5
60						
61	3,9	NSI	4,9	RNA	96,2	R5
62						

SI = syncytium inducing; FPR = false-positive rate; baseline RNA = plasma HIV-RNA before start of maraviroc; plasma RNA = plasma HIV-RNA of the sample on which the tropism assay is performed; subtype = HIV-subtype.

ESTA and 89.5% (17/19) for MT-2 compared to the Tropgene-V3 assay. In 10 (16.1%) patients no tropism assay was performed. Out of these patients, 7 patients started MVC as intensification therapy, 2 because of previous therapy failure and 1 because of toxicity of the earlier regimen.

Tolerability

In general, MVC containing regimens were well tolerated. Twelve patients (19.3%) discontinued MVC therapy for various reasons. Out of these, three of them restarted treatment after an average of 6 months. Three patients discontinued MVC because of side effects possibly due to MVC. In the remaining patients, MVC was stopped for various reasons. Although four patients developed increased ALT levels that could be classified as severe or very severe toxicity (grade 3-4), there seemed to be no relation to MVC therapy. In 5 patients severe or very severe (grade 3-4) increased plasma creatinin levels were found. However all five patients were known with renal insufficiency before the start of MVC, and no relation to MVC use was found. MVC was therefore continued.

Five patients (8%) deceased during follow up. Causes of death were pneumonia (2x), B-cell lymphoma, squamous cell carcinoma, cardiomyopathy in combination with pulmonary hypertension. All patients had extensive co-morbidity, present before the start of MVC. There was no relation between MVC therapy and any of these deaths.

Immunological and virological response

The total follow up was 174.2 years (140.2 years for patients with baseline plasma HIV-RNA>50 copies/ml, whereas 34.0 years of follow up for patients with baseline plasma HIV-RNA <50 copies/ml). Five patients temporarily discontinued MVC during the follow up period.

Forty patients with a detectable plasma HIV-RNA (median 4.7 (IQR 3.4-5.3) $\log_{10}$ copies/ml) started a MVC containing regimen (median GSS 2.0; *Figure 1a*). The plasma HIV-RNA of the majority of patients (59.06%) became suppressed (< 50 copies/ml) after 3 months, whereas after 12, 24 and 36 months, plasma HIV-RNA was suppressed in 82.1.0, 85.0 and 68.8%, respectively (*Figure 2a*). Of the patients who initiated MVC with a detectable plasma HIV-RNA (n=40), an incomplete virological response was present in three patients (7.5%). In the first patient this was due to a compliance issue, in a second patient insufficient backbone suscep-

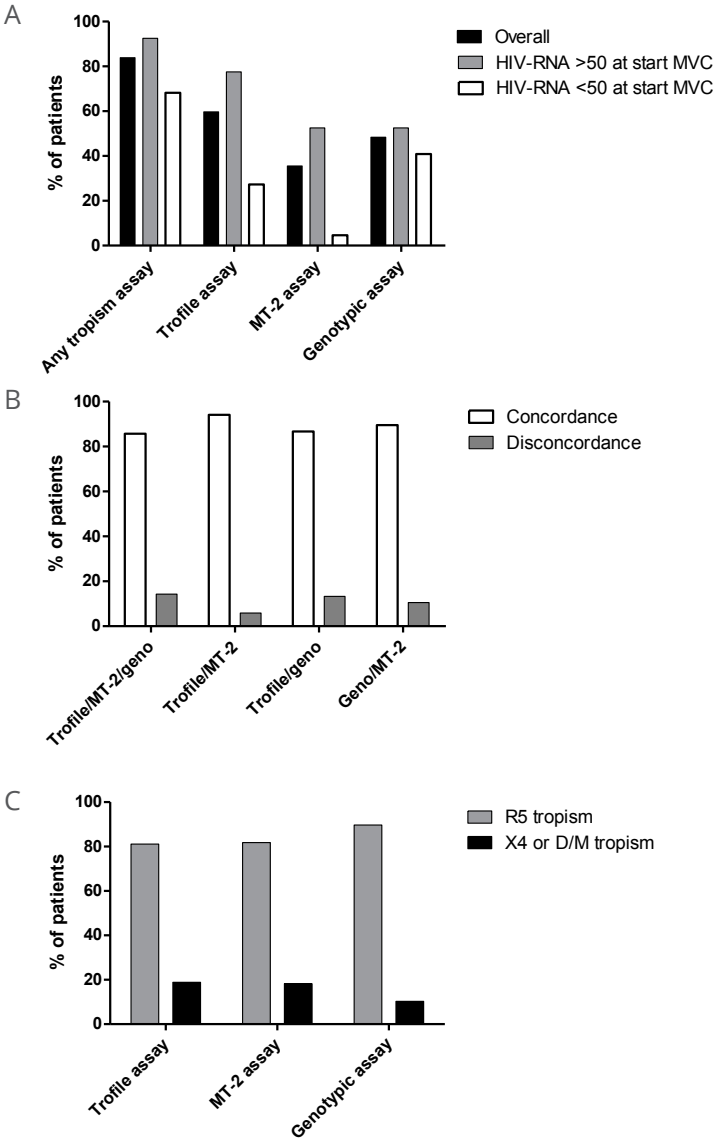

Figure 1. Overview and results of the three performed tropism assays (Trofile assay, MT-2 assay and genotypic assay). Overview of the percentage of patients in which the Trofile assay [OTA (original Trofile assay) or ESTA (Enhanced Sensitivity Trofile Assay)], MT-2 assay or genotypic tropism assay (Tropgene-V3) is performed in the total study population, patients with detectable and undetectable (<50 copies/mL) baseline plasma HIV-RNA (A). Observed concordance between Trofile, MT-2 and genotypic assay (geno); between Trofile and MT-2 assay; between Trofile and genotypic assay; and, between genotypic and MT-2 assay (B). Results of the Trofile assay, MT-2 assay and genotypic assay (C).

tibility due to extensive resistance (GSS of 0.5) resulted in incomplete virological response. In the third patient plasma HIV-RNA decreased substantially from 141,000 to 441 copies/ml at 12 months. Virologic rebound was observed in 4 patients (10%), of these one patient had an OBR with a GSS of 1 due to extensive previously selected resistance, another patient stopped cART various times at own initiative. In one patient with virologic rebound and in one with slow virological response after MVC initiation, the viral population was phenotypically assessed to be SI and had an extreme X4-prediction (FPR 1.1-1.8). Because of extensive previously selected resistance subsequent outcome of therapy switch did not result in virological suppression, however the patients remained clinically stable. Switching to a new antiretroviral regimen in the remaining three patients with viral rebound resulted in full virological suppression.

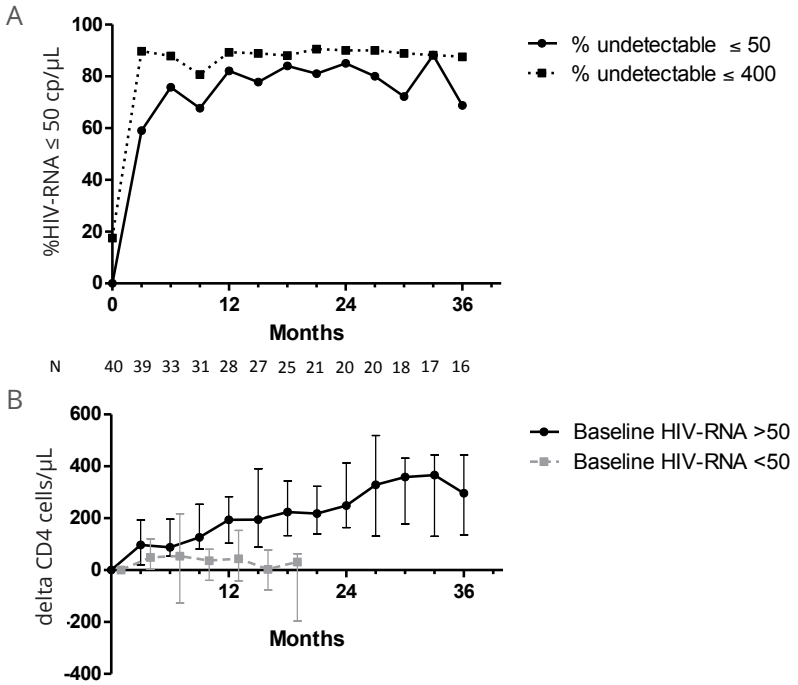

Figure 2. Virological and immunological response on a maraviroc containing regimen. Virological (percentage of patients <50 copies/mL) response on a maraviroc containing regimen of the patients with plasma HIV-RNA with a detectable plasma HIV-RNA at baseline (A; N = 40). Immunological response (Change in CD4 cell count (delta)) is given for those patients with detectable and undetectable baseline plasma HIV-RNA (B). N = number of remaining patients during follow up.

In the group of patients with an undetectable plasma HIV-RNA at baseline (n = 22), virologic rebound was not observed.
During follow-up a sustained immunological response was observed (*Figure 2*b). In the group with baseline plasma HIV-RNA > 50 copies/ml at the start of MVC, the median CD4 cell increase was 194 (IQR 104-283), 249 (IQR 164-412.5) and 296 (IQR 135-444) after 12, 24 and 36 months, respectively ($p < 0.0001$, $= 0.001$ and $= 0.0002$). In patients who switched to a MVC containing regimen while having a plasma HIV-RNA of < 50 copies/ml, no significant increase in CD4 cell count was observed.
Twelve patients started MVC as intensification therapy, with a median CD4 cell count increase at 12 months of 145 (IQR 57-196) cells/μL (p compared to baseline = 0.06).

Predictive value of the different tropism assays
We analyzed the predictive value of the different tropism assays with respect to virological and immunological outcome. When the virus was classified as R5 according to Trofile, in 92.3% of patients (with detectable viral load at baseline) plasma HIV-RNA was undetectable at month 24, compared to 90.0 and 100.0% according to respectively Tropgene-V3 and MT-2 (*Figure 3a*). The median CD4 cell count increase after 24 months in this group of patients was 274 (IQR 165-407) for R5 classification according to Trofile, compared to 323 (IQR 222-552) and 323 (IQR 213-552) according to respectively tropgene-V3 and MT-2 (*Figure 3b*).
Seven patients started an MVC containing regimen while harbouring a virus population classified as DM by Trofile at baseline. At 24 weeks in 60% (3 out of 5) of patients plasma HIV-RNA was suppressed. Out of the four patients with a virus population classified as SI by MT-2 assay, the two patients at 24 months follow up both had suppressed plasma HIV-RNA. The two patients harbouring a virus population predicted to be X4 by Tropgene-V3 had a very limited follow up period (only one patient had a follow up of 6 months and had suppressed plasma HIV-RNA at that time).

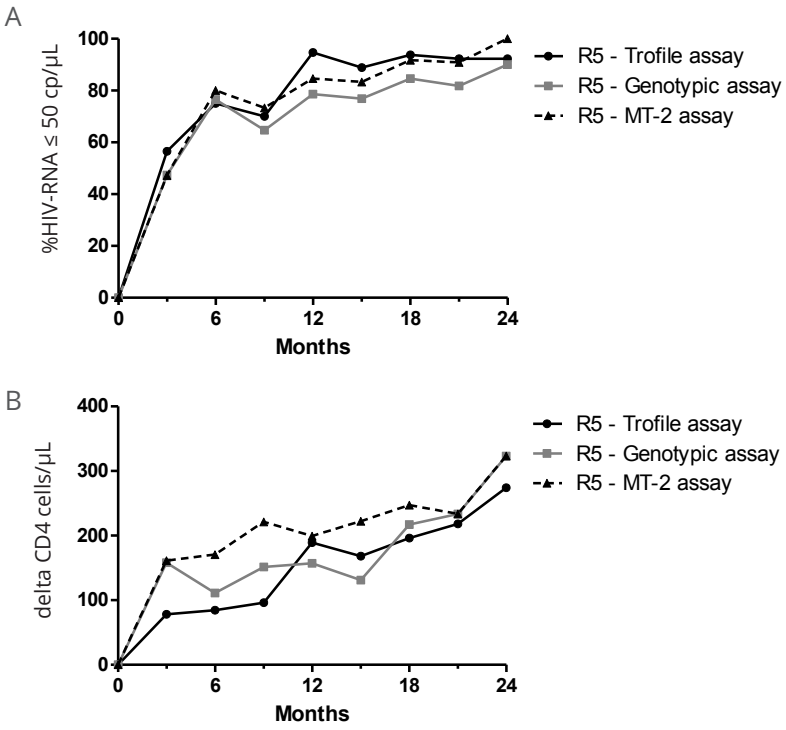

Figure 3. Virological and immunological outcome according to different tropism assays. Virological outcome (percentage of patients with plasma HIV-RNA <50 copies/mL) of those patients classified as R5-tropic by Trofile, genotypic (Tropgene-V3) or MT-2 assay (A). Immunological outcome (Change (delta) in CD4 cell count) of those patients classified as R5-tropic by Trofile, genotypic or MT-2 assay (B).

Discussion

The present study discusses the experiences and outcome of MVC containing therapy in clinical practice in a heterogeneous, heavily pretreated cohort of 62 HIV-1-infected patients. Treatment with MVC was tolerated well and resulted in a good immunological and virological response. The results generated by the different tropism assays correlated well with each other and clinical outcome.

Despite the heterogeneous and heavily antiretroviral treatment experienced cohort, 85.0% of patients who initiated MVC with a detectable

viral load at baseline had an undetectable plasma HIV-RNA and a median increase in CD4 cell count of 249 (IQR 164-412.5) cells/μL after 24 months. Although 3 (4.7%) of patients discontinued MVC because of possible related side effects, MVC therapy was well tolerated. None of the liver (6.4%) and kidney (8.1%) toxicity that was recorded seemed related to MVC therapy and while 5 patients (8%) deceased, none of these deaths were in some way related to MVC therapy. These toxicity characteristics merely are a reflection of the extensive co morbidity of the described cohort.

Although the quantity of reported efficacy and tolerability data of MVC containing regimens from routine clinical practice are very limited, our findings are in line with the few smaller studies that have been published. In a Spanish cohort 96.3% of patients (N = 46) had undetectable plasma HIV-RNA at week 48, with a mean CD4 cell count increase of 151 cells/μL [15]. In a German cohort (N = 44) 78% of patients had undetectable plasma HIV-RNA at month 6, whereas a median CD4 cell count increase of 124 cells/μL was observed. The major indication for MVC containing therapy in this heavily pre-treated cohort (median duration of cART 10.1 years; median number of previous drug combinations 9) was virological failure (56%) [16]. One prospective study from Italy has been published, where the efficacy and safety of the combination MVC, raltegravir and etravirine was evaluated in 28 antiretroviral therapy experienced patients [17]. All patients participated in the expanded access programs for these drugs (MK0518-023, raltegravir; A4001050, MVC; TMC125-C214, etravirine). At week 48, 93% of patients had a plasma HIV-RNA < 50 copies/mL, with a median CD4 cell count increase of 267 cells/μL. The regimens were well tolerated.

The reasons for initiating MVC in the present study were most commonly virological failure (54.8%), toxicity of the previous regimen (24.2%) and drug intensification strategy (19.4%). None of the patients that switched to a MVC containing regimen because of previous toxicity experienced virological failure during follow up, whereas the CD4 cell count of these patients remained stable. These data underline the fact that switching to a MVC containing regimen for toxicity reasons can be a good alternative. In twelve patients MVC was started as intensification of cART. Although a trend for an increase in CD4 cell count after 12 months was observed (145 (IQR 57-196) cells/μL), this was not

statistically significant (p = 0.06). This is in agreement with other intensification studies, that report minor or no changes in CD4 cell count after intensification of cART with MVC [18-21].
Since MVC is registered for treatment of patients infected with R5-tropic HIV-1, viral co-receptor tropism has to be assessed before start of treatment with MVC [8]. Various tropism assays are available for use in clinical practice.
To our knowledge, this is the first published study in which three different HIV coreceptor tropism assays have been used, namely two phenotypic (OTA/ESTA and MT-2 assay) and one genotypic assay (Trop-gene-V3). The concordance between the various tropism assays was very good, ranging from 85.7 to 94.1%. This is comparable or better than other comparisons between different tropism assays that have been reported so far [16, 22]. In addition, the predictive value of the different tropism assays with respect to virological and immunological outcome was excellent and correlated well with each other. MVC selected for extremely X4-predicted viral variants in two patients who experienced a viral rebound or a slow virological response during MVC containing therapy. In three out of the four patients with viral rebound, a subsequent switch to a new antiretroviral regimen resulted in adequate suppression of plasma HIV-RNA. The European guidelines for HIV-1 tropism recommend to use the population-based genotypic tropism test, mostly because of practical and costs issues. These guidelines advise to use a cut-off FPR of 10% in most situations for both viral RNA and pro-viral DNA. This may be a rather conservative approach since at virological failure only viral variants with an extremely low FPR were observed. Studies to prospectively explore the optimal cutt-off (i.e. FPR) should be performed.
Indeed, the data of the present study underline that a genotypic tropism test is a solid alternative for predicting HIV coreceptor tropism in clinical practice.
One of the strengths of this study is the heterogeneous group of patients described in this cohort, which makes the results more applicable to clinical practice. Other strengths are the larger sample size as compared to previously published studies and the relatively long period of follow-up. Besides the strengths, this study has some limitations. First, this study was of a retrospective nature. This could have resulted in uncontrolled confounders, because it was predetermined which pa-

tients received MVC. In this study MVC was in particular prescribed to heavily pre-treated patients what could have lead to an underestimation of the virological and immunological outcome and a higher rate of the adverse events. Second, this study was conducted at a single institution and therefore limits the generalisability of our results. Third, studies with a larger study population might generate more statistical power to detect differences in virological and immunological response. In conclusion, MVC containing therapy in a heterogeneous, mostly heavily pre-treated cohort, was tolerated well and resulted in good virological and immunological response during a substantial follow-up period. Moreover, the tropism data of this study show that prediction of HIV coreceptor tropism by a genotypic tropism assay is a reliable option in clinical practice.

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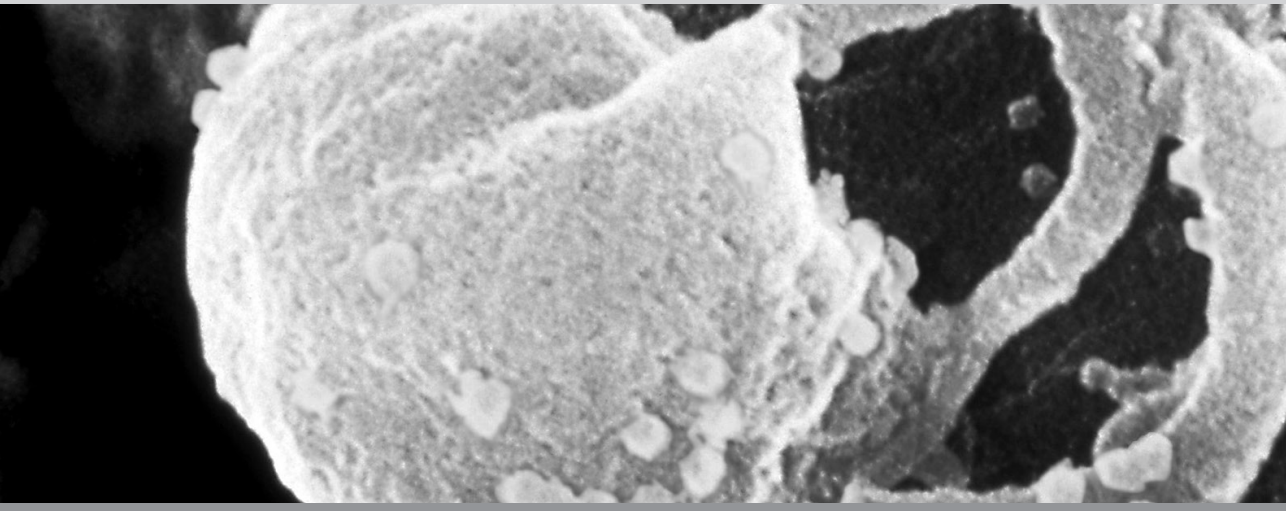
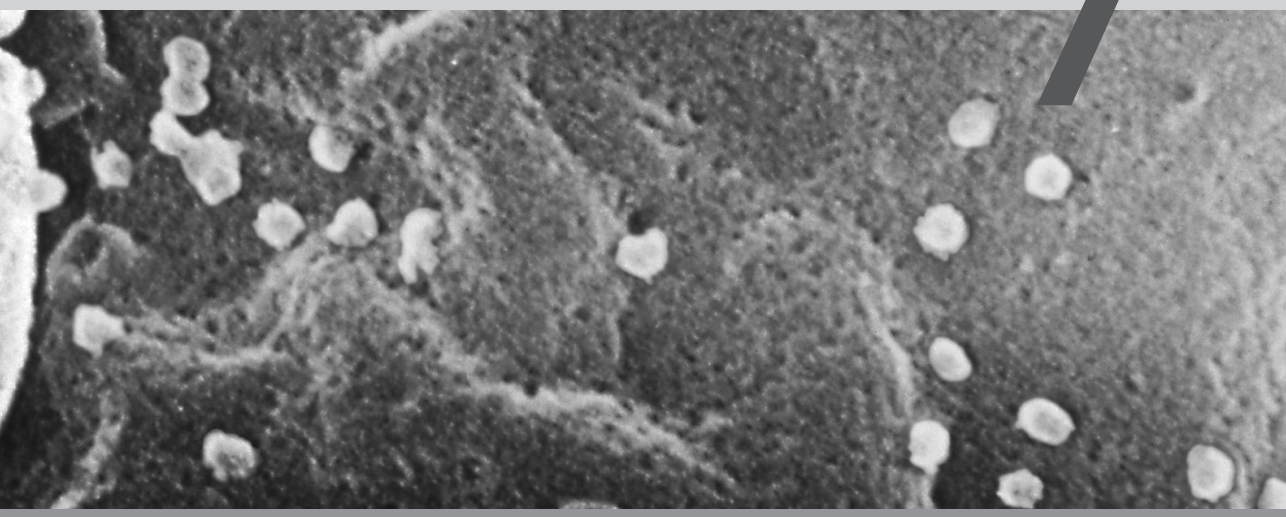


CHAPTER 7



Maraviroc is able to inhibit dual-R5 viruses in a dual/mixed HIV-1-infected patient

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Abstract

Objectives: Maraviroc is the first licensed chemokine coreceptor 5 (CCR5) coreceptor antagonist in clinical practice. It is currently being used in patients harbouring exclusively CCR5-tropic virus. The objective of the study was to investigate the impact of maraviroc on viruses with different coreceptor preferences in a patient with a dual/mixed (D/M) infection.

Methods: We present a case report of an HIV-1-infected patient infected with a D/M virus population. Coreceptor tropism was determined by phenotypic and genotypic tests. Biological clones from pre- and post-maraviroc therapy were generated. Tropism of these infectious clones was investigated in U373-MAGI cells expressing $CD4^{+}CCR5^{+}$ or $CD4^{+}CXCR4^{+}$. Maraviroc susceptibility and viral replication were determined using donor peripheral blood mononuclear cells (PBMCs).

Results: In-depth clonal genotypic analysis revealed the presence of both R5-tropic variants and X4-tropic viruses before the start of maraviroc. During maraviroc therapy all R5-predicted viruses were suppressed. Phenotypic analyses revealed that all biological clones before maraviroc therapy could infect both CCR5- and CXCR4-bearing U373-MAGI cells, demonstrating dual tropism. The baseline biological clones preferentially infected the CCR5 cell line and were fully susceptible to maraviroc in PBMCs (dual-R5). In contrast, during Maraviroc therapy the dual-R5-tropic viruses were replaced by more X4-tropic viruses (dual-X4), which could not be inhibited by maraviroc.

Conclusions: This case report demonstrates that dual-tropic viruses, capable of using both coreceptors in phenotypic assays, can be inhibited by maraviroc if they have a CCR5 coreceptor preference in vivo.

Introduction

HIV-1 entry into host cells requires binding of the viral envelope protein to the CD4 receptor and subsequently to a chemokine coreceptor (CCR5, CXCR4) [1,2]. Viral populations in an infected patient can be categorised by phenotypic tests as R5-tropic, X4-tropic or dual/mixed [D/M; use of both coreceptors by one virus (dual) and/or a mixture of CCR5-using (R5) and CXCR4-using (X4) viruses (mixed)] [3]. Genotypic tests predict viral coreceptor tropism based on the sequence of the viral envelope by means of interpretation algorithms (R5 or X4 prediction). Inhibition of coreceptor usage is a new antiretroviral strategy and multiple compounds are now being studied. Maraviroc is the first licensed CCR5 antagonist and in clinical trials demonstrated potent activity in patients in whom only CCR5-tropic viruses were detected [4,5]. In general, no added value of maraviroc with respect to viral efficacy was observed in patients harbouring D/M-tropic viral populations, which are capable of using the CXCR4 coreceptor [6]. Nevertheless, detailed genotypic analysis demonstrated that virological response could be achieved in a subset of these patients with 10% of X4-predicted viruses in their viral population [7]. Here we present a report of a patient with a D/M viral population where maraviroc inhibited not only R5-tropic viruses but also a range of dual-tropic viruses.

Material and Methods

The patient participates in the AIDS Therapy Evaluation in the Netherlands (ATHENA) observational cohort, which has been approved by local and national institutional review boards.

Genotypic analysis

Viral RNA was isolated from 200–1000 mL of plasma/serum as described previously [8]. The V3 region of the envelope was amplified using primers V3-1 (5' TATCCTTTGARCCAATTCCCAT-3') and V3-2 (5'-CAGTAGAAAAATTCCCCTCCACAA-3') (Superscript-III One-Step Platinum Taq, Invitrogen). Nested PCR using primers V3-3 (5'-AATTCCCCTCCACAATTAAAASTGTG-3') and V3-4 (5'-ACAGTACAATGTACACATGGAATTA-3') was performed (Expand High Fidelity PCR System, Roche). PCR-amplified products were ligated (pGEM-T Easy Vector; Promega) and sequenced using nested primers. Viral coreceptor tropism was predicted using

Geno2Pheno(coreceptor) [R5 prediction, .10%; and X4, ≤10% false positive rate (FPR)] and Web PSSM (where PSSM stands for position-specific scoring matrices) (R5 prediction, ≤26.69; and X4, ≥22.88; the 11/25 rule was applied at intermediate values) [9]. Genotypic sensitivity scores (GSSs) were calculated using the Stanford HIVdb algorithm [10].

Phenotypic analysis Cells

MT-2 cells were maintained in culture medium [CM; RPMI1640 with L-glutamine (BioWhittaker), 10% fetal bovine serum (FBS; Biochrom AG) and 10 mg/L gentamicin (Gibco)]. U373-MAGI cell lines were maintained as recommended by the NIH AIDS Research and Reference Reagent Program. Donor peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll–Paque density gradient centrifugation of heparinized blood from five HIV-seronegative donors (CCR5-homozygous wild-type for D32). The mix was stimulated for 2–3 days with phytohaemagglutinin (2 mg/L) in CM. Cells were incubated at 378C and 5% CO2.

MT-2 cell culture and generation of biological clones

Patient-derived PBMCs (1×10^6) prepared by Ficoll–Paque density gradient centrifugation were co-cultured in triplicate with 1×10^6 CXCR4+CCR52 MT-2 cells in CM [11]. Viral cultures were maintained for 3 weeks and monitored for syncytium formation. Positive viral cultures were used for the generation of biological clones by infecting 4×10^4 MT-2 cells/well in a 96-well plate in a 5-fold dilution series. In cases where less than one-third of viral cultures were positive, virus was harvested. These biological clones were expanded by infecting 1×10^6 MT-2 cells. Supernatant was harvested, and p24 was measured and sequenced. No nucleotide differences were observed after expansion and the biological clones corresponded to the dominant viral population at the specific timepoints. The 50% tissue culture infective dose ($TCID_{50}$) was determined on donor PBMCs. These biological clones were subsequently used in phenotypic analysis.

Coreceptor usage and inhibition in U-373 cells

At day 0, 1×10^4 cells/well of U373-MAGI-CCR5E or U373-MAGICXCR4$_{CEM}$, expressing $CD4^+CCR5^+CXCR4^-$ and $CD4^+CCR5^-CXCR4^+$, respectively, were plated into a 96-well plate in 100 mL of Dulbecco's modified Eagle's medium (DMEM; BioWhittaker) with 10% FBS and 10 mg/mL gentamicin. Subsequently, at day 1, medium was discarded and replaced

by 150 mL DMEM with 10% FBS and 10 mg/mL gentamicin, with a final concentration of 10 mM maraviroc, 1 mM AMD-3100 (a CXCR4 inhibitor) or no inhibitor. This was incubated for 1 h at 37ºC. Subsequently, 1 ng of p24 of the biological clones, Bal or HXB2 suspended in 50 mL of CM was added and incubation was continued for 2 days at 37ºC. Subsequently, luminescence was measured using the Galacto-StarTM b-Galactosidase Reporter Gene Assay System for Mammalian Cells (Applied Biosystems) according to the manufacturer's protocol using 20 mL of lysis buffer and 60 mL of reaction buffer. Background activity (cells without virus in the absence or presence of inhibitor) was subtracted from the activity of the wells containing virus.

Analysis of maraviroc and AMD-3100 susceptibility in PBMCs

Donor PBMCs were infected with a biological clone using a multiplicity of infection (moi) of 0.001 (PBMC titration) in CM with 5 U/mL IL-2 and incubated for 2 h at 37ºC, after which cells were washed twice. Subsequently, 0.2×10^6 cells/well were plated into a 96-well plate with 5 U/mL IL-2 in CM containing increasing concentrations of maraviroc or AMD-3100. p24 was analysed on days 0 and 7.

Analysis of viral replication capacity in PBMCs

Donor PBMCs (5×106) were infected with a biological clone (50 ng of p24) in 1 mL of CM, incubated for 2 h at 37ºC and washed twice. Cells were cultured in 10 mL of CM with 5 U/mL IL-2. Cells were incubated for 14 days and p24 was analysed daily. Viral replication of all biological clones was comparable.

Results

A 51-year-old man was diagnosed with HIV-1 subtype B infection in 1992. Zidovudine monotherapy was initiated and he was subsequently treated with multiple antiretroviral regimens, including integrase and fusion inhibitors. Initial antiretroviral monotherapy and add-on therapy together with intolerability to enfuvirtide, darunavir and other drugs resulted in frequent virological failure and selection of multidrug-resistant HIV (cumulative resistance profile: RT, 41L-67S-69del-74I/V-98G-103N-118I-Y181C-184V-190A-210W-215Y-219E; PR, 10I-20I- 36I-43T-46I-54V-62V-63P-71V-73S-82C-84V-90M; and IN, Q95K-V151I-N155H). Due to a lack of alternative treatment options, sustained virological

suppression was never achieved and immunological deterioration was observed ($CD4^+$ cell count, 50 cells/mm^3). In 2008, the patient received tenofovir, raltegravir and tipranavir/ritonavir and presented with a $CD4^+$ cell count of 4 cells/mm^3 and plasma HIV-RNA of 5.6×10^4 copies/mL. Efforts to design an effective antiretroviral regimen included determination of viral coreceptor tropism using phenotypic assays, namely Trofile (original Trofile assay and enhanced sensitivity Trofile assay); Monogram Biosciences] and MT-2 [11]. These assays demonstrated that the viral population was capable of using the CXCR4 coreceptor (D/M and syncytium-inducing, respectively, *Figure 1*).
However, genotypic analysis using two interpretation algorithms pre-

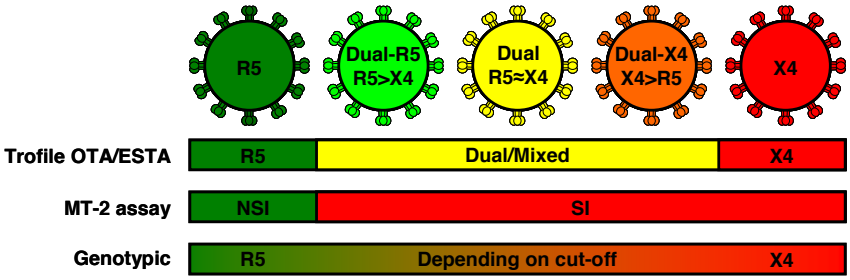
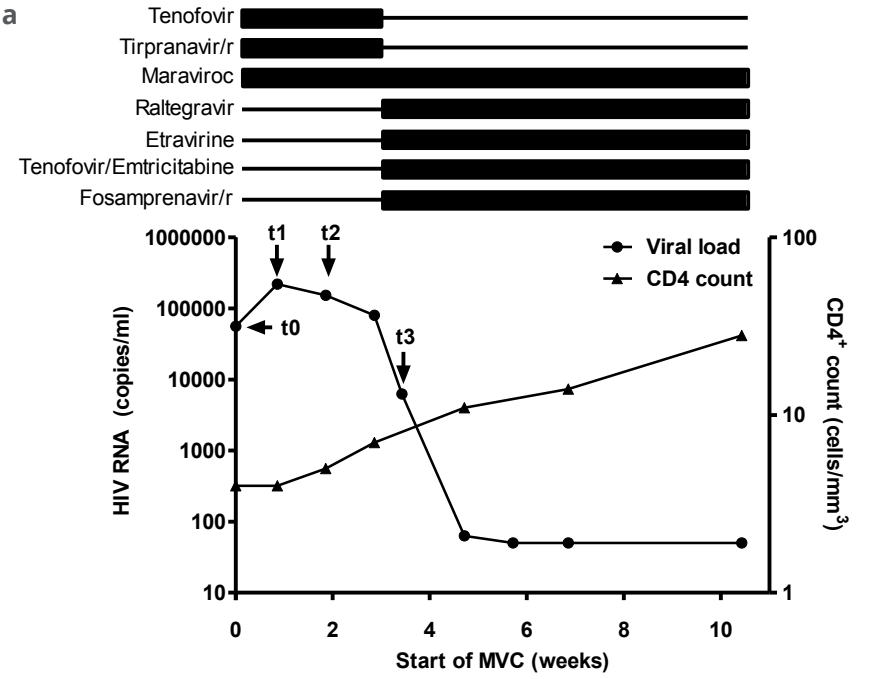


Figure 1. Schematic representation of HIV-1 tropism and corresponding phenotypic and genotypic test results. OTA, original Trofile assay; ESTA, enhanced sensitivity Trofile assay; NSI, non-syncytium-inducing (correlates to CCR5 usage); SI, syncytium-inducing (correlates to CXCR4 usage).

dicted CCR5 usage (Geno2Phenocoreceptor, 10.5% FPR; and Web PSSM, -8.61). Despite the ability of the viral population to use the X4 coreceptor in phenotypic tests, the potential value of maraviroc as an addition to the ongoing therapy was investigated, while raltegravir was discontinued to preserve future options. During 3 weeks of treatment intensification no clear effect on HIV-RNA concentration or $CD4^+$ cells was observed (*Figure 2a*). Nevertheless, the patient reported substantial improvement in his clinical condition and was motivated to continue with maraviroc as part of a mega-HAART (highly active antiretroviral therapy) regimen consisting of tenofovir/emtricitabine, etravirine, raltegravir and fosamprenavir/ritonavir. Despite the fact that the GSS of this therapy was only 1 based on the most recent genotype, or 0 ac-



b

AA position in V3 loop	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	FPR	PSSM
Consensus B V3	C	T	R	P	N	N	N	T	R	K	S	I	R	I	G	P	G	Q	A	F	Y	A	T	G	D	I	I	G	D	I	R	Q	A	H	C	49.3	-10.36
t0 plasma population	-	-	-	-	-	-	-	-	-	-	-	-	T	L	-	-	-	R	V	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5	-8.61
Plasma clones n=6	-	-	-	-	-	-	-	-	-	-	-	-	T	L	-	-	-	R	V	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5	-8.61
Plasma clone	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
Plasma clone	-	-	-	-	-	-	-	-	-	-	-	-	T	L	-	-	-	K	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	9.0	-7.65
MT2 culture population	-	-	-	-	-	-	-	-	-	-	-	-	T	L	-	-	-	R	V	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5	-8.61
Biological clones n=15	-	-	-	-	-	-	-	-	-	-	-	-	T	L	-	-	-	R	V	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5	-8.61
t1 Plasma population	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
Plasma clones n=9	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
Plasma clone	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	R	-	-	-	0.4	-3.30
MT2 culture population	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
t2 Plasma population	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
Plasma clones n=7	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
Plasma clone	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	G	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.80
Plasma clone	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	M	-	-	-	-	-	0.7	-1.78
MT2 culture population	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
Biological clones n=13	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
t3 Plasma population	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52
MT2 culture population	-	-	-	-	-	-	-	-	-	-	R	-	T	L	-	-	-	R	V	-	-	T	-	-	E	-	V	-	-	-	-	-	-	-	-	1.1	-4.52

Figure 2. (a) Plasma HIV-RNA levels and CD4⁺ cells/mm³ during maraviroc (MVC) therapy. (b) V3 loop sequence analysis at start of MVC (t0) and subsequent timepoints (t1–t3) during MVC therapy. Amino acid (AA) positions associated with HIV tropism are boxed, and amino acid changes as selected in the patient associated with X4 prediction are depicted in red italics. Genotypic interpretation of coreceptor tropism by Geno2Phenoco-receptor using an FPR of 10% and Web PSSM is indicated (R5 prediction is given in green and X4 prediction is given in red italics).

cording to the cumulative genotype, a rapid viral and immunological response was observed (*Figure 2a*). We set out to investigate the impact of maraviroc in this D/M-infected patient by performing in-depth in vivo and in vitro analysis. In-depth clonal genotypic analysis at baseline (t0) revealed the presence of X4-predicted viruses in plasma, in line with phenotypic tropism data (*Figure 2b*). However, these viruses constituted only a minority of the analysed clones, with most predicted to be R5-tropic. Furthermore, all viable viral variants generated from infected cells (biological clones) had V3 sequences identical to the dominant plasma population and were predicted to be R5-tropic. Within 1 week of maraviroc intensification, the R5-predicted viral population was replaced by X4-predicted viruses (*Figure 2b*, t1). Follow-up during mega-HAART revealed no additional changes in the viral V3 envelope and corresponding coreceptor tropism (*Figure 2b*, t3). In addition, no new resistance mutations were observed in reverse transcriptase and protease. Coreceptor usage of the biological clones at baseline (t0-I and t0-II) and at t2 (t2-I, t2-II and t2-III) was assessed in X4 and R5 cell lines. The biological clones at t0 (t0-I and t0-II) demonstrated a higher entry efficacy in the CCR5 cell line as compared with the CXCR4 cell line. In contrast, the biological clones obtained at t2 (t2-I, t2-II and t2-III) showed a higher entry efficacy in the CXCR4 cell line as compared with the CCR5 cell line (*Figure 3a*). Since all biological clones were able to use both coreceptors, we asked why the dominant viral population in the patient shifted from R5- to X4-predicted viruses after administration of maraviroc in vivo. To gain more insight into coreceptor preference, we investigated maraviroc susceptibility in the CCR5 and CXCR4 cell lines and in the natural target cells (PBMCs). Viral entry in the CCR5 cell line was inhibited by maraviroc, whereas addition of AMD-3100 did not affect viral entry (*Figure 3b*). Vice versa, viral entry in the CXCR4 cell line could be inhibited by AMD-3100, whereas maraviroc did not inhibit viral entry in this cell line (*Figure 3b*). Interestingly, in PBMCs in which both coreceptors were expressed, all baseline biological clones could be fully inhibited by maraviroc, indicating their CCR5 coreceptor preference in natural host cells, whereas the biological clones obtained during subsequent maraviroc exposure (t2) were only partly inhibited by maraviroc (*Figure 3c*). These maraviroc-resistant biological clones (t2) mainly used the CXCR4 coreceptor and not maraviroc-bound CCR5 coreceptor, as indicated by full inhibition by a CXCR4 coreceptor antagonist.

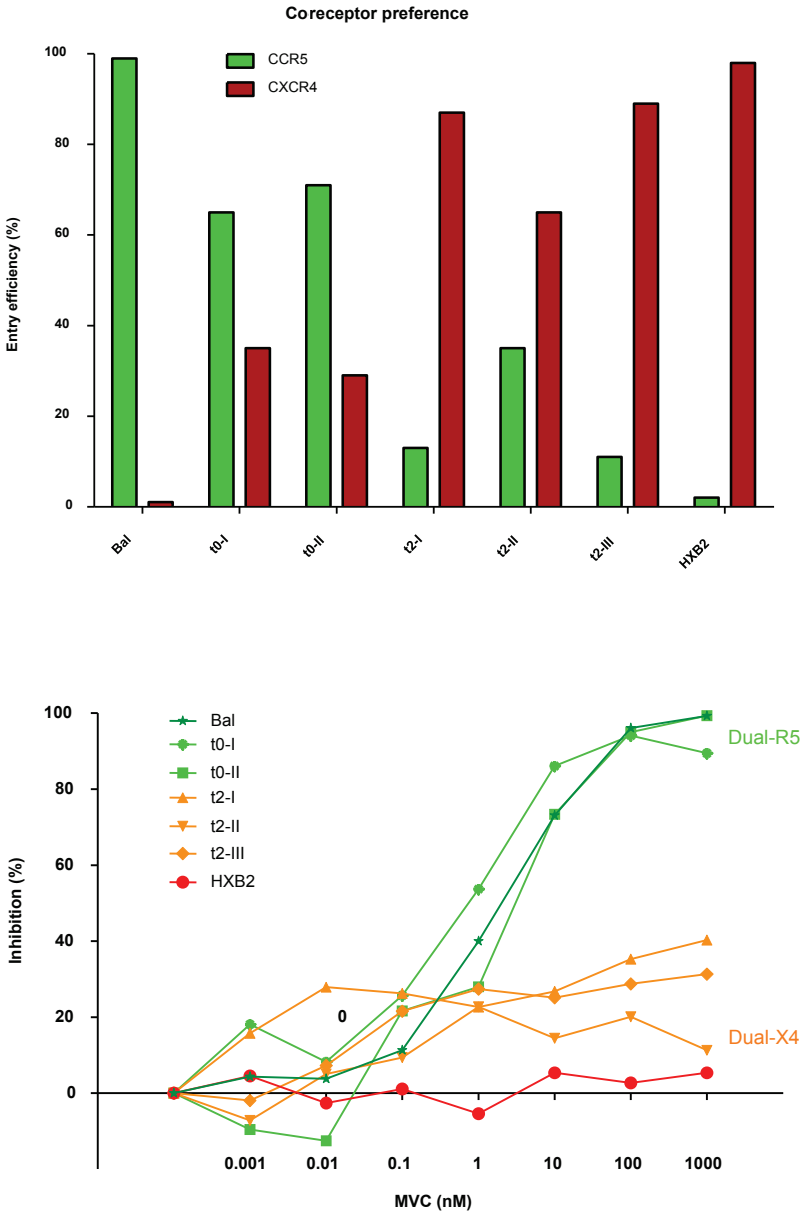


Figure 3. (a) Coreceptor preference in the CCR5 and CXCR4 cell lines (n=3). (b) Entry inhibition in the CCR5 and CXCR4 cell lines with no inhibitor, maraviroc (MVC) or AMD-3100. Standard error of the mean is depicted (n=3). (c) Representative MVC susceptibility assay in donor-derived PBMCs. Biological clones pre-MVC therapy (t0-I and t0-II), biological clones obtained during MVC therapy (t2-I, t2-II and t2-III) and the reference strains HXB2 and Bal were used.

Discussion

In this case report we describe the ability of maraviroc to inhibit a dual-tropic virus population in an HIV-1-infected patient. It has been reported earlier that certain dual-tropic viruses prefer usage of the CCR5 coreceptor and are typed as R5>X4 or dual-R5-tropic, whereas others use the CXCR4 coreceptor more efficiently (X4>R5 and dual-X4) in PBMCs [12,13]. In our case, the patient harboured a viral population that was reported to be D/M in the Trofile assays and syncytium-inducing in the MT-2 assay, but R5-tropic in two genotypic prediction algorithms. In vitro experiments demonstrated that this dominant baseline viral population could be considered R5>X4 or dual-R5 since it was capable of using the CXCR4 coreceptor, but preferentially used the CCR5 coreceptor in cell lines and natural target cells (PBMCs). Furthermore, entry of these dual-R5 viruses in the CCR5 cell line and in PBMCs could be inhibited by maraviroc. Also, in vivo these dual-R5 variants were suppressed by maraviroc, demonstrating their preferential usage of the CCR5 coreceptor. Unfortunately, in our patient no viral efficacy of maraviroc was observed, which could be explained by the presence of a dual-X4 minority at baseline that was rapidly selected in the absence of an active backbone regimen.
These results indicate that viruses capable of using both coreceptors in vitro may be inhibited by maraviroc. Further research is warranted to establish whether maraviroc in combination with an active backbone might be of added value in patients harbouring dual-tropic virus.

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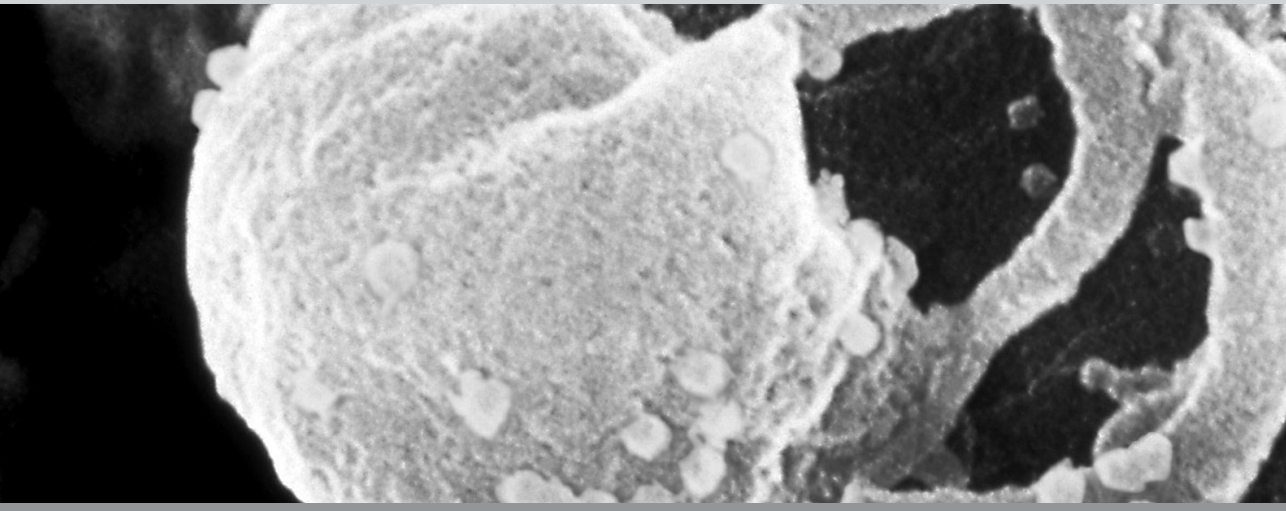
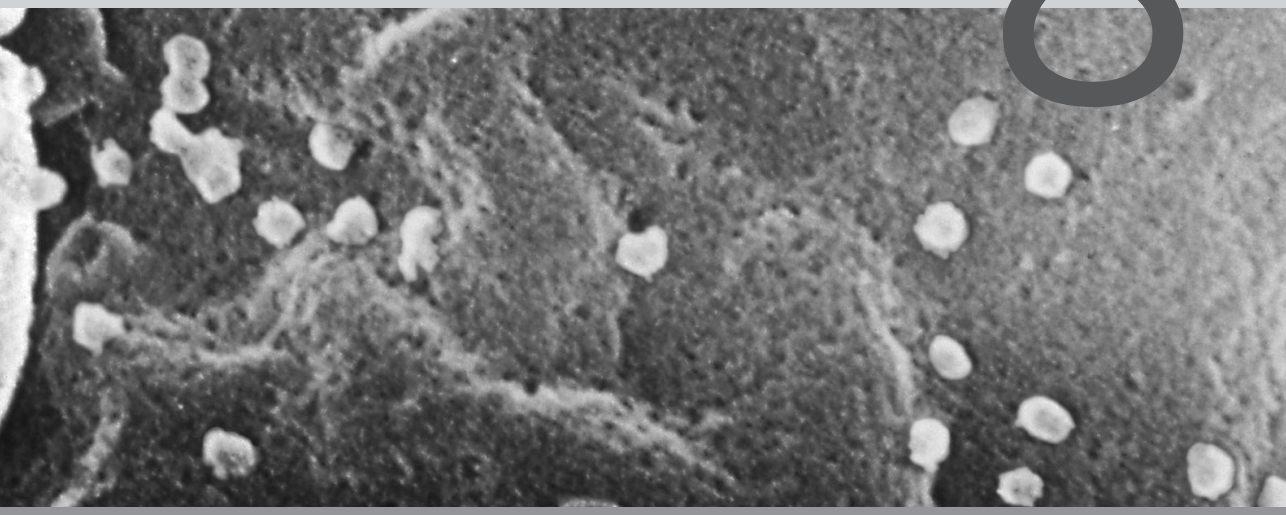


CHAPTER 8



Maraviroc intensification in patients with suboptimal immunological recovery despite virological suppressive cART: a 48-week, placebo-controlled trial

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Submitted

Background: Several trials have investigated whether cART intensification with the CCR5-antagonist maraviroc (MVC) in patients with suboptimal immunological recovery despite viral suppression increases $CD4^+$ T cell counts and reduces immune activation, with conflicting results. We performed a 48-week, double-blind, placebo-controlled trial to determine the effects of treatment intensification with MVC on $CD4^+$ T cell reconstitution and residual immune activation.

Methods: Major inclusion criteria were: CD4 count <350 cells/μL while at least two years on cART, or CD4 count <200 cells/μL while at least one year on cART, and viral suppression for at least the previous 6 months. HIV-infected patients were randomized to add MVC (42 patients) or placebo (43 patients) to their existing cART regimen for 48 weeks.
Primary outcome was the change in $CD4^+$ T cell count. Changes in T cell counts and other parameters were modeled using mixed effects models.

Results: Patient characteristics and baseline values were not different between the MVC and placebo arms. The median age of the patients was 49 years, 5 (6%) were women, the median duration of cART was 5.0 years and the median baseline $CD4^+$ T cell count was 237 cells/μL. At week 48, the increase in $CD4^+$ T cell count was +16.3 (95% confidence interval [-0.2; 32.8]) cells/μL in the placebo arm versus +22.6 (95% CI [9.8; 35.3]) cells/μL in the MVC arm ($p = 0.62$). Naive $CD4^+$ T cells increased in both arms, whereas the number of memory $CD4^+$ T cells did not change. MVC intensification decreased $CD8^+$ T cell apoptosis levels and counteracted the decrease of memory $CD8^+$ T cell numbers and the increase of $CD8^+$ T cell activation as observed in the placebo arm. No differences in levels of markers for $CD4^+$ T cell activation, T cell production, proliferation and microbial translocation were found between the arms.

Conclusions: After 48 weeks of treatment there were no significant differences in CD4cell reconstitution between the MVC and placebo arm. Minor differences in $CD8^+$ T cell activation and apoptosis were found between the arms. In general, there seems to be no immunological benefit of MVC intensification of cART in patients with suboptimal immunological recovery. Further studies might identify subgroups of patients for which MVC intensification might be beneficial.
ClinicalTrials.gov identifier: NCT00875368.

Introduction

Treatment of HIV-infection with combination antiretroviral therapy (cART) suppresses viral replication, leading to recovery of $CD4^+$ T-cells (CD4 cells). Unfortunately, 9-29% of the patients treated with cART experience a suboptimal immunological response, i.e. failure to restore cell count despite virological suppression [1-6]. Several studies have shown a worse long term clinical outcome in terms of death, AIDS and non-AIDS defining diseases in these patients [1, 2, 4, 7, 8].

The CCR5-antagonist maraviroc (MVC) was registered in 2008 for the treatment of antiretroviral treatment-naive (USA only) and –experienced HIV-1 infected patients [9]. Next to its established efficacy in suppressing plasma HIV-RNA, there has been much interest in the potential immunological effects of CCR5 antagonists. Molecular studies have shown that the CCR5 pathway can influence T cell trafficking, activation and apoptosis [10-12]. In line with these observations, genetic studies have shown that the CCL3L1-CCR5 genotype influences the degree of CD4 cell reconstitution during cART [13] and manipulation of this pathway might enhance CD4 cell recovery. For example, a MVC containing regimen leads to a larger increase in CD4 cell count, as compared to an efavirenz containing regimen[14].A meta-regression analysis of clinical trials investigating CCR5-antagonists in antiretroviral treatment-experienced patients found that the use of a CCR5-antagonist was associated with a significant increase in CD4 cell gain (+30 cells/μL [95% CI, 19-42]) [15]. Immune modulating properties of MVC have also been described in HIV-negative patients. Recently, a study showed that the addition of MVC to treatment of recipients of allogeneic hematopoietic stem-cell transplantation reduced the risk of visceral acute graft-versus-host disease, possibly by inhibition of lymphocyte trafficking [12].

We hypothesized that intensification of cART in patients with a suboptimal immunological response might result in an increase in the number of CD4 cells, which might be of clinical benefit for these patients. Therefore, we performed the 'Maraviroc Immune Recovery Study' (MIRS), a 48-week, double-blind, placebo-controlled trial studying the effect of MVC intensification of cART on $CD4^+$ T cell recovery.

Methods

Subjects

HIV-infected patients were recruited from 10 HIV treatment centers (4 University Medical Centres and 6 teaching hospitals) in the Netherlands. All subjects provided written informed consent. This study was approved by the Ethical Committee of the University Medical Centre Utrecht, The Netherlands (ClinicalTrials.gov identifier: NCT00875368; EudraCT number 2008-003635-20).

Inclusion criteria were: age 18 years and older; either a $CD4^+$ T cell count (CD4 count) <350 cells/μL while at least two years on cART, or $CD4^+$ T cell count <200 cells/μL while at least one year on cART; and, viral suppression (plasma HIV-RNA < 50 copies/ml) for at least the previous 6 months. Exclusion criteria were: previous use of MVC; HIV-2 infection; cART regimen containing a combination of tenofovir and didanosine; active infection treated with antimicrobial therapy; acute hepatitis B or C infection; chronic hepatitis B or C infection treated with (peg)interferon and/or ribavirine; immunosuppressive medication; and, radiotherapy or chemotherapy in the previous 2 years.

Study procedures

Included patients were randomized to add MVC or placebo to their existing cART regimen for 48 weeks. The MVC dose was 150-600 mg twice daily, depending on interactions with concurrent medication, as specified in the package insert. In case of virological failure (defined as two consecutive plasma HIV-1 RNA measurements of 50 copies/mL or higher), participants had to discontinue study medication. Subjects were seen for screening, at baseline, and at weeks 2, 4, 8, 12, 24, 36 and 48 of their study participation. At all visits patients were questioned for side effects and other complaints, physical examination (if indicated) was performed, and EDTA- and heparin-plasma was drawn. Adherence to study drug was measured at every visit by self-report, and at week 4, 12, 24, 36 and 48 by pill count.

Virological Analyses

Plasma HIV-RNA was measured in the participating sites using validated assays with a lower limit of detection (LLD) of 20-40 copies/mL.

Immunologic Analyses

Absolute CD4+ T cell counts were assessed by flow cytometry at the local-site laboratory at each visit. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood via density gradient centrifugation, cryopreserved and stored. Total $CD3^+CD8^+$ and subsets of $CD3^+CD8^+$ and $CD3^+CD4^+$ T cell (i.e. naive ($CD27^+$ $CD45RO^-$), memory ($CDRO^+$) and effector ($CD27^-CD45RO^-$) and levels of markers for T cell production (% $CD31^+$ within naive $CD27^+$ $CD45RO^-$ $CD4^+$ T cells), activation (% $CD38^+$/HLA-DR^+), proliferation (% Ki-67^+), apoptosis (% annexin-v^+), were determined for $CD4^+$ and $CD8^+$ T cells. Soluble CD14 was assessed as a measure for monocyte activation in plasma.

T cell analysis was performed on thawed material by flow cytometry as described previously [16]. Flow cytometry was performed using a FACS LSR II (BD Biosciences) and FACS Diva software (BD Biosciences). Lymphocytes were gate within the live gate based on forward and side scatter and subsets were identified based on expression of a combination of the indicated molecules (see above). The concentration of soluble CD14 was assessed on heparin plasma using a commercial ELISA kit (Gen-Probe Diaclone SAS).

Sample size calculation

To achieve 90% statistical power to detect a 30% difference in increase in CD4 count, 62 patients in each group were required (alpha=0.05 and beta=0.10). We therefore planned to enrol 130 patients (65 in each group), in order to account for potential losses to follow-up and early treatment discontinuations.

Statistical analyses

Primary outcome was the change in absolute $CD4^+$ T cell count. Patients were stratified in two groups: group I with CD4 count <200 and group II with CD4 count between 200-350 cells/μL at screening. Analysis of the primary endpoint was done by an intention-to-treat (ITT) procedure, defined as the analysis of CD4 cell counts of every patient from the moment study medication was started until the end of the planned study period. In case of premature discontinuation of the study, CD4 cell counts of these patients after discontinuation until week 48 were included for ITT analysis.

Continuous variables were compared using Student's t-test or the Mann-Whitney test, for categorical variables Chi-square or Fisher's exact test were used. Differences were considered statistically significant at $p < 0.05$.
Changes in biological markers were studied using linear mixed effects models. Trends in the evolution of markers were fitted using one or two slopes depending on the best fit (defined by Akaike criteria, the lower the better). The time taken for the slope to change was determined for all patients by a likelihood profile. To achieve normality and homoscedasticity of measurement error distributions, the fourth-root of markers was used instead of the natural markers when necessary. All statistical analysis were performed with SAS software (version 9.2, SAS institute Inc.).

Results

Study population

Between February 2009 and February 2011 one hundred and four patients were screened for eligibility (*Figure 1*), of whom 7 declined to participate, 10 failed to meet the inclusion criteria and 2 were excluded for other reasons. Because enrolment was slower than expected, 85 instead of the anticipated 130 patients were included. Twenty-two patients were included in group 1 (<200 CD4 cells/μL at baseline) and 63 in group 2 (200-350 CD4 cells/μL). These groups were taken together in order to perform an overall analysis. A modified intention-to-treat analysis was performed for the primary endpoint, because we analysed in the MVC arm one patient who was mistakenly treated with MVC instead of placebo during the entire study.
During the study one patient deceased (in the placebo arm) and 5 patients discontinued the study medication because of side effects (n = 3; gastro-intestinal complaints, dry mouth, itching and hallucinations), no effect on CD4 cell count (n = 1) or other reasons (n = 1). Seventy-nine patients completed the full study period.

Virological analyses

Viral load was measured during the entire study period, no virological failure was recorded.

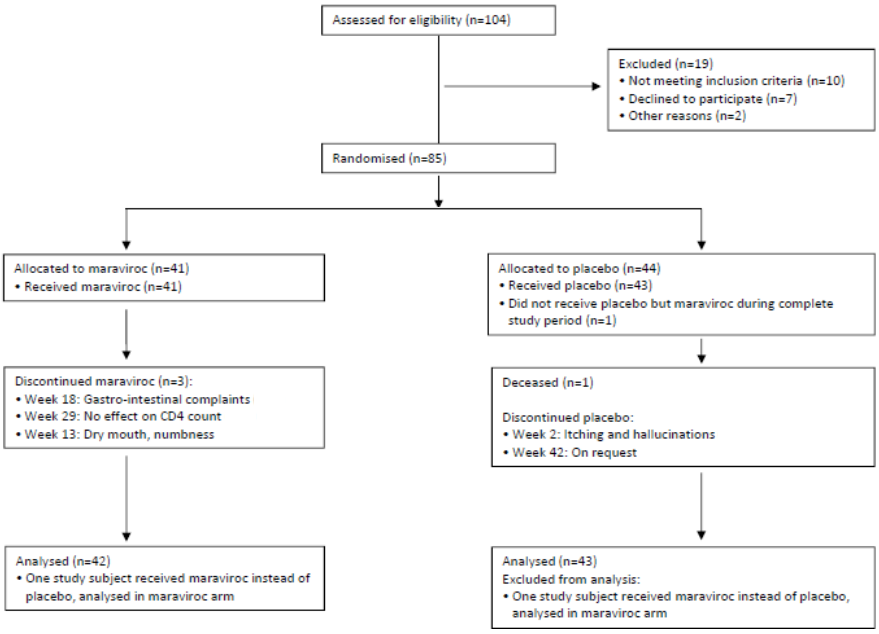

Figure 1. Study flow chart. 104 patients were screened, of whom 85 were included in the study. One patient died (in the placebo arm) and 5 patients prematurely discontinued the study; 79 patients finished the complete study protocol.

Safety

The study medication was well tolerated. During the total study duration 16 serious adverse events were registered in 12 study participants, 7 in the placebo and 9 in the MVC arm ($p = 0.55$). In two cases the study medication could not be ruled out as a causative factor. One participant reported gastro-intestinal side effects and discontinued the study medication during week 17. However, stool cultures later pointed out that she had suffered a gastro-enteristis caused by *Giardia Lamblia* infection, and she recovered completely after treatment. In the other participant plasma gamma-glutamyl transpeptidase (γGT), which was already highly elevated at the start of the study (800 U/L, >10 times upper limit of normal (ULN), temporarily increased to 1607 U/L. This study participant however, was known with a large alcohol intake. Liver biopsy showed steatotic hepatitis. It was therefore decided to continue his study medication, and after he decreased his alcohol intake the γGT returned to pre-study levels. One study participant in the placebo

arm deceased during the study. The reason of his death remained unknown, and was classified to be a natural death by the coroner.

Baseline characteristics

The median age of the patients was 49 years (interquartile range (IQR) 43-57), 5 (6%) were women, and the median overall baseline CD4⁺ T cell count was 237 (IQR 180-286) cells/μL. The median duration of cART was 5.1 (IQR 9.9-3.2) years, whereas the median overall nadir CD4 cell count was 40 (IQR 10-86). The clinical and immunological baseline characteristics were not different between the treatment arms (*Table 1*).

Change in CD4⁺, CD8⁺ T cell counts and subsets

The baseline T cell and subset counts are given in *table 1*. The median baseline CD4⁺ T cell count was 220 (IQR 176-300) in the placebo arm versus 240 (IQR 180-286) cells/μL in the MVC arm (p = 0.61). Mixed model analysis showed an increase of 16.3 (95% confidence interval (95% CI) [-0.2;32.8]; p = 0.02) in the placebo arm versus +22.6 (95% CI

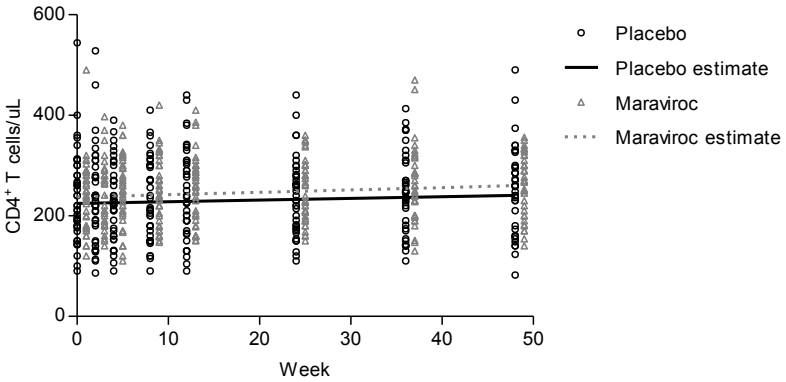

Figure 2. Change in CD4⁺ T cell count on intensification of cART with MVC

[9.8;35.3] p = 0.005) cells/μL in the MVC arm over the treatment period. The increases were not significantly different between both arms (p = 0.62; *Figure 2 and 3*).

Naive CD4⁺ T cell counts (*Figure 3*) increased in the placebo arm with 11.4 cells/μL (95% [CI 4.2;18.6]; p = 0.002), which was not significantly different from the MVC arm (p = 0.89; *Figure 3*). No increase in memory CD4⁺ T cell count was found for both arms during the study period

Table 1. Clinical and immunological baseline characteristics.

	Total	Placebo	Maraviroc	P-value[b]
N	85	43	42	
Age (years)	49 (43-57)	51 (43-60)	48 (41-54)	0.08
Male sex[1]	80 (94%)	42 (98%)	38 (90%)	0.16
Duration cART	5.1 (9.9-3.2)	5.0 (3.2-9.4)	5.8 (3.0-12.4)	0.49
Years undetectable	3.5 (5.7-2.2)	4.0 (2.2-7.7)	3.3 (2.1-5.5)	0.59
Nadir CD4 cell count (cells/μL)	40 (10-86)	30 (10-80)	44 (10-90)	0.71
Previous CDC-C events[a]	48 (56.5)	26 (60.5)	22 (52.4)	0.45
$CD4^+$ T cells (cells/μL)	237 (180-286)	220 (176-300)	240 (180-286)	0.61
Naive $CD4^+$ T cells (cells/μL)	49.4 (27.7-73.4)	45.1 (20.7-73.7)	49.7 (33.5-73.4)	0.38
Memory $CD4^+$ T cells (cells/μL)	169.4 (133.1-211.3)	172.3 (118.7-221.5)	166.5 (134.5-208.0)	1.00
Effector $CD4^+$ T cells (cells/μL)	3.5 (1.6-13.2)	3.1 (1.4-16.3)	5.4 (2.3-12.1)	0.41
$CD8^+$ T cells (cells/μL)	837.0 (597.2-1210.4)	765.5 (503.2-1107.4)	951.4 (706.3-1210.4)	0.11
Naïve $CD8^+$ T cells (cells/μL)	151.2 (94.8-212.8)	140.9 (62.0-261.7)	152.5 (117.5-183.5)	0.55
Memory $CD8^+$ T cells (cells/μL)	424.5 (220.6-652.7)	361.5 (209.8-583.6)	436.9 (309.9-652.7)	0.75
Effector $CD8^+$ T cells (cells/μL)	179.3 (74.6-385.5)	147.2 (54.1-258.6)	285.5 (97.5-495.3)	0.03
$CD38^+$ $CD4^+$ T cells (%)	3.1 (2.3-4.4)	3.1 (2.2-4.0)	3.1 (2.5-4.5)	0.78
$CD38^+$ $CD8^+$ T cells (%)	5.0 (2.8-8.1)	5.8 (2.8-10.2)	4.9 (3.0-7.1)	0.55
$Ki67^+$ $CD4^+$ T cells (%)	3.3 (2.0-4.3)	3.4 (2.2-4.3)	3.2 (1.9-4.2)	0.52
$Ki67^+$ $CD8^+$ T cells (%)	1.0 (0.7-1.6)	1.0 (0.7-1.9)	1.2 (0.8-1.6)	0.69
sCD14 (μg/L)	5.8 (4.9-10.0)	7.9 (5.7-9.5)	7.2 (5.8-10.3)	0.98
$CD31^+$ naive $CD4^+$ T cells (%)	56.3 (41.8-66.6)	57.7 (44.9-64.6)	54.1 (40.7-66.6)	0.81
Annexin-v^+ $CD4^+$ T cells (%)	20.4 (14.2-27.4)	19.0 (13.1-26.1)	20.7 (15.4-28.2)	0.39
Annexin-v^+ $CD8^+$ T cells (%)	34.5 (20.3-47.6)	33.7 (14.3-46.1)	35.7 (26.7-51.3)	0.27
$CCR5^+$ $CD4^+$ T cells (%)	4.7 (1.8-9.1)	5.0 (2.1-7.2)	4.3 (1.8-12.0)	0.69
$CCR5^+$ $CD8^+$ T cells (%)	12.9 (7.0-21.9)	14.1 (7.4-21.9)	11.5 (6.0-15.5)	0.41
$CXCR4^+$ $CD4^+$ T cells (%)	42.2 (27.3-62.1)	42.2 (28.0-65.0)	45.7 (24.9-61.6)	0.89
$CXCR4^+$ $CD8^+$ T cells(%)	34.5 (16.1-62.3)	32.6 (17.2-58.4)	36.3 (14.1-63.5)	0.83

Baseline clinical and immunological characteristics of the included patients. All values are given as median (intequartile reange), unless indicated otherwise. [a]Number of patients [N (%)].[b]P-value of maraviroc compared to placebo arm.

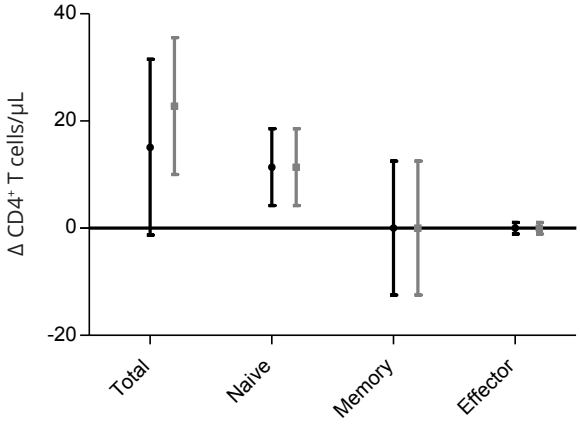

Figure 3. Change in total, naive,memory and effector CD4⁺ T cell count after 48 weeks of MVC treatment intensification (median [95% CI]). Black = placebo, grey = MVC.

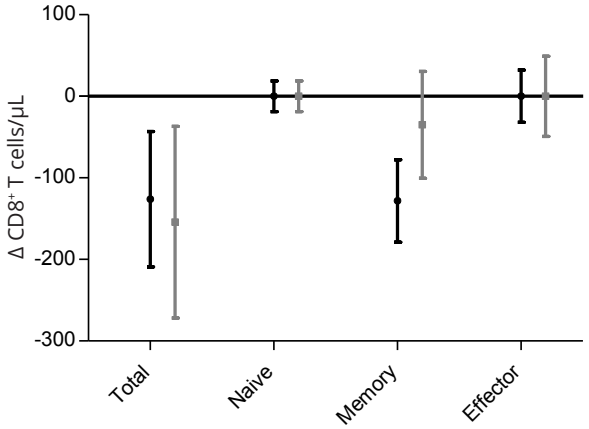

Figure 4. Change in total, naive, memory and effector CD8⁺ T cell count after 48 weeks of MVC treatment intensification (median [95% CI]). Black = placebo, grey = MVC.

(compared to baseline, p = 0.89; between arms, p = 0.70), neither for effector CD4⁺ T cells (compared to baseline, p = 0.27; between arms, p = 0.39).

For CD8⁺ T cells (*Figure 4*) a decrease of 126.1 (95% CI [-209.1;-43.1];p = 0.006) cells/µL was observed in the placebo arm, which was comparable to the MVC arm (p = 0.14). No difference in naive CD8⁺ T cell counts was found in both arms during the observation period (compared to

baseline, p = 0.24; between the arms, p = 0.72), neither for effector CD8$^+$ T cell counts (compared to baseline, p = 0.82; between the arms, p = 0.58).
In both study arms a decrease in memory CD8$^+$ T cells was observed during the trial period, however the decrease was more pronounced in the placebo arm (128.3 (95% CI [-179.0;-77.7]; compared to baseline, p < 0.0001) cells/µL than in the MVC arm 34.9 (95% CI [-100.5;30.6];) between the arms, p = 0.03). No difference in naive CD8$^+$ T cell counts was found in both arms during the observation period (compared to baseline, p = 0.24; between the arms, p = 0.72).

Effects on T cell characteristics
An extended analysis of the effects on T cells was performed in a subset of 66 patients. The percentages of cells expressing markers for proliferation (Ki67$^+$), activation (CD38$^+$ HLA-DR$^+$) and apoptosis (annexin-v$^+$) were measured for CD4$^+$ and CD8$^+$ T cells. As a measure of thymic T

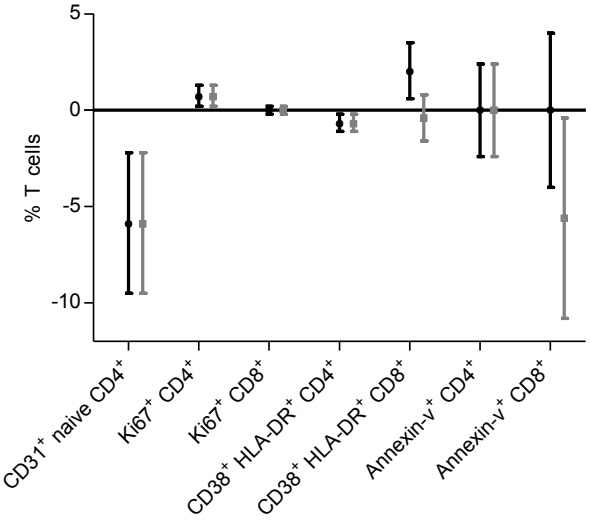

Figure 5. Change in expression of markers for T cell production (%CD31$^+$), proliferation (%Ki67$^+$), activation (%CD38$^+$/HLA-DR$^+$) and apoptosis (annexin-v$^+$) after 48 weeks of MVC treatment intensification (median [95% CI]). Black = placebo, grey = MVC.

cell production, the % $CD31^+$ within naive $CD4^+$ T cells was determined. Baseline values are shown in *table 1*, an overview of the observed changes are depicted in *Figure 5*.

The percentage of $CD31^+$ naive $CD4^+$ T cells decreased in with 5.9% (95% CI [-9.5;-2.2]) in the placebo arm (compared to baseline, p = 0.0003), which was not significantly different as compared to the MVC arm (p = 0.17).

An increase of 0.70% (95% CI [0.2;1.3]) $Ki67^+$ $CD4^+$ T cells was observed in the placebo arm (p = 0.02), comparable to the MVC arm (p = 0.15). The percentage of $Ki67^+$ $CD8^+$ T cells did not change significantly in either one of the arms (compared to baseline, p = 0.65; between the arms, p = 0.61).

The median baseline percentage of $CD38^+$ HLA-DR^+ $CD4^+$ T cells was 3.1% (IQR 2.2-4.0) in the placebo arm versus 3.1% (IQR 2.5-4.5) in the MVC arm (p = 0.78). A significant decrease of 0.70% (95% CI [-1.1;-0.2]) was found in the placebo arm (p = 0.006), however this was comparable to the MVC arm (p = 0.57).

The median baseline percentage of $CD38^+$ HLA-DR^+ $CD8^+$ T cells was 5.8% (IQR 2.8-10.2) in the placebo arm, versus 4.9% (IQR 3.0-7.1) in the MVC arm (p = 0.55). An increase of 2.0% (95% CI [0.6;3.5]) was found in the placebo arm (p = 0.02), whereas no change (0.4% (95% CI [-1.6;0.8]) was observed in the MVC arm (between the arms, p = 0.046).

With respect to T cell apoptosis, no significant change in annexin-v^+ $CD4^+$ T cells was observed in either one of the arms (compared to baseline, p = 0.56; between the arms, p = 0.07). However, whereas no change in annexin-v^+ $CD8^+$ T cells was observed in the placebo arm (p = 0.35), a decrease of 5.6 (95% CI [-10.8;-0.4]) was found in the MVC arm (between the arms, p = 0.04).

As a marker of bacterial translocation and monocyte activation we measured plasma levels of sCD14.The median baseline plasma concentration of soluble CD14 was 7.9 (IQR 5.7-9.5) µg/L in the placebo arm, versus 7.2 (IQR 5.8-10.3) in the MVC arm (p = 0.98). A decrease of 1.4 (95% CI [-1.9;-0.9]) µg/L was found in the placebo arm (p < 0.0001), which was not significantly different as compared to the MVC arm (p = 0.15).

CCR5⁺ expression

The median baseline percentage of CCR5⁺ CD4⁺ T cells was 5.0% (IQR 2.1-7.2) in the placebo arm, versus 4.3% (IQR 1.8-12.0) in the MVC arm (p = 0.69). While no change in the percentage of CD4⁺ T cells expressing CCR5 was observed during the trial period in the placebo arm, an increase of 1.5% (95% CI [0.0;3.5]) was observed in the MVC arm (compared to placebo, p = 0.03; *Figure 6*).

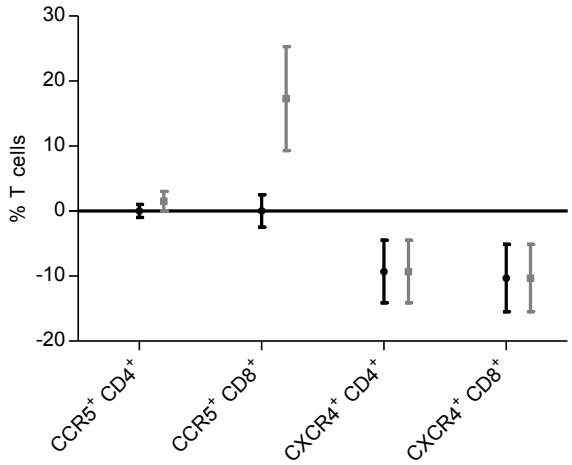

Figure 6. Change in CCR5⁺ and CXCR4⁺ T cells after 48 weeks of MVC treatment intensification (median [95% CI]). Black = placebo, grey = MVC.

The median baseline percentage of CCR5 expressing CD8⁺ T cells was 14.1% (IQR 7.4-21.9) in the placebo arm, and 11.5% (IQR 6.0-15.5) in the MVC arm (p = 0.41). An increase of 17.3% (95% CI [9.3;25.3]) was observed in the MVC arm, whereas no change was observed in the placebo arm (p = 0.0009). No differences in the percentage of CXCR4⁺ CD4⁺ and CD8⁺ T cells were observed between the arms.

Discussion

We performed randomized, placebo-controlled trial investigating the effect of MVC intensification on CD4⁺ T cell recovery in patients with a suboptimal immunological response despite viral suppression to test

our hypothesis that MVC intensification would aid $CD4^+$ T cell recovery and decrease immune activation. No effect on $CD4^+$ T cell gain after 48 weeks of MVC intensification of cART was observed. A concurrent effect of MVC treatment was however seen on the level of CCR5 expression on $CD8^+$ T cells, memory $CD8^+$ T cell numbers and activation and apoptosis levels of $CD8\ T^+$ cells.

Increased immune activation leading to an increase in lymphoid tissue fibrosis and T cell apoptosis is implicated in $CD4^+$ T cell loss in HIV infection [17-22]. Indeed, not only has the level of activated ($CD38^+$) T cells been recognized as a strong independent predictor for survival of HIV-infected men[23], it also has been shown that elevated levels of $CD38^+$ HLA DR^+ $CD4^+$ and $CD8^+$ T cells are associated with a lower CD4 cell gain during cART[24, 25].

Besides the fact that the CCR5 receptor is a co-receptor for HIV-entry [26, 27], this receptor has also been shown directly influence T cells. Binding of chemokines to CCR5 co-stimulates T cell activation [10, 11] and modulates apoptosis [28]. Effects of MVC on the immune system and in particular immune activation could thus act via further suppression of residual low level viral replication, via direct manipulation of T cells or a synergistic combination of both mechanisms. In our study we did find an effect of MVC treatment on $CD8^+$ T cells, whereas no effects on $CD4^+$ T cells were observed. MVC intensification decreased $CD8^+$ T cell apoptosis and counteracted the observed increase in $CD8^+$ T cell activation and decrease of the memory $CD8^+$ T cell count in the placebo arm. During the current study none of the patients experienced virological failure and although in the current study plasma HIV-RNA was not measured with a single copy assay, a previous MVC intensification study did not find a difference in plasma HIV-RNA between the MVC and placebo arm using this technique [29].

In addition, we observed an increase in $CCR5^+$ $CD4^+$ and $CD8^+$ T cells in the MVC arm. MVC induced CCR5 upregulation on T cells has been shown in vitro [30] and might be the consequence of disruption of CCL5-CCR5 interaction. This interaction has been shown to result in internalisation of the CCR5 receptor and to inversely correlate with CCR5 expression on the T cell surface. These results are in line with data showing an increase in CCR5 expression on T cells in healthy volunteers treated with the CCR5 antagonist vicriviroc [31] and with preliminary data of another MVC intensification study [29].

Collectively, this suggests that the observed alterations for $CD8^+$ T cells are the consequence of a direct blockade of CCR5 signalling on T cells and not an indirect effect of changes in plasma HIV-RNA by MVC intensification. In the present study the difference in CCR5 expression levels between $CD4^+$ and $CD8^+$ T cells might explain that no effects were found for (memory) $CD4^+$ T cells.

In general, our findings are in agreement with other MVC intensification studies, which report no or minor changes in CD4 cell count and markers for T cell activation as well [29, 32-35].

A recently published single-arm pilot trial in which MVC was added to cART of 34 patients for 24 weeks reported an estimated difference of +25.2 cells/μL per year (90% CI, +2.1 to +48.3) compared to the period before MVC intensification [33]. Markers of immune activation and apoptosis decreased during MVC intensification and reversed after discontinuation of MVC. A French single arm MVC intensification trial (57 patients, 24 weeks) reported an increase (+22.6 cells/mm3/y, p=0.04) in the CD4+ T cell slope in the on-treatment analysis (47 patients), whereas a trend was found in the intention-to-treat analysis (p = 0.08) [34]. Preliminary results of an Italian randomized (open label) trial (96 patients, 48 weeks) did not show a significant difference in $CD4^+$ T cell increase between the arms, but a significant increase in CD8 cell count in the MVC arm was reported. T cell activation decreased in both arms [35] in this study.

To our knowledge, the preliminary results of only one placebo-controlled randomized trial have been reported [29]. In this study 42 patients were randomized to add either MVC or placebo to their antiretroviral regimen for 24 weeks. Although no difference in CD4 cell count increase was found between the arms, a difference in T cell activation parameters was found. This difference was explained by the fact that T cell activation decreased in the placebo arm. The authors suggested that blocking of the CCR5 co-receptor would lead to an increased production of other chemokines, leading to an increase in T cell activation via other pathways.

In light of these different outcomes between above mentioned studies, pooling of data for subanalyses might be helpful to investigate whether specific subgroups that can benefit from MVC intensification exist, such as patients with high baseline levels of immune activation or patients with a specific genetic makeup. For example, it has previ-

ously been shown that the CCL3L1-CCR5 genotype influences $CD4^+$ T cell recovery on cART [13].
Several studies show a worse long term clinical outcome in terms of death, AIDS and non-AIDS defining diseases, in patients with a suboptimal immunological response on cART [1, 2, 4, 7, 8]. Since suppression of HIV-1 replication by cART is the only therapy currently available for increasing CD4 cell counts, there is a need for immunomodulating therapies for this particular group of patients. Next to MVC treatment intensification studies, trials with interleukin-2 and interleukin-7 have been performed. Subcutaneous recombinant interleukin-2 treatment in combination with antiretroviral therapy resulted in a substantial and sustained increase in CD4 cell count; however this treatment did not translate into an effect on clinical endpoints (opportunistic disease or death from any cause) and had substantial toxicity [36]. A recently published phase I/IIa trial investigating the effect subcutaneous recombinant interleukin-7 therapy on T cell recovery in antiretroviral treated patients with a suboptimal immunological response showed promising results in terms of CD4 cell count increase and toxicity, but larger trials with clinical endpoints are needed to establish its clinical utility [37]. In addition, it will be interesting to investigate whether interleukin-7 therapy will have effects on levels of T cell activation, apoptosis and plasma HIV-RNA in patients with as suboptimal immunological response on cART.
In the present randomised, placebo-controlled trial, we did not find a significant change in $CD4^+$ T cell count in the MVC arm. However, slight effects on memory $CD8^+$ T cell numbers and $CD8^+$ T cell activation and apoptosis markers where found, prompting the need for more research to elucidate the underlying mechanism and the clinical implications.

In conclusion, the data of this study do not support MVC intensification of cART in patients with a suboptimal immunological response in order to restore CD4 cell count. However, since in this and other studies some changes in T cell activation and apoptosis are found, further research is needed to investigate whether specific subgroups of patients can be identified who might benefit of MVC intensification of cART.

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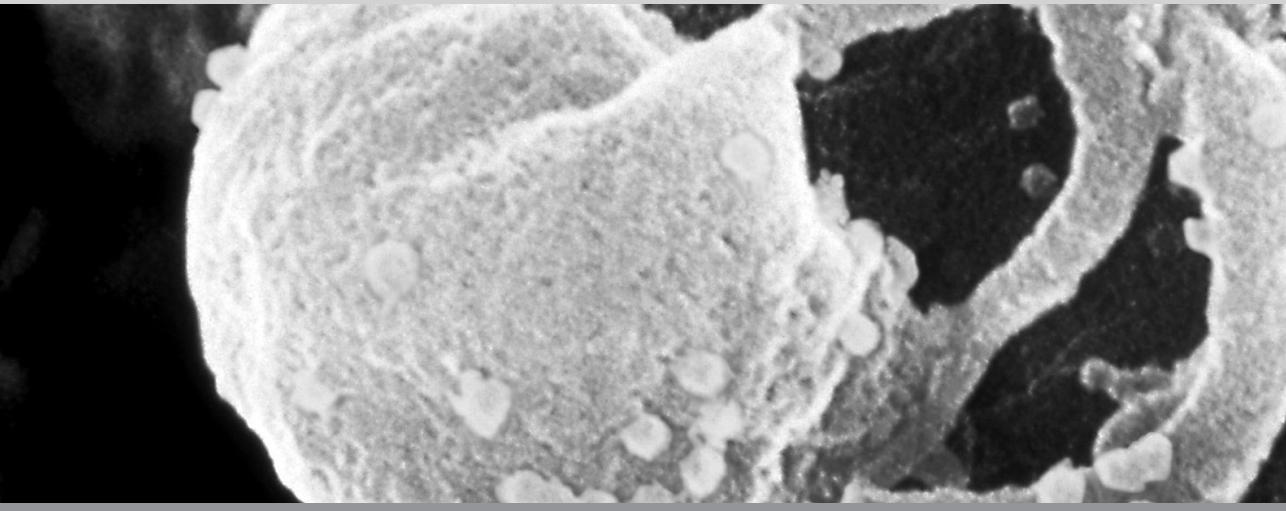
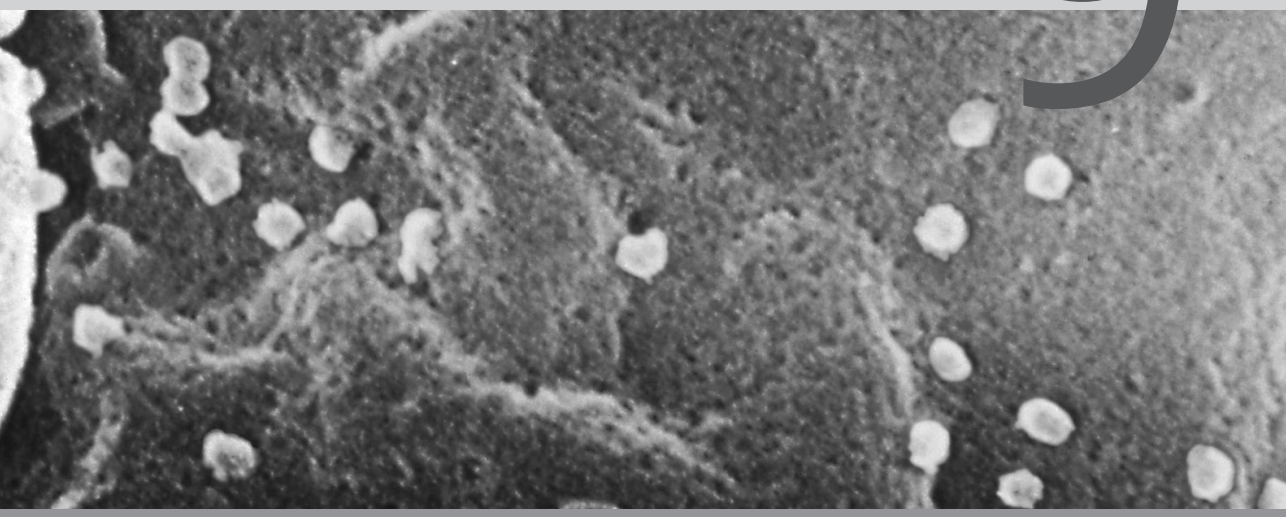


CHAPTER 9



Intensification of cART with maraviroc decreases T cell turnover in HIV-1 immunological non-responders

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Submitted

Abstract

The CCR5-antagonist maraviroc (MVC) has been implicated in optimising $CD4^+$ T cell recovery in HIV-1-infected patients with viral suppression but slow immune recovery. In a substudy of a double-blinded placebo controlled MVC intensification study in this type of patients, we performed *in vivo* labelling with deuterated water to determine the effect of MVC on T cell turnover. Comparison of the estimated life spans between placebo and MVC treated patients suggest that MVC intensification extends the life span of naive and memory $CD4^+$ and $CD8^+$ T cells.

Introduction

Combination antiretroviral therapy (cART) is aimed at suppression of HIV replication and disease progression. Successful cART with viral load reduction to < 50 copies/ml not only stops $CD4^+$ T cell depletion, but allows for $CD4^+$ T cell recovery to near normalized levels within 3 years. Unfortunately not all patients with a successful virological response have successful T cell immune reconstitution. Pre-therapy $CD4^+$ T cell numbers have been described to influence the increase in $CD4^+$ T cell numbers during cART [1] and patients that start cART with fewer than 350 $CD4^+$ T cells per µl blood frequently fail to increase their $CD4^+$T cell numbers during therapy, despite suppression of plasma HIV-RNA [2-4]. Other factors that are associated with a suboptimal immunologic response on cART are older age at start of cART, reduced thymic function, increased immune activation leading to an increase in lymphoid tissue fibrosis and T cell apoptosis, and human genetic polymorphisms [4-9]. Individuals that do not, or very slowly, increase $CD4^+$ T cell numbers despite undetectable plasma HIV-RNA are referred to as immunological non-responders. A percentage of 17.3% – 29% of all HIV infected individuals starting cART with less than 350 $CD4^+$ T cells has been reported to fall in this category [2, 3] .

One of the newest antiretroviral drug classes is the HIV entry inhibitors, which include the CCR5 antagonists. Currently, maraviroc (MVC, Celsentri ®) is the only registered CCR5-antagonist for the treatment of antiretroviral treatment-naive (USA only) and –experienced HIV-1 infected patients [10]. MVC selectively binds to the CCR5 coreceptor, thereby preventing HIV binding and inhibiting HIV-entry [11]. Next to its proven in vivo virological properties, intensification of cART with MVC has been implicated in $CD4^+$ T cell recovery. In a study by Cooper et al treatment with a MVC containing regimen led to a larger increase in $CD4^+$ T cell count, as compared to an efavirenz containing regimen [12]. Furthermore, a meta-regression analysis of clinical trials investigating CCR5-antagonists in antiretroviral treatment-experienced patients showed that the use of a CCR5-antagonist was associated with as significant increased $CD4^+$ T cell gain (+30 cells/µL [95% CI, 19-42]) [13]. These findings are in line with a study showing that the CCL3L1-CCR5 genotype influences the degree of $CD4^+$ T cell reconstitution during cART but could not be confirmed by more recent studies on the effect of MVC intensification [14, 15].

Several mechanisms have been proposed to explain the effect of MVC treatment on $CD4^+$ T cell gain. Redistribution of CCR5 expressing T cells could be involved, as was recently suggested in a study which showed that the addition of MVC to treatment of recipients of allogeneic hematopoietic stem-cell transplantation reduces the risk of visceral acute graft-versus-host disease, possibly by inhibition of lymphocyte trafficking [16]. But also reduction of the amount of immune activation, either by lowering residual viral load or by a direct effect via the CCR5 molecule expressed on T cells, could alter T cell turnover and recovery of peripheral T cell numbers.
We performed an in depth substudy within the MIRS [17], a double blinded study in immunological non responders, investigating the effect MVC intensification on T cell recovery. T cell turnover and death of naïve and memory $CD4^+$ and $CD8^+$ T cells were quantified using *in vivo* labeling with deuterated water and mathematical modeling in patients receiving placebo or MVC intensification. These studies showed a reduced T cell turnover in naive and memory $CD4^+$ and $CD8^+$ T cells after MVC intensification.

Materials and methods

Study population

Immunological non-responders were recruited from the 'Maraviroc Immune Recovery Study', a multicenter, randomized, placebo-controlled study investigating the effect of MVC intensification of cART on $CD4^+$ T cell count in patients with a suboptimal immunological response despite suppression of plasma HIV-RNA [17]. The labeling substudy was performed in the University Medical Center Utrecht only. All subjects provided written informed consent. This study was approved by the Ethical Committee of the University Medical Center Utrecht, The Netherlands (ClinicalTrials.gov identifier: NCT00875368; EudraCT number 2008-003635-20).
Inclusion criteria were: age 18 years and older; either a $CD4^+$ T cell count <350 cells/μL while at least two years on HAART, or $CD4^+$ T cell count <200 cells/μl while at least one year on HAART; and, viral suppression (plasma HIV-RNA < 50 copies/ml) for at least the previous 6 months. Exclusion criteria were: previous use of MVC; HIV-2 infection; HAART regimen containing a combination of tenofovir and didanosine;

active infection for which antimicrobial treatment; acute hepatitis B or C; chronic hepatitis B or C for which treatment with (peg)interferon and/or ribavirine; immunosuppressive medication; and, radiotherapy or chemotherapy in the previous 2 years.
Included patients were randomised to add MVC or placebo to their existing HAART regimen for 48 weeks. The MVC dose was 150-600 mg twice daily, depending on interactions with concurrent medication, as specified in the package insert. In case of virological failure (defined as two consecutive measurements of plasma HIV-1 RNA of 50 copies/mL or higher), participants had to discontinue the study medication.

Deuterium labeling protocol.
Study subjects drank a bolus of 7.5 ml deuterated water per kg body water (60% of body weight) at the first day of the protocol. During the labeling period of 9 weeks participants drank 1.25 ml deuterated water per kg body water daily. During the labeling period (5 times) and in the following 15 weeks after stop labeling (5 times), blood (50 ml) and urine samples were collected at the indicated time (*Figure 1*).

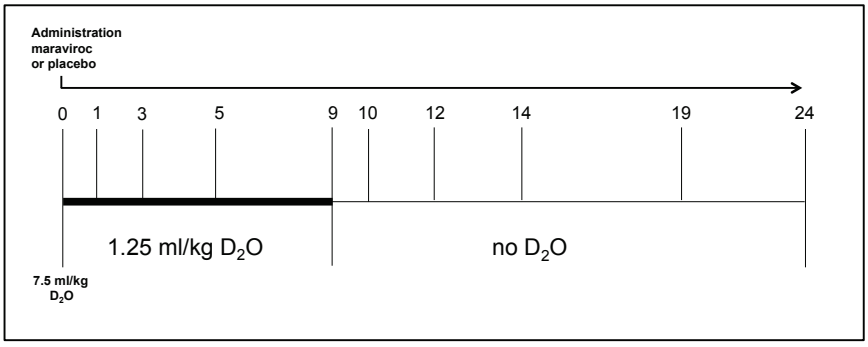

Figure 1: flow chart labeling protocol. The study-participants were subjected to the following study protocol. Administration of either maraviroc or placebo started at day 0 and continued throughout the entire protocol. Blood (50 ml) and a urine sample were collected at the indicated time points; day 0, week 1, week 3, week 5, week 9, week 10, week 12, week 14, week 19 and week 25. The indicated amounts of deuterated water were administered to the participants from day 0 – week 9 of the protocol.

Flow cytometry and cell sorting
PBMC were obtained by Ficoll-Paque density gradient centrifugation blood and either cryopreserved until further use or used directly. Absolute $CD4^+$ and $CD8^+$ T cell numbers were determined by dual-plat-

form flow cytometry, using TruCount tubes (BD Biosciences). Naive ($CD27^+CD45RO^-$), memory ($CD45RO^+$), $CD4^+$ T cells and $CD8^+$ T cells were assessed by flow cytometry. PBMC were incubated with monoclonal antibodies (mAb) to CD3 Pacific Blue, CD4 APC-cyy7 (eBioscience) and CD8 PE, CD45RO PE-Cy7 and CD27 APC (BD Biosciences). To measure T cell proliferation, PBMC were stained intracellularly with Ki67-FITC (DakoCytomation) after fixation and permeabilization with Cytofix/ Cytoperm and Perm/Wash according to the manufacturer's instructions (BD Biosciences). All experiments described above were analysed on a FACS Canto II of FACS LSR II (BD Biosciences) with FACS Diva software (BD Biosciences).
To measure the fraction of labeled cells within the naive ($CD27^+CD45RO^-$) and memory ($CD45RO^+$), $CD4^+$ and $CD8^+$ T cell population, PBMC were incubated with mAb to CD3 FITC, CD45RO-PE-Cy7 (BD Biosciences), CD27 APC, CD4 Pacific Blue (eBioscience) and CD8 V500 (Biolegend). The specified cell fractions were isolated by cell sorting on a FACSAria (BD PharMingen). Purity of the sorted cells was above 90% in the majority of the cases (84% of all samples analysed).

DNA isolation and measurement of deuterium enrichment
DNA isolation was performed using the QiaAMP DNA blood min kit (Qiagen) according to the manufacturer's instructions. Measurement of deuterium enrichment was performed according to Busch et al [18].

Mathematical modeling
Following Vrisekoop et al. [19], the availability of heavy water at any moment in time was calculated by fitting the deuterium enrichment in the urine of each individual to the following equations:

$$U(t) = f(1 - e^{-\delta t}) + \beta e^{-} \text{ during label intake } (t \le \tau) \qquad \text{(Equation 1a)}$$

and

$$U(t) = \left[f(1 - e^{-\delta \tau}) + \beta e^{-\delta \tau} \right] e^{-\delta (t - \tau)} \text{ after label intake } (t > \tau) \text{ (Equation 1b)}$$

where U(t) represents the fraction of 2H2O in urine at time t (in days), f is the fraction of 2H2O in the drinking water, labelling was stopped at $t = \tau$ days, δ represents the turnover rate of body water per day, and

β is the urine enrichment attained after the boost of label by the end of day 0. We incorporated these best fits when analyzing the enrichment in the different cell populations. Up- and down-labeling of the granulocyte population of each individual was analysed as described previously [19], to estimate the maximum level of label intake that cells could possibly attain. The label enrichment data of all cell subsets were subsequently scaled by the granulocyte asymptote of each individual.
Labeling data of the different T cell subsets were fitted with mathematical models that did or did not allow for kinetic heterogeneity between cells of the same population (Westera et al. submitted). Each kinetic sub-population i was modelled to contain a fraction αi of cells with turnover rate pi. Assuming a steady state for each kinetic subpopulation the fraction of labelled DNA of subpopulation i was modelled by the following differential equation:

$$\frac{dL_i}{dt} = p_i c U(t) - p_i L_i \qquad \text{(Equation 2)}$$

throughout the up- and down-labelling period, where Li represents the fraction of labelled DNA of subpopulation i, c is an amplification factor that needs to be introduced because the adenosine deoxyribose moiety contains seven hydrogen atoms that can be replaced by deuterium [19]. Basically, labelled adenosines in sub-population i are gained when a deuterium atom is incorporated with probability cU(t) in the DNA of cells that replicate at rate pi, and they are lost when cells of sub-population i are lost at rate pi. For naive T cells this replication may occur both in the periphery and in the thymus. The corresponding analytical solutions are:

$$L_i(t) = \frac{c}{\delta - p_i}\left[\delta f(1 - e^{-p_i t}) - p_i f(1 - e^{-\delta t}) + \beta p_i (e^{-p_i t} - e^{-\delta t})\right] \qquad \text{(Equation 3a)}$$

during label intake (t ≤ τ), and

$$L_i(t) = \frac{c}{\delta - p_i}\left[\delta f(e^{-p_i (t-\tau)} - e^{-p_i t}) - p_i f(e^{-\delta (t-\tau)} - e^{-\delta t}) + \beta p_i (e^{-p_i t} - e^{-\delta t})\right]$$

(Equation 3b)

after label intake (t > τ).

The fraction of labelled DNA in the total T cell population under investigation was subsequently derived from $L(t) = \sum \alpha_i L_i(t)$ and the averge turnover rate p was calculated from $L(t) = \sum \alpha_i L_i(t)$.

Statistical analyses

*V*ariables were compared using the Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$.

Results

Study population and baseline characteristics

Seven patients were enrolled in the labeling study, 4 in the MVC arm and 3 in the placebo arm. Comparison of the patient characteristics and baseline values (*Table 1*) showed no differences between the groups.

T cells in immunological non-responders show decreased turnover and lifespans upon treatment with MVC

To investigate whether the turnover of the MVC treated group differed from the placebo group, we pooled the enrichment data of each T cell subsets from study participants in the groups and performed a Wald

Table 1 Patient characteristics

	Placebo			Maraviroc			
Subject	1042	1044	2026	1043	2041	2042	2076
Age (years)	68	35	54	45	56	49	27
Sex	M	M	M	M	M	M	M
Duration cART (years)	9	2	5	3	12	2	2
nadir $CD4^+$ T cells count	4	1	153	47	86	33	56
$CD4^+$ T cell count at week 0	180	90	267	176	264	312	286
% naive $CD4^+$ T cells	5	48	26	41	47	28	45
% memory $CD4^+$ T cells	95	52	73	17	48	63	55
% naive $CD8^+$ T cells	5	61	35	72	22	36	38
% memory $CD8^+$ T cells	77	38	54	17	50	20	44
%$CD38^+$HLA-DR^+ $CD4^+$ T cells	18	2	3	6	3	4	4
%$CD38^+$HLA-DR^+ $CD8^+$ T cells	29	2	5	5	12	3	8
%$Ki67^+$ $CD4^+$ T cells	3	2	4	4	1	5	2
%$Ki67^+$ $CD8^+$ T cells	2	1	3	2	1	2	2
%CD31 in naive $CD4^+$ T cells	50	85	61	70	35	36	73

Demographic, clinical and immunological characteristics at baseline. Nadir $CD4^+$ T cell and week 0 $CD4^+$ T cell numbers are given as cells/μL, whereas T cell subsets, markers for T cell activation (CD38+HLA-DR^+), proliferation ($Ki67^+$) and production ($CD31^+$) are given as percentages.

test during the estimation by non-linear mixed effect models to estimate the turnover (*Figure 2* and *Table 2*).

The estimated average turnover in T cells in the MVC treated group was significantly lower in all cell subsets analysed. For naive T cells in the placebo treated group life span were 262 and 348 days, for $CD4^+$ and $CD8^+$ T cells respectively. In the MVC treated group, label incorporation was considerably lower and the estimated lifespan considerably longer. For naive $CD4^+$ T cells the estimated lifespan of 1184 days was 4 fold higher compared to the placebo group (p = 0.02).

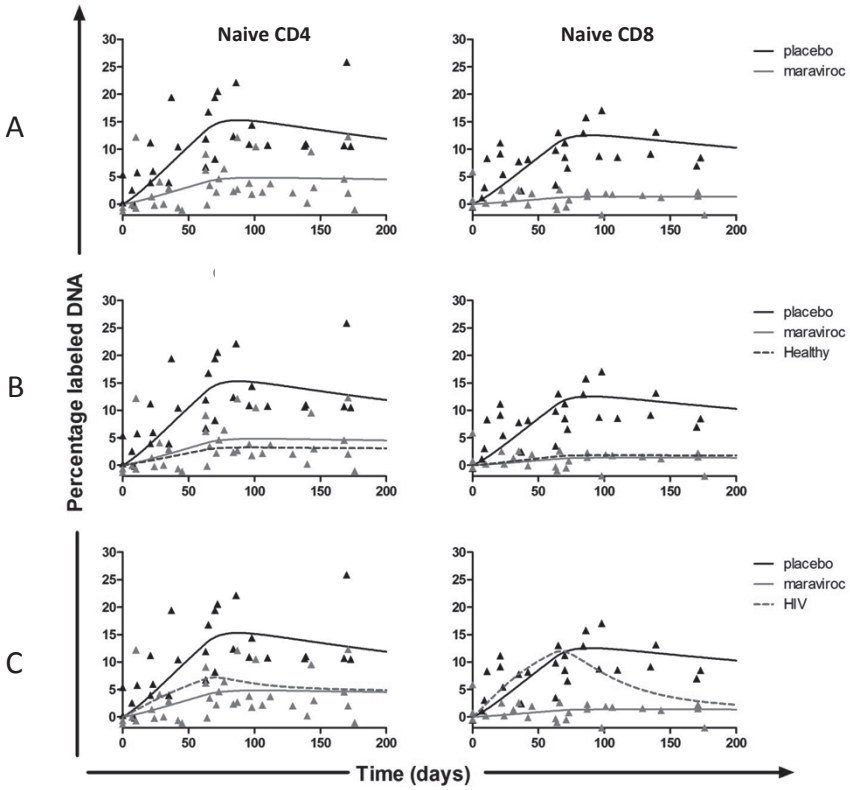

Figure 2: Average D2O labeling curves of T cell subsets in the MVC and the placebo arm. A. Estimated DNA enrichment curves of naive $CD4^+$ T-cells (left graphs) and naive $CD8^+$ (right graphs). T cells calculated with pooled data of the individuals of the MVC (n=4, ▲, grey line) and the placebo group (n=3, ▲, black line) B. Comparison with estimated enrichment curves of naive T cells of healthy individuals (dashed line). C. Comparison with estimated enrichment curves of naive T cells of untreated HIV infected individuals (dashed line).

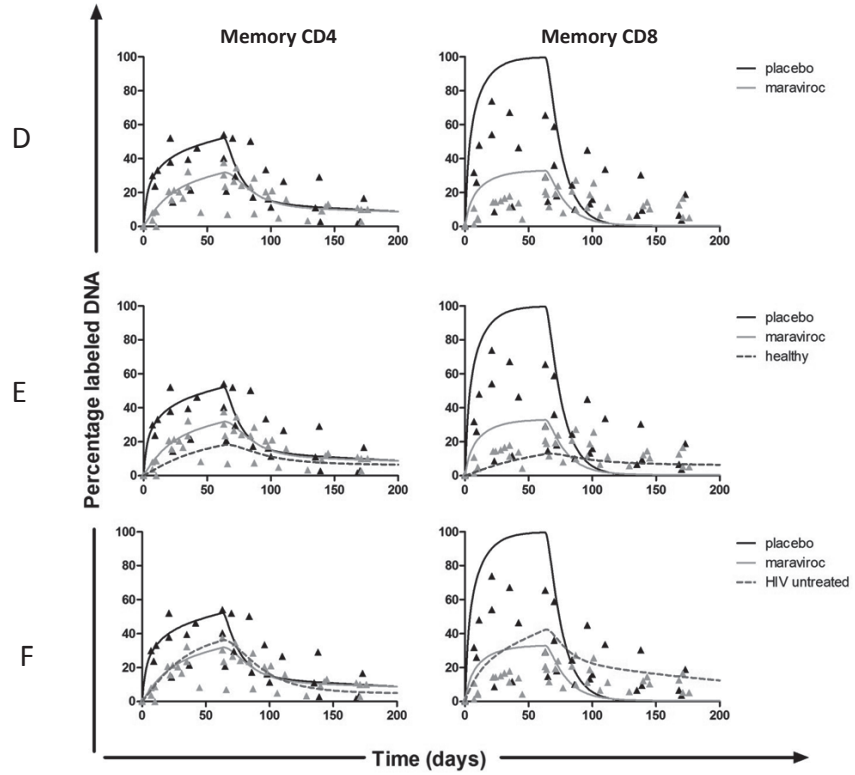

Figure 2 continued: D. Estimated DNA enrichment curves of memory $CD4^+$ T cells (left graphs) and memory $CD8^+$ (right graphs). T cells calculated with pooled data of the individuals in the MVC (n=4, ▲, black line) and the placebo group (n=3, ▲, grey line). E. Comparison with estimated enrichment curves of memory T cells of healthy individuals (dashed line). F. Comparison with estimated enrichment curves of memory T cells of untreated HIV-infected individuals (dashed line).

Table 2 Estimated life spans of $CD4^+$ and $CD8^+$, naive and memory T cells

	Naive $CD4^+$ T-cells	Naive $CD8^+$ T-cells	Memory $CD4^+$ T-cells	Memory $CD8^+$ T-cells
Placebo	262	348	15	3
Maraviroc	1148	2407	55	79
Healthy	2000	3333	164	119
Untreated	625	270	61	53

The estimated life span of $CD4^+$ and $CD8^+$, naive and memory T cells in MVC treated individuals, individuals in the placebo group, untreated HIV infected individuals and healthy individuals in days.

For naive $CD8^+$ T cells the estimated average life span of 2407 days was 7 times higher compared to the placebo group ($p = 0.01$; *Figure 2A*). When we compared these results with data from healthy individuals [19], we observed a remarkable resemblance between individuals that were treated with MVC and healthy individuals (*Figure 2B*). The labeling curves of naive T cells in MVC treated individuals and healthy individuals were nearly identical. Furthermore, we compared the results of the present study to data available from untreated HIV infected individuals (Vriesekoop, submitted) and, observed a similarity in the up-labeling curve of naive $CD8^+$ T cells of untreated HIV infected individuals and our placebo group (*Figure 2C*). The loss of label during the downlabeling phase in naive $CD8^+$ T cells, however, was much faster in untreated HIV infected individuals, as compared to the placebo group.

Label incorporation in memory $CD4^+$ and $CD8^+$ T cells was substantially similarly lower in the MVC treated group. For memory T cells in the placebo treated group life spans were 15 and 3 days, for $CD4^+$ and $CD8^+$ T cells, respectively. In the MVC treated group, label incorporation was considerably lower and the estimated lifespan considerably longer. For memory $CD4^+$ T cells the estimated average lifespan of 55 days was 4 fold higher compared to the placebo group ($p < 0.0001$). For memory $CD8^+$ T cells the estimated average life span of 79 days was 26 times higher compared to the placebo group ($p < 0.0001$; *Figure 2D*). The down-labeling curve of memory T cells in the placebo group was much steeper, meaning that more labeled cells were lost (*Figure 2D*). Labeled memory $CD4^+$ and CD8 T cells were mainly lost during the first 30-40 days of the down-labeling phase in both the placebo and the MVC group. After this period, a population of labeled CD4 + T cells remained that was still present at day 200, the last day of the protocol (*Figure 2D*). In the memory $CD8^+$ T cell pool all labeled memory $CD8^+$ T cells had disappeared at day approximately 125 of the protocol (day 55 of the down-labeling phase, *Figure 2D*). When these data were compared to data from healthy and untreated HIV infected individuals, we observed that memory T cell labeling curves of the placebo and MVC group were lower than the curves of the healthy individuals (*Figure 2E, F*) and the MCV treated group had curves comparable to untreated HIV infected individuals. The down-labeling curve of memory $CD4^+$ T cells in the MVC group was also very similar to that of untreated HIV infected individuals. In memory $CD8^+$ T cells label was lost faster in both the

MVC and placebo group, compared to the HIV untreated group (*Figure 2*F).

D2O label incorporation is diminished in nearly all MVC treated individual patients

We next examined deuterium labeling curves of T cells of individual patients within one group and estimated the individual average lifespans of the different T cell populations (*Figure 3 and 4*). Naive $CD4^+$ and $CD8^+$ T cells T cells of the four individuals of the MVC group all incorporated less label than individuals of the placebo group and their estimated lifespans were lower. In fact label enrichment in naive CD8 T cells of one of the individuals of the MVC treated group was so low that no lifespan could be estimated. Despite the low number of individuals in the groups the lifespan of the placebo and MVC treated groups tended to be different ($p=0.057$ and 0.058 for naive $CD4^+$ and $CD8^+$ T cells respectively). For memory cells the combined analysis showed that label incorporation in both memory $CD4^+$ and $CD8^+$ T cells is lower in the MVC group, as compared to the placebo group (figure 2D and 2E). When analyzing individual participants we observe a comparable pattern, except for participant 1043 of the placebo group, who falls within the range of individuals in the MVC group.

Ki67 levels do not differ between the MVC and the placebo group

To identify determinants of T cell turnover we measures markers of cell division (Ki67), T cell activation ($CD38^+$, HLA-DR+) and microbial translocation (soluble CD14) within the placebo and MVC treated group and performed linear regression analysis. We found no association between the turnover of the specific subsets with Ki67 expression within the subsets (*Figure 5*), activation of total $CD4^+$ or $CD8^+$ T cells or sCD14 levels (data not shown). Analysis of T cells numbers in these restricted patients groups also showed no association, however when all groups were compared (untreated HIV infected, and healthy) a weak association between naive $CD4^+$ but not $CD8^+$ T cells was found.

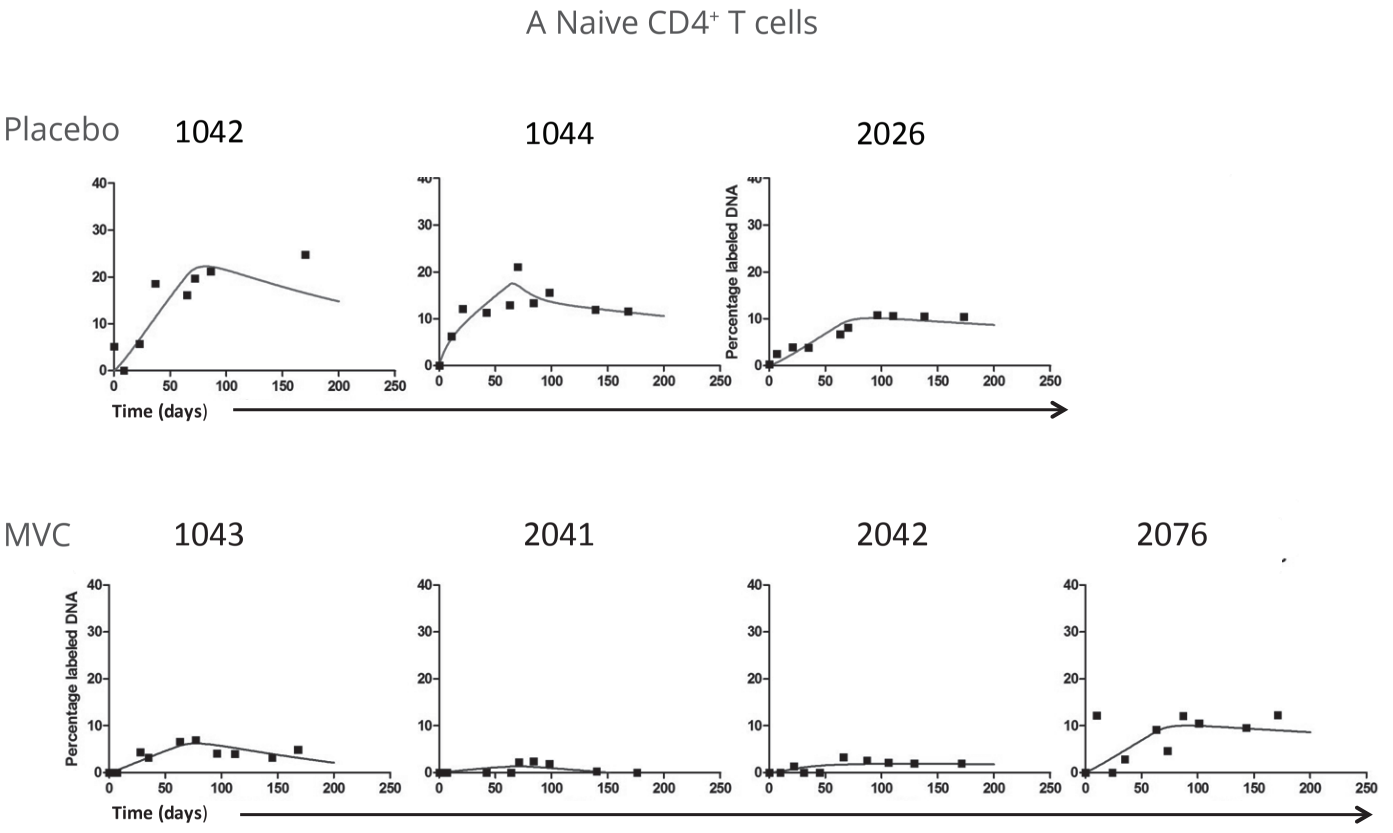

Figure 3: Individual D2O labeling curves of T cell subsets in individuals in the MVC and the placebo group.
A. DNA enrichment curves of naive CD4+ T cells of individual patients in the MVC and the placebo group. The upper graphs represent individuals in the placebo group and the lower graphs represent individuals in the MVC group.

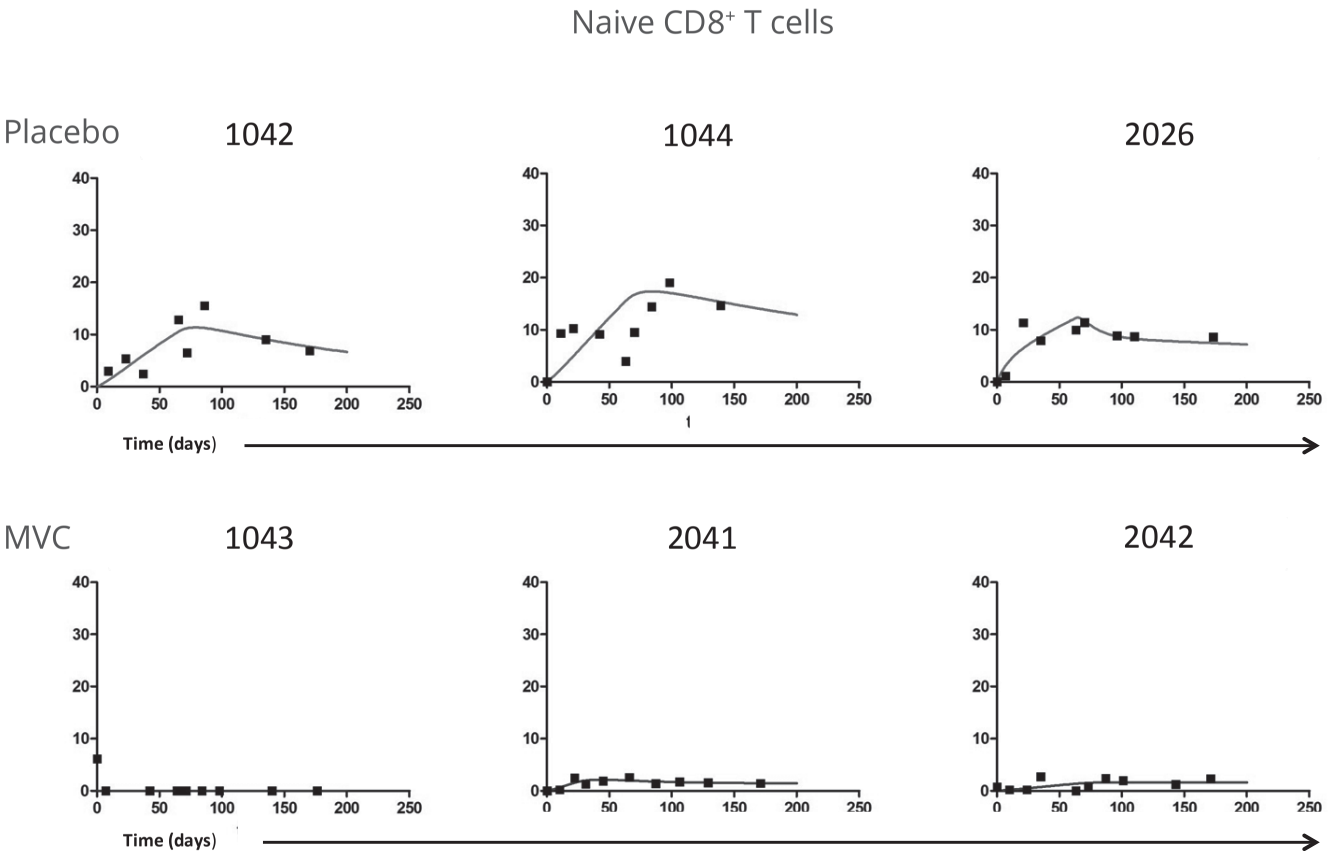

Figure 3B. DNA enrichment curves of naive CD8⁺ T cells of individual patients in the MVC and the placebo group. The upper graphs represent individuals in the placebo group and the lower graphs represent individuals in the MVC group.

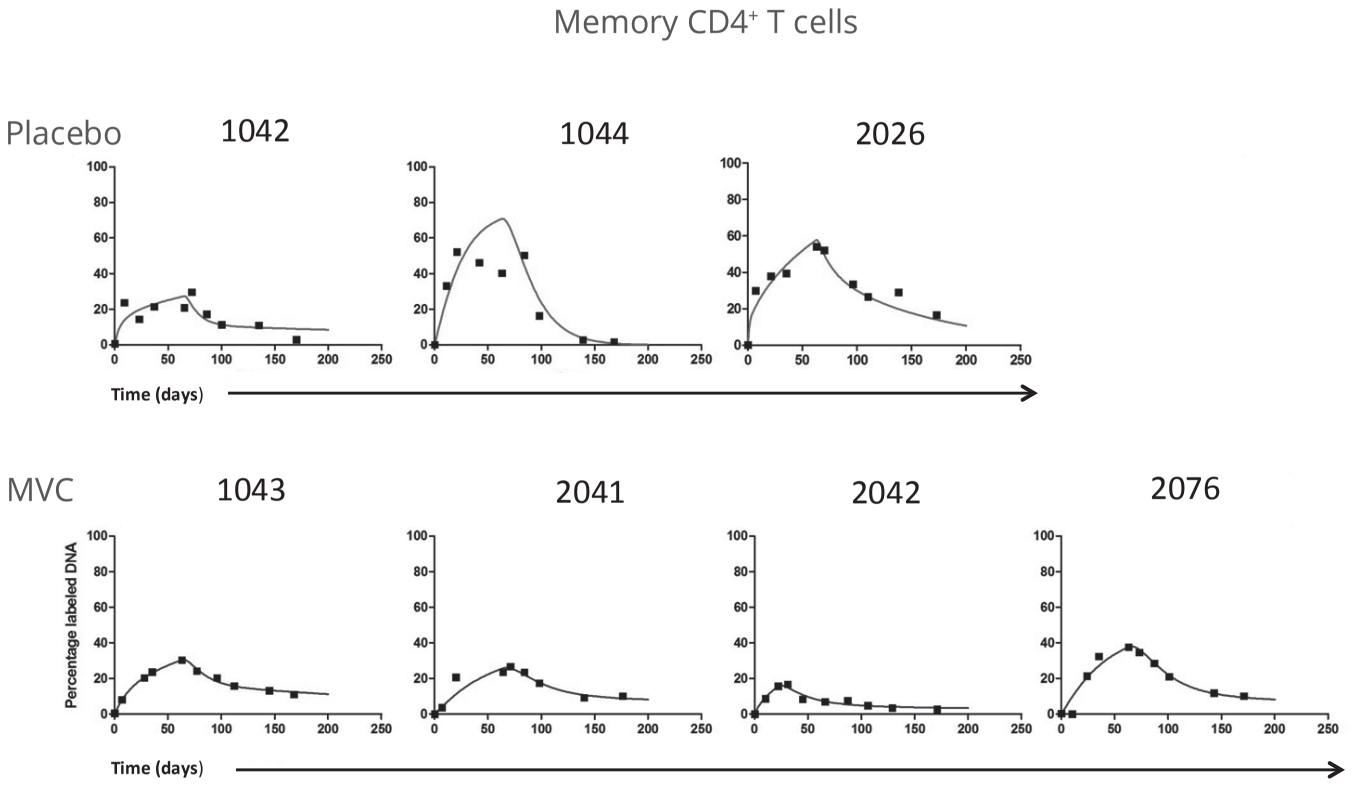

Figure 3C. DNA enrichment of memory CD4+ T cells of individual patients in the MVC and the placebo group. The upper graphs represent individuals in the placebo group and the lower graphs represent individuals in the MVC group.

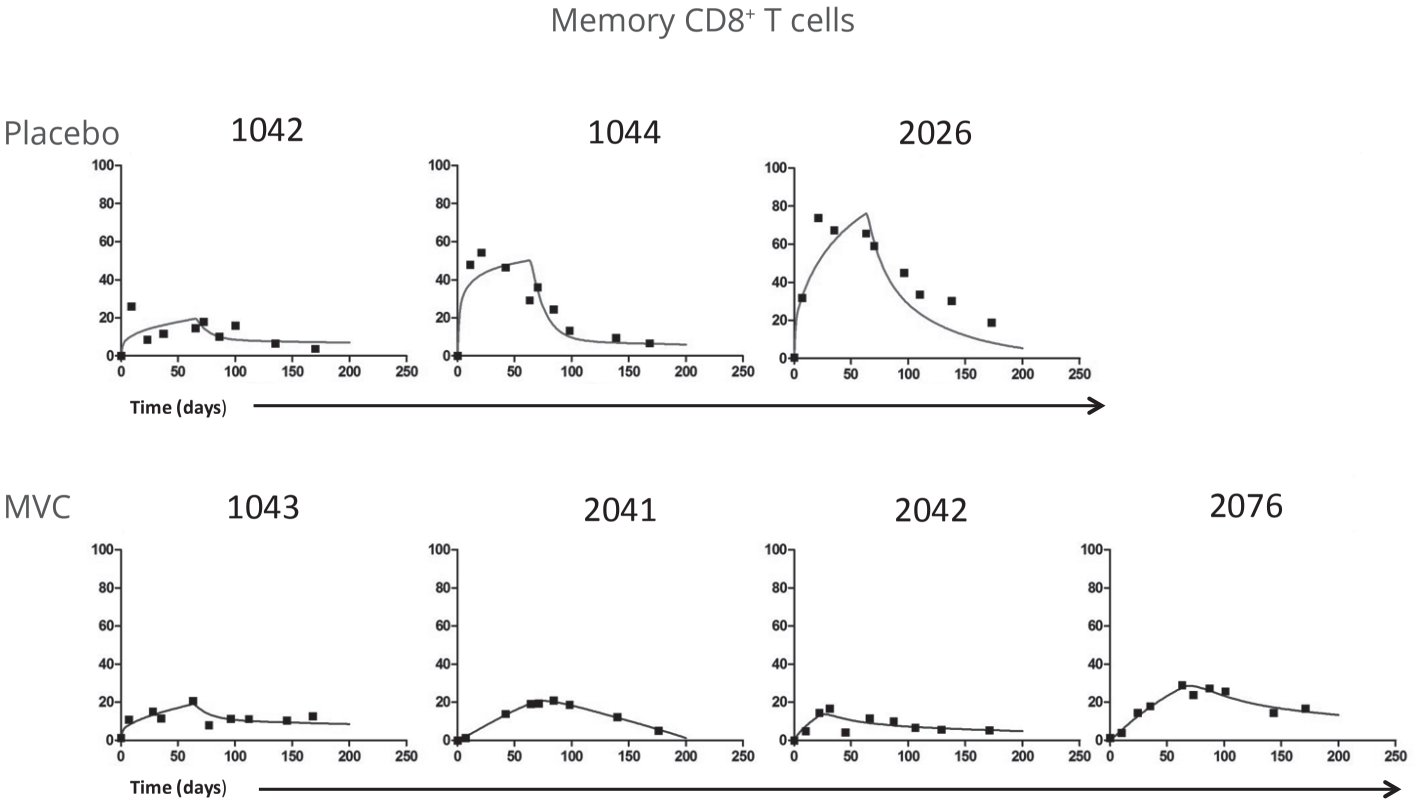

Figure 3D. DNA enrichment of memory CD8+ T cells of individual patients in the MVC and the placebo group. The upper graphs represent individuals in the placebo group and the lower graphs represent individuals in the MVC group.

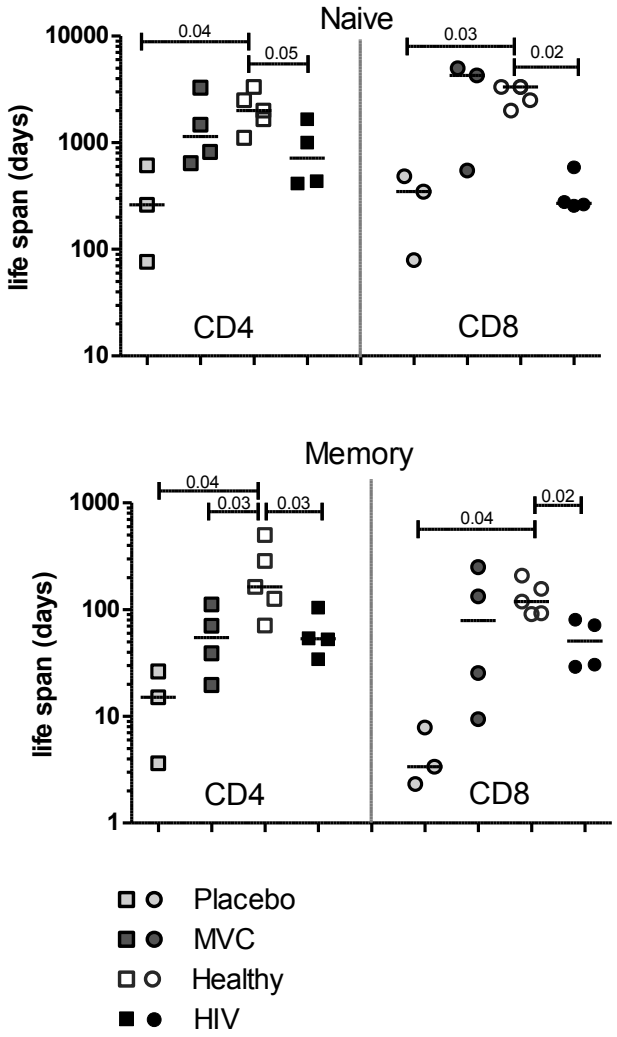

Figure 4: Average estimated life span of naive and memory T cells in the MVC group and the placebo group compared to healthy and untreated HIV infected individuals.
A. Estimated life span (days) of naive $CD4^+$ T cells (circles) and memory $CD8^+$ T cells (squares) of individuals in the placebo group (●, ■) and MVC (●, ■) compared to reference groups(o, □ healthy individuals, ●, ■ HIV-1 infected individuals). B. Estimated life span (days) of memory $CD4^+$ T cells (circles) and memory $CD8^+$ T cells (squares) of individuals in the placebo group (●, ■) and MVC (●, ■) compared to reference groups(o, □ healthy individuals, ●, ■ HIV-1 infected individuals). Significant p-values between the groups are given.

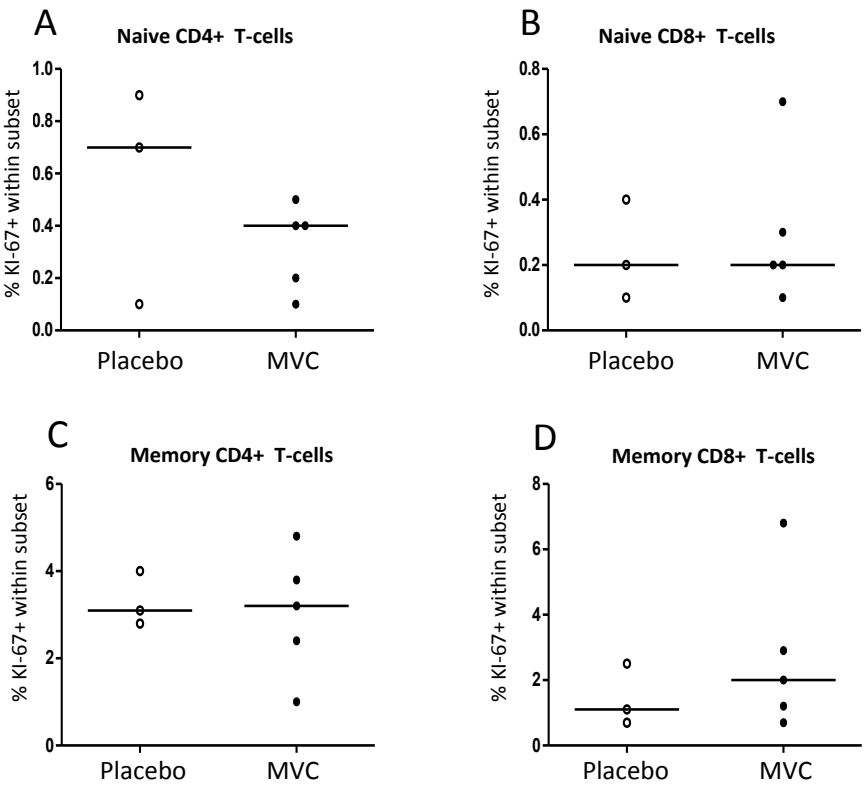

Figure 5: Ki67 expression in T cell subsets in MVC and placebo group.
Open dot represent individuals in the MVC group, filled dots represent individuals in the placebo group. A. Relative expression of Ki67 within naive $CD4^+$ T cells. B. Relative expression of Ki67 within naive $CD8^+$ T cells. C. Relative expression of Ki67 within memory $CD4^+$ T cells. D. Relative expression of Ki67 within memory $CD8^+$ T cells.

Discussion

We studied the effects of MVC intensification of cART in immunological non-responders on T cell kinetics. By using deuterium labeling we were able to show an increased T cell life span in MVC treated individuals, as compared to placebo treated individuals. These data suggest that MVC treatment alters T cell production and death in treated HIV infected immunological non responder patients.

Diminished T cell apoptosis, due to blockade of CCR5 signaling, may explain the increased life span that we observed in the MVC treated group. A role of CCR5 in the induction of apoptosis has previously been described [20-24]. CCR5 ligands were shown to induce cell death in certain CCR5 expressing cell types [21] and CCL5 aggregates, which form at high ligand concentrations, have been described to induce apoptosis in T cell lines as well as in primary human T cells in a CCR5-dependent manner [23]. These observations are supported by the notion that susceptibility to activation-induced cell death (AICD) and Fas-mediated apoptosis was selectively increased in $CD4^+CCR5^+$T cells compared with $CD4^+CCR5^-$ and $CD4^+CXCR3^{-/+}$ T cells in humans [22]. In subjects with acute primary HIV infection, high levels of apoptosis were observed in T cells with increased expression levels of CCR5 [24] and interaction of CCR5 and R5 env was described to activate the Fas pathway and caspase-8 as well as triggering FasL production in HIV infected primary human $CD4^+$ T cells [20]. If CCR5 indeed is important in the induction of apoptosis, the apoptosis levels might decrease in cells in which CCR5 activation is blocked by MVC, which would in turn result in a longer life span. In line with this suggestion apoptosis levels as measured by annexin-V expression were lower in the MVC arm compared to the placebo arm in our MVC intensification study. CCR5 has been described to be expressed on both naïve and memory $CD4^+$ and $CD8^+$ T cells [25], albeit higher on memory T cells compared to naïve T cells. Therefore, if decreased apoptosis would play a role in increasing the life span of T cells in MVC treated individuals, this could indeed be observed in all T cell subsets investigated.

Apart from its proposed role in T cell apoptosis, CCR5 has also been described to be involved in the activation of T cells. In humans, the CCR5 ligand CCL5 is capable of activating T cell clones and inducing IFN gamma production. Also, ligation of CCR5 with CCL3, CCL4 and CCL5 has been described to increase IL-2 production and cell proliferation [26]. If binding of MVC to CCR5 indeed does prevent this ligation to take place, this would result in a decreased T cell turnover in the MVC group. In the present study we were not able to detect decreased proliferation in the MVC treated group by means of Ki67 staining (*Figure 5*). However, due to the limited number of participants and the low level of Ki67 expression (*Table 1*) this effect might be too subtle to measure.

CCR5 has been described to be recruited to the immunological synapse during the interaction of T cells and antigen presenting cells and to induce a second activation signal, next to TCR triggering, in T cells in vitro [26-28]. Furthermore, CCR5 density on $HLA^{-}DR^{+}$ $CD4^{+}$ T cells has been reported to be positively correlated with the percentage of CD38 expressing $CD8^{+}$ T cells and HLA-DR expressing $CD4^{+}$ T cells in HIV infected individuals, independent of HIV viral load[25]. However, conflicting results have been published regarding the effects of MVC intensification on T cell activation [14, 15, 28-30].

MVC intensification has also been suggested to influence T cell dynamics by lowering residual viral load and concomitantly immune activation. In our patients plasma HIV-RNA was suppressed (< 50 copies/ml) and no viral blips or reactivations were observed. An effect below the detection limit of 50 copies HIV-RNA /ml plasma however cannot be excluded, but is less likely since two recent studies could not find a decrease of plasma HIV-RNA below 50 copies/ml after MVC intensification [14, 28].

Despite our observed changes in T cell lifespans in naive and memory $CD4^{+}$ and $CD8^{+}$ T cells our MVC intensification study only showed differences in CD8 memory counts and a reduction in $CD8^{+}$ T cell activation [17]. Positive effects on MVC intensification on $CD4^{+}$ T cell gain and immune have been observed, but effects are quite variable. A recently published single-arm pilot trial where MVC was added to cART of 34 patients for 24 weeks reported only a slight increase of 12 CD4 cells/µl [30]. However, markers of immune activation and apoptosis decreased during MVC intensification and reversed after discontinuation of MVC. A recent French single arm MVC intensification trial (57 patients, 24 weeks) found a slight significant increase (+22.6 cells/µl/y) in the $CD4^{+}$ T cell slope in the on-treatment analysis (47 patients), whereas a trend was found in the intention-to-treat analysis [29]. Although the preliminary results of an Italian randomised (open label) trial (96 patients, 48 weeks) did not show a significant difference in $CD4^{+}$ T cell increase between the arms, a significant increase in $CD8^{+}$ T cell count in the MVC arm was reported. In this study T cell activation decreased in both arms [15].

The question remains why, despite decreased turnover and an increased life span, T cell numbers do not increase in the MVC group. An altered balance between T cell activation and apoptosis may alter

dynamics but leave numbers unchanged. A difference in T cell distribution between tissue, blood and lymphoid organs may also play a role. In this regard, a recent study showed that the addition of MVC to treatment of recipients of allogeneic hematopoietic stem-cell transplantation reduced the risk of visceral acute graft-versus-host disease, possibly by inhibition of lymphocyte trafficking [16]. We tested whether duration of cART, CD4 count at start cART or age differed between the groups or correlated with the observed life span of the different T cell subsets, but found no indications that these factors might explain the differences between the placebo and MVC groups.
Strengths of this study are the randomised, placebo controlled design and the new approach to study the effects of MVC intensification on T cell kinetics. A limitation is the relatively small sample size, mainly due to the labor intensive nature of this study. In addition, the follow up of 24 weeks in the current study might be too short to fully appreciate the effects of MVC intensification on T cell kinetics. More studies using the deuterated water labeling technique are necessary to confirm our findings.

In conclusion, we hypothesize that the lower turnover and increased T cell lifespan in MVC treated individuals is a direct result of blocking CCR5, resulting in diminished T cell proliferation and apoptosis rates. Based on our findings, MVC intensification could therefore be beneficial in the long run, however further studies are necessary to confirm our results and to investigate the clinical benefits.

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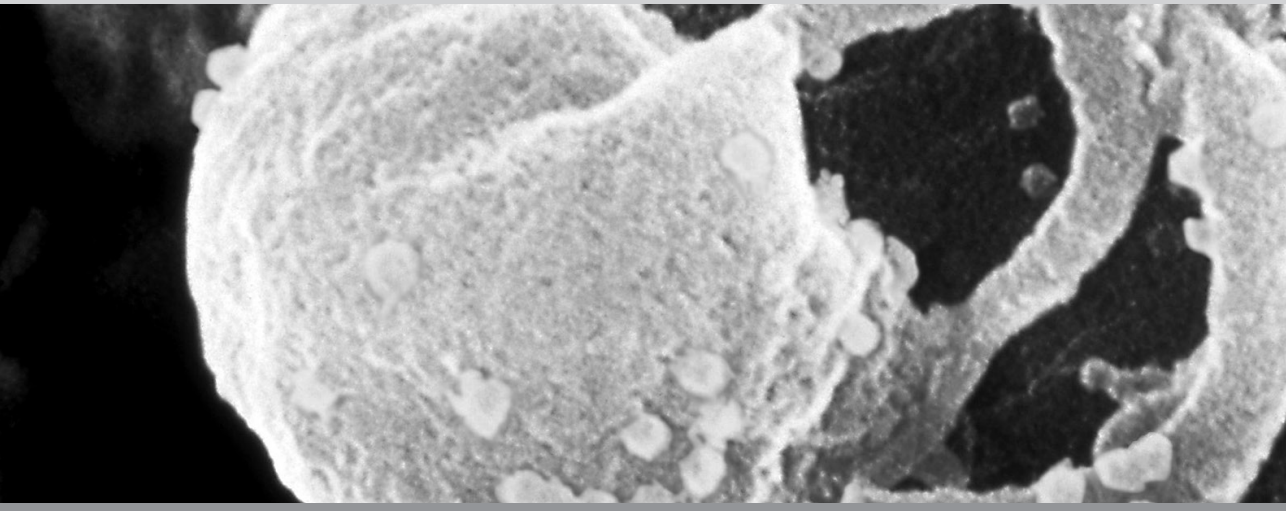
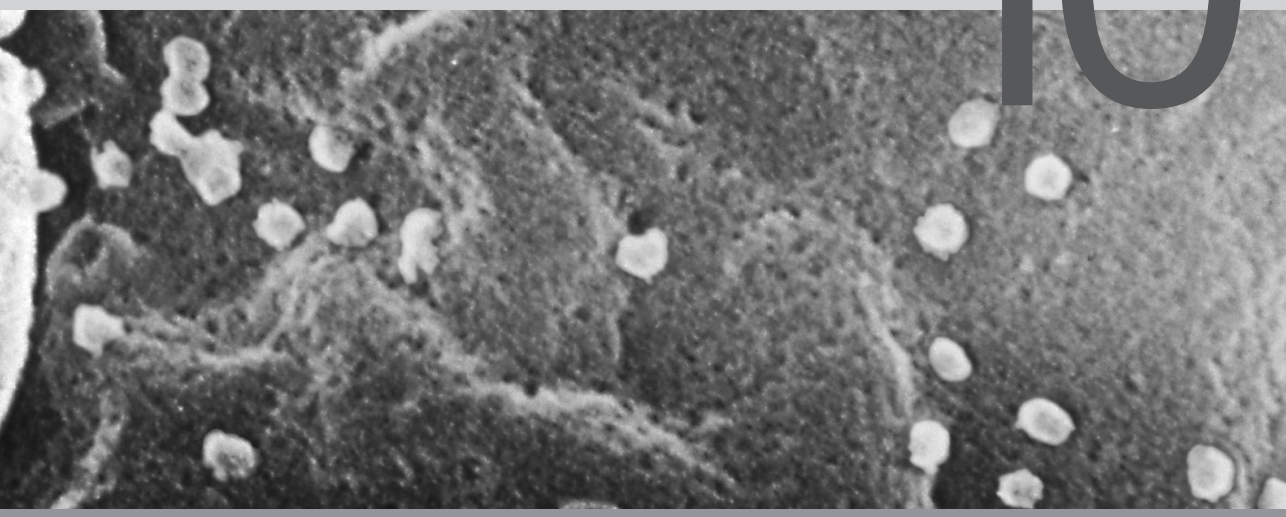


CHAPTER 10



Summary, general discussion and future perspectives

Summary and general discussion

The introduction of combination antiretroviral therapy (cART) has led to a spectacular decrease in morbidity and mortality of HIV-infected patients in the Western world [1], resulting in a life expectancy that seems comparable to those having other chronic medical diseases (e.g. diabetes mellitus) [2] and in some cohorts even to the general population [3, 4]. In general, however, life expectancy of HIV-infected patients is still shorter as compared to the general population [5-7]. Limited data is available on the clinical outcome of serious illnesses in HIV-infected patients in current times. We therefore performed an analysis of the long term outcome of HIV-infected patients admitted to the intensive care unit (ICU) in **chapter 3.** Previously, it had been reported that since the beginning of the cART era the spectrum of illnesses of HIV-infected patients admitted to the ICU has changed and non-AIDS related ICU admissions are seen more often [8, 9]. Although the grim mortality rates of the early days of the HIV pandemic have improved and short term mortality rates in the range of 8.6-32% have been reported, data on long term mortality of HIV-infected patients admitted to the ICU are scarce and originate from the early years of the cART era [9, 10]). In our cohort of ICU admitted HIV-infected patients, we found improvement in both one-year (71% in pre-cART era to 53% in the cART era, p = 0.05) and five-year mortality rates (87% in the pre-cART era to 59% in the early-cART period, p = 0.005). Moreover, five-year survival after hospitalization was 80%, which is in range of reported survival of non-HIV infected patients (83.7%) [11], underlining the fact that HIV-infection is not a reason to withhold intensive care treatment.

Despite the described improvement of morbidity and mortality, evidence is accumulating that in patients with HIV-infection even during viral suppression by cART, the process of aging is occurring much faster as compared to the general population, resulting in a higher incidence of malignancies and cardiovascular diseases (CVD) [12-14]. Although combination antiretroviral therapy (cART) does reduce the risk for CVD, it remains still higher as compared to HIV-negative controls [15]. The mechanism for this increased risk of CVD is probably multifactorial, and includes traditional cardiovascular risk factors and antiretroviral drug related toxicities. It is further hypothesized that chronic inflammation (which is a well accepted CVD risk factor in the general popu-

lation), induced by HIV related (residual) immune activation, may account for early atherosclerosis in these patients [16, 17].
Not only levels of inflammation, but also T cell activation is markedly elevated in patients with untreated HIV-infection and only partially reversed by cART [18]. Moreover, T cell activation is increased in patients with a suboptimal immunological outcome despite viral suppression by cART [18-21]. The role of activated T cells and their inflammatory products in the pathogenesis of atherosclerosis has been well studied in the general population [22], and data of studies confirming the relation between systemic T cell activation in HIV-infected patients and vascular changes have been published [23-25]
In light of these findings it is therefore not surprising that several studies report a relationship between low $CD4^+$ T (CD4) cell counts and occurrence of cardiovascular events; Triant et al. reported low CD4 cell count as an independent predictor for myocardial infarction [26], whereas data of the HIV Outpatient Study (HOPS) showed a relationship between latest CD4 cell count and cardiovascular disease [27]. Generally, treatment of HIV-infection with cART suppresses viral replication, leading to recovery of CD4 cells. Unfortunately, in 9-29% of patients treatment with cART fails to achieve an adequate CD4 cell count despite virological suppression [28-33], known as 'immunological non response'. In this particular group of patients, reduced thymic function, increased immune activation leading to lymphoid tissue fibrosis and T-cell apoptosis, and human genetic polymorphisms are other factors reported to be involved in the mechanism of a suboptimal immunological response [19, 34-37].
Since the amount of published data on long term clinical complications of a suboptimal immunological response on cART is limited, we set out to analyse these factors in a large retrospective cohort study which is described in **chapter 4**. In this study, we found a suboptimal immunological response in 27.5% of patients on virological successful cART. We found that an adequate immunological response (>500 CD4 cells/μL) was associated with a lower risk for a composite endpoint of death, AIDS, malignancies, liver cirrhosis and cardiovascular events (HR 0.54 (95% CI 0.33-0.87); p = 0.01) and a trend for lower risk for cardiovascular events in particular (HR 0.52 (95% CI 0.22-1.20); p = 0.12). In addition, age was a strong independent predictor for clinical complications.

These findings clearly stress the importance of achieving an adequate restoration of the immune system, to minimize the risk of serious diseases such as cardiovascular events. Our data further underline that starting cART at higher CD4 cell counts, especially in older aged patients, is warranted.

During the past years interest in the neurocognitive complications of HIV-infection and the possible relation to central nervous system (CNS) penetration of antiretroviral drugs has increased enormously [38-49]. CCR5 inhibitors are a promising option for patients with viral replication in the CNS, since HIV population in the CSF has generally been found to be R5-tropic [50, 51]. In **chapter 5** we discuss the potential complications of treatment of HIV-infection with cART with low level penetration in the CNS. During apparently successful maraviroc (MVC) and enfuvirtide containing salvage therapy with adequate suppression of plasma viremia we observed viral replication in the CNS of a heavily pre-treated patient. The fusion inhibitor enfuvirtide is known not to penetrate in the CNS sufficiently, but for MVC good CSF drug levels have been reported. Unfortunately, we could only detect very low levels of both entry inhibitors in the CSF of our patient, resulting in the detection of enfuvirtide resistant HIV in the CSF. Subsequently, we convincingly demonstrated leakage of this resistant virus to the plasma resulting in an unfortunate loss of overall viral suppression in this compartment as well. This case study is the first to show that selection of drug resistant HIV in the CNS may affect general therapy success and underlines the potential danger of low level penetration of antiretroviral drugs into the central nervous system.

In light of the complications as discussed above, and in combination with the observation that a large proportion of new diagnosed HIV-infected patients in Western-Europe still present with advanced disease (CD4 cell count of <200 cells/μL) [52, 53], life expectancy of HIV-infected patients is in general still shorter compared to the general population [5]. Therefore, a need for more optimal treatment strategies exists. Side effects of current cART and selection and transmission of drug resistance further enhance to the need for the development of antiretroviral compounds with new mechanisms of action [54-57].

Maraviroc (MVC [Celsentri®]) is the first and only drug of the new class of CCR5 antagonists registered for treatment for HIV-1-infected antiretroviral therapy experienced patients. Registration was based on

the 'Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients (MOTIVATE) 1 and 2 studies', which compared the efficacy and tolerability of MVC in combination with optimized background therapy to placebo. In **chapter 2** we review these MOTIVATE studies as well as other data regarding MVC containing antiretroviral therapy, and demonstrate that a MVC containing regimen is effective in antiretroviral experienced patients infected with R5-tropic HIV-1 infection; moreover, side effects are mild [58-60]. Since MVC is registered for treatment of patients infected with R5-tropic HIV-1, viral coreceptor tropism has to be assessed before start of treatment with MVC [61]. 'Coreceptor tropism' refers to the ability of HIV to enter $CD4^+$ cells using the CCR5 coreceptor ('R5-tropic virus'), the CXCR4 coreceptor ('X4-tropic virus'), or both coreceptors ('dual-tropic virus') [62]. Some patients are infected with a population of viruses with different coreceptor tropism ('mixed-tropic virus'). For assessment of HIV coreceptor tropism, various phenotypic and genotypic assays are available. At present, the European guidelines for HIV-1 tropism recommend using population-based genotypic tropism assays, mostly because of practical and costs issues. These guidelines advise to use a cut-off FPR of 10% in most situations for both viral RNA and pro-viral DNA. This may be a rather conservative approach since the optimal cut-off (i.e. FPR) for R5-classification is not yet fully explored.

Our experiences and the clinical outcome of patients treated with a MVC containing regimen in the UMC Utrecht are discussed in **chapter 6**. Despite the fact that these patients were extensively pre-treated with cART, the majority had experienced previous antiretroviral therapy failure, and had long-standing HIV infection with various comorbidity, MVC containing regimens were tolerated well and resulted in a good immunological and virological response (median increase of 249 (IQR 164-412.5) CD4 cells after 24 months, whereas 85.0% of patients had suppressed plasma HIV-RNA below 50 copies/ml). In addition, we were able to compare three different HIV coreceptor tropism assays: the phenotypic Trofile ('original Trofile assay' [OTA] and 'enhanced sensitivity Trofile assay' [ESTA]), the MT-2 assay, and a population-based genotypic tropism assay. Comparison of these three assays resulted in a good concordance, ranging from 85.7 to 94.1%. These data confirm that the genotypic tropism test is a solid alternative for predicting HIV coreceptor tropism in

clinical practice, as compared to the Trofile assay, which is performed in San Francisco only and not readily available anymore in Europe.
As mentioned above, MVC is currently registered for patients infected with a R5-trope virus population. However, it seems that HIV coreceptor tropism and MVC therapy is not just a matter of black and white. For example, a small fraction X4-tropic virus in the total virus population of an HIV-1-infected patient can result into a D/M or X4 tropism classification, especially in case of the ESTA but also when using a genotypic tropism assay. It is currently unknown whether a small fraction X4-tropic virus (and what size of the fraction) has clinical relevance, especially since patients are generally treated with a combination of MVC and other active antiretroviral drugs. Moreover, in **chapter 7** we report the results of a case study of a patient that was treated with a MVC containing regimen, despite the fact that he harboured a dual/mixed-tropic virus population, as classified by ESTA. We found that MVC was able to inhibit these dual-tropic viruses. These preliminary data point out that although viral variants are classified as dual-tropic by a phenotypic tropism assay, they may preferably use the CCR5 coreceptor in vivo and can be inhibited by MVC. This suggests that MVC therapy in these patients might still be of benefit if combined with adequate backbone therapy; however more studies are necessary to confirm these initial results.
The chemokine (C-C motif) receptor 5 (CCR5 coreceptor) is a G-protein-coupled transmembrane receptor that is able to bind CC-chemokines, such as CCL3 (MIP-1α), CCL4 (MIP-1β) en CCL5 (RANTES). CCR5 can be found on many different types of cells, such as $CD4^+$ T cells, dendritic cells, monocytes and macrophages [63]. Besides the fact that this receptor, next to the chemokine (C-X-C motif) receptor 4 (CXCR4), has been recognized as a coreceptor for HIV-entry[64, 65], it has also been shown to affect T cells. Binding chemokines to CCR5 is a co-stimulatory step leading to T cell activation [66, 67]. Moreover, the CCR5 coreceptor and its ligands are involved in lymphocyte trafficking, as has recently been showed in HIV-negative allogeneic hematopoietic stem-cell transplantation recipients [68].
Several studies show a poorer long term clinical outcome in terms of death, AIDS and non-AIDS defining diseases, in patients with a suboptimal immunological response to cART [28, 29, 31, 69]. Since suppres-

sion of HIV-1 replication by cART is the only currently available therapy for increasing CD4 cell counts, there is a need for immunomodulating therapies for this particular group of patients.
We hypothesized that mariviroc intensification of cART in patients with a suboptimal immunological response might lead to an increase in CD4 cell counts, via direct manipulation of T cells. In addition, suppression of residual low level viral replication might further decrease immune activation, potentially resulting in an increase in CD4 cell count. However, intensification studies with other antiretroviral drugs (mostly the integrase inhibitor raltegravir) have reported conflicting results [70-72].
In **chapter 8**, we discuss the results of the 'Maraviroc Immune Recovery Study', which investigated the effects of MVC intensification in patients with a suboptimal immunological recovery on cART despite successful viral suppression. In this randomized, placebo-controlled trial, we did not find a significant change in CD4 cell count in the MVC arm as compared to placebo. However, slight effects on memory $CD8^+$ T cell numbers and $CD8^+$ T cell activation and apoptosis markers where found, prompting the need for more research to elucidate the underlying mechanism and the possible clinical implications. In general our findings are in agreement with other MVC intensification studies, which report no or minor changes in CD4 cell count and markers for T cell activation as well [73-77].
Concluding, the data of this study do not support MVC intensification of cART as a general approach in patients with a suboptimal immunological response in order to restore CD4 cell count. However, since the fact that in this and other studies some changes in T cell activation and apoptosis are found combined with the clinical perception that some patients did indeed show a good CD4 cell count increase on MVC intensification therapy, further research is needed to investigate whether specific subgroups of patients (and their pre-intensification characteristics) can be identified who might benefit of MVC intensification of cART.
A novel way to investigate T cell kinetics *in vivo* in HIV-infected patients is the deuterated water (D2O) labeling protocol which we use to study the effects of MVC intensification of cART of patients with a suboptimal immunological response (**chapter 9**). During intake of D2O, deuterium

is incorporated into the deoxyribose moiety of newly synthesized DNA, thereby labeling the DNA of newly produced cells. The presence of deuterium in the DNA can be detected using gas chromatography and mass spectrometry (GC/MS) [78-80]. Up- and downlabeling profiles of cell population can be analysed mathematically resulting in estimates of T cell life spans. Remarkably, we found a lower turnover and increased T-cell lifespan in MVC treated individuals. We hypothesize this is a direct result of blocking CCR5, resulting in diminished T cell proliferation and apoptosis rates. Moreover, these combined effects could explain the lack of change in CD4 cell numbers as well. Based on our findings, MVC intensification could therefore be beneficial in the long run, however further studies are necessary to confirm our results and to investigate the clinical benefits.

Future perspectives

It has now become apparent that long term complications of HIV-infection such as cardiovascular disease and non-AIDS defining malignancies are probably related to ongoing (residual) immune activation and chronic inflammation [12]. Besides the fact that the level of immune activation has been recognized as a strong independent predictor for survival of HIV-infected men in general, the level of immune activation and chronic inflammation is reported to be higher in patients with a poor immunological response on HAART, despite viral suppression [18, 18-21, 81]. As a result, these patients are at higher risk for non-AIDS defining diseases as long term complications of HIV-infection, which has been shown by several studies [82-86]. Therefore, efforts have to be undertaken to develop strategies to reduce the level of immune activation in HIV-infected patients in general, but in particular in this population of patients. Two key issues can be identified.

First, we and others have shown that age and nadir CD4 cell count are strong predictors for a successful immunological response on cART. Unfortunately, it has previously been reported that in Western-Europe 31-39% of new diagnosed HIV-infected patients in Western-Europe present with a CD4 cell count of <200 cells/uL [52]. In addition, according to the monitoring report 2012 of the Stichting HIV Monitoring, 43% of patients entering clinical care in 2011 were 'late presenters' (CD4 cell count <350 cells/μL or an AIDS-defining event) [53]. Therefore, more

aggressive screening policies need to be implemented in order to diminish the number of patients with a suboptimal immunological response and at risk for long term complications.

Second, the development of new therapeutic strategies that reduce the level of immune activation is needed. Several studies have been undertaken to intensify cART in order to reduce the level of immune activation, either by aiming to further decrease the level of viremia below the cut-off of 50 copies/ml (e.g. raltregravir intensification studies [70-72]), to reduce immune activation by other mechanisms, or both (e.g. MVC intensification studies [70, 74, 75, 77]). Although the results of the various MVC intensification studies (including the MIRS study, **chapter 8**) are not spectacular and conflicting as well, slight changes in immune activation and apoptosis levels have been observed in our and other studies [74, 75, 77]. Therefore, further subanalyses of these studies and even pooling of data of different studies might be helpful to further identify the immunomodulatory effects of MVC intensification. One could argue that these effects – if they exist – would be so minor that there would be no clinical benefit for these patients. However although this might be true initially, it is of importance to first unravel the possible mechanism and from there on pursue further improvements in therapeutic strategies.

Another interesting field for further research is the effects of MVC on endothelial function in HIV-infected patients. Because of the important role of inflammation and immune cells in the aetiology of atherosclerosis, it is not surprising that many chemokines and chemokines receptors have been linked to this disease, such as the CCR5-receptor and its ligands [87].

The CC chemokine ligand-5 (CCL5) and its receptor (CCR5-coreceptor) seems to play an important role in atherosclerosis. The expression of CCL5 can be detected in atherosclerotic plaques and in smooth muscle cells, whereas the CCR5-receptor is expressed on various cell types involved in atherosclerosis, e.g. monocytes/macrophages and T-lymphocytes. They are involved in CCL5-triggered arrest, transendothelial diapedesis and in detrimental effects of emigrated cells at lesion sites. In humans, the CCR5delta 32 polymorphism, resulting in reduced expression or absence of the CCR5-coreceptor, has been linked with reduced susceptibility for cardiovascular disease. In addition, experimen-

tal studies in mice have shown that genetic inactivation or blocking of the CCR5-receptor in protects against atherosclerosis [88-95]. For example, administration of the CCR5-receptor antagonist Met-RANTES reduced the progression of atherosclerosis in a hypercholesterolemic mouse model [96] and in another study treatment of apoE-deficient mice RANTES markedly reduced neointimal plaque area and macrophage infiltration [97].

Thus, therapy aiming at blocking the CCR5-receptor might be a good approach to decrease ongoing inflammatory processes. By this mechanism CCR5-blockade may improve endothelial function and, ultimately, decrease the risk for atherosclerosis.

Therefore we have initiated the "Maraviroc Abacavir STudy: effects on Endothelial Recovery" (MASTER) study in the UMC Utrecht, which investigates the effects of MVC intensification on endothelial function in HIV-infected patients on abacavir-containing therapy (ClinicalTrials.gov Identifier: NCT01389063).

Another interesting field of future research is the possible effects of statin therapy in HIV-infected patients, not only to reduce cardiovascular events but other non-AIDS defining complications as well. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors ("statins") are a class of drugs that have been found to inhibit several pro-inflammatory processes independent of their cholesterol-lowering effects. They decrease pro-inflammatory cytokine levels, acute phase proteins, neutrophil migation and also appear to reduce T cell activation [98-101]. Evidence is available that, in addition to their beneficial effect on atherosclerosis, statins may have other therapeutic benefits because of these immune modulating properties. For example, a reduced risk for and mortality of infectious complications such as pneumonia, and sepsis has been reported [102-106]. However, whether statin treatment really does reduce the risk for and mortality of infections in immunocompetent persons is currently controversial [107].

Only very limited data on the relation between statin treatment and complications as mentioned above in HIV-infected persons is available. It has been shown in two studies that treatment with atorvastatin is associated with reduction of immune activation, in patients without cART treatment [98] and with cART treatment [99]. One observational study showed that in a cohort of HIV-infected persons (n=1538) statin use

was associated with a reduced risk for mortality [108]. Preliminary results of an observational cohort study performed in the UMC Utrecht, do not show a beneficial effect of statin therapy on all-cause mortality or the occurrence of pneumonia [109]. Large prospective studies are warranted to further assess the possible beneficial effects of statins in HIV-infected patients.

Besides its possible immunological properties, MVC has been proven to be effective as part of an antiretroviral regimen for treatment of HIV-1 (see **chapter 2**) and the experiences in clinical practice with this antiretroviral drug in the UMC Utrecht are excellent (**chapter 6**). However, MVC is not used frequently as part of antiretroviral treatment of HIV-infected patients. As of December 7, 2012, in the Netherlands only 105 patients are registered to use MVC, of which 52 (50%) are treated in the UMC Utrecht (SHM, personal communication). Several issues have hampered the use of this drug in the Netherlands, such as the fact that MVC has been registered for treatment-experienced patients only, the logistic issues surrounding tropism testing, and the fact that MVC has to be taken twice daily. The introduction of the genotypic tropism test has now improved and simplified the logistics surrounding tropism testing significantly, making this less of an issue. The optimal cut-off FPR for R5-classification is not fully explored, with the risk that MVC treatment is unnecessary withheld in patients. Studies designed to investigate the predictive value of different tropism assays and especially to explore whether the optimal cut-off level for X4-prediction can be decreased are needed during the next years. Furthermore, clinical trials investigating the possibility of once daily MVC are ongoing, such as the MODERN trial that compares the efficacy of MVC with boosted darunavir once daily, as compared to emtricitabine/tenofovir with boosted darunavir, in antiretroviral treatment naïve patients (www.clinicaltrials.gov, NCT01345630). This trial will analyse the predictive value of different tropism assays as well. If once daily dosing appears to be safe and effective, this would expand the therapeutic possibilities of MVC.

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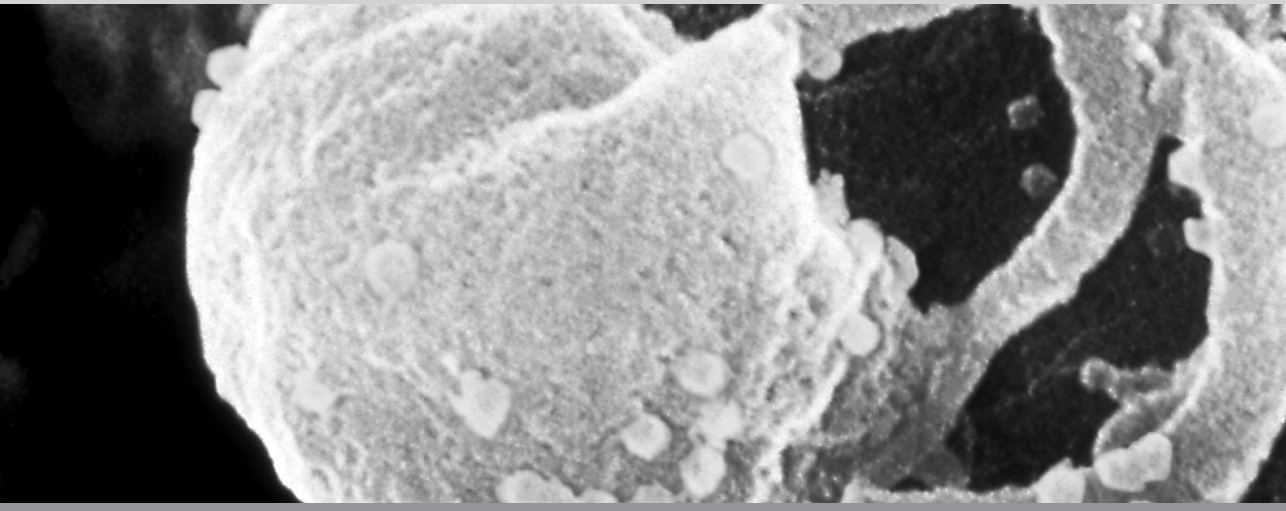
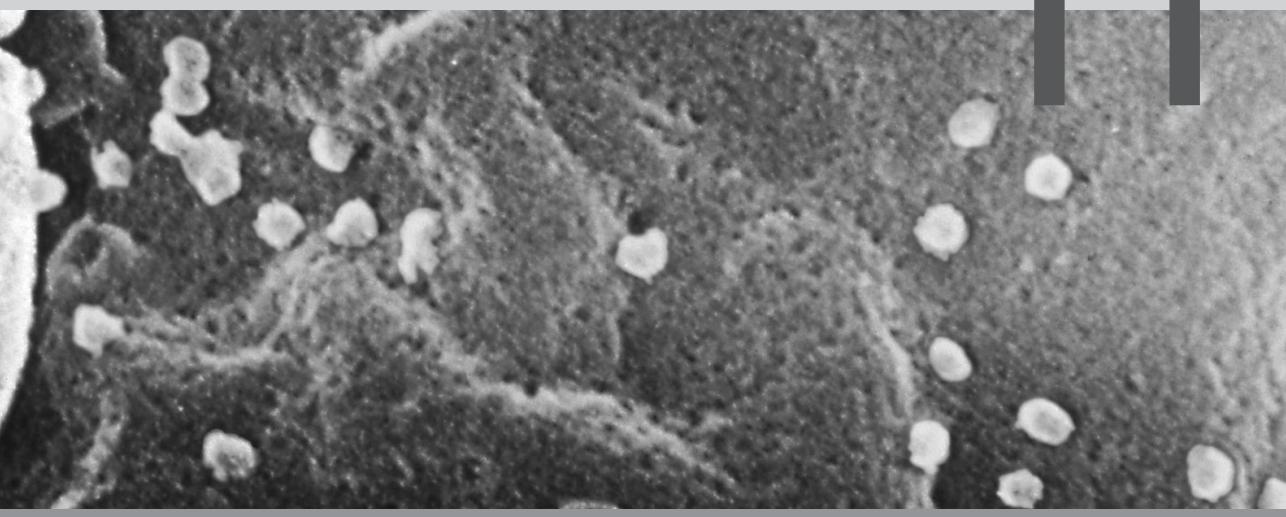


CHAPTER 11



Nederlandse samenvatting

De introductie van de 'combinatie antiretrovirale therapie' (cART) in 1996 heeft geleid tot een enorme verbetering van de levensverwachting van HIV-geïnfecteerde patiënten, zodanig dat deze nu vergelijkbaar is met die van patiënten met andere chronische ziekten, zoals diabetes mellitus. Een van de vragen die wij ons stelden is of deze verbeterde behandeling zich ook heeft vertaald in een verbeterde korte en lange termijn overleving van HIV patiënten die met levensbedreigende aandoeningen worden opgenomen op een intensive care afdeling (IC). Gegevens hierover waren beperkt en dateerden vooral uit het begin van het cART tijdperk. In **hoofdstuk 3** presenteren wij de resultaten van een retrospectieve cohortstudie naar de korte en lange termijn overleving van alle HIV-geïnfecteerde patiënten die opgenomen zijn geweest op de IC van het Universitair Medisch Centrum Utrecht (UMCU) in de periode 1990-2008. Wij vonden in het cART tijdperk zowel een verbetering van de sterfte na 1 jaar als na 5 jaar. Daarnaast was de overleving van deze patiëntencategorie 5 jaar na ontslag uit het ziekenhuis vergelijkbaar met die van de algemene populatie. Deze studie onderstreept dat HIV-infectie absoluut geen reden is om af te zien van opname op de IC.
Hoewel sommige onderzoeken suggereren dat de levensverwachting van bepaalde cohorten van HIV-geïnfecteerde patiënten vergelijkbaar zijn met die van de niet geïnfecteerde populatie, lijkt dit in het algemeen (nog) niet zo te zijn. Er zijn aanwijzingen dat het proces van veroudering versneld optreedt in HIV-geïnfecteerde patiënten. Zo wordt bijvoorbeeld gezien dat hart- en vaatziekten en maligniteiten vaker voorkomen bij deze patiënten in vergelijking met de algemene populatie. Dit lijkt gerelateerd te zijn aan een overmatige activatie van het immuunsysteem ('immuunactivatie'), wat gepaard gaat met verhoogde mate van chronische ontsteking ('inflammatie'). Hoewel cART de mate van immuunactivatie en inflammatie sterk vermindert, blijft dit nog altijd verhoogd in vergelijking met de algemene populatie. Atherosclerose, het proces dat ten grondslag ligt aan het optreden van hart- en vaatziekten, is een inflammatoire aandoening. In het licht van deze bevindingen is het daarom niet verwonderlijk dat hart- en vaatziekten vaker voorkomen bij HIV-geïnfecteerde patiënten.
Succesvolle behandeling met cART leidt tot een daling van de hoeveelheid van het virus in het bloed tot zeer lage of ondetecteerbare waarden en herstel van het immuunsysteem (wat gemeten wordt aan de hand

van het aantal $CD4^+$ T lymfocyten [CD4 cellen], een bepaald type witte bloedcellen). Helaas treedt een adequaat herstel van het immuunsysteem niet altijd op en blijft het aantal CD4 cellen in 10-30% van de patiënten verlaagd ('suboptimale immunologische respons'), ondanks het feit dat het virus in het bloed van deze patiënten ondetecteerbaar is. Er zijn aanwijzingen dat er bij deze groep patiënten een extra hoge mate van immuunactivatie aanwezig is. In **hoofdstuk 4** onderzochten wij of een suboptimale immunologische respons op cART daarom gepaard gaat met een toename van het risico op lange termijn complicaties, zoals hart- en vaatziekten. In dit retrospectieve cohortonderzoek bij ruim 3000 patiënten vonden wij dat een suboptimale immunologische respons ondanks suppressie van het HIV-virus in het bloed voorkwam bij 27.5% van de patiënten. Belangrijke oorzaken hiervan waren onder andere het laat starten van cART (bij een laag CD4 cel aantal) en het starten van cART op oudere leeftijd. Een adequaat herstel van het immuunsysteem (> 500 CD4 cellen/μL) bleek inderdaad geassocieerd te zijn met een lager risico op lange termijn complicaties in het algemeen (gecombineerd eindpunt van dood, AIDS, maligniteiten, levercirrhose en hart- en vaatziekten) en een trend voor een lager risico op hart- en vaatziekten. Oudere leeftijd was een zeer sterke voorspeller voor het optreden van lange termijn complicaties. Deze studie onderstreept daarom het belang van goed herstel van het immuunsysteem naast onderdrukking van het virus in het bloed, om het risico op lange termijn complicaties te verminderen.

De interesse in de neurocognitieve ('hersen') complicaties van HIV-infectie en de mogelijke relatie met de mate van doordringen van HIV-('antiretrovirale') geneesmiddelen in het centrale zenuwstelsel (CZS) is de laatste jaren enorm toegenomen. Het blijkt dat het HIV-virus de 'bloed-hersenbarrière' kan passeren, maar dat is niet voor elk antiretroviraal geneesmiddel in gelijke mate het geval. Indien de gebruikte antiretrovirale geneesmiddelen slecht doordringen in het in het CSZ (een zogenaamde 'HIV-sanctuary site') kan het daarom voorkomen dat het HIV-virus niet goed onderdrukt wordt in het CZS. In **hoofdstuk 5** worden de mogelijke complicaties besproken van behandeling van HIV-infectie met cART met slechte penetratie in het centraal zenuwstelsel. Een patiënt die al lang en met veel verschillende antiretrovirale geneesmiddelen werd behandeld met wisselend succes (wat resulteerde in uitgebreide resist-

entie tegen verschillende klassen van antiretrovirale geneesmiddelen), werd behandeld met een maraviroc en enfuvirtide bevattende combinatie van HIV-geneesmiddelen. Wij konden in het hersenvocht (liquor) van deze patiënt het HIV-virus detecteren en aantonen dat het virus zich vermenigvuldigde, ondanks het feit dat het virus in het bloed onderdrukt was. Van enfuvirtide is het bekend dat het slecht doordringt in het centraal zenuwstelsel, maar voor maraviroc zijn adequate liquor concentraties gerapporteerd. Helaas werden van beide geneesmiddelen slechts zeer lage spiegels in de liquor aangetroffen, wat resulteerde in het ontstaan van virus dat resistent was tegen enfuvirtide. Vervolgens konden wij aantonen dat dit resistente virus terug 'gelekt' of getransporteerd is naar het bloed, wat leidde tot vermenigvuldiging in het bloed en algeheel therapiefalen. Deze casus illustreert, als een van de eersten, dat het ontwikkelen van resistent virus in het centraal zenuwstelsel het succes van therapie (nadelig) kan beïnvloeden en onderstreept het potentiële gevaar van slechte penetratie van antiretrovirale geneesmiddelen in het centrale zenuwstelsel.
Vanwege het feit dat de algemene levensverwachting van HIV-patiënten nog steeds lager is dan die van de algemene populatie, de bijwerkingen van (sommige van de) huidige antiretrovirale geneesmiddelen en het ontwikkelen van resistentie tegen antiretrovirale geneesmiddelen, is er nog steeds een noodzaak tot het ontwikkelen van antiretrovirale geneesmiddelen met een nieuw werkingsmechanisme. Maraviroc (Celsentri®) is het eerste en tot nu toe enige antiretrovirale geneesmiddel van de klasse van de CCR5 antagonisten en is in Nederland geregistreerd voor de behandeling van HIV-geïnfecteerde patiënten die al eerder zijn behandeld met andere antiretrovirale geneesmiddelen. Registratie van maraviroc is gebaseerd op de the 'Maraviroc plus Optimized Background Therapy in Viremic, ART-Experienced Patients (MOTIVATE) 1 and 2 studies', die de effecticiteit van maraviroc in combinatie met optimaal gekozen andere antiretrovirale geneesmiddelen vergelijkt met placebo. In **hoofdstuk 2** bespreken wij deze en andere studies met maraviroc. Het blijkt dat een maraviroc bevattende combinatie van antiretrovirale geneesmiddelen een uitstekende optie is voor de behandeling van HIV-infectie vanwege een goede effectiviteit en een mild bijwerkingenprofiel.

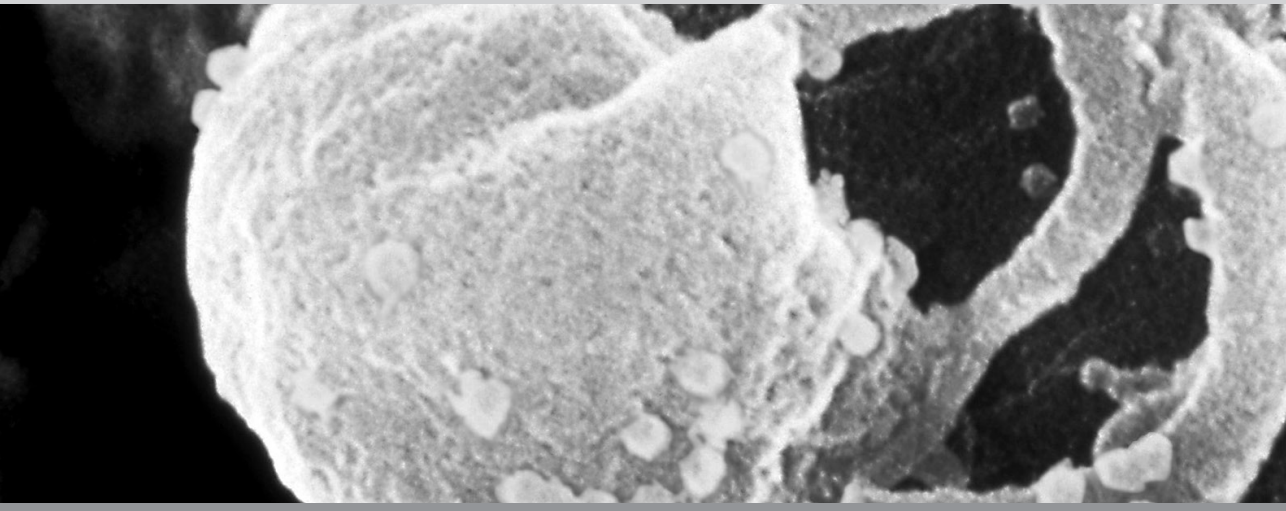
Maraviroc blokkeert de CCR5 coreceptor, wat naast de CXCR4 coreceptor een van de receptoren ('toegangspoort') is die het HIV-virus kan gebruiken om de cel binnen te dringen en zichzelf te vermenigvuldigen. Het 'tropisme' van HIV verwijst naar de mogelijkheid van het virus de cel binnen te dringen door middel van het gebruiken van 'een toegangspoort', de CCR5 coreceptor('R5-troop virus'), de CXCR4 coreceptor ('X4-troop'), of beide coreceptoren ('duaal-troop'). Het HIV-virus vermenigvuldigt zich enorm snel en gaat gepaard met veel 'foutjes', waardoor bij mensen korte tijd na infectie in feite een 'zwerm' van verschillende virusvarianten aanwezig is ('populatie'). Soms zijn er in een viruspopulatie zowel virussen aanwezig die R5-troop zijn als virussen die X4-troop zijn, wat aangeduid wordt als een 'mixed-troop' viruspopulatie. Omdat maraviroc de CCR5 coreceptor blokkeert, is dit geneesmiddel alleen geregistreerd voor de behandeling van patiënten geïnfecteerd met R5-troop virus. Voor het bepalen van het tropisme zijn verschillende soorten testen beschikbaar. Op dit moment wordt hiervoor door de Europese richtlijn de zogenaamde genotypische tropisme test aanbevolen. Bij deze methode wordt aan de hand van de aminozuur volgorde van de 'V3-loop', een specifiek gebied van het gp120 eiwit (een glycoproteine dat zich aan de buitenkant van het HIV-virus bevindt en bindt aan de coreceptor), een voorspelling gedaan over het tropisme van een viruspopulatie.

Ondanks de goede resultaten van maraviroc therapie, wordt het niet vaak voorgeschreven. In Nederland heeft het UMCU de meeste ervaring met het behandelen van HIV-infectie met maraviroc, ongeveer de helft van de patiënten die hiermee worden behandeld wordt begeleid in het UMCU. De ervaringen met en de resultaten van behandeling van deze patiënten worden beschreven in **hoofdstuk 6**. Ondanks het feit dat deze patiënten in het verleden veel zijn behandeld met antiretrovirale geneesmiddelen wat bij hen vaak heeft geleid tot uitgebreide resistentie van het virus, en het feit veel patiënten tevens andere aandoeningen hadden, werd maraviroc goed verdragen en was er sprake van een goede immunologische (gemiddelde stijging van het aantal CD4 cellen) en virologische respons (percentage van patiënten waarbij het virus ondetecteerbaar werd in het bloed). Daarnaast hebben wij drie verschillende soorten tropisme testen vergeleken, de Trofile test, de MT-2 test en de genotypische test. Voor en vlak na registratie van maraviroc

werd voor het testen van tropisme de Trofile test gebruikt, probleem is echter dat deze alleen wordt verricht in San Francisco, USA. Onze resultaten laten zien dat de genotypische tropisme test inderdaad een goed alternatief is.
Wat echter belangrijk is om te realiseren is dat het tropisme van een viruspopulatie niet zwart-wit is, maar een dynamisch gebeuren. Er kunnen altijd enkele virusvarianten in een populatie aanwezig zijn, die een ander tropisme hebben dan de rest van de populatie. Waar moet de grens worden gelegd, hoeveel X4-troop virus in een populatie is klinisch relevant? Is het relevant dat bijvoorbeeld 0.5% van de viruspopulatie X4-troop is, terwijl de rest van de populatie R5-troop is? Toekomstig onderzoek zal verder moeten aanwijzen wat de beste afkappunten van de tropisme testen zullen zijn.
Hierop voortbordurend, hebben we in **hoofdstuk 7** de virologische effecten onderzocht van behandeling met onder andere maraviroc van een patiënt die geïnfecteerd was met een duaal-troop viruspopulatie. Dit werd gedaan omdat het virus van deze patiënt tegen alle klassen van antiretrovirale geneesmiddelen resistent was, en er daarom als laatste mogelijkheid een 'reddingscombinatie' van geneesmiddelen gegeven werd. Opvallend was dat maraviroc in staat bleek deze duaal-trope virussen te remmen. Dit zou betekenen dat ondanks het feit dat virusvarianten door een tropisme test geclassificeerd worden als duaal-troop, ze in vivo een voorkeur kunnen hebben voor het gebruik van de CCR5 coreceptor en dus geremd kunnen worden door maraviroc. Dit suggereert dat maraviroc therapie in deze patiënten toch van nut kan zijn, indien gecombineerd met krachtige andere antiretrovirale geneesmiddelen; echter, meer onderzoek is nodig om deze initiële resultaten te bevestigen.
De CCR5 coreceptor is aanwezig op het oppervlak van veel verschillende cellen van het immuunsysteem, zoals $CD4^+$ T lymfocyten, dendritische cellen, monocyten en macrofagen. Naast de hierboven besproken rol van de CCR5 coreceptor bij HIV-infectie, speelt de CCR5 coreceptor een belangrijke rol in de activatie van T lymfocyten en daarom in de immuunactivatie die optreedt bij HIV-infectie.
Zoals hierboven besproken, gaat een suboptimaal herstel van het immuunsysteem ondanks adequate suppressie van het HIV-virus door cART gepaard met een grotere kans op overlijden, AIDS, maar ook

op niet aan AIDS gerelateerde aandoeningen. Omdat op dit moment suppressie van het HIV-virus de enige manier is om CD4 cellen te verhogen, zijn dus nieuwe immuunmodulerende therapieën nodig voor deze specifieke groep patiënten.
Daarom wilden wij onderzoeken of het toevoegen van maraviroc aan de cART van deze patiënten zou leiden tot een stijging van het aantal CD4 cellen. Dit op grond van de hypothese dat het HIV-virus mogelijk verder onderdrukt zou worden, dat door directe beïnvloeding van T cellen de mate van immuunactivatie verminderd zou worden of door een combinatie van deze mechanismen. In **hoofdstuk 8** bespreken wij de resultaten van de 'Maraviroc Immune Recovery Study', een studie die het effect van 'intensificatie' (toevoegen) van cART met maraviroc onderzoekt in deze patiënten. In dit gerandomiseerde, placebogecontroleerde, multicenter, onderzoek vonden wij geen stijging van het aantal CD4 cellen in de maraviroc intensificatie groep in vergelijking met de placebo groep. Wel werden er bescheiden invloeden gevonden op het aantal memory $CD8^+$ T lymfocyten, immuunactivatie en celdood. In het algemeen kan er gesteld worden dat op basis van deze onderzoeksresultaten het niet nuttig is om maraviroc toe te voegen aan de cART van patiënten met een suboptimale immunologische respons, met het doel om het aantal CD4 cellen te doen toenemen. Echter, omdat in dit en andere onderzoeken enige veranderingen in immuunactivatie en celdood zijn gevonden, is meer onderzoek noodzakelijk om te onderzoeken of er wellicht subgroepen van patiënten zijn die wel baat hebben bij maraviroc intensificatie van cART.
Een nieuwe methode om de levensduur van T lymfocyten te onderzoeken is de zogenaamde 'zwaar water (dideuteriumoxide, D2O) labeling' techniek, die wij hebben gebruikt om de effecten van maraviroc intensificatie van cART van patiënten met een suboptimale immunologische respons te onderzoeken (**hoofdstuk 9**). Zwaar water is een stabiel isotoop van gewoon water, en na het drinken van D2O wordt deuterium ingebouwd in het DNA van nieuw gevormde cellen, waardoor deze nieuwe cellen worden gelabeld. T lymfocyten kunnen geïsoleerd worden uit het bloed en de aanwezigheid van deuterium kan gedetecteerd en gekwantificeerd worden. Als er bij patiënten bloed wordt afgenomen in een periode waarin zij achtereenvolgens D2O wel en niet drinken, dan kan de toename en vervolgens afname van de hoeveel-

heid deuterium in T lymfocyten bepaald worden. Aan de hand daarvan kan dan via wiskundige modellen de levensduur van T lymfocyten berekend worden. In deze pilotstudy vonden wij een langere levensduur van T lymfocyten in patiënten die behandeld werden met maraviroc in vergelijking met placebo. Mogelijk is dit een direct gevolg van blokkeren van de CCR5 receptor, met als gevolg een vermindering van immuunactivatie en celdood van deze lymfocyten. Deze gecombineerde effecten zouden tevens verklaren waarom het aantal CD4 cellen niet stijgt bij deze patiënten. Op basis van deze bevindingen zou maraviroc intensificatie op de lange termijn mogelijk van nut kunnen zijn, maar meer onderzoek is nodig om deze resultaten te bevestigen en het klinische nut te onderzoeken.



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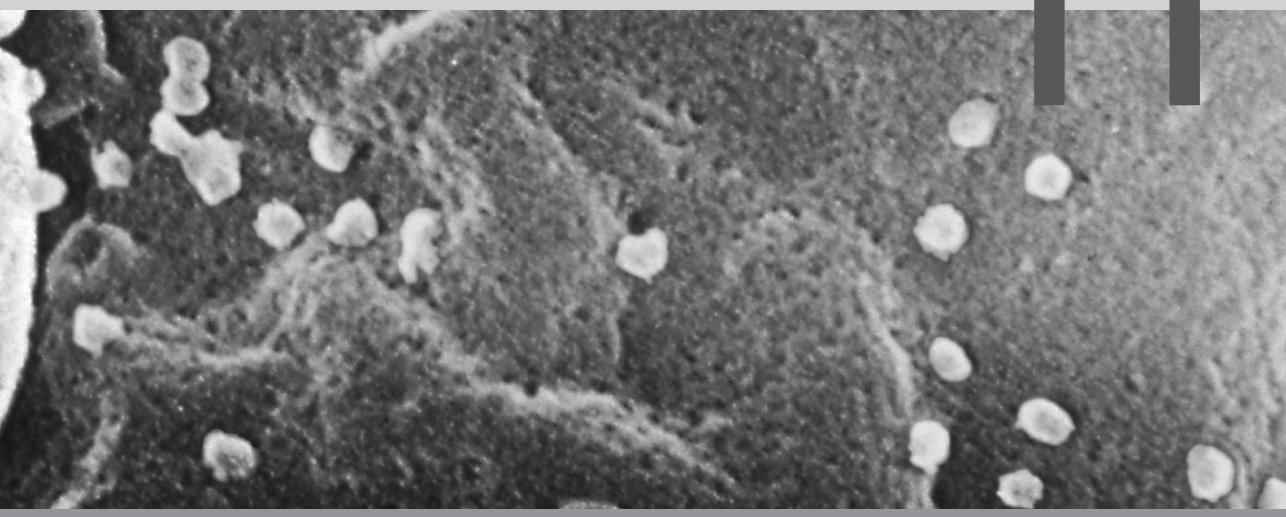
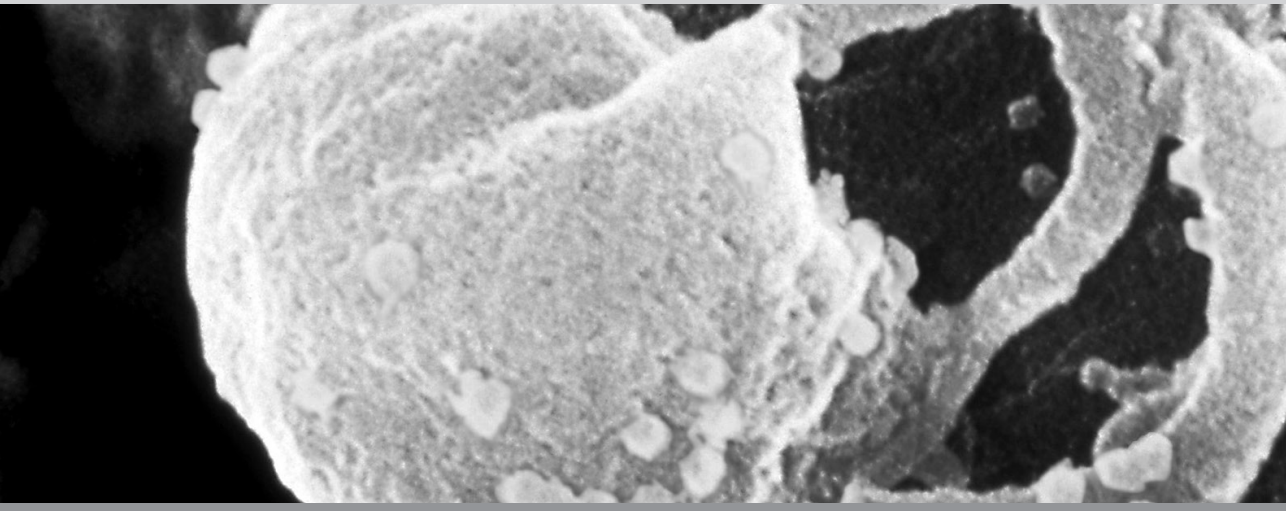


Curriculum vitae

Steven van Lelyveld was born on June 9th, 1975 in Breda, the Netherlands. He moved at a young age to the north of the country, where he graduated in 1993 from the 'Praedinius Gymnasium' in Groningen. After studying at the medical school of the Catholic University in Leuven for one year, he continued his studies at the State University Groningen. During his studies he performed a research elective of 7 months at the department of Immunology at the University of Massachusetts Medical Center, Worcester, USA (supervisor Dr. K.L. Rock), and a clinical elective of 4 months at the Paediatric department of the State Hospital Winhoek, Namibia. He obtained his medical degree in 2001, and subsequently started working as a house officer Internal Medicine at the Diakonessenhuis in Utrecht, where he started his specialisation in Internal Medicine in September 2002 (supervisor Dr. W.M.N. Hustinx). In September 2005 he continued his training in Internal Medicine at the University Medical Center Utrecht (supervisors Prof. dr. E. van der Wall and Prof. dr. D.H. Biesma). After working for two months as a registrar at the Infectious Diseases department of the Tygerberg Hospital, Cape Town, South Africa, he started with his specialisation in Infectious Diseases in March 2007 (supervisor Prof. dr. A.I.M. Hoepelman) which he finished in December 2008. He continued to work on the research projects described in this thesis at the departments of Infectious Diseases, Immunology and Virology (Medical Microbiology) in the UMC Utrecht. Since November 2012 he works as an Internal Medicine specialist at the Reinier de Graaf Gasthuis in Delft.
Steven van Lelyveld is married to Lenneke Haas since May 16th, 2007, and together they have two sons: Kas (2007) and Jorre (2010).



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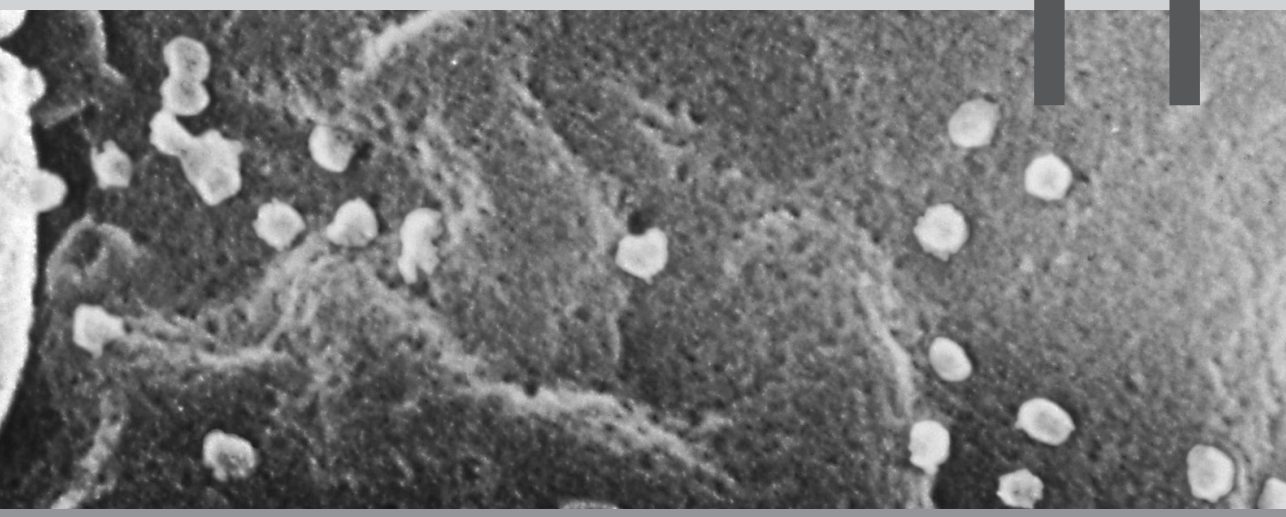

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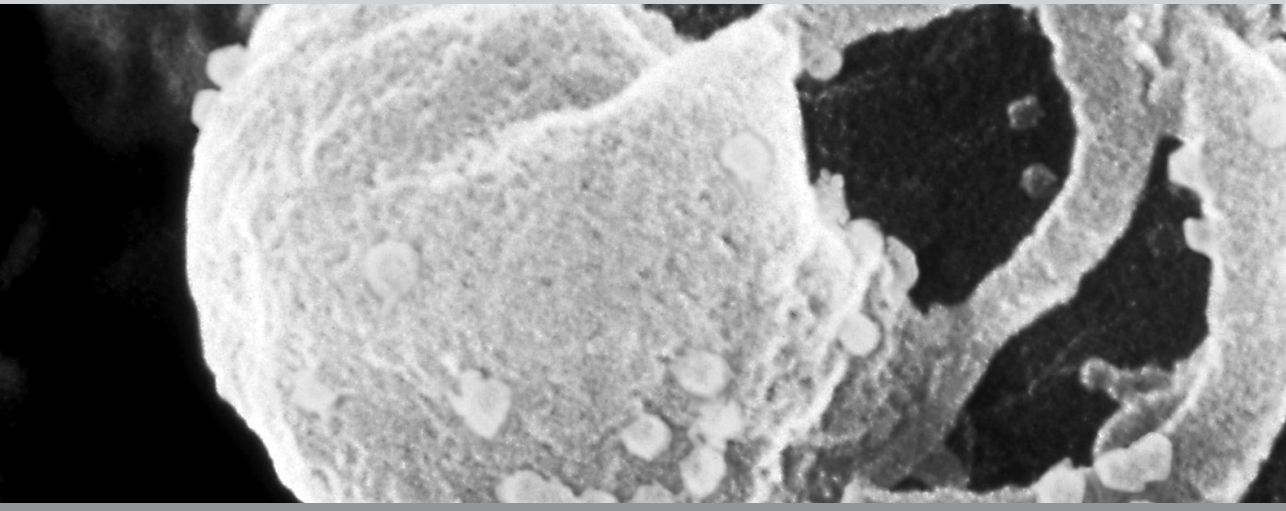

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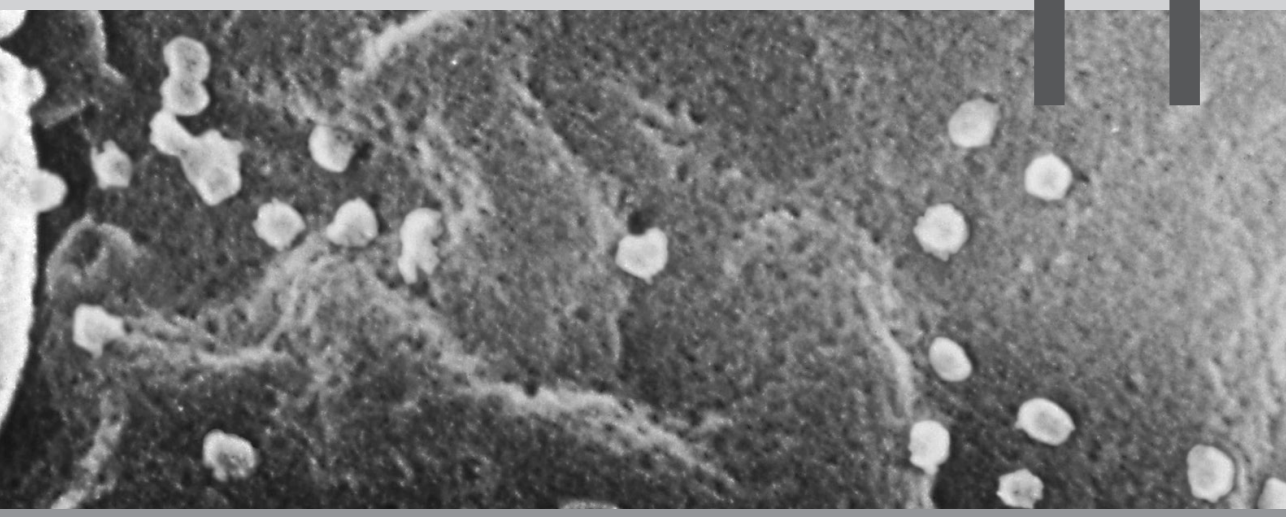

Dankwoord

Eindelijk, het proefschrift is af! Ik heb de afgelopen jaren met erg veel mensen samen gewerkt, graag wil ik hen bij deze hiervoor bedanken.

In de eerste plaats alle patiënten die deelgenomen hebben aan de verschillende onderzoeken, en hier veel tijd en moeite in hebben gestoken. Zonder hen waren deze onderzoeken natuurlijk nooit mogelijk geweest. Hopelijk draagt dit proefschrift bij aan de verbetering van de behandeling van HIV-patiënten.

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