Treatment of primary HIV infection
Grijsen, M.L.

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To date, clinical guidelines do not offer a definitive answer whether to intervene with (temporary) combination antiretroviral therapy (cART) during primary HIV infection (PHI) or not, and if so, for how long. In this thesis we studied the treatment of PHI. Early cART lowered the viral setpoint and deferred the need for restart of cART during chronic HIV infection. Even though the exact mechanisms explaining the lowering of the viral setpoint after early cART remain unsolved, we observed a clear clinical benefit of temporary treatment during PHI. Additionally, early cART had a positive impact on patients’ quality of life and did not affect the subsequent virologic response to long-term cART. In case early cART is considered, it should be given for a duration of 24 weeks and contain a boosted protease inhibitor, at least until resistance testing results are available.
Treatment of primary HIV infection

Marlous L. Grijsen
Treatment of primary HIV infection

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het college voor promoties
ingeschikte commissie,
in het openbaar te verdedigen in de Agnietenkapel
op donderdag 28 februari 2013, te 12:00 uur

door

Marianne Louise Grijsen

egenomen te Emmeloord
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Faculteit der Geneeskunde
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PART I

INTRODUCTION
chapter ONE

General introduction
This general introduction will provide a brief overview of HIV/AIDS and will then narrow down to primary HIV infection, including a description of its pathogenesis, clinical manifestations, diagnosis and treatment. This will be followed by a summary of the outline of the thesis.

**HIV/AIDS**

Acquired Immunodeficiency Syndrome (AIDS) was first identified in San Francisco, USA, in the early 1980s [1], after which the discovery of a novel retrovirus, named human immunodeficiency virus (HIV), soon followed [2]. Of all problems in global health, not many have had such a devastating impact as HIV/AIDS, and are as unequally distributed across the world and certain subpopulations: men who have sex with men (MSM), sex workers, injecting-drug users. To date, some 60 million people have been infected with HIV since the beginning of the pandemic. HIV/AIDS has claimed the lives of more than 25 million people. Sub-Saharan Africa is the region most heavily affected and is home to more than half of all people living with HIV/AIDS worldwide [3].

The introduction of combination antiretroviral therapy (cART) has curbed the growth of the epidemic with a reduction in HIV/AIDS-related mortality and, as part of a public health strategy, has also reduced the spread of new infections [4-6].

HIV is an enveloped retrovirus that belongs to the genus of *Lentiviruses*. The virus is subdivided into two types: HIV-1, causing the majority of all HIV infections, and HIV-2, a less pathogenic variant concentrated in West Africa. HIV is transmitted through sexual intercourse, exposure to contaminated blood or from mother-to-child, including through breastfeeding. The transmission of HIV is inefficient: the probability ranges between 0.0001 to 0.004 per coital act [7]. This percentage is evidently influenced by factors which can either increase or decrease the transmission rate, i.e., the stage of HIV infection [8], the presence of sexually transmitted infections [9], or circumcision[10]. HIV is divided into four distinct stages, i.e. primary infection, asymptomatic chronic infection, symptomatic chronic infection, and AIDS. The silent asymptomatic period may last for many years. During this period the virus interferes with the immune system, causing a gradual decline of CD4+ T cells. If left untreated, the cell-mediated immunity will eventually be lost and the patient will become susceptible to opportunistic infections and neoplasms (AIDS), which will ultimately lead to death [11].

**Primary HIV infection**

**Pathogenesis**

Acute or primary HIV infection (PHI) is the earliest stage of HIV infection and refers to the first six months following HIV acquisition [12]. Transmission of HIV typically takes place at the genital mucosa. The first cellular targets of HIV are submucosal dendritic cells, macrophages and CD4+ T lymphocytes, followed by the spread of HIV to regional lymph nodes, and ultimately plasma [13]. Viral entry into these cells is mediated by the CD4 receptor and the CCR5 coreceptor. Once HIV infects a cell, the virus will integrate its HIV-DNA into the genetic material of the host and will either initiate viral replication or remain inactive, causing latent infection in resting T cells, lymphoid tissue and other sequestered sites throughout the body.
The rapid viral replication in actively infected cells results in widespread dissemination of the virus to lymphoid tissues and organs [15-18]. This stage is characterized by an exponential rise of viral replication: virus populations may double every six to ten hours, causing a peak plasma viremia of millions of RNA molecules per millilitre after approximately three to four weeks post-exposure [19]. During this stage several critical events occur, including the massive depletion of CD4+ T cells in the gastrointestinal tract [20-22], the irreversible destruction of T-helper cell reservoirs, the establishment of viral reservoirs [23, 24] and the development of host immune responses against the virus. A crucial immunologic response is the activation of virus-specific CD8+ cytotoxic T lymphocytes (CTLs), which coincides with a sharp decline in the plasma RNA levels until it reaches a steady state known as the viral setpoint approximately six months after infection [25-27]. This setpoint is a prognostic factor for disease progression [28-31]. Host polymorphisms in the human leukocyte antigens class I alleles (i.e. HLA-B27 en B57) are also key genetic determinants on the outcome of HIV disease progression and are associated with a more effective HIV control [32].

Symptoms
PHI involves a dynamic relationship between the host and the virus. Forty to ninety percent of patients with PHI develop an acute retroviral syndrome (ARS), which often coincides with the high-level viraemia and the initial immunologic response of the host [33, 34]. The acute illness typically occurs two to six weeks after initial exposure and usually lasts less than 14 days [35]. The most common signs and symptoms include fever, fatigue, skin rash, pharyngitis, weight loss, night sweats, lymphadenopathy, myalgias, headache, nausea, and diarrhoea [36, 37]. The majority of patients seek medical care during this acute phase, yet the nonspecific nature of the symptoms makes the ARS often not being recognized [38]. This makes the diagnosis of PHI challenging to health care providers and emphasizes the importance of an accurate history of previous sexual exposures and high-risk behaviour. The severity and the duration of an ARS is associated with a more rapid disease progression [39-42].

Diagnosis
The diagnosis of PHI may easily be missed, because at the time of clinical symptoms HIV antibodies have usually not been formed, and a standard serologic enzyme-linked immunosorbent assay (ELISA) used for chronic HIV infection will be negative [43, 44]. Diagnostic markers to establish a PHI are a p24 antigen assay or a detectable pVL, along with a negative or indeterminate Western blot [45, 46]. p24 antigen is a viral core protein that transiently emerges during the acute phase, approximately 17 days after transmission, and before the development of detectable HIV antibodies [47]. Recently, a novel ELISA, which can detect both p24 antigen and anti-viral antibodies, has been developed and approved for clinical use [48, 49]. Further, it is anticipated that a rapid point-of-care test will also be developed to detect an acute infection. The implementation of these two latter tests will increase the number of patients diagnosed with PHI who otherwise might have been missed [23].
Rationale for treatment of primary HIV infection

PHI is associated with intense viral replication and the development of an initial immune response against the virus, which makes this stage very different from chronic HIV infection and provides a unique opportunity for therapeutic and public health interventions [50]. Although thus far no consensus exists, evidence is accumulating that temporary early cART during PHI has a beneficial effect on early and late disease progression [51-53]. Observational studies have suggested that treatment during PHI may preserve HIV specific immunity [54-61], limit viral evolution and restrict the establishment of viral reservoirs [62, 63]. An important immunological study observed that intermittent cART during PHI led to vigorous HIV specific CD4 and CD8 T cell responses, which were similar to those seen in long-term nonprogressors. It was hypothesized that treatment interruptions enhanced the immune response by reexposure to HIV antigens [58]. Early cART may have a clinical benefit by lowering of the viral setpoint [64-66] and by reducing the rate of CD4 T cell decline [67, 68], and thereby delaying the need for long-term cART during chronic HIV infection. An additional rationale for early cART may be that current treatment guidelines recommend earlier therapy in chronic HIV infection (CD4 T cell count below 500 cells/mm³), to reduce the development of co-morbidities such as cardiovascular and metabolic diseases [69, 70], so a relevant point may be ‘why wait to treat?’ Especially since studies have shown that PHI-patients, who are often symptomatic, have a faster drop in CD4 T cell counts than expected and will rapidly meet the criteria for treatment initiation [71, 72]. The additional time spent on cART will then only be limited as compared to the total life-time spent on cART [73]. Finally, treatment of PHI may have significant public health implications by reducing the spread of HIV transmission, as patients are extremely infectious during this period, are often unaware of their HIV status, and if accompanied with high-risk behaviour, are prone to transmit the virus to others [74, 75].

In contrast, not all studies suggest a benefit on viral setpoint [76-79], and some cohort studies reported a similar or even faster CD4 T cell decline after interruption of early treatment [80, 81]. One could also argue that the early treatment is provided too late to have a major impact, since virus-induced immunopathogenesis has already taken hold [82].

In spite of the earlier mentioned benefits, there are also potential disadvantages of temporary early cART. An important concern is the drug-related (long-term) toxicity, although most cART regimens nowadays are better tolerated, have fewer side effects and are more convenient than older regimens, and its potential negative impact on the quality of life. Another reason not to intervene in PHI may be the risk of developing drug resistance mutations in non-adherent patients, which might be more common in PHI as patients are often physically and emotionally distressed and may have more difficulty with strict adherence to cART, which could compromise future treatment options. However, thus far, this fear has not been substantiated [83, 84]. Finally, the cost effectiveness of temporary early cART has not been studied, although modelling studies have shown that earlier initiation of therapy is a cost-effective strategy because it prevents the person from more severe stages of HIV infection [85, 86]. Moreover, early cART may be cost-effective as it decreases the spread of new infections.

To date, clinical guidelines do not offer a definitive answer whether to intervene with (temporary) cART during PHI or not, and if so, for how long [87, 88]. The first randomized
General introduction

Controlled trial was performed in the early nineties and compared six months of zidovudine monotherapy with placebo in patients with PHI, and this study reported a significant reduction of minor opportunistic infections after early cART [89]. A second randomized trial with zidovudine monotherapy demonstrated similar immunological and virological benefits [90]. It lasted another fourteen years before results of randomized trials that were performed in the current cART era became available.

In 2003 we initiated the Primo-SHM trial, a multi-centre trial in which patients with laboratory evidence of PHI were randomized to receive no treatment, 24 or 60 weeks of early cART [91]. The aim of the study was to assess the clinical benefit of temporary early cART, measured by the time that patients could remain off therapy until subsequent (re)start of cART was indicated based on current treatment guidelines, and to assess the optimal duration of such early treatment. Patients were recruited in 13 HIV treatment centres in the Netherlands between May 2003 and March 2010. The large cohort of PHI-patients that was established during this time frame is the basis of most studies described in this thesis.

Scope of this thesis

The core of the thesis comprises three parts that include a randomized trial on the effect of temporary early cART during PHI (Part II), studies on quadruple or triple-class therapy in patients with primary and chronic HIV infection (Part III), and studies on bone mineral density during PHI (Part IV).

As an introduction to this thesis Chapter 2 reports an illustrative case study of a patient who presented with Kaposi’s sarcoma during PHI. Part II focuses on the clinical management of PHI. Chapter 3 describes the results of the Primo-SHM trial, an open-label randomized controlled trial comparing no treatment with 24- or 60-weeks of early cART during PHI. The aim of this study was to assess the clinical benefit of early cART, measured by the time that patients could remain off therapy until subsequent (re)start of cART was indicated based on current treatment guidelines, and to assess the optimal duration of such early treatment. In the Primo-SHM substudy, we compared the impact of temporary cART during PHI with no treatment on health-related quality of life, over a study period of 96 weeks (Chapter 4). In Chapter 5 we assess the effect of early cART on the subsequent virologic response to long-term cART, in patients who participated in the Primo-SHM trial. To this end, we compare the viral decay and the time to viral (re)suppression between the early treated patients who reinitiated cART and the patients in whom treatment was deferred until conventional criteria to start long-term cART had been reached. Finally, Chapter 6 addresses the pathogenic mechanisms of the lower viral setpoint that we observed after early cART and Chapter 7 investigates the effect of dual HIV infections (co- or superinfection) on disease progression in a well-defined cohort of 37 MSM with PHI and a subtype B virus.

Part III involves two clinical studies on the virologic response in primary and chronic HIV infected patients receiving triple-class quadruple antiretroviral therapy. When we started the Primo-SHM trial, we decided to initiate cART consisting of a quadruple, triple-class regimen, given the often very high pVL in PHI and the fear that standard triple therapy would easily result in virological failure, and that drug resistance test results were seldom available before
the initiation of cART. To examine whether indeed quadruple therapy has an advantage over standard-of-care triple therapy, Chapter 8 explores whether quadruple or triple-class therapy provides a more rapid pVL decline and an improved virologic response compared with standard-of-care dual-class triple therapy in treatment-naive patients with very high viraemia. An unanswered question so far is whether the virologic response to cART in PHI is comparable to that in CHI. The second study therefore compares the time to viral suppression in our relatively large cohort of PHI-patients treated with triple-class therapy with that of a cohort of naive CHI-patients with comparable treatment and initial pVL (Chapter 9).

Low bone mineral density (BMD), including osteopenia and osteoporosis, and bone fractures appears to be common in HIV infected persons. Osteopenia is defined as the thinning of bone mass which may precede osteoporosis [92]. Osteoporosis is characterized by severe bone loss and structural deterioration of bone tissue and is associated with susceptibility for bone fractures [93]. A systematic review of cross-sectional studies revealed a 6.4-fold increased odds of osteopenia in HIV infected patients and a 3.7-fold increased odds of osteoporosis compared to the general population [94]. A recent study confirmed the higher prevalence of bone fractures in HIV infected adults compared to uninfected controls [95]. The causes of low BMD are multifactorial and represent a complex interaction between HIV infection itself, the use of cART, low vitamin D levels, and traditional risk factors, which might be more prevalent among HIV infected persons (smoking, alcohol and recreational drug use) or are exacerbated by the consequences of chronic HIV infection (e.g., low body weight, poor nutrition) [96]. HIV itself induces a direct effect on the cells of the bone and bone marrow microenvironment, activates T cells and produces an abnormal cytokine reaction, such as tumor necrosis factor-α and interleukin-1, which affect osteoblast and osteoclast function [97]. The subsequent bone demineralization results from a negative imbalance between osteolytic activities of osteoclasts and regenerative activities of osteoblasts. Every reduction of one standard deviation in vertebral BMD, results in a two-fold increased risk of vertebral fracture [98].

In order to gain further insight into the contribution of HIV infection per se on BMD, Part IV explores the BMD during PHI, which provides a unique opportunity to investigate BMD in HIV infected populations, because of the limited duration of HIV infection and the absence of exposure to cART. Chapter 10 studies the BMD and biochemical markers relevant for bone metabolism in a cohort of untreated PHI-men and Chapter 11 compares the BMD and biochemical markers relevant for bone metabolism of untreated primary and chronically HIV infected MSM with those of a control group of HIV negative MSM.

In the summary and general discussion, the main results of this thesis are summarized and discussed, followed by clinical recommendations and suggestions for future research.
References


A case of Kaposi’s sarcoma during primary HIV-1 infection

Marlous L. Grijsen¹, Marion Cornelissen², Jan M. Prins¹

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Abstract
The majority of cases of Kaposi’s sarcoma (KS) occur at low CD4 T-cell counts during chronic HIV-1 infection. We present a case of KS, which was diagnosed during primary HIV-1 infection. This report aims to draw attention that KS may occur early in the course of HIV-1 infection and that primary HIV-1 infection may rapidly progress to AIDS.
Kaposi’s sarcoma during primary HIV infection

Introduction
Kaposi’s sarcoma (KS) is a low-grade vascular tumour caused by human herpesvirus 8 (HHV8). It is one of the most common neoplasms in HIV-1 infected individuals. With the introduction of combination antiretroviral therapy (cART), the incidence of KS has decreased significantly. Traditionally, a low CD4 T-cell count during chronic HIV-1 infection was the most important factor associated with the development of KS, although recent studies have shown that the majority of KS now occurs at higher CD4 T-cell counts. We report a patient who presented with KS during primary HIV-1 infection (PHI).

Case Report
A 48-year-old homosexual man was diagnosed with PHI in June 2009, based on an indeterminate Western blot (p24 and gp120/160 antibodies were detected), and a plasma viral load (pVL) of 267,479 cps/ml. Three weeks prior to the diagnosis, the patient had experienced symptoms which were compatible with an acute retroviral syndrome: fever, sore throat, a dry cough, myalgia, fatigue and weight loss of 5 kg. Physical examination revealed no abnormalities. Laboratory investigations showed a mild anaemia, thrombocytopenia and elevated liver enzymes. CD4+ T-cell count was 210 cells/mm³, CD8+ 2310 cells/mm³ and CD4/8 ratio 0.09. No transmitted drug resistance mutations were present and the HIV-1 strain belonged to subtype B. Using MT-2 assay to define HIV-1 tropism, no CXCR4-tropic virus was detected. Hepatitis B/C and syphilis serology were negative.

11 weeks after HIV diagnosis, the patient discovered a small purple-brown lesion on his left forearm which was clinically suspect for KS (Figure 1). The diagnosis was confirmed with a skin biopsy. The CD4+ T-cell count had increased to 500 cells/mm³ and the pVL had dropped significantly to 64,218 cps/ml. The HHV8 load was 799 cps/ml. Retrospectively, sequential HHV8 loads were performed on stored plasma samples and HHV8 load at the time of PHI diagnosis was 163 cps/ml (Figure 2). Other possible underlying diseases like Castleman’s disease, another HHV8 related disease, were excluded.

Since the patient was recently diagnosed with PHI and was recovering from the PHI-associated transient low CD4+ T-cell count and peak pVL, we decided to defer cART to await spontaneous regression. During the following months, the KS lesion on the arm remained stable and no new lesions occurred. 36 weeks after HIV diagnosis, the CD4+ T-cell count had dropped to 310 cells/mm³, the pVL was 18,138 cps/ml and the HHV8 load had increased to 10,600 cps/ml (Figure 2). cART was initiated. The patient responded well to therapy. Within eight weeks, HIV-1

Figure 1. Kaposi’s sarcoma on the forearm of the patient.
viral suppression in plasma was achieved, CD4+ T-cell count increased to 430 cells/mm³, and plasma HHV8 became undetectable. The KS lesion disappeared within the following six months.

Discussion

The natural history of HIV-1 infection varies widely between patients and may be affected by viral and/or host factors. Rapid progression to AIDS shortly after PHI has been described previously, including a case of KS diagnosed 24 months after PHI. Our patient was symptomatic during PHI, which is a strong predictor of AIDS progression. To our knowledge, this is the first patient in whom KS was diagnosed during PHI.

Unfortunately we were not able to determine the IgM and IgG antibody titers against HHV8 to distinguish between an acute HHV8 infection or a reactivation of an existing HHV8 infection. Since the patient had a transient immunosuppression and a peak pVL as a result of PHI, reactivation of KS seems likely, caused by the immunodeficiency itself and/or by direct effects of HIV-1.

This case demonstrates that KS may occur early in the course of HIV-1 infection and that symptomatic PHI may rapidly progress to AIDS.

Contributors

MLG drafted the manuscript. MLG and JMP treated the patient. MC retrieved the HHV8 results. All authors read and approved the final manuscript. The authors wish to thank prof. dr. Henry J.C. de Vries for his useful comments on the manuscript.

Consent

Informed consent for publication was obtained from the patient.
References


PART II

Treatment during primary HIV infection
No treatment versus 24 or 60 weeks of antiretroviral treatment during primary HIV infection: the randomized Primo-SHM trial

Marlous L. Grijsen, Radjin Steingrover, Ferdinand W. N. M. Wit, Suzanne Jurriaans, Annelies Verbon, Kees Brinkman, Marchina E. van der Ende, Robin Soetekouw, Frank de Wolf, Joep M. A. Lange, Hanneke Schuitemaker, Jan M. Prins, on behalf of the Primo-SHM Study Group

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* These authors contributed equally to this work

Abstract

Background
The objective of this study was to assess the benefit of temporary combination antiretroviral therapy (cART) during primary HIV infection (PHI).

Methods and Findings
Adult patients with laboratory evidence of PHI were recruited in 13 HIV treatment centers in the Netherlands and randomly assigned to receive no treatment or 24 or 60 wk of cART (allocation in a 1:1:1 ratio); if therapy was clinically indicated, participants were randomized over the two treatment arms (allocation in a 1:1 ratio). Primary endpoints were (1) viral setpoint, defined as the plasma viral load 36 wk after randomization in the no treatment arm and 36 wk after treatment interruption in the treatment arms, and (2) the total time that patients were off therapy, defined as the time between randomization and start of cART in the no treatment arm, and the time between treatment interruption and restart of cART in the treatment arms. cART was (re)started in case of confirmed CD4 cell count <350 cells/mm³ or symptomatic HIV disease.

In total, 173 participants were randomized. The modified intention-to-treat analysis comprised 168 patients: 115 were randomized over the three study arms, and 53 randomized over the two treatment arms. Of the 115 patients randomized over the three study arms, mean viral setpoint was 4.8 (standard deviation 0.6) log₁₀ copies/ml in the no treatment arm, and 4.0 (1.0) and 4.3 (0.9) log₁₀ copies/ml in the 24- and 60-wk treatment arms (between groups: \( p < 0.001 \)). The median total time off therapy in the no treatment arm was 0.7 (95% confidence interval 0.0–1.8) years compared to 3.0 (1.9–4.2) and 1.8 (0.5–3.0) years in the 24- and 60-wk treatment arms (log rank test, \( p < 0.001 \)). In the adjusted Cox analysis, both 24 wk (hazard ratio 0.42 [95% CI 0.25–0.73]) and 60 wk of early treatment (0.55 [0.32–0.95]) were associated with time to (re)start of cART.

Conclusions
In this trial, temporary cART during PHI was found to transiently lower the viral setpoint and defer the restart of cART during chronic HIV infection.

Trial registration
Introduction

The optimal management of primary HIV infection (PHI) and the possible impact of temporary combination antiretroviral therapy (cART) on clinical outcome are controversial [1,2]. Reported benefits of temporary early cART from previous observational studies include lowering of the viral setpoint [3–5], a slower decline of CD4 T cells [6], partial normalization of CD4 T cell subsets [7], preservation of HIV specific immune responses [8–10], and limitation of viral reservoirs established during the first few weeks after transmission [11]. However, other cohort studies have not confirmed an effect on viral setpoint [12–15] or have reported a similar or faster decline of CD4 T cells in patients treated during PHI [16,17].

From a clinical perspective, an important question is whether patients who are treated during PHI remain off treatment longer than patients in whom treatment is deferred until indicated based on their CD4 cell count or clinical condition. A randomized controlled trial of 6 months of zidovudine monotherapy in patients with PHI reported a reduction of minor opportunistic infections during the first year of follow-up [18]. Results of two randomized controlled trials in the cART era suggested a clinical benefit of temporary treatment during PHI [19,20]. Preliminary results of the SPARTAC trial, which compared 12 and 48 wk of cART with no therapy during PHI, reported a modest delay in disease progression after 48 wk of cART [19]. The SETPOINT study aimed to compare 36 wk of cART with deferred therapy in early HIV infection, but was prematurely stopped in June 2009 by the Data Safety and Monitoring Board, because of a higher rate of disease progression in the untreated arm [20].

We conducted the Primo-SHM trial, in which patients with PHI were randomized between no treatment and 24 and 60 wk of early cART. The aim of the study was to assess the clinical benefit of temporary CART initiated during PHI, measured by the time that patients could remain off therapy until subsequent (re)start of cART was indicated based on current treatment guidelines, and to assess the optimal duration of such early treatment.

Methods

Study Population

Inclusion criteria were age over 18 years and laboratory evidence of PHI infection, defined as a negative or indeterminate Western blot in combination with detectable plasma HIV-1 RNA (Fiebig stage I–IV) or, in case of a positive Western blot, a documented negative HIV screening test in the previous 180 d (Fiebig stage V–VI [21]). Women were counseled to use adequate contraception; pregnant and breast-feeding women were excluded. The study was approved by the Medical Ethics Committee of each participating site, and written informed consent was obtained from all participants. The study protocol and the CONSORT checklist are provided as Text S1 and Text S2, respectively.

Design

The Primo-SHM trial was a multicenter, open-label randomized controlled trial comparing temporary early cART (24 or 60 wk) with no treatment. Patients were recruited in 13 HIV treatment centers in the Netherlands. Participants were randomly assigned to receive no
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treatment or 24 or 60 wk of cART (three-way randomization). In cases where treatment was clinically indicated based on severe clinical symptoms (e.g., HIV related meningitis) or the patient insisted on starting early cART, participants were randomized over the 24- and 60-wk treatment arms only (two-way randomization).

The study was designed to evaluate (1) the effect of temporary treatment during PHI on the viral setpoint, defined as the plasma viral load (pVL) at 36 wk after randomization in the no treatment arm and at 36 wk after treatment interruption (TI) in the treatment arms, and (2) the total time that patients were off therapy after randomization (treatment-free period). The analyses of the viral setpoint and the total time off therapy were restricted to patients who were randomized over all three study arms. A second comparison evaluated the optimal duration of early cART by comparing all patients who were treated with 24 or 60 wk of cART, including patients from both the three- and two-way randomization.

Recruitment started on 1st of May 2003 and continued until 31st of March 2010. The present analysis includes follow-up data until 14th of September 2011.

Randomization
Patients were allocated to one of the three study arms using a computerized minimization algorithm with stratification for the stage of PHI (Fiebig stage I–II, III–IV, or V–VI). The Dutch HIV Monitoring Foundation performed the randomization procedure, had no interaction with study participants, and was responsible for data management. Randomization results were sent by fax to the clinical investigators, who were unaware of the allocation procedure.

Procedures
Early cART consisted of a triple-class regimen of zidovudine/lamivudine (300/150 mg bid), efavirenz (600 mg qd), and lopinavir/ritonavir capsules (533/133 mg bid). The last was discontinued when the pVL dropped below 50 copies/ml. Changes to this regimen were allowed in case of transmitted drug resistance or if one of the drugs was contraindicated or not tolerated. After 28th of January 2008, zidovudine/lamivudine was replaced by tenofovir/emtricitabine (245/200 mg qd), according to the Dutch standards of care, and lopinavir/ritonavir tablets (600/150 mg bid) replaced the capsules. Patients on early cART were required to reach viral suppression below 50 copies/ml in plasma before interrupting therapy.

Baseline evaluations included a medical history including the presence of symptoms compatible with an acute retroviral syndrome, a physical examination, and collection of blood for routine hematology and chemistry, CD4 and CD8 cell counts, pVL, hepatitis B and C serology, and storage of peripheral blood mononuclear cells. All study sites used a sensitive HIV RNA assay with comparable accuracy and a lower limit of detection of 40 or 50 copies/ml [22]. The assays that were used were Amplicor HIV-1 Monitor ultrasensitive RNA assay (Roche), Amplicor HIV-1 Monitor, Cobas Amplicor, Cobas TaqMan HIV-1 (Roche Diagnostics), m2000rt HIV RNA (Abbott), NucliSens EasyQ (bioMérieux), Quantiplex bDNA 3.0 (Chiron), and Versant HIV RNA 3.0 Assay (Siemens Healthcare Diagnostics). Standard HIV-1 genotyping and subtyping were performed at most participating sites. Drug-resistance mutations were identified according to the World Health Organization Surveillance Drug-Resistance Mutation list [23], using the calibrated population resistance tool of the Stanford University HIV Drug Resistance Database
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In patients recruited at the Academic Medical Center of the University of Amsterdam, additional viral and host genetic analyses were performed: the presence of CXCR4-using viruses at baseline, CCR5 Δ32 genotyping, and human leucocyte antigen (HLA) typing. CXCR4 coreceptor usage was determined using the MT-2 assay [25].

Randomization was performed at week 0, and patients randomized to receive cART started treatment. Patients were scheduled for follow-up visits at weeks 2, 4, 8, and 12, and every 12 wk thereafter for the duration of the study. Additional visits were scheduled at weeks 4, 8, and 12 following TI. During each follow-up visit, blood was collected for routine hematology and chemistry, CD4 and CD8 cell counts, pVL, and storage of peripheral blood mononuclear cells.

Primary Endpoints
The primary efficacy endpoints were (1) the viral setpoint, defined as pVL at 36 wk after randomization in the no treatment arm and pVL at 36 wk after TI in the treatment arms, and (2) the total time that patients were off therapy, defined as the time between randomization and start of cART in the no treatment arm, and as the time between TI and restart of cART in the treatment arms. We chose the viral setpoint as the pVL at 36 wk to allow for stabilization of the pVL in untreated and treated patients [3,5,13,14] and to minimize drop out due to rapid disease progression. (Re)start of cART was defined as the moment when the criteria for (re)start of cART were met - a CD4 cell count below 350 cells/mm³ on two consecutive occasions, severe constitutional symptoms, or the occurrence of an AIDS-defining event [26] - or if the physician or patient insisted on (re)initiating cART, whichever occurred first. We also explored the time to (re)start of cART with a CD4 cell count threshold of 500 cells/mm³ or less on two consecutive occasions [27,28]. In the no treatment arm, the CD4 count criterion was not applied during the first 12 wk after randomization, because of the transiently low CD4 cell counts often observed during PHI.

Statistical Analysis
At the time the protocol was developed in 2002, insufficient data were available to make a reliable estimation of the sample size needed to detect a clinically significant difference between the study arms in viral setpoint and time to (re)start of cART. An estimated number of 30 patients were expected to be enrolled annually, and in the protocol amendment of 28th of January 2008, the end of study inclusion was set at March 2010, when we expected to have enrolled approximately 200 patients.

As defined in the study protocol, we conducted a modified intention-to-treat (mITT) analysis: participants who withdrew consent because they insisted on starting early cART while randomized to the no treatment arm or those who declined therapy after being randomized to one of the treatment arms were excluded from the analysis. In a standard intention-to-treat analysis, patients who are randomized to the no treatment arm but insist on starting treatment immediately have 0 d of survival since they reach the study endpoint immediately at initiation of treatment, and patients who are randomized to one of the treatment arms but refuse to start early treatment reach the study endpoint only at the moment they eventually start cART. This results in a strong and unnecessary bias in favor of the treatment arms. Therefore, we report the mITT analysis, in which patients who did not start with the allocated treatment plan were excluded.
Participants who were lost to follow-up while still on early cART were not included in the analyses. Patients who discontinued early cART before completing the scheduled period of 24 or 60 wk remained in the mITT analysis. Participants who did not discontinue early cART at the scheduled TI date or were lost to follow-up after randomization/TI were considered having reached the (re)start endpoint. Per protocol analyses were performed excluding patients who discontinued cART earlier than planned and patients who did not discontinue cART at the scheduled TI date but instead elected to continue cART for reasons other than a low CD4 count or symptomatic disease. Patients who had not yet (re)started cART were censored at the last follow-up visit.

Reported results are data from the three-way randomized patients unless stated otherwise. Viral setpoint and the CD4 cell count measured at viral setpoint were compared using one-way ANOVA. The evolution of pVL and CD4+ T cell count decline following randomization/TI in the no treatment/treatment arms were analyzed using linear mixed models incorporating repeated measurements. Both the pVL and CD4 evolution showed a tri-phasic pattern with distinct slopes from week 0 to 8, week 8 to 36, and week 36 to 144. The total time off therapy for the no treatment versus the treatment arms was analyzed using the mITT population of the three-way randomized patients. For the second comparison, which evaluated the optimal duration of early cART, we combined the data of all treated patients, including those participants who were randomized over the two treatment arms. In both analyses, the total time off therapy was compared across the study arms using Kaplan-Meier plots and log rank tests.

We fitted a series of multivariable Cox regression models of time to (re)start of cART using the mITT population and the per protocol population of the three-way randomized patients separately and of the three- and two-way randomized patients combined. Because the sample size of our study was relatively small, the study arms might by chance be imbalanced with regard to certain prognostic factors. To reduce potential bias, we weighted all Cox regression analyses using propensity score weights [29]. Weights were calculated separately for each analysis using multivariable logistic regression with a generalized logit link function. The models included potential confounders that from the literature are known to influence HIV disease progression and that were measured at baseline: gender, age, country of origin, HIV transmission risk category, baseline CD4 cell count, stage of PHI, symptomatic during PHI, resistance profile, HIV-1 subtype B/non-B, protective HLA alleles, X4/R5-tropism, heterozygosity for CCR5 Δ32 deletions, and viral hepatitis. All models using data from the three- and two-way randomized patients combined were additionally adjusted for the randomization scheme of each patient. The proportional hazards assumption was checked.

We hypothesized that the proposed mechanism by which early cART may result in a clinical benefit was by lowering of the viral setpoint and/or by increasing the CD4 cell count during the treatment period. Therefore, we also fitted Cox models that included the viral setpoint at 36 wk after randomization/TI in the no treatment/treatment arms and the CD4 cell count measured at viral setpoint. To further explore which factors might be associated with time to (re)start of cART, other than temporary early cART, viral setpoint, and CD4 cell count at viral setpoint, we performed a final sub-group analysis for which we selected all patients who had been randomized to one of the early treatment arms and fitted a multivariable Cox model using a stepwise selection process using all aforementioned variables. Data were analyzed using SPSS.
version 18.0 (SPSS) and SAS version 9.1.3 (SAS Institute). All reported \( p \)-values were two-sided and considered statistically significant when less than 0.05.

Results

Patient Characteristics

The patient enrollment is summarized in Figure 1: 238 eligible PHI patients were screened, of whom 173 were enrolled. Four participants withdrew consent and one patient developed an acute hepatitis B infection and was withdrawn from the study by the treating physician immediately following randomization. These five patients were excluded from all analyses. The mITT population consisted of 168 patients: 115 were randomized over the three study arms, and 53 were randomized over the two treatment arms only. Four patients were lost to follow-up while on early cART and were not included in the survival analyses. Of the three-way randomized patients, five interrupted cART earlier than planned, and three did not interrupt cART as planned, of whom two had a low CD4 cell count at the scheduled TI date. Of the two-way randomized participants, one interrupted cART earlier than planned, and five participants did not discontinue early cART as planned, of whom one had a low CD4 cell count at the scheduled TI date. These 14 patients remained, according to the protocol, in the analysis (Figure 1). Participants were followed for a period between 3 and 400 wk, with a median follow-up of 100 (interquartile range [IQR] 60–160) wk.

Demographic and baseline characteristics of the three-way randomized patients are summarized in Table 1. Most patients were men who have sex with men, had a negative or indeterminate Western blot (Fiebig stage I–IV), and were symptomatic during PHI. The mean baseline CD4 cell count was 458 (standard deviation [SD] 183) cells/mm\(^3\) in the no treatment arm, and 584 (230) cells/mm\(^3\) and 483 (241) cells/mm\(^3\) in the 24- and 60-wk treatment arms. The multivariable Cox regression analyses were adjusted for this baseline difference. Nine of 99 patients (9%) for whom HIV genotyping was available harbored transmitted drug-resistance mutations: in eight patients a single mutation in reverse transcriptase or protease was present, and one patient harbored a mutation in both reverse transcriptase and protease. All participants were treated with at least three active drugs during early cART. The presence of CXCR4-using viruses at baseline, CCR5 Δ32 heterozygosity, and HLA B27 or B57 were equally distributed between the three study arms. One untreated patient had a chronic hepatitis B infection, and none had hepatitis C infection. Patients on early cART had a median delay of 2 (IQR 0–6) d between randomization and start of early cART. The study drugs were well tolerated and caused no serious adverse events.

No differences in baseline characteristics were seen between the three- and two-way randomized treated patients, with the exception of the nucleoside backbone (zidovudine/lamivudine versus tenofovir/emtricitabine) used during early cART (Table 2).

Treatment Interruption, Viral Setpoint, and CD4 Cell Count Changes

Of the three-way randomized patients, 66/76 treated participants (87%) had a pVL below 50 copies/ml at the time of TI. Five patients interrupted early cART on the scheduled TI date even though their pVL was not below 50 copies/ml (median pVL 161 [range 74–262] copies/ml).
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Figure 1. Consort Flow Diagram. cART, combination antiretroviral therapy; HBV, hepatitis B virus; LTFU, loss to follow-up; mITT, modified intention-to-treat; PHI, primary HIV-1 infection; TI, treatment interruption.
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| Table 1. Baseline characteristics of 115 patients randomized over 3 study arms |
|---------------------------------|------------------|------------------|------------------|
|                                  | No treatment     | 24 weeks cART    | 60 weeks cART    |
|                                  | (N=36)           | (N=40)           | (N=39)           |
| Age (years), mean (SD)           | 42 (11)          | 40 (10)          | 39 (9)           |
| Male                             | 36 (100)         | 36 (90)          | 38 (97)          |
| MSM                              | 31 (86)          | 31 (78)          | 34 (87)          |
| Born in the Netherlands          | 33 (92)          | 33 (83)          | 32 (82)          |
| Stages of PHI:                   |                  |                  |                  |
| - Fiebig I-IV                    | 27 (75)          | 27 (68)          | 30 (77)          |
| - Fiebig V-VI                    | 9 (23)           | 13 (32)          | 9 (23)           |
| Acute retroviral syndrome        | 30 (83)          | 31 (78)          | 34 (87)          |
| CD4 cell count (cells/mm³), mean (SD) | 458 (183)      | 584 (230)        | 483 (241)        |
| Plasma HIV-1 RNA (log_{10} copies/ml), mean (SD) | 5.1 (0.9)      | 5.1 (0.9)        | 4.9 (0.9)        |
| Genotypic resistance mutations   | 1 (3)            | 5 (15)           | 3 (9)            |
| Subtype B virus                  | 29 (91)          | 30 (91)          | 31 (91)          |
| HLA B27 or B57                   | 1 (5)            | 1 (5)            | 1 (4)            |
| CCR5Δ32 heterozygosity           | 1 (8)            | 3 (14)           | 5 (29)           |
| CXCR4-using virus                | 0 (0)            | 0 (0)            | 1 (6)            |
| Interval between diagnosis and randomization (weeks), median (IQR) | 4 (2-6)        | 4 (3-6)          | 4 (2-6)          |
| Early cART nucleoside backbone:  |                  |                  |                  |
| - zidovudine/lamivudine          | n.a.             | 19 (48)          | 22 (56)          |
| - tenofovir/emtricitabine        | n.a.             | 21 (52)          | 17 (44)          |

Data are n (%) unless indicated otherwise. cART, combination antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; PHI, primary HIV infection; SD, standard deviation.

* 16 patients with missing data.
1 51 patients with missing data.
5 65 patients with missing data.
4 60 patients with missing data.

The remaining five participants had interrupted early cART before the scheduled TI date and had a median pVL of 977 (range 95–100,000) copies/ml.

Twelve weeks after TI, 69/71 participants (97%) for whom a pVL was available had a viral rebound. Following TI, the pVL rebounded during the first 8 wk, with an estimated increase of 0.29 and 0.23 log_{10} copies/ml/wk in the 24- and 60-wk treatment arms, respectively (p = 0.05). From week 8 to 36 after randomization/TI the pVL did not change significantly in the no treatment and 24-wk treatment arms, but increased by 0.01 log_{10} copies/ml/wk in the 60-wk treatment arm (p = 0.02; Figure 2A). The mean viral setpoint, 36 wk after randomization/TI, was 4.8 (SD 0.6) log_{10} copies/ml in the no treatment arm, and 4.0 (1.0) and 4.3 (0.9) log_{10} copies/ml in the 24- and 60-wk treatment arms, respectively (comparing all three study arms, p < 0.001). Seven patients in the 24- and 60-wk treatment arms had a pVL < 1,000 copies/ml at viral setpoint, of whom four had a pVL below 100 copies/ml, as compared to none in the no treatment arm. From week 36 to 144 the pVL increased by 0.18 and 0.21 log_{10} copies/ml/y (p = 0.9) in the 24- and 60-wk treatment arms until the pVL in the three study arms slowly converged 2–3 years after TI.
The mean CD4 cell count at TI in the 24- and 60-wk treatment arms was 737 (SD 245) and 672 (245) cells/mm$^3$, respectively ($p = 0.3$). After TI the CD4 cell counts showed an initial rapid decline during the first 8 wk, with an estimated loss of 6.3 and 10.7 cells/mm$^3$/wk in the 24- and 60-wk treatment arms, respectively ($p = 0.3$). From week 8 to 36 and week 36 to 144 after randomization/TI, the CD4 count decline was similar between all three study arms ($p = 0.9$ and $p = 1.0$ for both periods respectively; Figure 2B). The mean CD4 count at viral setpoint was 383 (SD 158) cells/mm$^3$ in the no treatment arm, and 584 (202) and 503 (254) cells/mm$^3$ in the 24- and 60-wk treatment arms, respectively (comparing all three study arms, $p < 0.001$).
Figure 2. pVL and CD4 cell count after randomization/treatment interruption in the no treatment and treatment arms. Modelled mean plasma viral load (pVL; 2A) and CD4+ T cells (2B) over time after randomization/treatment interruption in respectively the no treatment and the 24- and 60-week treatment arms of the patients randomized over the three study arms. Graphs show the estimates (± standard error of the mean) from the linear mixed models. The box below each graph shows the number of pVL and CD4+ T-cell measurements at each time point used for fitting the linear mixed models.
Total Time Off Therapy

At the time of analysis, 32/36 patients (89%) in the no treatment arm had initiated cART, while 22/38 (58%) in the 24-wk treatment arm and 24/38 (63%) in the 60-wk treatment arm had restarted cART ($p = 0.008$). 54/78 participants (69%) (re)started cART according to the CD4 count criteria, four patients (5%) because of severe symptoms/AIDS diagnosis, and 20 (26%) patients because of a preference by physician or patient to restart cART. Of note, 14 of these 20 patients had already had one CD4 count <350 cells/mm$^3$. The mean CD4 cell count at (re)start was 294 (SD 126) cells/mm$^3$ in the no treatment arm, and 322 (114) and 317 (127) cells/mm$^3$ in the 24- and 60-wk treatment arms ($p = 0.7$). The median time off therapy after randomization/TI was 0.7 (95% confidence interval [CI] 0.0–1.8) years in the no treatment arm, and 3.0 (1.9–4.2) and 1.8 (0.5–3.0) years in the 24- and 60-wk treatment arms, respectively (log rank test comparing all three study arms, $p < 0.001$; Figure 3A). Using a CD4 cell count threshold of 500 instead of 350 cells/mm$^3$ for (re)start of cART, 34/36 (94%) patients in the no treatment arm, 26/38 (68%) in the 24-wk treatment arm, and 27/38 (71%) in the 60-wk treatment arm would have (re)initiated cART ($p = 0.01$). The median time off therapy after randomization/TI would have been 0.5 (95% CI 0.4–0.6) years in the no treatment arm, and 2.0 (1.2–2.9) and 0.7 (0.3–1.0) years in the 24- and 60-wk treatment arms, respectively (log rank test, $p = 0.002$).

Combining all treated patients ($n = 128$), including those participants who were randomized over the two treatment arms, the median time off therapy was 3.1 (95% CI 2.1–4.0) and 2.1 (1.1–3.1) years in the patients treated for 24- and 60-wk, respectively ($p = 0.03$). However, 6/128 patients discontinued cART earlier than planned, and 5/128 did not discontinue cART at the scheduled TI date even though their CD4 cell counts were high (range 480–863 cells/mm$^3$; Figure 1). Excluding these 11 patients in the per protocol analysis, the median time off therapy was 3.0 (95% CI 2.1–3.9) and 2.2 (1.1–3.3) years in the patients treated for 24- and 60-wk, respectively (log rank test, $p = 0.1$; Figure 3B).

Factors Associated with Time to (Re)Start of cART

We fitted six Cox regression models to compare the time to (re)start of cART between the patients in the no treatment and 24- and 60-wk treatment arms (Table 3). To account for potential bias, including the differences in baseline CD4 cell count between the three study arms, all Cox models were adjusted for differences between groups in potential confounding factors by propensity score weighting. In the adjusted Cox model for the mITT population of the three-way randomized patients, the hazard ratio for (re)start was significantly smaller for both treatment arms compared to the no treatment arm (Model 1; Table 3). There were no significant differences between the 24- and 60-wk treatment arms ($p = 0.45$). Repeating the analysis for the per protocol population of the three-way randomized patients yielded similar results (Model 2). Then we pooled the three-way and two-way randomized patients and performed Cox analyses for both the mITT population and the per protocol population, adjusting both analyses for the randomization scheme of each patient. The hazard ratio for time to (re)start of cART was significantly smaller in both treatment arms as compared to the no treatment arm (Models 3 and 4), and there were no significant differences between the two treatment arms ($p = 0.77$ and $p = 0.66$, respectively).
Figure 3. Probability of remaining off treatment in the no treatment and treatment arms. Kaplan-Meier curves of the probability to remain off-treatment, for the no treatment arm versus the 24- and 60-week treatment arms in the patients randomized over the three study arms using the mITT-population (3A), and for the 24 versus the 60-week treatment arms, including both treated patients randomized over all three study arms and patients randomized over the two treatment arms, using the per-protocol population (3B).
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To explore the contribution of the lowering of the viral setpoint and the increase in CD4 cell count during early treatment to the timing of (re)start of cART, we added these two parameters to the Cox models. Both the viral setpoint and the CD4 count at setpoint were strong and independent predictors of time to (re)start of cART in both the three-way randomized patients and the three- and two-way randomized patients combined (Models 5 and 6). In these two models, early cART was no longer significantly associated with time to restart of cART, suggesting that the lowering of the viral setpoint and the increase of the CD4 cell count during the early treatment period explained for the most part why early cART resulted in a clinical benefit.

In an attempt to identify further factors that predict a longer time to restart of cART, we performed a final sub-group analysis of all patients who had been treated with temporary early cART. This model included both the viral setpoint and CD4 count at setpoint. We found that the stage of PHI, the self-reported occurrence of an acute retroviral syndrome, baseline pVL, and all other investigated factors were not statistically significantly associated with time to (re)start of cART.

Discussion
The present randomized trial provides the strongest evidence to date of a clinical benefit of temporary cART initiated during PHI. Early cART transiently lowered the viral setpoint by 0.5–0.8
log10 copies/ml, increased the CD4 cell count, and deferred the need for initiation of cART during chronic HIV infection by 1.1–2.3 years. Both viral setpoint and CD4 cell count measured at viral setpoint were associated with time to (re)start of cART. The duration of temporary early cART was not predictive, suggesting that 24 wk of cART would be sufficient. The time off therapy was longer in the 24-wk treatment arm than in the 60-wk treatment arm, but in the per protocol analysis, and in the Cox models, which were adjusted for possible confounders, there was no statistically significant difference between the 24- and 60-wk treatment arms.

Overall, these findings are in agreement with the data of the SETPOINT study and SPARTAC trial, in which, respectively, 36 and 48 wk of cART during early HIV infection modestly delayed the need for subsequent initiation of cART [19,20].

An important strength of our study is that it showed a significant benefit of temporary early cART as compared to deferred therapy even using conservative mITT analyses, in which patients who discontinued cART earlier than planned remained in the analyses and those who did not discontinue cART at the scheduled TI date were considered as having reached the study endpoint.

The study has several limitations. First, despite the random allocation of patients to the different study arms, the mean baseline CD4 cell count was by chance higher in the 24-wk treatment arm, which might have affected the time to restart in this group. However, the mean baseline CD4 cell count was not different between the no treatment and 60-wk treatment arms, whereas the time off therapy was significantly longer in the patients treated for 60 wk (p = 0.02). More importantly, after adjusting for baseline CD4 cell count in the Cox models, temporary early cART remained associated with a longer time to subsequent reinitiation of cART. Second, by providing the option of randomizing patients over the two treatment arms only, we might have introduced selection bias, as patients with more severe symptoms might have been less likely to be enrolled in the three-way randomization. However, we observed that the baseline characteristics, viral setpoint, CD4 cell count measured at viral setpoint, and the time to (re)start of cART did not differ between the three- and two-way randomized treated patients. Additionally, the adjusted Cox models also showed that the time to (re)start of cART was not different for the treated patients randomized over three or two study arms. Third, according to current treatment guidelines zidovudine/lamivudine was replaced by tenofovir/emtricitabine halfway through the trial. The proportion of PHI patients treated with both drugs was however comparable in both treatment arms (Table 1).

The rate of HIV disease progression in our study was high: the median time off therapy in the no treatment arm was less than 1 y. More than 80% of patients presented with an acute retroviral syndrome, which is a strong predictor of HIV disease progression [30], suggesting that our results may not be generalizable to asymptomatic seroconverters. Our findings are consistent with a German cohort study in which 56 patients with untreated PHI had a median time to CD4 cell count <350 cells/mm³ of 8.3 months after seroconversion [31]. Data from the CASCADE cohort, a collaboration of international cohorts of patients with a well-estimated date of HIV seroconversion [32], demonstrated that in 179 untreated seroconverters the median time to initiation of cART or reaching a CD4 cell count below 350 cells/mm³ was approximately 1.5 years [6]. However, it was not reported whether patients were symptomatic or not during the acute stage of the disease.
The stage of PHI was not associated with time to (re)start of cART. This is in contrast with findings from two previous cohort studies. In one study, initiation of cART within 2 wk of antibody seroconversion was associated with viral and immunological benefits during at least 24 wk after TI as compared to starting therapy between 2 wk and 6 months after HIV seroconversion [3]. The second study reported that if early cART was initiated within 60 d after estimated HIV infection, it resulted in reduced pVL and proviral HIV DNA levels as compared to later starters (between 61 and 120 d) or untreated controls for more than 1 y after TI [33]. The treated patients in our study had a median delay of 5 (IQR 3–7) wk between the diagnosis of PHI and the start of early cART, which means that the golden hour, in which the greatest benefits of early cART could have been achieved, was possibly already missed. Nevertheless, even with this delay we observed a clear benefit of initiating cART during PHI.

Temporary cART initiated during PHI deferred the subsequent restart of cART, which, according to the Cox models, was most likely caused by the effects of the CD4 gain during treatment and the transient lowering of the viral setpoint. The question remains how the latter might be explained. We know that two critical events in PHI are the massive destruction of CD4 T cells in the gastrointestinal tract and the establishment of latent HIV reservoirs. Early treatment may result in viral suppression and immune restoration in gut-associated lymphoid tissue [34]. Alternatively, it may have an effect on the cellular HIV proviral load and limit the size of the viral reservoirs [35,36]. Others have postulated that early treatment enables virus-specific CD8+ T cells to mature into fully differentiated effector cells, which might be important in viral control [37]. Early treatment is also suggested to help preserve specific anti-HIV responses [9,38], though we previously found that HIV specific CD4 T cell responses provided no explanation for the lower viral setpoint in patients treated during PHI [39]. Recently, we reported that HIV-1 dual infection (coinfection or superinfection) was the main factor associated with CD4 cell count decline in a cohort of 37 untreated men with subtype B PHI [40]. A potential benefit of temporary early cART may therefore be the prevention of early HIV-1 superinfection.

The gain in treatment-free years must be weighed against potential disadvantages of starting cART in the acute stage of the disease, a period in which patients are often physically and emotionally distressed and adherence may be suboptimal. In addition, early cART is often initiated before baseline resistance test results are available and therefore may require “overtreatment” to ensure an effective drug regimen, which may increase the risk of drug toxicity. In our study, patients were started on a triple-class regimen. In any case, if early treatment is considered, it should at least include a boosted protease inhibitor until resistance testing results are available [1]. Another concern may be the risk of developing drug resistance after TI. Extended follow-up studies will be needed to address this question.

Structured TI studies in chronic HIV infection have fallen into disfavor after the SMART trial demonstrated that CD4-guided TI was associated with adverse outcomes and a rapid CD4 cell count decline as compared to continuous therapy [41]. The question is whether this also holds true for patients who have initiated cART shortly after seroconversion, before the development of severe immunological dysfunction [42]. The ANRS PRIMO cohort reported that a larger increase in CD4 cells during early cART was associated with a markedly steeper decline after TI, and the benefit of a limited course of cART was questioned [17]. In our study, after an initial
rapid, but limited CD4 cell decline during the first 8 wk following TI in both the 24- and 60-wk treatment arms, the slope of CD4 cell decline was comparable between all three study arms.

Nonetheless, even in the patients in our study who received temporary early cART, the total time off therapy was relatively short. The lowering of the viral setpoint was transient, suggesting loss of protective immune functions and the emergence of viral escape mutants. Therefore, a reasonable question is whether early cART should not be interrupted but continued for life, given the concern that uncontrolled HIV replication and chronic immune activation carry an increased risk of morbidity and mortality at all stages of HIV infection [27]. Additionally, the continuation of cART may have a public health benefit as it decreases infectiousness [43–45].

In conclusion, this randomized study demonstrates a clear clinical benefit of temporary cART initiated during PHI. Early cART transiently lowered the viral setpoint and deferred the need for restart of cART during chronic HIV infection. Although extended follow-up studies are needed to evaluate the long-term benefits of such early treatment, starting cART when the patient is ready to do so seems the most reasonable advice for patients with PHI.

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Editors’ Summary

Background Every year, nearly three million people become infected with HIV, the virus that causes AIDS. The first stage of HIV infection—primary HIV infection—lasts a few weeks and often goes undetected, although most individuals develop a short, flu-like illness. During this stage of infection, the immune system begins to make antibodies to HIV. The second stage of HIV infection, which lasts many years, also has no major symptoms but, during this stage, HIV slowly destroys immune system cells, including CD4 cells, a type of lymphocyte. Eventually, the immune system is unable to fight off other infections and patients enter the third phase of HIV infection—symptomatic HIV infection. The final stage—AIDS—is characterized by the occurrence of one or more AIDS-defining conditions, which include severe but unusual infections and several types of cancer. Early in the AIDS epidemic, most HIV-positive people died within ten years of infection. Nowadays, although there is still no cure for HIV infection, HIV has become a chronic disease because of the availability of combination antiretroviral therapy (cART; cocktails of several powerful drugs). This means that many HIV-positive people have a near-normal life span.

Why Was This Study Done? It is currently recommended that people start cART when their CD4 count falls below 350 CD4 cells per cubic milliliter (cells/mm3) of blood, when they develop severe constitutional symptoms such as fever lasting longer than a month, or when they develop an AIDS-defining condition. But could a short course of cART during primary HIV infection be clinically beneficial? Some, but not all, nonrandomized studies have shown that such treatment reduces the viral set point (the stabilized viral load that
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is reached after the immune system begins to make antibodies to HIV; the viral load is the amount of virus in the blood) and slows the decline of CD4 cell count in patients. In this randomized trial (the Primo-SHM trial), the researchers assess the clinical benefit of temporary cART initiated during primary HIV infection by measuring its effects on the viral set point and on when patients have to restart cART during chronic HIV infection. In a randomized controlled trial, patients are assigned by the play of chance to receive different treatments and then followed to compare the effects of these treatments.

What Did the Researchers Do and Find? The researchers assigned 168 patients with primary HIV infection to receive no treatment, 24 weeks of cART, or 60 weeks of cART. They measured the viral set point (the viral load in the blood 36 weeks after randomization in the no treatment arm and 36 weeks after cART interruption in the treatment arms) and determined the time off therapy (the time between randomization and the start of cART in the no treatment arm, and the time between treatment interruption and restart of cART in the treatment arms) for each patient. cART was (re)started following two consecutive CD4 counts below 350 cells/mm³ or when symptomatic HIV disease developed. The average viral set point was lower in the patients who received early cART than in those who had no treatment. Moreover, on average, the patients in the no treatment arm started cART 0.7 years after randomization whereas those in the 24- and 60-week treatment arms restarted cART after 3.0 and 1.8 years, respectively. There was no statistically significant difference between the 24-week and 60-week treatment arms in time off therapy.

What Do These Findings Mean? These findings suggest that temporary cART during primary HIV infection can transiently lower the viral set point and can delay the need to restart cART during chronic HIV infection. They also suggest that 24 weeks of cART during primary HIV is as effective as 60 weeks of treatment. These findings need to be confirmed in other settings, and follow-up studies are needed to evaluate the long-term benefits of early temporary cART, but given the short time between cART interruption and treatment restart, the researchers suggest that not interrupting early cART, but instead continuing it for life, should be considered. However, they add, because patients are often physically and emotionally distressed at this stage of HIV infection, adherence to cART during primary HIV infection may be suboptimal, and so patients with primary HIV infection should be advised to start cART only when they feel ready to start treatment.

Additional Information Please access these websites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.1001196.

- Information is available from the US National Institute of Allergy and Infectious Diseases on HIV infection and AIDS, including information on the clinical progression of HIV infection
- NAM/aidsmap provides information about HIV/AIDS, including a factsheet on primary infection
- Information is available from Avert, an international AIDS charity on many aspects of HIV/AIDS, including detailed information on HIV treatment and care and on the stages of HIV infection (in English and Spanish)
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- The World Health Organization’s 2010 antiretroviral therapy guidelines provide recommendations on when to initiate cART
- Information about Primo-SHM is available
- Patient stories about living with HIV/AIDS are available through Avert and through the charity website Healthtalkonline
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References


Early treatment of primary HIV infection


Temporary antiretroviral treatment during primary HIV-1 infection has a positive impact on health-related quality of life: data from the Primo-SHM cohort study

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Abstract

Objectives
The aim of the study was to compare health-related quality of life (HRQL) over 96 weeks in patients receiving no treatment or 24 or 60 weeks of combination antiretroviral therapy (cART) during primary HIV-1 infection (PHI).

Methods
A multicentre prospective cohort study of PHI patients, with an embedded randomized trial, was carried out. HRQL was assessed with the Medical Outcomes Study Health Survey for HIV (MOS-HIV) and a symptom checklist administered at weeks 0, 8, 24, 36, 48, 60, 72, 84 and 96. Mixed linear models were used for the analysis of differences in HRQL among the three groups.

Results
A total of 112 patients were included in the study: 28 received no treatment, 45 received 24 weeks of cART and 39 received 60 weeks of cART. Over 96 weeks of follow-up, the groups receiving 24 and 60 weeks of cART had better cognitive functioning than the no-treatment group \( (P = 0.005) \). Patients receiving 60 weeks of cART had less pain \( (P = 0.004) \), better role functioning \( (P = 0.001) \), better physical functioning \( (P = 0.020) \) and a better physical health summary score \( (P = 0.006) \) than the groups receiving no treatment or 24 weeks of cART. Mental health was better in patients receiving 24 weeks of cART than in patients in the no-treatment group or the group receiving 60 weeks of cART \( (P = 0.020) \). At week 8, patients in the groups receiving 24 and 60 weeks of cART reported more nausea \( (P = 0.002) \), diarrhoea \( (P < 0.001) \), abdominal pain \( (P = 0.023) \), stomach pain \( (P = 0.049) \) and dizziness \( (P = 0.011) \) than those in the no-treatment group. These differences had disappeared by week 24.

Conclusions
Temporary cART during PHI had a significant positive impact on patients’ HRQL as compared with no treatment, despite the initial, short-term occurrence of more physical symptoms, probably related to drug toxicity.
Quality of life during primary HIV infection

Introduction

The impact of temporary combination antiretroviral therapy (cART) during primary HIV-1 infection (PHI) on viral setpoint and HIV disease progression has recently been studied in three randomized clinical trials (RCT) and showed that early cART provided a clinical benefit [1-3]. In the Primo-SHM trial, an open-label RCT comparing no treatment with 24- or 60-weeks of cART during PHI, we demonstrated that temporary early cART lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV-1 infection [1]. Both the SPARTAC trial, which compared no therapy with 12 or 48 weeks of cART in PHI, and the SETPOINT study, which compared no therapy with 36 weeks of cART, reported that a period of 48 and 36 weeks of cART, respectively, modestly delayed disease progression [2, 3]. However, during the acute stage of HIV-1 disease patients are often physically and emotionally distressed, and the initiation of cART may have a negative impact on their health-related quality of life (HRQL) as a result of pill burden, the need for strict adherence to cART and potential drug-related adverse events and toxicity [4, 5]. Conversely, early cART may also have a positive effect on patients’ HRQL, by delaying disease progression, lowering the plasma viral load and because patients may feel they are actively “doing something” about the PHI [6]. In chronic HIV infection the potential negative effects of cART on patients’ HRQL are generally offset by positive effects [7-10]. The aim of the current Primo-SHM substudy was to compare the impact on HRQL of 24- or 60-weeks of cART during PHI versus no treatment, over a study period of 96 weeks.

Methods

Patients

Patients were selected between May 2003 and April 2010 from the Primo-SHM cohort, a multi-center prospective cohort study in the Netherlands, with an embedded RCT, that investigates the natural course of HIV-1 infection, and the effects of 24- and 60-weeks of early cART in PHI-patients [1, 11]. For the present substudy, we included both patients from the cohort and the RCT. Main inclusion criteria were age ≥ 18 years and laboratory evidence of PHI, defined as having a negative or indeterminate Western Blot in combination with detectable plasma HIV-1 RNA, or, in case of a positive Western Blot, a proven negative HIV screening test result within the previous 180 days. Early cART consisted of a triple-class regimen of zidovudine/lamivudine (300/150 mg BID), efavirenz (600 mg QD), and lopinavir/ritonavir capsules (533/133 mg BID). Lopinavir/ritonavir was discontinued when the pVL dropped below 50 copies/ml. After January 2008, zidovudine/lamivudine was replaced by tenofovir/emtricitabine (245/200 mg QD), and lopinavir/ritonavir tablets (600/150 mg BID) replaced the capsules. Patients needed to have sufficient fluency in Dutch or English to complete a self-administered HRQL-questionnaire. Recruitment of participants and study design have been described previously [1, 11]. The study was approved by the Medical Ethics Committee of each participating site and written informed consent was obtained from all participants.

Quality of life measurement

Patients received a self-report questionnaire measuring HRQL when attending the outpatient clinic for the study visits at weeks 0, 8, 24, 36, 48, 60, 72, 84 and 96. The questionnaire consisted
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of two parts: the Medical Outcomes Study Health Survey for HIV (MOS-HIV) and a symptom checklist. The MOS-HIV is a widely used questionnaire comprising 10 subscales [12]. Physical health (PHS-) and mental health summary (MHS-) scores can be calculated on the basis of these subscale scores [13]. Higher scores indicate a better HRQL.

The symptom checklist consisted of 14 items referring to symptoms related to PHI or to side-effects of cART, i.e.: difficulty with sleeping, lack of appetite, nausea, vomiting, diarrhoea, abdominal or stomach pain, fever, flu-like symptoms such as myalgia or chills, tingling of hands or feet, numb feeling in fingers or toes, dizziness, itchiness and skin changes. These items were derived from the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire – Core 30 and an HIV/AIDS-specific questionnaire [9]. The questions related to the experience of symptoms during the past week. Symptoms were scored on a four-point scale with the response categories ‘not at all’, ‘a little’, ‘quite a bit’, and ‘very much’. The 4-point scale scores were linearly transformed to a scale of 0 to 100, with higher scores indicating more symptoms.

Statistical analyses
We included patients who completed a HRQL-questionnaire at baseline and at least one questionnaire during follow-up. Baseline characteristics were compared using Chi-squared tests for categorical variables and general linear models or Kruskal-Wallis tests for continuous variables. Linear mixed effect models for repeated measurements were used to test for differences in MOS-HIV and symptoms scores during follow-up between the three groups, with baseline values included as covariate. Model results were summarized by the estimated mean values during follow-up for the three groups, adjusted for baseline measurements. To investigate potential short-term toxicity of cART, we also compared the symptom scores among the three groups at week 8 using general linear models, with the baseline measurement included as covariate.

To increase the sample size, we also included untreated patients who were not randomized in the trial but were enrolled in the Primo-SHM cohort. To assess a potential difference between randomized and non-randomized untreated patients, we compared their baseline characteristics using Chi-squared tests or Kruskal-Wallis tests, if appropriate, and their HRQL at baseline and at each follow-up visit using student t-tests. Additionally, we repeated the mixed linear models including only those patients who were enrolled in the RCT. Analyses were according to intent-to-treat, regardless of treatment changes or discontinuation. Two-sided P-values <0.05 were considered statistically significant. Data were analyzed using SPSS version 16.0 (IBM Corporation, USA).

Results
Patient characteristics
Of 168 participants enrolled in the Primo-SHM RCT, 100 (60%) were included in the present study: 16 in the no treatment, 45 in the 24-weeks of early cART and 39 in the 60-weeks of early cART group. For 25 of the 168 participants (15%), no baseline HRQL-questionnaire was available, and they were therefore excluded from further analyses. The reasons for excluding the other 43 participants (26%) were that the patient had insufficient language skills or did not want to complete the HRQL questionnaires, or that the specific study site did not participate in this
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A substudy. Twelve of the 16 eligible non-randomized untreated patients in the Primo-SHM cohort completed HRQL-questionnaires and were included in the present analysis.

A total of 631 questionnaires were completed, with a median of 5 (IQR: 4-8) per patient. Most patients (85%) were men who have sex with men (MSM), 71% had a negative or indeterminate Western blot (Fiebig stage I-IV) and 80% were symptomatic during PHI. Patient characteristics are summarized in Table 1.

**Quality of life**

At baseline, patients receiving no treatment had significantly lower mental health scores \( (P = 0.02) \), lower energy/fatigue scores \( (P = 0.03) \) and lower MHS-scores \( (P = 0.04) \) than patients receiving 60-weeks of cART. Model results were adjusted for these baseline differences.

We found a significant difference between the three groups in five of the ten MOS-HIV subscales and in the PHS-score over the follow-up period of 96 weeks. Patients receiving 24- and 60-weeks of early cART showed better cognitive functioning than patients receiving no treatment \( (P = 0.005, \text{Figure 1A}) \). Participants receiving 60-weeks of early cART experienced less pain \( (P = 0.004) \), and showed better role \( (P = 0.001) \) and physical functioning \( (P = 0.02) \) and had a better PHS-score \( (P = 0.006) \) than patients receiving no treatment or 24-weeks of early cART (Figure 1B-E). Patients receiving 24-weeks of early cART showed a better mental health than patients receiving no treatment or 60-weeks of early cART \( (P = 0.02, \text{Figure 1F}) \). Social functioning, health distress, overall quality of life, energy/fatigue and the MHS-score improved significantly from baseline to 96 weeks of follow-up irrespective of the treatment group (data not shown).

Four symptoms differed significantly between the three groups over the follow-up period of 96 weeks. Patients receiving 24-weeks of early cART more often reported tingling in the hands or

**Table 1. Patient characteristics of the 112 PHI-patients at study entry**

<table>
<thead>
<tr>
<th></th>
<th>No treatment (N=28)</th>
<th>24-weeks of early cART (N=45)</th>
<th>60-weeks of early cART (N=39)</th>
<th>( P )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27 (96)</td>
<td>40 (89)</td>
<td>36 (92)</td>
<td>0.5</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>44 (10)</td>
<td>41 (9)</td>
<td>39 (9)</td>
<td>0.1</td>
</tr>
<tr>
<td>Born in the Netherlands</td>
<td>22 (79)</td>
<td>39 (87)</td>
<td>34 (87)</td>
<td>0.6</td>
</tr>
<tr>
<td>MSM</td>
<td>26 (93)</td>
<td>37 (82)</td>
<td>32 (82)</td>
<td>0.4</td>
</tr>
<tr>
<td>Stage of PHI:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fiebig I-IV</td>
<td>19 (68)</td>
<td>34 (76)</td>
<td>27 (69)</td>
<td>0.7</td>
</tr>
<tr>
<td>- Fiebig V-VI</td>
<td>9 (32)</td>
<td>11 (24)</td>
<td>12 (31)</td>
<td></td>
</tr>
<tr>
<td>Acute retroviral syndrome</td>
<td>22 (79)</td>
<td>34 (76)</td>
<td>34 (87)</td>
<td>0.4</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml), median (IQR)</td>
<td>5.2 (4.6-5.8)</td>
<td>5.0 (4.6-5.7)</td>
<td>5.1 (4.4-5.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>CD4 count (cells/mm(^3)), median (IQR)</td>
<td>500 (333-670)</td>
<td>550 (420-735)</td>
<td>430 (310-650)</td>
<td>0.2</td>
</tr>
<tr>
<td>Early cART nucleoside backbone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- zidovudine/lamivudine</td>
<td>n.a.</td>
<td>26 (58)</td>
<td>24 (62)</td>
<td>0.7</td>
</tr>
<tr>
<td>- tenofovir/emtricitabine</td>
<td>n.a.</td>
<td>19 (42)</td>
<td>16 (41)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data are n (%) unless indicated otherwise. cART, combination antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; PHI, primary HIV-1 infection; SD, standard deviation.

* P-value based on \( \chi^2 \) tests for proportions and general linear models or Kruskal-Wallis tests for continuous variables.
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Figure 1. A: Cognitive functioning, B: Pain, C: Role functioning, D: Physical functioning, E: Physical Health Summary score, F: Mental health.

---·---: no treatment
---+---: 24-weeks of early cART
---Δ---: 60-weeks of early cART

feet ($P = 0.02$) and a numb feeling in fingers or toes ($P = 0.01$) than patients receiving 60-weeks of early cART or no treatment. Patients receiving no treatment reported more itchiness ($P = 0.001$) and skin changes ($P = 0.04$) than patients receiving 24- or 60-weeks of early cART. At week 8, patients receiving 24- or 60-weeks of early cART reported more nausea ($P = 0.002$), diarrhoea ($P < 0.001$), abdominal pain ($P = 0.02$), stomach pain ($P = 0.049$) and dizziness ($P = 0.01$) than patients receiving no treatment (Figure 2). These differences had disappeared at week 24.
No differences in patient characteristics and HRQL at baseline and during follow-up were seen between the randomized (n=16) and non-randomized (n=12) untreated patients, except that the randomized patients were more often born in the Netherlands (15/16 (94%) versus 7/12 (58%), \( P = 0.02 \)). When we repeated the mixed linear models including only the RCT patients, the significant differences in HRQL between the three groups disappeared for cognitive functioning and mental health, even though the trend remained similar. The differences in pain, physical functioning, role functioning and the PHS-score remained significant. For these scales, patients receiving 60-weeks of early cART had significantly better HRQL than patients receiving 24-weeks of early cART. The differences seen in reported symptoms remained the same.

Discussion

The present study was set up as a substudy of the Primo-SHM RCT, which demonstrated a clinical benefit of 24- and 60-weeks of cART initiated during PHI [1]. This substudy provides the first data on the effects on HRQL of temporary treatment during PHI. Early cART did not have a negative impact on patients’ HRQL over a study period of 96 weeks as compared with no treatment. Overall, patients receiving 60-weeks of cART showed a better HRQL than patients in whom treatment was deferred. Although the patients on early cART initially suffered more from physical symptoms, which were probably related to drug toxicity, this seemed to have minor effects on their HRQL perception. This is in agreement with a previous study in which chronic HIV positive persons on cART made distinctions between symptoms caused by HIV itself or by drug toxicity when evaluating HRQL. Disease-related symptoms, but not side effects, were related to perceptions of general health. [14]. Regardless of cART intervention, social functioning, health distress, overall quality of life, energy/fatigue and the MHS-score improved significantly during

![Figure 2](image_url)

Figure 2. Five symptoms were significantly different between the three groups at week 8.

- •••: no treatment
- •••: 24-weeks of early cART
- ••••: 60-weeks of early cART
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the 96 weeks of follow-up in all groups. This might be explained by initial psychological distress as a consequence of being diagnosed with PHI and its acceptance over time. In addition, the symptoms occurring during PHI will also diminish without early treatment over time.

In the Primo-SHM trial, receiving 60-weeks of early cART offered no additional benefit over 24-weeks of early cART with respect to lowering the viral setpoint and the total time off therapy [1]. An unexpected finding of the present study was that patients receiving 60-weeks of early cART had a better HRQL on some of the physical MOS-HIV subscales than patients receiving 24-weeks of early cART. Because this is the first study to report the impact of early cART during PHI on HRQL, we cannot relate this finding to previous studies. This result can either be a real finding or may be the consequence of selection bias, because not all participants enrolled in the RCT completed HRQL-questionnaires. Clearly, this finding should be corroborated in future studies.

The limitation of this substudy is that we included non-randomized untreated PHI-patients to increase the sample size of the no treatment group. However, no differences were observed in HRQL between randomized and non-randomized untreated patients. Additionally, we found a similar trend in results when analyzing only the randomized patients.

In conclusion, in addition to the clinical benefit of temporary cART initiated during PHI, this substudy demonstrates that temporary early cART had a significant positive impact on patients’ HRQL over a study period of 96 weeks, despite the initial, short-term occurrence of physical symptoms, which were most likely related to drug toxicity. These findings provide important additional support for early intervention in patients with PHI and should be taken into account when considering early cART in patients with PHI.

Acknowledgments
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Contributors
MLG, JMP and PTN drafted the manuscript. MLG, RS and JMP established the cohort and together with MGA, MK and CJK were responsible for patient enrolment and trial conduct at each study site. GK performed the data entry and PTN conducted the statistical analysis. All authors provided valuable input into protocol development and interpretation of data, and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
References


Temporary treatment during primary HIV infection does not affect virologic response to subsequent long-term treatment

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Submitted
Abstract

Temporary combination antiretroviral therapy (cART) during primary HIV infection (PHI) did not affect the subsequent virologic response to long-term cART. Concerns for developing drug resistance mutations after TI have not been substantiated: even after interrupting early treatment, which was given during a period of 24 or 60 weeks shortly after HIV acquisition, our study patients still had a good response after subsequent reinitiation of long-term cART. This study contributes to the increasing data supporting temporary cART during PHI.
Introduction

In the Primo-SHM trial, a multicenter randomized trial comparing no treatment with 24- or 60-weeks of combination antiretroviral therapy (cART) during primary HIV infection (PHI), we recently demonstrated that temporary early cART lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV infection [1]. Two other randomized studies also observed a modest delay in disease progression after a short course of cART in PHI [2, 3]. However, an important concern of temporary early cART, and of structured treatment interruptions (TI) in general, is the risk of developing drug resistance mutations after TI, especially in the case of NNRTI-based regimens, which compromise future treatment options. The aim of this study was to assess the effect of temporary cART during PHI on the subsequent virologic response to long-term cART in patients who previously participated in the Primo-SHM trial. To this end, we compared the viral decay and the time to viral (re)suppression between the early treated patients who reinitiated cART and the patients in whom treatment was deferred until conventional criteria to start long-term cART had been reached.

Methods

Between May 2003 and March 2010 168 patients with laboratory evidence of PHI were randomized in the Primo-SHM trial to receive no treatment (naive patients, n=36) or 24 or 60 weeks of early cART (early treated patients, n=132) [4]. PHI was defined as a negative or indeterminate Western blot combined with a detectable plasma viral load (pVL), or, in case of a positive Western blot, a negative HIV screening test result ≤ 180 days. Early cART consisted of a quadruple triple-class regimen containing two NRTIs (zidovudine/lamivudine 300/150 mg bid), an NNRTI (efavirenz 600 mg qd) and a boosted PI (lopinavir/ritonavir capsules 533/133 mg bid). The latter was discontinued when the pVL had dropped <50 copies/ml. After January 2008 zidovudine/lamivudine was replaced by tenofovir/emtricitabine (245/200 mg qd) and lopinavir/ritonavir tablets (600/150 mg bid) replaced the capsules. Changes to this regimen were allowed in case of transmitted drug resistance or if one of the drugs was not tolerated. The study protocol required patients to reach a pVL <50 copies/ml before interrupting therapy as scheduled. Long-term cART was (re)started in case of two consecutive CD4 cell counts below 350 cells/mm³, severe constitutional symptoms, the occurrence of an AIDS defining event, or if the patient preferred on (re)initiating cART. Follow-up visits after (re)start of cART were scheduled according to standard treatment guidelines, i.e. after four weeks of treatment and every three months thereafter.

In the current study, we included the 94 out of 168 participants (56%) who had started or restarted long-term cART by September 2011 and who had at least one pVL measurement after (re)initiation of cART. (Re)Start regimens were at the discretion of the treating physician. Resistance testing was performed at diagnosis of PHI. To investigate possible acquired resistance during or after stopping of early cART, we performed resistance testing of the reverse transcriptase gene retrospectively in the first stored plasma sample with a pVL above 3.0 log₁₀ c/ml after TI, in the subset of early treated patients who were treated with an NNRTI
in the early phase and reinitiated long-term cART with a boosted PI. For the patients who were treated with an NNRTI during PHI and reinitiated with an NNRTI, we assumed that if NNRTI resistance had been acquired during early treatment, it would result in virological failure after restart with an NNRTI-based regimen. Data of the 24- and 60-weeks early treated patients were combined in all analyses because the viral decay was not significantly different between the two groups (data not shown). Sociodemographic characteristics and laboratory data at (re)start of long-term cART were compared between the naive and early treated patients using chi-square, Fisher’s exact and Kruskal-Wallis tests where appropriate.

Viral decay after start/restart of cART in naive/early treated patients was analysed using linear mixed models incorporating repeated measurements, which showed a tri-phasic pattern with distinct slopes from week zero to four, week four to eight and from week eight onwards. For this analysis patients were censored once they reached a pVL <50 copies/ml. A similar analysis was done for the early treated group, comparing viral decay during early initial cART with the decay after subsequent restart of cART. Time to viral (re)suppression, defined as a pVL <50 copies/ml, was compared between the two groups using Kaplan-Meier plots and multivariable Cox regression analysis. All analyses ignored modifications of treatment regimens, but censored patients at the moment of interruption of cART for more than two weeks. Data were analyzed using SAS version 9.2 (SAS institute, USA).

Results

Of the 36 naive and 132 early treated participants in the Primo-SHM trial, 31 (86%) and 63 (48%) had (re)initiated long-term cART by September 2011, respectively. In 52/63 early treated patients all antiretroviral drugs had been stopped simultaneously at TI: at that moment 31/63 (49%) were receiving dual-class NNRTI-based therapy, 15/63 (24%) dual-class boosted PI-based therapy, and 6/63 (10%) triple-class therapy. In the remaining 11/63 patients (17%) a staggered TI method was used, in which the NNRTI was stopped prior to the NRTI-backbone. Six early treated patients (6%) did not have a pVL <50 c/ml at TI (range 58-1882 copies/ml). The median time between TI and restart of long-term cART was 1.9 (IQR 0.9-3.1) years.

89/94 (re)starting participants (95%) were men. Mean age and CD4 count at (re)start were 44 (SD 9) years and 290 (110) cells/mm³, respectively, and were not significantly different between the naive and early treated patients. The naive patients had a higher mean pVL at (re)start 5.0 (SD 0.7) versus 4.7 (0.7) log₁₀ c/ml; P=0.07) and less transmitted drug resistance mutations at the moment of PHI-diagnosis (0 versus 8 (14%); P=0.048). Naive patients initiated long-term cART more often with an NNRTI-containing regimen than early treated patients (24 (77%) versus 37 (59%); P=0.10). Four naive (13%) and 23 early treated patients (37%) (re)started long-term cART with a boosted PI (P=0.03), and three (10%) versus three (5%) patients, respectively, (re) initiated with triple-class therapy (P=0.40).

Drug resistance testing was performed after TI in 20/23 early treated participants who had been treated with an NNRTI and restarted long-term cART with a boosted PI. None of these patients had developed a drug resistance mutation. Of the remaining three patients, one harboured a 103N mutation, which was already present at diagnosis of PHI (this patient had
Viral resuppression after early cART started with the standard quadruple triple-class regimen before baseline resistance testing results were known, once results were available the regimen was adapted and the NNRTI was removed), and for two patients no stored samples were available. The median interval between TI and the timepoint of resistance testing was 4 (IQR 3.7-8) weeks. Other patient characteristics (transmission route, ethnicity, history of CDC-events, virus subtype) were comparable between the two groups. One early treated patient was lost-to-follow-up after restart of long-term cART and he was censored at this visit. Patient characteristics are summarized in Table 1.

### Table 1. Patient characteristics at (re)start of treatment

<table>
<thead>
<tr>
<th></th>
<th>Total (N=94)</th>
<th>No treatment during PHI (N=31)</th>
<th>Early cART during PHI (N=63)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>44 (9)</td>
<td>44 (10)</td>
<td>43 (8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Men</td>
<td>89 (95)</td>
<td>31 (100)</td>
<td>58 (92)</td>
<td>0.2</td>
</tr>
<tr>
<td>MSM</td>
<td>78 (83)</td>
<td>26 (84)</td>
<td>52 (83)</td>
<td>0.9</td>
</tr>
<tr>
<td>Born in the Netherlands</td>
<td>84 (89)</td>
<td>28 (90)</td>
<td>56 (89)</td>
<td>1.0</td>
</tr>
<tr>
<td>History of CDC C-event</td>
<td>11 (12)</td>
<td>2 (7)</td>
<td>9 (14)</td>
<td>0.3</td>
</tr>
<tr>
<td>CD4 count (cells/mm³), mean (SD)</td>
<td>290 (110)</td>
<td>273 (133)</td>
<td>299 (96)</td>
<td>0.3</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log₁₀ copies/ml), mean (SD)</td>
<td>4.8 (0.7)</td>
<td>5.0 (0.7)</td>
<td>4.7 (0.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Subtype B virus</td>
<td>75 (88)</td>
<td>25 (89)</td>
<td>50 (88)</td>
<td>1.0</td>
</tr>
<tr>
<td>Genotypic resistance mutations at diagnosis of PHI</td>
<td>8 (9)</td>
<td>0 (0)</td>
<td>8 (14)</td>
<td>0.048</td>
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</table>

<table>
<thead>
<tr>
<th>Initiation of cART during chronic HIV-infection</th>
<th>Total (N=94)</th>
<th>No treatment during PHI (N=31)</th>
<th>Early cART during PHI (N=63)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>- triple-class therapy</td>
<td>6 (6)</td>
<td>3 (10)</td>
<td>3 (5)</td>
<td>0.4</td>
</tr>
<tr>
<td>- dual-class NNRTI</td>
<td>61 (65)</td>
<td>24 (77)</td>
<td>37 (59)</td>
<td>0.1</td>
</tr>
<tr>
<td>- dual-class PI</td>
<td>27 (29)</td>
<td>4 (13)</td>
<td>23 (37)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are n (%) unless indicated otherwise. cART, combination antiretroviral therapy; MSM, men who have sex with men; (N)NRTI, (non-)nucleoside reverse transcriptase inhibitor; PHI, primary HIV infection; PI, boosted protease inhibitor.

a 9 missing patients: 3 in the non-early treated and 6 in the early treated group.
b 5 patients carried a M41L mutation, 2 patients a M46I mutation of whom one also had a T215S mutation and one carried a K103N mutation.

All naive and early treated patients achieved viral (re)suppression. Viral decay after treatment (re)initiation was similar between the naive and early treated patients: during the first four weeks the pVL decreased with 0.62 and 0.58 log₁₀ copies/ml/week respectively (P=0.32), from week four to eight with 0.087 and 0.13 log₁₀ copies/ml/week (P=0.37), and from eight weeks onward with 0.043 and 0.027 log₁₀ copies/ml/week (P=0.23) (Figure 1A). Adjusting the viral decay for the difference in pVL at (re)start of long-term cART also showed no significant differences between the two groups (data not shown). The median time to viral (re)suppression in naive and early treated patients was 16.4 (IQR 9.6-20.6) and 16.6 (8.7-21.0) weeks, respectively (log-rank, P=0.72). In the Cox analysis early treatment during PHI as compared to no treatment was not associated with time to viral resuppression (HR 0.81 (95% CI 0.27-2.39); P=0.70). As expected, a higher vireamia at (re)start of long-term cART was predictive for a longer time to viral suppression (HR 0.37 per 1 log₁₀ copies/ml increase (95% CI 0.25-0.56); P<0.001). Other
Viral resuppression after early cART

parameters, including reinitiating with an NNRTI-based regimen or with triple-class therapy, were not associated with time to viral (re)suppression (data not shown).

For the early treated group, we additionally compared viral decay during the early treatment episode and subsequent restart of long-term cART (Figure 1B). During the first four weeks the pVL decreased with 0.52 and 0.57 log_{10} copies/ml/week, respectively (P=0.16), from week four to eight with 0.13 and 0.14 log_{10} copies/ml/week (P=0.70), and from eight weeks onward with 0.048 and 0.021 log_{10} copies/ml/week (P=0.003).

Discussion

Temporary cART during PHI was not associated with a diminished virologic response after subsequent reinitiation of long-term cART, when we compared early treated with naive patients and when we compared both treatment episodes in early treated patients. This suggests that temporary early cART did not select for clinically relevant drug resistance and supports the use of early treatment during PHI. Of note, the slower decline of the pVL from 8 weeks onward in

Figure 1. Viral decay after treatment (re)initiation. Viral decay after treatment (re)initiation of long-term cART in naive and early treated patients (1A) and the viral decay after treatment (re)initiation of early cART and subsequent long-term cART in the early treated patients (1B).
the second treatment period of the early treated patients is probably an artefact of the model, because patients had a lower baseline pVL at restart than during early treatment and were usually already undetectable by week 8.

We did not perform resistance testing after TI in all our patients. We therefore cannot exclude with certainty that there might have been selection of drug resistance after TI. This is in particular relevant for patients who had interrupted an NNRTI-based regimen, because of the long half-life of NNRTIs. However, in all patients, irrespective of the regimen, the pVL was resuppressed upon restart, which virtually excludes clinically important mutations. Early treated patients reinitiated long-term cART more often with a boosted PI than naive patients, which may have overcome possible acquired NNRTI mutations. We therefore retrospectively performed genotypic resistance testing after TI in 20/23 of these patients and did not detect any new drug resistance mutation. The reason for the preference of a PI-containing regimen was usually that patients preferred not to restart an NNRTI because of side effects they had experienced previously during the early cART period. Therefore, there is no indication of acquired resistance in our patients.

Our study is supported by another study in which 37 PHI-patients were treated with temporary early cART and no drug resistance was observed after TI [5]. However, in this study the NNRTI was stopped 96h before the NRTI-backbone. Because NNRTIs have a slower metabolism and a low genetic barrier to resistance, simultaneous TI of an NNRTI-containing regimen may result in a period of NNRTI-monotherapy, which may select for drug resistance mutations [6]. NNRTI-drug resistance mutations that were selected after intrapartum exposure to single-dose nevirapine in HIV infected women have been associated with decreased virologic response after subsequent treatment with an NNRTI-containing regimen [7, 8]. Noteworthy, the pVL in these women exposed to single-dose nevirapine was much higher than the pVL in a controlled TI-setting in which patients have an undetectable pVL. In the SMART trial [9], NNRTI-drug resistance mutations were more common in case of simultaneous TI than in case of a staggered or a switched interruption, in which the NNRTI is replaced by a boosted PI [10]. However, in SMART most drug combinations included a zidovudine/lamivudine-backbone in combination with an NNRTI [9], whereas in our trial half of the patients were using a tenofovir-containing regimen, which has a longer half-life [11], and together with an NNRTI forms a more balanced regimen that is less prone to development of drug resistance when treatment is discontinued simultaneously.

To date, there is no clear consensus how to stop cART regimens [12]. In our study we found no indication for selection of drug resistance after interrupting all drugs simultaneously.

In conclusion, temporary cART during PHI was not associated with a reduced virologic response after subsequent reinitiation of long-term cART. Concerns for developing drug resistance mutations after TI have not been substantiated: even if patients interrupt early treatment, they still have a good response after subsequent reinitiation of cART. This study contributes to the increasing data supporting temporary cART during PHI.
Acknowledgments


The authors wish to thank the study participants for helping to establish the Primo-SHM cohort.

Contributors

MLG and JMP established the cohort and together with SJ, FPK, EFS and PK, they were responsible for patient enrolment and trial conduct at each study site. LG assisted with the data retrieval and MLG and FWNMW conducted the statistical analysis. MLG drafted the manuscript and JMP and JL critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
References


Early HIV treatment preserves cytotoxic T-cell functionality

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Submitted
Abstract

Background
In HIV infection, low viral setpoints correlate with slow disease progression. The Primo-SHM trial, a multicenter randomized trial comparing no treatment with 24- or 60-weeks of combination antiretroviral therapy (cART) during primary HIV infection (PHI), recently demonstrated that temporary early cART lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV infection. We studied whether the beneficial effect of early treatment was caused by preservation of immunological responses.

Methods
Twenty-six treated and thirteen untreated PHI-individuals participating in the Primo-SHM study were compared at viral setpoint: 36 weeks after treatment interruption (TI) and randomization, respectively. We studied i) effector T-cell formation and function, by measuring terminal differentiation and ex vivo expression of the cytolytic molecules perforin and granzyme B in CD4 and CD8 T cells by flow cytometry, ii) poly-functionality of CD8 T cells, by measuring the cytokines TNFα, IFNγ, and IL-2, and the chemokine Mip1β after stimulation with an overlapping peptide-pool of the HIV-gag protein, and iii) regulation of the cellular immune response by measuring various inhibitory and regulatory markers on T, B and NK cells and dendritic cells. We also assessed, by measurement of the gut homing marker α4β7, whether early treatment prevented severe CD4 T cell depletion in the GALT and thereby prevented excessive immune activation.

Results
The polyfunctionality of the HIV specific T-cell response was more preserved in treated individuals; a median of 32% of Gag-specific CD8 T cells of treated individuals produced more than 1 cytokine whereas in untreated PHI individuals this was merely 12%. Also, approximately 14% of the total response was made up of 3 functions in treated individuals compared to 1% in the untreated individuals. In contrast, no differences due to treatment were found at the level of regulation, senescence, activation or effector function of the T cell response, nor on preservation of CD4 T cells in the gut.

Conclusions
Treatment during PHI led to the preservation of a more polyfunctional HIV-gag specific T cell response. These data suggest that early treatment may preserve important CTL functions, which are crucial in control of HIV viraemia.
Early treatment preserves T-cell functionality

Introduction
In HIV infection, a low viral setpoint correlates with slow disease progression [1]. The Primo-SHM trial, a multicenter randomized study comparing no treatment with 24- or 60-weeks of combination antiretroviral therapy (cART) during primary HIV infection (PHI), recently demonstrated that temporary early cART transiently lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV infection [2]. Factors such as baseline CD4 T-cell count, stage of PHI, virological features, and HLA-background could not explain the differences observed in viral load [2, 3].

There are many immunological parameters that can play a role in HIV infection. Foremost, there is compelling evidence that gag-specific CTL responses correlate with HIV viral load and the rate of HIV disease progression [4-12]. Current consensus in the field is that a ‘protective’ T cell response in HIV infection is comprised of two elements, namely effective cytolytic killing and production of multiple cytokines. Recent work from Soghoian et al. highlights the importance of vigorous cytolytic activity by HIV specific CD4 T cells in controlling disease progression [9]. The presence of HIV specific CD4 T cells, which are able to express both perforin and high levels of granzyme A in PHI, was found to be highly predictive of slower disease progression and clinical outcome. Likewise, polyfunctionality (the capacity of cells to secrete multiple cytokines and chemokines upon antigenic stimulation) is a hallmark of a highly functional T cell response. For instance, HIV nonprogressors preferentially maintain more polyfunctional HIV specific T cell responses during infection [5]. In addition, a study by Almeida et al. demonstrated that superior control of HIV infection by CD8 T cells was reflected by a polyfunctional and high avidity phenotype [4].

The effect of these immune responses can be severely limited during disease progression, for the persistent viraemia has deleterious effects on HIV specific CD4 and CD8 T-cell immunity [7]. For instance, chronic antigen exposure leads to a terminally differentiated phenotype of the CD4 and CD8 T cells, which have upregulated CD57 expression and a diminished functional proliferative capacity [13]. HIV viraemia prevents the establishment of highly functional memory CD4 T cells that retain the capacity to proliferate upon antigen stimulation [14, 15]. And finally, chronic antigenic stimulation induces upregulation of inhibitory receptors, most notably PD-1 and CTLA-4, which may interfere with HIV specific T-cell responses and ultimately lead to T-cell anergy and loss of HIV specific T cells [16, 17].

One of the earliest effects of HIV infection is a massive depletion of central memory CD4 T cells from the gut associated lymphoid tissue (GALT) [18]. The instant and massive early injury to the gut immune system, together with the subsequent damage exacted to the gut epithelial cells, is thought to induce gut permeability and translocation of microbial products such as LPS. This is thought to contribute to the systemic immune activation which characterizes HIV infection [19]. It is widely accepted that chronic immune activation drives progression to AIDS [20-23]. In fact immune activation is more strongly associated with the rate of CD4 T-cell loss in HIV infected individuals than viral load [20]. We and others hypothesize that viral suppression in PHI might prevent the excessive depletion of the GALT and the subsequent chronic immune activation, and would thereby contribute to the delay in CD4 T cell loss found in early treated individuals. Depletion of CD4 T cells in the GALT can indirectly be assessed in the blood, by measuring the
Early treatment preserves T-cell functionality

level of α4β7 expression on T cells. Indeed, in primary SIV infection the reduction of α4β7high CD4 T cells in the peripheral blood was shown to parallel the reduction of CD4 T cells in intestinal tract biopsies [24, 25]. Likewise, the finding that α4β7high CD4 T cells are preferentially depleted in the blood in PHI [26] likely reflects the depletion of CD4 T cells of the GALT.

To determine the cause of the lower viral setpoint in individuals treated during PHI, we evaluated whether i) effector T-cell formation, ii) inhibitory receptor expression on immune cells, or iii) effectivity of the T cell response (in terms of cytolytic function, or polyfunctionality) were altered in individuals treated during PHI. Furthermore, to determine whether viral suppression in PHI caused preservation of CD4 T cells in the GALT we evaluated whether expression of the gut homing receptor α4β7 on CD4 T cells was altered in individuals treated during PHI. In concordance we assessed whether early treatment prevented excessive T cell activation and may have thereby contributed to a delay in disease progression.

Materials and Methods

Study Population

Blood samples were obtained from participants of the Primo-SHM trial. The Primo-SHM study was a multicentre, open-label randomized controlled trial comparing temporary early cART (24 or 60 wk) with no treatment. Detailed procedures have been described elsewhere [2]. Briefly, inclusion criteria were age over 18 years and a laboratory evidence of PHI, defined as a negative or indeterminate Western blot in combination with detectable plasma HIV-1 RNA (Fiebig stage I–IV) or, in case of a positive Western blot, a documented negative HIV screening test in the previous 180 days (Fiebig stage V–VI [21]).

Thirteen untreated and 26 treated (for either 24 or 60 weeks) PHI-individuals were selected based on sample availability and their immunological parameters were measured at randomization/treatment interruption (TI) and at ‘viral setpoint’, defined as 36 weeks after randomization/TI, respectively. In addition blood samples of 13 healthy donors were obtained via the ‘mini donor dienst’ of the UMC Utrecht. This was approved by the Medical Ethics Committee of the UMC Utrecht, and written informed consent was obtained from all donors.

T cell activation and phenotype

Expression of activation markers on CD4 and CD8 T cells was measured after staining of the cells with antiCD4-APC, antiCD8-PerCP, antiCD38-FITC and antiHLA-DR-PE monoclonal antibodies (BD). The phenotype of the cells was determined using antiCD27- APC-Cy7 (Biolegend) and antiCD45RO-PECy7 (BD). In the same sample, cells were also stained with antiPD-1-FITC. All incubations were performed at 4°C (20 minutes) after which cells were fixed in cellfix (BD) and analysed with the LSRII flow cytometer.

Ex vivo T cell function

Ex vivo surface staining was performed with antiCD3-eFluor450 (eBioscience), antiCD8-V500, antiα4β7-APC, antiCD57-FITC, antiCD45RO-PE-Cy7 (all BD) and antiCD27-APC-Cy7 (Biolegend) monoclonal antibodies for 20 min at 4°C. After fixation and permeabilisation (permeabilisation reagents, BD) for 10 min, cells were stained for cytotoxic molecules with antigranzyme
Early treatment preserves T-cell functionality

B-Pe (Sanquin) and antiperforin-PerCP-Cy5.5. Hereafter cells were fixed in cellfix (BD) and flowcytometry was performed.

**CD8 T cell stimulation and intracellular cytokine staining**

Cryopreserved PBMC were thawed and aliquotted at 2x10^6 cells per ml in round bottom tubes (Becton Dickinson (BD), San Jose, California). CD8 T-lymphocytes were stimulated for 6 hours with a Gag-peptide pool (15mers with 11 overlap, final concentration of the individual peptides was 2 µg/ml, Consensus B 2007, NIH AIDS Research and Reagent program, Bethesda, Maryland, United States). As a positive control, PMA and ionomycin (Sigma-Aldrich, Zwijndrecht, The Netherlands; 5 ng/ml and 1 µg/ml respectively) were used. After 1.5 hours, Brefeldin A (3 µM, Sigma-Aldrich, Zwijndrecht, The Netherlands) was added. Surface staining was performed with antiCD3-PerCP, antiCD8-V500, antiα4β7-APC (all BD) and antiCD27-APC-Cy7 (Biolegend) monoclonal antibodies for 20 min at 4°C. After fixation and permeabilisation (permeabilisation reagents, BD) for 10 min, cells were stained with antiIFNγ-Pe-Cy7 (eBioscience), antiTNFα-FITC, antiMIP1β-PE and antiIL-2-PB (BD) for 20 min at 4°C. Cells were fixed in cellfix (BD) and flowcytometry was performed.

**Characterisation of inhibitory markers**

Expression of inhibitory markers was assessed on CD4 and CD8 T cells, B cells, NK cells and dendritic cells. A surface staining was performed for CD4 and CD8 T cells (antiCD3-eFluor450 (eBioscience), antiCD8-V500 (BD)), B cells (antiCD19-PerCP (BD)), NK cells (antiCD56-APC (BD)) and dendritic cells (antiHLA-DR-APC-Cy7 (BD), antiCD11c-PE-Cy7 (BD)). These sets were completed with either antiCD31–PE (BD)/3D3 antisirl-FITC or antiLAIR-PE/antiILT4-FITC or antiIREM-1-PE/antiKLRG-1-FITC or isoptype controls. After staining for 20 minutes at 4 °C, cells were fixed in cellfix (BD) and flowcytometry was performed.

**Flow cytometry analysis**

At least 100.000 events were acquired after phenotypical staining and at least 300.000 events were acquired after intracellular cytokine stainings, using the LSRII flow cytometer (BD). Data were analyzed using the DIVA software (BD). The events were gated for either lymphocytes or monocytes in a FSC-A versus SSC plot. Following this, events were gated using the markers described above. T-cell polyfunctionality was analysed by Flowjo software (version 9.2). After determining the lymphocyte gate in a FSC-A versus SSC plot, cells were sequentially gated for CD3 and CD8. Subsequently, within the CD8 T cell population a gate was created for the 4 respective functions; IFNγ, TNFα, MIP1β, and IL-2. Hereon a Boolean gating was performed resulting in 20 different combinations. All data were background-subtracted using the unstimulated samples.

**Statistical analysis**

Differences between the 2 groups were analyzed using the Mann-Whitney test. Groups of >2 were analysed with a Kruskal –Wallis test. All statistical analyses were performed using the software program SPSS 19.0 (SPSS Inc, Chicago, Illinois).
Results

Patient characteristics
The baseline characteristics and the viral setpoint measurements of the patients selected for this study (Table 1) resembled those of the Primo-SHM trial. The median viral loads, CD4 and CD8 T cell counts were similar at baseline in the treated and untreated patients. The viral load at setpoint was significantly lowered by treatment during PHI: a median 4.4 versus 5.2 log_{10} copies/ml for treated and untreated individuals, respectively. At that moment, median CD4 T cell counts were significantly higher in treated patients than in untreated patients: 645 (range 290-950) versus 325 (180-560) CD4 T cells/mm³ respectively. CD8 T cell counts were also significantly higher in early treated individuals.

No change in T cell subsets after early treatment
To investigate the immunological cause of the lower viral setpoint in individuals treated during PHI, we determined whether T cells of treated individuals had a less mature and/or less exhausted phenotype, and could consequently form a more effective immune response. Therefore we analyzed the level of terminal differentiation (based on the markers CD45RO and CD27) and replicative senescence (based on the marker CD57) of the CD4 and CD8 T cells of treated and untreated individuals (Figure 1). We found that irrespective of treatment, CD4 T cells of HIV infected individuals had a more terminally differentiated phenotype (CD45RO-CD27-) and increased replicative senescence (CD57+) than healthy volunteers (p=0.0002 and p<0.0001, respectively). The level of senescence of CD8 T cells was also higher in HIV infected individuals compared to healthy volunteers (p=0.008). However, no differences could be observed in treated versus untreated individuals. To reveal whether the HIV specific cells also

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>A. Patient characteristics at baseline</th>
<th>Treatment (N=26)</th>
<th>No Treatment (N=13)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>43 (30-59)</td>
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<tr>
<td>Male</td>
<td>24 (92%)</td>
<td>13 (100%)</td>
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<tr>
<td>MSM</td>
<td>20 (76%)</td>
<td>12 (92%)</td>
<td>0.388</td>
</tr>
<tr>
<td>Caucasian</td>
<td>22 (84%)</td>
<td>12 (92%)</td>
<td>0.648</td>
</tr>
<tr>
<td>Stage of PHI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fiebig I–IV</td>
<td>20 (77%)</td>
<td>11 (85 %)</td>
<td>0.694</td>
</tr>
<tr>
<td>- Fiebig V–VI</td>
<td>6 (23%)</td>
<td>2 (15%)</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count (cells/mm³), median (range)</td>
<td>510 (280-1050)</td>
<td>455 (200-680)</td>
<td>0.163</td>
</tr>
<tr>
<td>CD8 cell count (cells/mm³), median (range)</td>
<td>1285 (300-1050)</td>
<td>1350 (380-1940)</td>
<td>0.639</td>
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<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml), median (range)</td>
<td>5.28 (2.91-6.70)</td>
<td>5.45 (2.49-6.14)</td>
<td>0.438</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Patient characteristics at viral setpoint</th>
<th>Treatment (N=26)</th>
<th>No Treatment (N=13)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CD4 cell count (cells/mm³), median (range)</td>
<td>645 (290-950)</td>
<td>325 (180-560)</td>
<td>0.026</td>
</tr>
<tr>
<td>CD8 cell count (cells/mm³), median (range)</td>
<td>1270 (380-1800)</td>
<td>885 (560-1080)</td>
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<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml), median (range)</td>
<td>4.37 (2.36 -5.35)</td>
<td>5.16 (4.30-5.44)</td>
<td>0.036</td>
</tr>
</tbody>
</table>

*Treatment consists of either 24 or 60 weeks of cART. MSM, men who have sex with men; PHI, primary HIV infection.*
Early treatment preserves T-cell functionality

Displayed differences in replicative capacity, an in vitro proliferation assay was performed. Cells were stimulated with an overlapping gag-peptide pool and after 6 days the stimulation index was determined. Early treatment had no effect on the gag-specific proliferative capacity of either CD4 or CD8 T cells (data not shown).

Ex vivo cytolytic T cell activity is not enhanced after treatment

Next, direct ex vivo cytolytic T cell functionality was assessed by measurement of the levels of granzyme B and perforin expression in the total CD4 and CD8 T-cell pool. The percentage of cells expressing perforin, granzyme B and a combination of both was elevated in CD4 T cells of HIV infected individuals compared to healthy individuals (Figure 2). Granzyme B expression in CD8 T cells of HIV infected individuals was also increased. However, no differences were found between untreated and treated individuals, suggesting that treatment did not preserve the cytolytic activity of T cells.

Preservation of a more polyfunctional T cell response due to treatment

Another measure for an effective T cell response is its polyfunctionality. We performed a stimulation assay with an overlapping gag-peptidepool (Figure 3A) and measured which proportion of cells produced one or more of the cytokines TNFa, MIP1b, IFNy and IL-2. No
Early treatment preserves T-cell functionality

differences were observed in the total amount of CD8 T cells that produced cytokines/chemokines to HIV gag in treated and untreated PHI individuals (median 1.0 % vs 0.5% of CD8 T cells, respectively; NS, data not shown). When we compared the functional profiles of the CD8 T-cell responses by expressing each functional component as a proportion of the total response [5], we found that treated individuals exhibited a more polyfunctional response than untreated PHI-individuals (Figure 3A). In treated individuals, a median of 32% of the gag-specific CD8 T cells displayed more than 1 function compared to 12 % of CD8 T cells in the untreated individuals. Moreover, a median of 14% of the responding CD8 T cells in treated individuals produced 3 functions simultaneously compared to 1% in the untreated individuals. The differences in the T cell polyfunctionality profiles were HIV-gag specific, for when the cells were stimulated with the ‘superstimuli’ PMA and ionomycin (Figure 3B), both treated and untreated PHI individuals showed a similar functional profile to that of healthy controls.

The separate responses showed distinct cytokine profiles when stimulated with specific antigen. For instance, in treated individuals IL-2 production by HIV specific CD8 T cells made up approximately 20% of the total response while its median contribution did not exceed 4% in untreated individuals.

No changes in expression of regulatory and inhibitory molecules after early treatment

In addition we analysed markers of regulation of the immune response. We selected a panel of ‘well recognized’ but also recently characterized and largely unexplored inhibitory receptors

Figure 2. Ex vivo cytolytic T cell activity is not enhanced after treatment. The ex-vivo effector functions of CD4 (figure 2A) and CD8 T cells (figure 2B) in terms of perforin and/or granzyme B production in treated (n=10, square), untreated (n=8, open circle) and healthy (n=12, triangle) individuals was assessed. Dots represent individuals, with a bar at the median. **P-value =0.01-0.001, ***P-value<0.001
Early treatment preserves T-cell functionality

Figure 3. Preservation of a more polyfunctional T cell response due to treatment. PHI- individuals with treatment (n=10), no treatment (n=8) and uninfected healthy donors (n=6) were compared. The piecharts depict the relative contribution of the number of cytokines/chemokines that are produced by CD8+ T cells in response to either HIV-gag (figure 3A) or PMA/ionomycin (figure 3B). The graphs zoom in on the relative contribution of the cytokine (Mip1β, INFγ, TNFα and IL-2) combinations to the total CD8 T cell response. In all graphs medians (with ranges) are shown.
Early treatment preserves T-cell functionality

with different expression aspects [27] and analysed their level of expression on CD4 and CD8 T cells, B cells, NK cells and dendritic cells.

Pecam-1 (CD31), known to inhibit apoptosis [28], was expressed less in HIV infected individuals compared to healthy controls in all measured cell types (Figure 4 & S1 first panel). Similarly, a distinct down regulation of LAIR-1 (known to inhibit cytolytic function) in HIV infected individuals was seen on T-, B- and dendritic cells (Figure 4 & S1 third panel). KLRG-1, an NK cell inhibitor, was increased on CD4 T cells in HIV infected individuals, reflecting an exhausted phenotype. On dendritic cells no difference was seen for ILT4 expression, a molecule that inhibits CTL function (Figure 4 & S1 forth panel). IREM-1, which inhibits TLR signalling in dendritic cells (Figure 4 & S1 fifth panel), was the only receptor that showed a significant effect of early treatment. However, instead of mirroring the healthy controls, the expression of this receptor was even more decreased in the treated group compared to the untreated group (median 78 vs 88% of dendritic cells positive for IREM-1, respectively). Finally, an upregulation of SIRL-1 was found on dendritic cells and B cells in HIV infected individuals (Figure 4 & S1 second panel). Remarkably, so far, this receptor was described as an inhibitory signaling molecule found solely on monocytes and neutrophils. No differences were found in the well characterized PD-1 expression in treated compared to untreated HIV infected individuals (data not shown).

No effect on GALT T cell depletion and T cell activation after early treatment

To study whether treatment in PHI could overcome the rapid depletion of T cells from the GALT that is typically observed in untreated HIV infection, we evaluated the level of expression of the gut homing receptor α4β7 on CD4 and CD8 T cells of treated and untreated individuals (Figure 5A & B). Both treated and untreated HIV infected individuals exhibited a marked decrease in the percentage of α4β7 high cells in the naive CD4 T cell compartment compared to healthy controls. Also, in the effector CD4 T cell compartment the percentage of α4β7 expression was lower in treated individuals than healthy controls. In contrast, no significant differences in α4β7 expression were found on CD8 T cells. Despite the lower viral setpoint in patients treated during PHI, there were no significant differences between treated and untreated individuals in the percentages of α4β7 high cells; thus treatment did not lead to the preservation of the α4β7 high CD4 T-cells.

Because immune activation levels are known to be even more predictive of the rate of disease progression than viral loads, we analyzed whether treatment affected the overall level of immune activation, by measuring CD38 and HLA-DR expression on T cells. Again, no differences could be found between treated and untreated individuals in the expression of CD38 and HLA-DR on CD4 or CD8 T cells (data not shown).

Discussion

We investigated whether the reported beneficial effect of treatment during PHI [2] was caused by preservation of immunological responses, and whether early treatment could overcome the rapid depletion of CD4 T cells from the GALT, and its immune activating effect. Treatment in PHI individuals seemed to preserve the quality of HIV specific CD8 T cell responses in terms of their cytokine polyfunctionality, which could contribute to a lower viral setpoint. In contrast, our results
Figure 4. No changes in regulatory and inhibitory molecules after early treatment. To assess the level of inhibitory receptor expression, the % (y-axis) of inhibitory receptors on CD4+ T cells, CD8+ T cells, B cells, NK cells and DC’s was determined. The parameters were compared between; healthy individuals (n=13, black), treated individuals (n=8, dark grey) and untreated individuals (n=10, white). Bars represent the median with range.*P-value = 0.01-0.05, **P-value =0.01-0.001, ***P-value<0.001
Early treatment preserves T-cell functionality

We were surprised that treatment did not decrease the level of senescence of the effector T cells. As previously, chronic antigen exposure was shown to lead to a terminally differentiated phenotype of CD4 and CD8 T cells, with a diminished functional proliferative capacity [13]. In addition, neither levels of inhibitory receptor expression (after correction for multiple testing) or cytolytic function of T cells were significantly altered in treated compared to untreated individuals.

If so, we have missed the effect of treatment on the immune response by measuring too late. Though, for the cytolytic T-cell functions, the discrepancy between our results and existing literature is likely to be found in the T cell subsets measured. In the literature, proliferated HIV

Figure 5. No differences in T cell activation between treated and untreated patients. The expression of gut homing marker a4b7 within the T cell compartments (naïve, central memory, effector memory and effector) on CD4 T cells (figure 5A) and CD8 T cells (figure 5B), is depicted. The mean fluorescent intensity of a4b7 was compared for treated (n=8, light gray), untreated (n=10, dark grey) and healthy (n=12, black) individuals. Bars represent the median with range. *P-value = 0.01-0.05, **P-value =0.01-0.001

show no differences due to treatment at the level of i) effector T cell formation or replication capacity of the T cells, ii) the overall cytolytic T cell response iii) regulation of various T, B and NK cells or dendritic cells, iv) preservation of CD4 T cells in the GALT and v) immune activation.
specific CD8 T cells of long term non-progressors were more prone to produce perforin in comparison with the T cells of progressors [7]. Also, increased granzyme production after HIV specific stimulation was predictive of outcome [9]. We, on the other hand, looked into perforin and granzyme expression of the total CD4 and CD8 T cell pool. Additionally, changes might have been missed due to our limited sample size.

Equally, treatment had no effect on preservation of CD4 T cells in the GALT and subsequent overall immune activation. As there is an acute and severe depletion of CD4 T cells in the GALT due to HIV infection, we expected treatment in PHI to prevent this. However, the initiation of treatment might have been too late to preserve gut immunity, even though all individuals in the Primo-SHM study were recruited within 100 days of HIV infection and 73% was even recruited within 30 days. In SIV infection severe depletion of CD4 T cells in the GALT (up to 65%) was seen within days [29] and not weeks.

Reflecting the unaffected CD4 T cell depletion of the GALT, systemic immune activation was also not diminished after treatment. However, immune activation is not only induced by gut permeability and translocation of microbial products such as LPS, but also HIV itself is a strong driver of immune activation [22]. Therefore, one would expect a lower viral load to have an impact on the level of immune activation. Immune activation is an even stronger predictor of disease progression than viral load, and a diminished immune activation would therefore strongly contribute to delay of cART, as seen in the Primo-SHM study [2].

Finally, treatment in PHI-individuals seemed to preserve the quality of HIV specific CD8 T cell responses, in terms of their cytokine polyfunctionality. So far, not one single function of HIV specific CD8 T cells has been proven to correlate with control of HIV infection. Therefore, the current consensus is that the more functions a CD8 T cell performs (HIV specific), the more protective it must be. Indeed, HIV specific CD8 T cells of long term non-progressors are more prone to perform 5 functions simultaneously (IL-2, IFN-γ, TNF-α, MIP-1β, and CD107a) compared to HIV progressors [5]. Moreover, the cytolytic response is thought to be more antiviral when producing a distinct profile of perforin and various granzymes against HIV-gag [9]. In addition, while the antigen-specific polyfunctional CD8 T cells do not produce more of a single cytokine per cell basis, overall they produce more cytokines on a per cell basis than monofunctional CD8 T cells [30]. Therefore, it seems that the preservation of a polyfunctional HIV specific CD8 T cell response could be involved in lowering the viral setpoint as a result of treatment.

In conclusion, our results showed that early treatment during PHI might preserve a more polyfunctional HIV-gag T-cell response. These data thereby suggest that early treatment may preserve important CTL functions, which are crucial in control of HIV viraemia.
SIX Early treatment preserves T-cell functionality

References


Early treatment preserves T-cell functionality


HIV-1 dual infection is associated with faster CD4+ T cell decline in a cohort of men with primary HIV infection

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Abstract

Background
In vitro, animal, and mathematical models suggest that HIV co- or superinfection would result in increased fitness of the pathogen and, possibly, increased virulence. However, in patients, the impact of HIV-1 dual infection on disease progression is unclear, because parameters relevant for disease progression have not been strictly analysed. The objective of the present study is to analyse the effect of HIV-1 dual infections on disease progression in a well-defined cohort of men having sex with men (MSM).

Methods
Between 2000 and 2009, 37 MSM who had primary infection with HIV-1 subtype B, no indication for immediate need of combination antiretroviral therapy (cART), and sufficient follow-up, were characterized with regard to single- or dual infection, HLA-I type, CCR5 genotype and coreceptor usage. Patients were followed to estimate the effect of these parameters on clinical disease progression, as defined by the rate of CD4+ T-cell decline and the time to initiation of cART.

Results
Four patients presented with an HIV-1 coinfection, six patients acquired an HIV-1 superinfection, on average 8.5 months from their primary infection; and twenty-seven patients remained infected with a single strain. Slopes of longitudinal CD4+ T-cell counts and time-weighed changes from baseline were significantly steeper for patients with dual infection compared with patients with single infection. Multivariate analysis showed that the most important parameter associated with CD4+ T-cell decline over time was dual infection (p=0.001). Additionally, patients with an HIV-1 coinfection had a significantly earlier start of cART (p<0.0001).

Conclusions
HIV-1 dual infection is the main factor associated with CD4+ T-cell decline in untreated, primary HIV-1 infected MSM with subtype B.
Introduction
HIV-1 coinfections (i.e., infection with a second virus strain before seroconversion) and superinfections (infection with a second virus strain after seroconversion) are reported with increasing frequency. Superinfections were first reported in 2002 [1-3], and a serial superinfection case was published in 2005 [4]. In many cases, infection with more than one virus strains was associated with disease progression. However, HIV-1 superinfections have also been reported in long-term non-progressors [5-7], making it unclear whether superinfection is harmful to the individual patient. The clinical effects of HIV-1 coinfections are also largely unknown.

Infection of cats with feline immunodeficiency virus suggest that both co- and superinfection lead to the rapid outgrowth of viruses with increased replication capacity and virulence [8], as is also suggested by a general mathematical model describing the evolution of parasitic virulence [9]. Another mathematical model suggested that superinfection with a more virulent HIV-1 strain leads to faster disease progression [10]. In vitro superinfection of T-cell lines with different HIV-1 strains resulted in an increase in virus production and cell mortality [11]. Analysis of two HIV superinfected patients showed that the second strain had a greater replicative fitness [12], which could lead to greater virulence. Furthermore, superinfection could compromise the patient’s immune system by potentiating immune escape [3]. Immune escape, as well as increased replicative fitness or drug resistance, could rapidly be acquired by the virus through recombination between the two strains after dual infection. Recombination events expand the evolutionary potential of HIV-1 and pose one of the dangers of dual infection, both for the individual patient as well as for the epidemic [13].

The reported clinical effects of HIV dual infections are largely anecdotal. In fact, many HIV-1 superinfections were detected retrospectively because of unfavourable changes in clinical parameters. Disease outcome was studied in dual HIV-1 infected patients from three cohorts, in whom dual infection was associated with rapid disease progression in five individuals [14]. However, these patients were infected with HIV-1 well before the year 2000, and were not matched for sex, HIV subtype, HLA type, or CCR5 genotype, while the seroconversion date and date of superinfection were approximated. Studies have shown that recent HIV-1 strains are increasing in replicative fitness [15] and in virulence by adapting to protective HLA-I types [16,17] and by escaping from antibody neutralization [18].

To study the effect of HIV dual infections in recent years and to control for other variables relevant to disease progression, we assessed disease progression in a prospective study involving 37 well-characterized patients with primary HIV infection (PHI).

Materials and Methods
Patients
Thirty-seven PHI-patients who were seen at the Academic Medical Center (AMC) in Amsterdam, The Netherlands, between 2000 and 2009 were selected from the Primo-SHM cohort, a multicentre prospective cohort study in the Netherlands with an embedded randomized trial that investigates the natural course of HIV-1 infection and the effects of early combination antiretroviral therapy (cART) in patients with PHI [19]. PHI was defined as a negative or
Significance of HIV-1 dual infection

indeterminate Western blot combined with either a positive test for p24 antigen or a detectable HIV-1 RNA concentration, or as a negative result of an HIV screening test within 6 months before seroconversion. At their first visit to the AMC, eligible patients were invited to join the Primo-SHM cohort, and if included, were randomized with regard to the study arm (i.e., to receive early treatment or not). Inclusion criteria for the present analysis were laboratory evidence of PHI with subtype B (as determined by genotyping of the pol gene), male sex, no immediate indication for cART, and at least two years of follow-up, unless 2 CD4+ T cell counts of ≥ 350 × 10⁶ cells/ml were reached and/or cART was started before that time. Eight enrolled patients, who were later all determined to be and remain infected with a single strain, presented with CD4+ T-cell counts <350 × 10⁶ cells/ml, but low CD4+ T-cell counts were not an exclusion criterion. In most cases, CD4+ T-cell counts increased spontaneously after the first measurement. Clinical parameters, including treatment, CD4+ T cell count, and plasma viral load (pVL) were collected at enrollment and during follow-up visits every 3 months. Follow-up was terminated at the start of cART or at the end of the study (September 1, 2010). The research protocol was approved by the medical ethical committee of the AMC and all participants gave written informed consent.

Assays
Plasma viral load measurements were performed using the Versant HIV-1 RNA 3.0 assay (Bayer Diagnostics Division Tarrytown, NY, USA) from January 2000-February 2007 and from February 2007 onwards the Abbott RealTime HIV-1 assay (Abbott Molecular Inc, IL, USA) was used. The lower limits of detection of these assays were 50 and 40 HIV-1 copies/ml, respectively. HIV-1 protease/reverse transcriptase gene sequences were generated using the ViroSeq HIV-1 genotyping kit version 2 (Celera Diagnostics, Alameda, CA, USA). HLA typing was performed and CCR5 haplotypes were analysed for the presence of the CCR5-Δ32 deletion allele as previously described [20].

Polymerase chain reaction amplification and sequence analysis
RNA was isolated from 200 µl of blood plasma using silica and guanidine thiocyanate [21]. One-fifth of the isolate was then used to reverse-transcribe, amplify, clone and sequence the env V3-V4 fragment (nt 6949-7519 of the HXB2 reference sequence, GenBank acc. no. K03455) [22]. At least 16 clones were analysed per sample.

Phylogenetic analysis
Sequences were aligned with the CLUSTAL W sequence alignment tool implemented in BioEdit Sequence Alignment Editor Version 7.0.9 [23]. Alignments were manually adjusted to preserve in-frame insertions and deletions. Phylogenetic analyses were performed with the MEGA 5 software package [24] and with the parallel version of MrBayes 3.1 [25] as described [22].

Definition of HIV co- or superinfection
HIV-1 coinfection was defined as having two separate env sequence clusters in a phylogenetic tree already in the first sample obtained during PHI. HIV-1 superinfection was defined as having an infection with a second HIV-1 strain that clusters separately in a phylogenetic tree at a time point after PHI. For all patients, a second sample from the end of the follow-up period
was analysed to investigate whether an HIV-1 superinfection had occurred after the primary infection period. In case superinfection was identified in the second (= last) sample, all earlier samples from that patient were amplified and sequenced to accurately determine the moment of superinfection. Although it is possible that a superinfection is missed when analysing only one follow-up sample, and only env region sequences are cloned [26], we identified 90% of dual infections correctly with this strategy.

Coreceptor usage
HIV-1 env-clones were genotyped for CCR5/CXCR4 coreceptor tropism with the Geno2pheno algorithm [27].

Statistical analysis
Variables were compared between the single and dual infected groups, and for some analyses, the dual infected group was separated into co- and superinfected groups. Mann-Whitney tests were used to compare continuous variables, and proportions were compared using the Fisher’s exact test. Clinical progression was defined as the time between PHI and one of the two endpoints of the study: a CD4+ T cell count <350 cells/ml on two consecutive occasions, or starting cART. Clinical progression was compared across the groups using Kaplan-Meier plots and log rank tests.

Longitudinal slopes of CD4+ T-cell count in individual patients were calculated by linear regression, and time-weighted changes (TWC) from baseline were calculated by linear trapezoidal integration. Parameters associated with CD4+ T cell count slope in univariate analyses with p values <0.1 were included in multivariate analyses (General Linear Model (GLM); full factorial). Linear mixed models with patient as a random factor and time from baseline as a fixed covariate were used to compare the changes from baseline of the CD4+ T cell count between the patient groups, and to estimate the longitudinal slopes of CD4+ T cell count in the patient groups. For pVL, log10-transformed values were used. Linear mixed models and GLM were performed by using PASW Statistics 18 [28], and all other statistical tests were performed by using GraphPad Prism 5.01 [29]. All statistical tests were two-sided. P values <0.05 were considered statistically significant.

Results
Patient characteristics
Patient characteristics are summarized in Table 1. All 37 patients were men having sex with men (MSM) and 36 (97%) were Caucasian. Twenty-four (65%) reported symptoms compatible with an acute retroviral syndrome. Follow-up was on average 33 (range 9-80) months, whereby 19 patients (51%) reached a study endpoint before the intended follow-up of 2 years. Four patients had a HIV-1 coinfection, six were superinfected with a second HIV-1 strain on average 8.5 months (range 3.5-15.5 months) after the PHI, and 27 remained infected with a single HIV-1 strain until the end of follow-up. Three dually infected patients were identified earlier [30,31,12]. The remaining seven patients were discovered during additional analysis of samples with a high degenerate base code count in the genotyping sequence [30], or were found during the present study. Although all PHI occurred with subtype B strains, one patient was co-infected
with a subtype G strain, while two superinfections occurred with CRF01_AE, a third with CRF02_AG, and a fourth with a subtype A strain (Figure 1).

Patient age, the occurrence of an acute retroviral syndrome during PHI, HLA-I haplotype, and host CCR5 genotype did not differ significantly between the single and dual infected groups (Table 1). All primary virus strains were predicted to use the CCR5 coreceptor, except for the first strain infecting a later superinfected patient who presented with an exceptionally low pVL [12]. However, as the Geno2pheno algorithm is known to have only approximately 75% sensitivity for CXCR4-using viruses [32], some might have been missed.

Figure 1. Phylogenetic tree of HIV-1 env sequences, generated with the neighbour-joining method implemented in MEGA 5 software package (available at www.megasoftware.net, accessed February 8, 2011) and bootstrap resampling with 1000 replicates. Shown are env sequences of 27 single infected patients, and two env sequences each from the ten dual infected patients (open symbols depict sequences from coinfected patients, closed symbols depict sequences from superinfected patients). Bootstrap values >85 are indicated. Reference sequences were from the Los Alamos National Laboratory HIV database (available at http://hiv-web.lanl.gov, accessed March 3, 2011). HIV-1 env sequences have been deposited in the GenBank database with accession numbers JN647642-JN647688.
At enrollment, a marginally significant difference in pVL was seen between single and dual infected patients \( (p=0.05) \), which could be attributed to a difference between single- and future superinfected patients \( (p=0.03) \), but not to differences between single and coinfected individuals \( (p=0.58) \), although the number of patients was low (Table 1). Patients that were going to experience an HIV-1 superinfection at a later time point had a lower pVL at baseline. At study endpoint, there was no significant difference in plasma viral load between the groups \( (p=0.51) \).

**Disease progression and CD4 dynamics**

Time to reach CD4+ T cell levels ≤ 350 × 10^6 cells/ml was not significantly different between dual- and single infected patients \( (p=0.88) \) (Figure 2A, left panel), possibly because 4/10 dual infected patients were censored as cART was initiated at higher CD4+ T cell counts. Splitting dual infected patients into co- and super-infected groups did not show differences either (Figure 2A, right panel).

Next, we looked at start of cART, which is not only driven by CD4 count, but also by clinical symptoms. Of the 37 patients, 31 patients started cART within two years. Six started before

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (N=37)</th>
<th>Single infection (N=27)</th>
<th>Dual infection (N=10)</th>
<th>P-value(^a)</th>
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<tbody>
<tr>
<td>Age (years), median (IQR)</td>
<td>37.0 (29.0 – 42.0)</td>
<td>36.0 (29.0 – 42.0)</td>
<td>39.0 (24.8 – 41.3)</td>
<td>0.86</td>
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<td>Acute retroviral syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>64.9 (24/37)</td>
<td>66.7 (18/27)</td>
<td>60.0 (6/10)</td>
<td>0.72</td>
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<tr>
<td>No</td>
<td>35.1 (13/37)</td>
<td>33.3 (9/27)</td>
<td>40.0 (4/10)</td>
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</tr>
<tr>
<td>Plasma viral load (log(_{10}) copies/ml), median (IQR)</td>
<td>5.24 (2.25-7.15)</td>
<td>5.51 (3.34-7.15)</td>
<td>4.52 (2.25-5.89)(^b)</td>
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</tr>
<tr>
<td>CCR5-Δ32 genotype</td>
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<tr>
<td>Homozygous CCR5-Δ32</td>
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<td>11.1 (3/27)</td>
<td>0.0 (0/10)</td>
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<tr>
<td>HLA-A haplotype(^c)</td>
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<td></td>
</tr>
<tr>
<td>Homozygous</td>
<td>73.3 (22/30)</td>
<td>78.3 (18/23)</td>
<td>57.1 (4/7)</td>
<td>0.34</td>
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<tr>
<td>Heterozygous</td>
<td>26.7 (8/30)</td>
<td>21.7 (5/23)</td>
<td>42.9 (3/7)</td>
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<tr>
<td>HLA-B haplotype(^d)</td>
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<tr>
<td>Alleles associated with slow progress(^e)</td>
<td>27.6 (8/29)</td>
<td>22.7 (5/22)</td>
<td>42.9 (3/7)</td>
<td>0.36</td>
</tr>
<tr>
<td>Alleles associated with rapid progress(^e)</td>
<td>34.5 (10/29)</td>
<td>31.8 (7/22)</td>
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</tr>
</tbody>
</table>

Data are n (%) unless indicated otherwise.

\(^a\) Mann-Whitney tests were used for continuous variables and Fischer’s exact tests for proportions.

\(^b\) The difference in plasma viral load seen between patients with single infection and those with dual infection \( (p=0.05) \) at enrollment could be attributed to a difference between patients with single infection and those with future superinfection \( (p=0.03) \), as no difference between patients with single infection and those with coinfection was observed \( (p=0.58) \).

\(^c\) Data are for 30 patients \( (23\) with single infection and 7 with dual infection).

\(^d\) Data are for 29 patients \( (22\) with single infection and 7 with dual infection).

\(^e\) According to Goulder and Watkins [33], HLA-B alleles associated with slow progression are: HLA-B*1202, HLA-B*2205, HLA-B*3101, HLA-B*501/02/03, HLA-B*5801, and HLA-B*8101; HLA-B alleles associated with rapid progression are: HLA-B*1801, HLA-B*3502/03, and HLA-B*5802.
their CD4+ T cell counts reached ≤350 × 10^6 cells/ml: four were dual infected and two single infected (p=0.035). Seven patients started according to treatment guidelines (six single- and one superinfected patients), and 18 patients started treatment at lower CD4+ T cell counts (average 219 cells/ml, range 100–320 cells/ml, average treatment delay 13.5 months, range 1–33 months). Of the latter patients, 14 were single infected (mean treatment delay 15.4 months), and four were dual infected (mean treatment delay 7.0 months), suggesting that dual infections are associated with more constitutional symptoms. However, an AIDS-defining opportunistic illness was not documented for any patient. At the time of cART initiation, the treating physician was unaware of the (single or dual) infection status of the patient, which could otherwise

Figure 2. Kaplan-Meier analysis of time to reach a repeated CD4+ T cell count <350 cells/ml (A) or to start of cART (B) according to single or dual (left panels) or single, super-, or coinfection (right panels). P-values were calculated using the log-rank test.
possibly have influenced the decision to start treatment. Analysis of the time to start of cART in single and dual infected groups resulted in a significantly earlier start of therapy for coinfected patients compared to single infected individuals (p<0.0001, Figure 2B, right panel), but not between single and superinfected patients (p=0.56), or between the single and dual infected patients (p=0.065) (Figure 2B, left panel).

However, the time to CD4+ T cell counts ≤ 350 × 10^6 cells/ml and time to start of cART in superinfected patients should be interpreted with caution, as superinfected patients were initially single infected, and remained single infected for a substantial period of follow-up. To accurately compare disease progression between the super- and single infected patients, we set an adjusted baseline at 8.5 months post-infection (the mean time to superinfection in the superinfected patients) for the single and coinfected patients. This will be further referred to as “adjusted baseline”. The baseline for the superinfected patients was set at their identified superinfection moment. For one co- and two single infected patients, no CD4+ T cell counts post-adjusted baseline could be included, as these patients started cART before that point.

Whereas the CD4+ T cell counts at adjusted baseline were not significantly different between the dual and single infected patients (p=0.15), longitudinal slopes post-adjusted baseline and the TWC from the adjusted baseline of CD4+ T cell counts were significantly different (p=0.003 and p=0.047, respectively) (Figure 3A-C, left panels), indicating that dual infection is associated with faster disease progression. TWC of CD4+ T cell counts were also significantly different between super- and single infected patients (p=0.013), whereas analogous difference for the slopes reached only a marginal significance (p=0.054) (Figure 3B-C, right panels). The CD4+ T cell count slopes of coinfected patients were significantly different from those of single infected patients (p=0.0075). It was not possible to calculate CD4+ T cell slopes before and after a superinfection event, as superinfections occurred mostly close to the acute phase of infection, with CD4+ T cell counts not yet having reached set-point values.

Next, we estimated changes of CD4+ T cell counts relative to adjusted baseline during the follow-up period by fitting the linear mixed models, taking into account correlations of repeated measurements within the individuals (Figure 3D). Median changes from the adjusted baseline were -180 × 10^6 cells/ml for super-, -110 × 10^6 cells/ml for co-, and -70 × 10^6 cells/ml for single infected patients (p=0.001 for the comparison between dual vs. single infected patients, p=0.003 for super- vs. single infected, p=0.09 for co- vs. single infected patients). Linear mixed modelling was also used to estimate the longitudinal slopes of CD4+ T cell count in the patient groups. As shown in Figure 3D, the slope was steeper in dual vs. single infected patients, and was especially steep in the coinfected patients. This analysis confirmed the earlier observed association of dual infection with accelerated disease progression.

Finally, we looked at whether the observed effect of dual infection on disease progression could be confounded by other variables. As shown in Table 2, neither age at infection, occurrence of an acute retroviral syndrome, or homozygosity for HLA-A or -C haplotypes, was significantly associated with the slope of CD4+ T cell count, in contrast to CCR5-Δ32 heterozygosity and presence of an HLA-B allele associated with rapid progression (p=0.045 and p=0.02, respectively) [33]. Plasma viral load at month 12 post-infection was associated with CD4+ T cell count slope with marginal significance (p=0.06 by linear regression). In a
Figure 3. Effects of dual infection on CD4+ T cell count. (A) CD4+ T cell counts at the adjusted baseline. (B) Linear regression slopes of CD4+ T cell counts relative to the adjusted baseline. (C) Time-weighted changes (TWC) of CD4+ T cell counts from the adjusted baseline. Horizontal lines show the median values; groups were compared using the Mann-Whitney test (A-C). (D) Changes of CD4+ T cell counts relative to adjusted baseline as estimated by linear mixed modelling. Dots on the graphs represent changes of CD4+ T cell counts from adjusted baseline at all follow-up time points. The horizontal lines on the Y-axes depict the median changes from the adjusted baseline in the corresponding patient groups. P-values of comparisons of changes from baseline between the groups were calculated by fitting linear mixed models. The diagonal lines show the longitudinal slopes of CD4+ count, as estimated by the linear mixed modelling.
Significance of HIV-1 dual infection

The multivariate model, dual infection and HLA-B haplotype remained significantly associated with the CD4+ T cell count slope (p=0.001 and p=0.006, respectively) (Table 2).

### Table 2. Variables associated with CD4+ T cell count slope

<table>
<thead>
<tr>
<th>Variable</th>
<th>CD4+ T cell count slope</th>
<th>P-value</th>
<th>P-value (multivariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at infection, years (n=34)</td>
<td>Slope = -0.16 ± 0.48; R² = 0.00</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Acute retroviral syndrome (n=34)</td>
<td>Yes (n=22) -8.2 (-11.6 to -3.4)</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No (n=12) -7.2 (-12.7 to -5.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual infection (n=34)</td>
<td>Yes (n=9) -17.8 (-33.8 to -8.1)</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>No (n=25) -6.9 (-9.7 to -2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viral load, log₁₀ copies/ml, by time</td>
<td>6 months after infection, (n=34) Slope = 0.70 ± 6.44; R² = 0.00</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 months after infection (n=32) Slope = -3.65 ± 1.89; R² = 0.11</td>
<td>0.06</td>
<td>0.59</td>
</tr>
<tr>
<td>CCR5-Δ32 genotype (n=34)</td>
<td>Homozygous (n=31) -8.3 (-11.9 to -5.2)</td>
<td>0.045</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Heterozygous (n=3) -2.1 (-4.6 to -1.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-A haplotype (n=27)</td>
<td>Homozygous (n=7) -6.9 (-13.5 to -5.2)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterozygous (n=20) -8.5 (-11.4 to -2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-B haplotype: alleles associated with slow progression (n=26)</td>
<td>Yes (n=7) -7.6 (-10.6 to -5.2)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No (n=19) -8.3 (-11.9 to -4.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-B haplotype: alleles associated with rapid progression (n=26)</td>
<td>Yes (n=8) -11.7 (-23.4 to -7.4)</td>
<td>0.02</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>No (n=18) -7.1 (-10.4 to -2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-C haplotype (n=27)</td>
<td>Homozygous (n=6) -9.2 (-21.3 to -0.9)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterozygous (n=21) -7.6 (-11.3 to -4.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For associations of CD4+ T cell count slope with continuous variables, linear regression slopes and R² values are shown; for associations of CD4+ count slope with discrete variables, medians and quartiles of CD4+ T cell count slope in each group are shown.

* Determined by linear regression for continuous variables, and by the Mann-Whitney test for discrete variables.

* Determined by fitting the general linear model.

* Cells/ml per month.

### Discussion

We evaluated the effect of co- or superinfection versus single infection on disease progression in a Dutch MSM cohort with subtype B PHI acquired between 2000 and 2009. Multivariate analysis showed that dual HIV-1 infection, resulting either from co- or superinfection, was the most important factor influencing CD4+ T cell decline over time, and was the major disease-accelerating characteristic in this cohort, suggesting that the viral virulence is increased in these patients, as predicted by model systems [9,34,8]. Unfavourable HLA-B alleles were also significantly associated with a more rapid CD4+ T cell decline, which is in agreement with findings from previous studies [33]. Of the patients with an HIV-1 dual infection, those with a coinfection had the most rapid CD4+ T cell decline. In line with these findings, patients with an HIV-1 coinfection started cART significantly earlier compared with patients with a single
infection. Patients acquiring an HIV-1 superinfection also had a decline in CD4+ T cell count that was significantly greater than that for individuals with a single infection, but less so than that for patients with a coinfection. Most likely, competition between virus strains is more severe when the moment of infection with the second strain is closer to the first infection point.

The strength of this study is that disease progression was studied in a well-characterized recently infected cohort of PHI-patients. The limitations are that, in this cohort, four of the six superinfections occurred with a non-B HIV-1 subtype. However, none of the subtypes involved have been associated with increased disease progression. Second, because this study has been performed in a cohort with PHI in which early superinfections occurred, it is unclear whether the findings are also applicable to patients who acquire an HIV-1 superinfection in a later phase of the infection. It is possible that susceptibility to a second HIV-1 infection is increased during the acute phase, as the cellular immunity is less developed at that time, and that the second infection is also more damaging during this period. Of note, 65% of patients presented with an acute retroviral syndrome, which is a strong predictor of HIV-1 disease progression [35] suggesting that our results may not be generalized to unrecognized PHI. Finally, as dual HIV-1 infections are infrequent events and PHI is usually not recognized, the sample size of our study is relatively small, so that replication of these results in an independent cohort would strengthen our findings.

In conclusion, this study demonstrates that a recent HIV-1 dual infection is independently associated with increased disease progression. Our findings suggest that serosorting (e.g. practising unsafe sex only with partners who have the same HIV serostatus) should be discouraged, especially in patients with a recognized PHI, as superinfections increase disease progression as shown by a faster CD4+ T cell decline in this study. Finally, temporary cART during PHI might have clinical benefits, although randomized data are not yet available [36]. Apart from potential other benefits, an additional advantage of (temporary) treatment might be the prevention of early HIV-1 superinfection.
Significance of HIV-1 dual infection

References


PART III

Quadruple therapy in patients with primary and chronic HIV infection
No advantage of quadruple or triple-class antiretroviral therapy as initial treatment in patients with very high viraemia

Marlous L. Grijzen, Rebecca Holman, Luuk Gras, Ferdinand W.N.M. Wit, Andy I.M. Hoepelman, Guido E. van den Berck, Frank de Wolf, Jan M. Prins for the ATHENA national observational cohort study

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Abstract

Background
We assessed whether quadruple or triple-class therapy for the initial treatment of HIV-1 infection provides a virological benefit over standard triple therapy in patients with a very high plasma viraemia.

Design
National observational HIV cohort in the Netherlands.

Methods
Inclusion criteria were age $\geq 18$ years, treatment-naïve, plasma viral load (pVL) $\geq 500,000$ copies/ml and initiation of quadruple or triple therapy between 2001-2011. Time to viral suppression, defined as pVL $< 50$ c/ml, was compared between the two groups using Kaplan-Meier plots and multivariate Cox regression analysis.

Results
675 patients were included: 125 (19%) initiated quadruple and 550 (81%) triple therapy. Median pVL was 5.9 (IQR 5.8-6.1) log$_{10}$ c/ml in both groups ($P=0.49$). 22 (18%) patients on quadruple and 63 (12%) on triple therapy interrupted the treatment regimen because of drug-related toxicity ($P=0.06$). Median time to viral suppression was 5.8 (IQR 4.6-7.9) and 6.0 (4.0-9.4) months in the patients on quadruple and triple therapy (log rank, $P=0.42$). In the adjusted Cox analysis, quadruple therapy was not associated with time to viral suppression (HR 1.07 (95% CI 0.86-1.33), $P=0.53$). Similar results were seen when comparing triple- versus dual-class therapy (n=72 vs. n=601, respectively).

Conclusions
Initial quadruple or triple-class therapy was equally effective as standard triple therapy in the suppression of HIV-1 in treatment-naïve patients with very high viraemia and did not result in a faster pVL decline, but did expose patients to additional toxicity.
Introduction

A higher baseline plasma HIV-1 RNA is an independent predictor of virological treatment failure [1, 2]. Plasma viral load (pVL) levels above 100,000 copies/ml are associated with a slower pVL decline, a reduced probability of achieving virologic suppression and an increased risk of mortality [1, 3, 4]. Dual- or triple-class quadruple therapy has been suggested to increase the antiretroviral activity of cART. Several randomized and nonrandomized studies have compared the potency of quadruple therapy with that of standard-of-care triple therapy in treatment-naive patients and found inconsistent results with regard to virologic response [5-15]. In most studies quadruple therapy consisted of a regimen in which the fourth drug was an older generation unboosted PI, questioning its relevance to current clinical practice. Furthermore, the effectiveness of quadruple/triple-class therapy has not yet been answered in the subgroup of patients with very high viraemia (≥500,000 c/ml). We assessed whether quadruple or triple-class therapy provides a more rapid pVL decline and an improved virologic response compared with standard dual-class triple therapy in treatment-naive patients with very high viraemia.

Methods

Data used in this study were selected from the Dutch observational HIV cohort (ATHENA) [16]. Inclusion criteria were: age ≥18 years, treatment-naive, a pVL of more than 500,000 copies/ml at start of therapy and initiation of quadruple or triple therapy between January 2001 and June 2011. Patients with primary HIV infection were excluded. The decision to initiate quadruple or triple therapy was at the discretion of the treating physician.

Quadruple and triple therapy was defined as cART with four and three effective drugs, respectively, including at least two different drug classes. Triple- and dual-class therapy was defined as cART containing three and two effective drug classes. Ritonavir-boosted PIs (PIs/r) were considered a single drug.

The primary endpoint was the time to viral suppression, defined as the time to the first of two consecutive pVL measurements below 50 c/ml, and the proportion of patients with a pVL below 50 c/ml after the first year of treatment. Secondary endpoints were the tolerability of the regimens and the number of patients experiencing virological failure (pVL >1000 c/ml) after initial viral suppression (pVL <50 c/ml).

Demographic characteristics, clinical and laboratory data were compared between the two groups using Kruskal-Wallis, chi-square or Fisher’s exact tests where appropriate. Time to viral suppression was compared between quadruple and triple therapy using Kaplan-Meier plots and multivariate Cox regression analysis. Patients who discontinued cART for more than two weeks or were lost-to-follow-up were censored in de survival analyses. All variables listed in Table 1 were considered potential confounders and entered into the Cox model. Analyses were repeated for triple- versus dual-class therapy. Data were analyzed using SAS version 9.2.
Results

Quadruple versus triple therapy

The study population consisted of 675 patients of whom 125 (19%) initiated quadruple therapy and 550 (81%) triple therapy. Patient characteristics prior to treatment are summarized in Table 1. The median pVL was 5.9 (IQR 5.8-6.1) log₁₀ c/ml in both groups (P=0.95). The median CD4 count was significantly lower in the patients initiating quadruple therapy (P=0.009). All patients, except one, initiated cART with at least two NRTIs. Patients on quadruple therapy received in addition to these NRTIs a regimen containing a third NRTI plus an NNRTI (18%), a third NRTI plus a PI/r (25%), an NNRTI plus a PI/r (50%), or an integrase inhibitor plus a NNRTI or PI/r (6%). Of the patients on triple therapy, 60% initiated a regimen of NRTIs with an NNRTI, 39% NRTIs with a PI/r and 1% NRTIs with an integrase inhibitor. One patient on triple therapy initiated with one NRTI, an NNRTI and a PI/r (Table 1). HIV genotyping was available for 221 (33%) of the patients. Two patients (5%) on quadruple and 10 patients (6%) on triple therapy harboured one or more transmitted drug resistance mutations in reverse transcriptase or protease (P=1.0) [17]. As a result, two patients on triple therapy were retrospectively treated with an ineffective triple regimen and were therefore excluded from further analyses. Participants were followed for a median of 50 (IQR 27-82) months.

Table 1. Patient characteristics at start of therapy

<table>
<thead>
<tr>
<th></th>
<th>Quadruple therapy (N=125)</th>
<th>Triple therapy (N=550)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>110 (88)</td>
<td>454 (83)</td>
<td>0.14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40 (35-46)</td>
<td>41 (35-48)</td>
<td>0.39</td>
</tr>
<tr>
<td>Native Dutch residents</td>
<td>78 (62)</td>
<td>322 (59)</td>
<td>0.43</td>
</tr>
<tr>
<td>HIV transmission route</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual</td>
<td>77 (62)</td>
<td>307 (56)</td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>39 (31)</td>
<td>208 (38)</td>
<td></td>
</tr>
<tr>
<td>Injecting drug use or blood-blood</td>
<td>3 (2)</td>
<td>8 (1)</td>
<td>0.50</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (5)</td>
<td>27 (5)</td>
<td></td>
</tr>
<tr>
<td>Co-infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>7 (6)</td>
<td>29 (5)</td>
<td>0.88</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>3 (2)</td>
<td>22 (4)</td>
<td>0.39</td>
</tr>
<tr>
<td>History of CDC-C event</td>
<td>65 (52)</td>
<td>234 (43)</td>
<td>0.05</td>
</tr>
<tr>
<td>CD4 count (cells/mm³)</td>
<td>80 (40-191)</td>
<td>125 (41-230)</td>
<td>0.009</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log₁₀ c/ml)</td>
<td>5.9 (5.8-6.1)</td>
<td>5.9 (5.8-6.1)</td>
<td>0.49</td>
</tr>
<tr>
<td>Drug resistance mutations</td>
<td>2 (5)</td>
<td>10 (6)</td>
<td>1.0</td>
</tr>
<tr>
<td>cART regimen including</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNRTI</td>
<td>23 (18)</td>
<td>328 (60)</td>
<td></td>
</tr>
<tr>
<td>Boosted PI</td>
<td>31 (25)</td>
<td>214 (39)</td>
<td></td>
</tr>
<tr>
<td>NNRTI plus boosted PI</td>
<td>63 (50)</td>
<td>1 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Integrase inhibitor</td>
<td>8 (6)</td>
<td>7 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Data are no. of patients (%) or medians (inter quartile ranges).

* P-value based on the Kruskal-Wallis test for continuous variables and χ² or Fisher’s exact tests for proportions.

a 3 patients and b 16 patients with missing data.

HIV genotyping was available for 43 patients on quadruple and 178 patients on triple therapy.
The median time spent on the first treatment regimen was 105 (IQR 28-240) days for patients on quadruple therapy and 415 (156-978) days for patients on triple therapy (P<0.001). Twenty-two patients (18%) on quadruple therapy switched to an alternative regimen within a year because of drug-related adverse events, as compared to 63 patients (12%) on triple therapy (P=0.06). Seventy-nine patients (63%) on quadruple therapy simplified the regimen to triple therapy during the first year. Seven patients (6%) on quadruple and 51 (9%) on triple therapy interrupted treatment for more than two weeks or were lost-to-follow-up in the first year of treatment before reaching viral suppression and were censored in the survival analyses.

The median time to viral suppression after initiation of therapy was 5.8 (IQR 4.6-7.9) months in the patients on quadruple therapy and 6.0 (4.0-9.4) months in the patients on triple therapy (log rank, P=0.42; Figure 1A). The KM-estimates of the proportion of patients that had achieved a viral suppression <50 c/ml after the first year of treatment were 104/118 (88%) for patients on quadruple and 418/497 (84%) for triple therapy. 10/97 (10%) and 20/397 (5%) patients on quadruple and triple therapy for whom follow-up pVL measurements were available experienced virological failure (pVL>1000 c/ml) after initial viral suppression (P=0.05), after a median time of 12 (IQR 8-23) months. In the adjusted Cox regression analysis, quadruple therapy was not associated with time to viral suppression (HR 1.07 (95% CI 0.86-1.33), P=0.53). As expected, a regimen containing an integrase inhibitor was associated with a more rapid time to viral suppression (HR 1.90 (95% CI 1.13-3.18), P=0.02).

**Triple- versus dual-class therapy**

A total of 72/673 patients (11%) initiated triple-class therapy and 601/673 patients (89%) started dual-class therapy. Thirteen patients (18%) on triple-class therapy and 72 (12%) on dual-class therapy switched to an alternative regimen because of drug-related adverse events (P=0.14). The median time to viral suppression after initiation of triple- versus dual-class therapy was 5.7 (IQR 4.7-7.6) and 6.0 (4.0-9.3) months, respectively (P=0.32; Figure 1B). 62/69 (90%) and 460/546 (84%) patients initiating triple or dual-class therapy achieved viral suppression within the first year. In the adjusted Cox analysis, triple-class therapy was not associated with time to viral suppression (HR 1.10 (95% CI 0.84-1.44), P=0.48), but the use of an integrase inhibitor was (HR 1.87 (1.11-3.15), P=0.02).

**Discussion**

The present study demonstrates that quadruple/triple-class therapy was equally effective as standard-of-care triple therapy in treatment-naïve patients with a pVL above 500,000 copies/ml, although it did expose patients to more drug-related adverse events. These results provide no evidence of benefit of adding an additional fourth drug or third drug-class to standard triple therapy.

Reviewing the literature, our work is supported by several studies in which no differences were seen in viral suppression between treatment-naïve patients on quadruple/triple-class versus dual-class triple therapy [10-15]. Three randomized studies however demonstrated a virological benefit after initiation of triple-class therapy [5-7]. The difficulty in interpreting and comparing these findings is that some of these studies compared triple therapy with a dual-class quadruple regimen [10, 12-14], included an older generation unboosted PI as the
No advantage of quadruple therapy

Fourth drug or third drug-class [5-9, 11, 14, 15], did not include treatment-naïve patients [6, 7] or contained only a small number of patients on quadruple therapy [8-10, 13, 14]. In all studies, the median baseline pVL was also significantly (at least ≥0.4 log_{10} c/ml) lower than in ours.

Two nonrandomized studies showed a faster decline to pVL <50 c/ml after triple-class quintuple therapy [9] as compared to standard triple therapy, with an improved reduction of low-level viraemia (pVL 5-50 c/ml) after 144 weeks [8]. From our study, we cannot exclude that quadruple/triple-class therapy resulted in reduced low-level viral replication and a stronger long-term suppression of pVL when compared to dual-class triple therapy. Moreover, in the above two studies patients received prolonged triple-class therapy, whereas in our study more than half of patients on quadruple therapy switched to an alternative, often simplified regimen within the first year.

Figure 1. Kaplan-Meier curves of the probability of achieving a viral suppression. Kaplan-Meier curves of the probability to achieve a viral suppression below 50 c/ml for patients on quadruple and standard of care triple therapy (1A), and patients on triple- and dual-class therapy (1B).
The current study has limitations, which are inherent to observational cohort studies evaluating the effectiveness of cART. First, the preference of physicians to prescribe quadruple or triple therapy was not random and was possibly influenced by prognostic factors and therefore susceptible to bias [18]. The patients on quadruple therapy had a significantly lower CD4 count prior to treatment. We additionally adjusted for this difference by doing a propensity score weighted Cox regression analysis with weights [19] and found similar results (data not shown). In spite of this, unmeasured, residual confounding might have biased our results. Second, follow-up visits including pVL measurements were scheduled arbitrarily and may differ between the physicians and HIV treatment centers, possibly resulting in a less accurate estimate of the time to viral suppression for patients who did not come for regular check-ups. However, we adjusted for this in an additional survival model using Weibull distribution and found similar results (data not shown). Third, HIV genotyping before the initiation of therapy was not available for more than half of the patients. Finally, our results are not adjusted for nonadherence to therapy, which is an important determinant of virologic response [1] and may have compromised the effectiveness of the treatment regimen. This might explain the higher rate of virological failure in the patients on quadruple therapy, since they were exposed to a higher pill burden and more drug toxicity, and may therefore have been at an increased risk of nonadherence.

In conclusion, this study provides no evidence to support the use of quadruple/triple-class therapy in treatment-naïve patients with very high viraemia. Quadruple/triple-class therapy did not improve the antiretroviral activity of cART, yet did expose patients to additional drug toxicity.

Acknowledgments

The authors wish to thank Ard van Sighem for assisting with the data retrieval and Peter Reiss for critically reviewing the manuscript. The ATHENA national observational cohort has been made possible through the collaborative efforts of the following physicians (*site coordinating physicians): Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam: Prof. dr. J.M. Prins*, Prof. dr. T.W. Kuipers, Dr. H.J. Scherpber, Dr. K. Boer, Dr. J.T.M. van der Meer, Dr. F.W.M.N. Wit, Dr. M.H. Godfried, Prof. dr. P. Reiss, Prof. Dr. T. van der Poll, Dr. F.J.B. Nellen, Prof. dr. J.M.A. Lange, Dr. S.E. Geerlings, Dr. M. van Vugt, Drs. D. Pajkrt, Drs. J.C. Bos, Drs. M. van der Valk, Drs. M.L. Grijzen, Dr. W.J. Wiersinga. Academisch Ziekenhuis Maastricht, Maastricht: Dr. G. Schreij, Dr. S. Lowe*, Dr. A. Oude Lashof, Dr. D. Posthouwer. Catharina-ziekenhuis, Eindhoven: Drs. M.J.H. Pronk*, Dr. B. Bravenboer. Erasmus Medisch Centrum, Rotterdam: Dr. M.E. van der Ende*, Drs. T.E.M.S. de Vries-Sluijs, Dr. C.A.M. Schurink, Drs. M. van der Feltz, Dr. J.L. Nouwen, Dr. L.B.S. Gelinck, Dr. A. Verbon, Drs. B.J.A. Rijnders, Dr. L. Slobbe, Dr. E.C.M. van Gorp. Erasmus Medisch Centrum–Sophia, Rotterdam: Dr. N.G. Hartwig, Dr. G.J.A. Driessen. Flevoziekenhuis, Almere: Dr. J. Branger*. HagaZiekenhuis, Den Haag: Dr. E.F. Schippers*, Dr. C. van Nieuwoop, Drs. J.W. Bouwhuis. Kenemer Gasthuis: Prof. dr. R.W. ten Kate*, Dr. R. Soetekouw. Leids Universitair Medisch Centrum, Leiden: Dr. F.P. Kroon*, Prof. dr. P.J. van den Broek, Prof. dr. J.T. van Dissel, Dr. S.M. Arend, Drs. C. van Nieuwoop, Drs. M.G.J. de Boer, Drs. H. Jolink. Maastadziekenhuis, Rotterdam: Dr. J.G. den Hollander*, Dr. K. Pogany. Medisch Centrum Alkmaar, Alkmaar: Drs. G.
Contributors
MLG, JMP and FdW conceived the study. RH, LG and FWNMW conducted the statistical analysis. MLG and JMP provided valuable input into interpretation of data. MLG drafted the manuscript and JMP critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
No advantage of quadruple therapy

EIGHT

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Similar virologic response after initiation of triple-class antiretroviral therapy in primary and chronic HIV infection

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Abstract
We compared the time to viral suppression between a cohort of 70 primary HIV infected (PHI-) patients treated with triple-class therapy and a cohort of 80 naive chronic HIV infected (CHI-) patients with comparable treatment and plasma viral load (≥100,000 copies/ml) at start of cART. The time to viral suppression after initiation of triple-class therapy was comparable for PHI and CHI, suggesting that the virologic response to therapy is not related to the stage of HIV infection.
Studies comparing the virologic response to cART between persons with primary (PHI) and chronic HIV infection (CHI) have shown inconsistent results [1-5]. In a recent issue of this journal, a more rapid plasma viral load (pVL) decline was observed in PHI as compared to CHI after initiation of a standard NNRTI-based regimen [1]. We compared the time to viral suppression in our cohort of 70 PHI-patients treated with triple-class therapy with that of 80 naïve CHI-patients with comparable treatment and initial pVL.

PHI-patients were selected from the Primo-SHM cohort, a prospective cohort study in the Netherlands with an embedded randomized trial which investigated the effects of 24 or 60 weeks of cART during PHI [6]. PHI was defined as a negative or indeterminate Western blot combined with a detectable pVL, or, in case of a positive Western blot, a negative HIV screening test result ≤ 180 days. CHI-patients were selected from the Dutch observational HIV cohort (ATHENA) [7]. Inclusion criteria for PHI- and CHI-patients in the present study were: age ≥18 years, treatment-naïve, a pVL of ≥100.000 c/ml, and start of triple-class cART between January 2002 and June 2010.

Time to viral suppression, defined as a pVL <50 c/ml, was compared between the two cohorts using Kaplan-Meier plots and multivariate Cox regression analysis, the last adjusted for the pVL before treatment and regimens containing an integrase inhibitor. Analyses were intention-to-treat, regardless of treatment changes. Patients were censored when lost-to-follow-up or when cART was interrupted for more than two weeks. Chi-squared, Fisher’s exact and Kruskal-Wallis tests were used where appropriate.

Sixty-four of 70 PHI-patients (91%) and 72 of 80 CHI-patients (90%) were men (P=0.8). The PHI-patients were younger (median age 38 (IQR 31-45) versus 42 (36-49) years; P=0.02), were more often men who have sex with men (59 (84%) versus 50 (63%); P=0.003), and had a higher median CD4 count prior to treatment than CHI-patients (470 (IQR 300-550) versus 97 (39-215) cells/mm³; P=0.001). Median baseline pVL was similar in both groups: 5.7 (IQR 5.3-6.1) versus 5.7 (5.4-6.1) log₁₀ c/ml (P=0.4). Fifty-seven PHI-patients (81%) had a negative or indeterminate Western blot, 67 (96%) were symptomatic during PHI, and the median time between HIV diagnosis and start of early cART was 4 (IQR 3-7) weeks. Twenty-nine CHI-patients (36%) had experienced a CDC-C event before treatment initiation.

All 70 PHI-patients and 69 of 80 CHI-patients (86%) initiated a triple-class regimen containing two NRTIs, an NNRTI and a boosted PI. The remaining 11 CHI-patients (14%) received an integrase inhibitor in addition to two NRTIs plus an NNRTI (n=5) or boosted PI (n=6). HIV genotyping was available for 111 patients (74%): three of 54 PHI- (6%) and six of 57 CHI-patients (11%; P=0.5) harboured one or more transmitted drug resistance mutations in reverse transcriptase or protease [8], of whom one PHI-patient and two CHI-patients were judged as being treated with an ineffective triple-class regimen and therefore excluded from further analyses.

A total of 47 of 69 PHI-patients (68%) and 59 of 78 CHI-patients (76%) switched to an alternative or simplified regimen before 24 weeks (P=0.3). Median time to viral suppression was comparable between both groups (log-rank, P=0.5; Figure 1). This was confirmed by the adjusted Cox regression analysis (HR PHI versus CHI 1.08 (95% CI 0.74-1.58), P=0.7). After 24 weeks 50% of the patients in both groups had a pVL ≤50 copies/ml, and after 48 weeks this was 82% in PHI- and 93% in CHI-patients.
Virologic response in primary and chronic HIV infection

Figure 1. Kaplan-Meier curves of the probability of achieving a viral suppression. Kaplan-Meier curve of the probability to achieve viral suppression (pVL <50 copies/ml) for primary and chronic HIV-infected patients with a high viraemia (pVL >100,000 copies/ml) initiating triple-class therapy.

The time to viral suppression in PHI was comparable to CHI after initiation of triple-class therapy. This is in contrast with a recent report, in which phase II viral decay was faster in PHI than in CHI, which resulted in a shorter time to viral suppression after start of treatment with dual-class, NNRTI-based therapy [1]. A much smaller study, which was designed to assess the effect of raltegravir on viral dynamics, also demonstrated a faster time to viral suppression in PHI than in CHI, but did not adjust for the higher baseline pVL in the CHI-patients [2]. In contrast to our study, both latter studies compared patients receiving standard triple therapy [1, 2], and the question is whether this explains why these two studies found a difference between PHI- and CHI-patients. A prospective cohort study observed that PHI-patients receiving quadruple dual-class therapy had a faster phase II viral decay and a non-significant shorter time to viral suppression than PHI-patients on triple therapy [9]. However, it is not clear how this differential effect of the regimen on HIV suppression should explain a shorter time to viral suppression in PHI- versus CHI-patients for dual-class [1], as opposed to triple-class therapy.

In summary, our results demonstrate that PHI- and CHI-patients with high viraemia are equally rapidly suppressed after initiation of triple-class therapy, suggesting that the virologic response to cART is not related to the stage of HIV infection.

Acknowledgments

The ATHENA national observational cohort has been made possible through the collaborative efforts of the following physicians (*site coordinating physicians): Academisch Medisch Centrum, University of Amsterdam, Amsterdam: Prof. dr. J.M. Prins*, Prof. dr. T.W. Kuijpers, Dr. H.J. Scherpier, Dr. K. Boer, Dr. J.T.M. van der Meer, Dr. F.W.M.N. Wit, Dr. M.H. Godfried, Prof. dr. P. Reiss, Prof. Dr. T. van der Poll, Dr. F.J.B. Nellen, Prof. dr. J.M.A. Lange, Dr. S.E. Geerlings, Dr. M. van Vugt, Drs. D. Pajkrt, Drs. J.C. Bos, Drs. M. van der Valk, Drs. M.L. Grijsen, Dr. W.J.
Virologic response in primary and chronic HIV infection


Contributors
MLG and JMP conceived the study. RH, LG and FWNMW conducted the statistical analysis. MLG and JMP provided valuable input into interpretation of data. MLG drafted the manuscript and JMP critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
References


PART IV

Bone mineral density during primary HIV infection
High prevalence of reduced bone mineral density in primary HIV-1 infected men

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Abstract

Objective
To assess the bone mineral density (BMD) in a cohort of men with primary HIV-1 infection (PHI).

Methods
Thirty-three men with PHI had a DXA of the lumbar spine, femoral neck and total hip. Osteopenia and osteoporosis were defined according to WHO criteria as T-scores between -1 and -2.5 and ≤ -2.5, respectively. The association between clinical and laboratory parameters and BMD was investigated using multivariable linear regression analysis.

Results
Mean age was 38 (SD 9) years and mean body mass index (BMI) 22.7 (SD 3.3) kg/m². Twenty-four men (73%) had a negative or indeterminate Western blot, 32 men (97%) were cART naive. Mean plasma HIV-1 RNA was 5.0 (SD 1.2) log₁₀ copies/ml. Mean lumbar spine T (-0.8, SD 1.3, P=0.001) and Z-scores (-0.7, SD 1.3, P=0.004) and femoral neck T-score (-0.5, SD 0.9, P=0.003) were significantly lower compared to the reference population. 15/33 men (45%) had osteopenia and 2/33 (6%) osteoporosis. Markers of bone turnover did not differ between patients with or without osteopenia/osteoporosis. Age was negatively associated with femoral neck (β-coefficient= -0.05; P<0.001) and total hip T-scores (β= -0.03; P=0.04). BMI was associated with lumbar spine (β=0.3), femoral neck (β=0.2) and total hip (β=0.2) T-scores (P<0.001) and thyroid stimulating hormone (TSH) with lumbar spine (β=0.5; P=0.045) and femoral neck T-scores (β=0.4; P<0.005). Increased plasma viral load was associated with lower total hip T-scores (β= -0.2; P=0.02).

Conclusions
Reduced BMD was prevalent in PHI-men and was associated with increased age, lower BMI and TSH levels, and higher levels of HIV-1 viraemia.
Background

Multiple cross-sectional studies have shown an increased prevalence of reduced bone mineral density (BMD) among HIV infected individuals [1, 2]. A systematic review revealed a 6.4-fold increased odds of reduced BMD in HIV infected subjects and a 3.7-fold increased odds of osteoporosis compared to HIV uninfected controls [2]. A recent population based study confirmed a higher prevalence of bone fractures in HIV infected men and women compared to HIV uninfected controls [3].

Although the aetiology and pathogenesis underlying this bone loss are not completely understood, the reduced BMD in HIV infected individuals is most likely of multifactorial origin [4]. Besides conventional risk factors, which might be more prevalent among HIV infected persons, HIV infection itself and combination antiretroviral therapy (cART) might each contribute [5-8]. In order to gain further insight into the contribution of HIV infection per se, we assessed BMD in a cohort of men with primary HIV-1 infection (PHI).

Methods

Study population

We evaluated the BMD of 33 men with PHI who presented at the Academic Medical Center (AMC) in Amsterdam between February 1st 2008 and October 31st 2009. All participants were enrolled in the Primo-SHM cohort, a multi-centre prospective cohort study in the Netherlands, with an embedded randomized trial, that investigates the natural course of HIV-1 infection, and the effects of early cART in patients with PHI [9]. Main inclusion criteria are age ≥ 18 years and laboratory evidence of PHI, defined as having a negative or indeterminate Western Blot in combination with detectable plasma HIV-1 RNA, or, in case of a positive Western Blot, a proven negative HIV screening test result within the previous 180 days. Exclusion criteria for the present study were medical conditions known to affect bone metabolism (e.g. hypercalcaemia), and corticosteroid therapy for ≥3 months. The study was approved by the Medical Ethics Committee of the AMC. All participants provided written informed consent.

Screening procedures at enrolment

A questionnaire was administered evaluating sociodemographic characteristics, risk factors for reduced BMD and occurrence of symptoms compatible with an acute retroviral syndrome (ARS). Blood was collected for HIV related parameters, hepatitis B/C and syphilis serology and biochemical markers of bone metabolism, including bone formation markers (total alkaline phosphatase, osteocalcin, and procollagen type 1 N-terminal propeptide) and bone resorption markers (C-terminal telopeptide of type 1 collagen and C-telopeptide crosslink of type 1 collagen).

BMD of the lumbar spine, femoral neck and total hip were measured by dual energy X-ray absorptiometry (DXA) using a Hologic QDR 4500W densitometer, software version 12.4. BMD was expressed in T- and Z-scores. T-scores refer to the difference in standard deviations (SD) between a patient’s BMD and that of young healthy adults matched for gender and ethnic group. Z-scores represent the difference in SD compared with an age-matched reference population. Osteopenia and osteoporosis were defined according to the WHO criteria as T-scores measured at lumbar spine, femoral neck and/or total hip between -1 and -2.5 SD and ≤-2.5 SD, respectively.
Low bone density in primary HIV-1 infected men

The overall fracture risk was assessed by FRAX®, a tool that calculates the 10-year probability of a major osteoporotic (spine, humerus, wrist) or hip fracture, based on validated clinical risk factors and the femoral neck T-score [10]. FRAX algorithms have not specifically been validated for relatively young HIV infected persons. Since FRAX was not available for the Netherlands, we used the German algorithm (www.shef.ac.uk/FRAX).

Data analysis
Mean T- and Z-scores of lumbar spine, femoral neck and total hip were calculated and compared to the reference population by one sample T-tests. Data of osteopenic and osteoporotic patients were combined in further analyses. Sociodemographic characteristics and laboratory values were compared between patients with normal and reduced BMD using independent sample T-tests and Wilcoxon rank sum tests for continuous data and chi-squared tests for categorical data. The association of the various parameters with lumbar spine, femoral neck and total hip T-scores was examined using multivariable linear regression analysis. All variables were evaluated separately and those associated (p<0.1) with reduced BMD were stepwise entered into a linear regression model for all three measured bone sites. All models were adjusted for patient age and BMI. Median FRAX scores were calculated for the total group of PHI-men and for patients with a normal or reduced BMD. Data were analyzed using SPSS statistical software version 16.0 (SPSS Inc., Chicago, Illinois, USA). P-values <0.05 were considered statistically significant.

Results
Patient characteristics
Most subjects (91%) were men who have sex with men (MSM). 73% were diagnosed with PHI based on a negative or indeterminate Western blot combined with detectable plasma HIV-1 RNA. One patient had been on cART since 9 days, the remaining 32 patients were antiretroviral therapy naïve. Five patients (15%) were hospitalized for several days during the ARS, none had been exposed to IV-drug use or was co-infected with syphilis or hepatitis B/C. Patient characteristics and biochemical markers relevant for bone metabolism are summarized in table 1. None of the patients had reduced levels of testosterone or vitamin D metabolites. Ten patients, equally distributed among patients with normal and reduced BMD, had low levels of osteocalcin, possibly indicating a decrease in bone formation. Levels of bone formation and resorption markers were not significantly different between patients with or without reduced BMD.

Prevalence of reduced bone density
The median number of days between the first HIV-1 positive test and the DXA-scan was 32 (IQR 20-45) days. Mean lumbar spine T (-0.8, SD 1.3, P=0.001) and Z-scores (-0.7, SD 1.3, P=0.004) and mean femoral neck T-score (-0.5, SD 0.9, P=0.003) were significantly lower compared to the reference population. The mean total hip T-score (-0.3, SD 0.8), femoral neck (-0.1, SD 0.8) and total hip (-0.1, SD 0.8) Z-scores were also lower than the reference values, but the differences did not reach statistical significance. Of the 33 men, 17 had reduced BMD of whom 15 (45%) had osteopenia and 2 (6%) had osteoporosis. For those patients with low BMD, bone loss predominated at the lumbar spine (mean T-score -1.8, SD 0.8).
Table 1. Patient characteristics of 33 PHI-men

<table>
<thead>
<tr>
<th>Demographic data and risk factors for BMD</th>
<th>Total (N=33)</th>
<th>Normal BMD (N=16)</th>
<th>Reduced BMD(^a) (N=17)</th>
<th>P-value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>38 (9)</td>
<td>37 (9)</td>
<td>39 (10)</td>
<td>0.7</td>
</tr>
<tr>
<td>Weight (kg), mean (SD)</td>
<td>76.0 (12.1)</td>
<td>78.3 (13.4)</td>
<td>73.8 (10.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>BMI (kg/m(^2)), mean (SD)</td>
<td>22.7 (3.3)</td>
<td>24.7 (3.4)</td>
<td>22.0 (2.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>Caucasian race, n (%)</td>
<td>26 (79)</td>
<td>13 (81)</td>
<td>13 (77)</td>
<td>0.7</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>18 (55)</td>
<td>9 (56)</td>
<td>9 (53)</td>
<td>0.8</td>
</tr>
<tr>
<td>Alcohol (≥ 3 units/day), n (%)</td>
<td>7 (21)</td>
<td>2 (13)</td>
<td>5 (29)</td>
<td>0.2</td>
</tr>
<tr>
<td>Current drug use, n (%)</td>
<td>22 (67)</td>
<td>12 (75)</td>
<td>10 (59)</td>
<td>0.3</td>
</tr>
<tr>
<td>Dairy food intake (≥ 3 products /week), n (%)</td>
<td>30 (91)</td>
<td>15 (94)</td>
<td>15 (88)</td>
<td>0.6</td>
</tr>
<tr>
<td>Multivitamin use, n (%)</td>
<td>17 (52)</td>
<td>8 (50)</td>
<td>9 (53)</td>
<td>0.9</td>
</tr>
<tr>
<td>History of bone fracture, n (%)</td>
<td>12 (36)</td>
<td>6 (38)</td>
<td>6 (35)</td>
<td>0.9</td>
</tr>
<tr>
<td>Strenuous physical activity (20 min ≥ 3x/week), n (%)</td>
<td>19 (58)</td>
<td>9 (56)</td>
<td>10 (59)</td>
<td>0.9</td>
</tr>
<tr>
<td>Stage of PHI(^c), n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- I</td>
<td>24 (73)</td>
<td>11 (69)</td>
<td>13 (76)</td>
<td>0.6</td>
</tr>
<tr>
<td>- II</td>
<td>9 (27)</td>
<td>5 (31)</td>
<td>4 (24)</td>
<td></td>
</tr>
<tr>
<td>Symptoms during PHI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Acute retroviral syndrome, n (%)</td>
<td>27 (82)</td>
<td>13 (81)</td>
<td>14 (82)</td>
<td>0.9</td>
</tr>
<tr>
<td>- Weight loss ≥ 5kg, n (%)</td>
<td>8 (24)</td>
<td>4 (25)</td>
<td>4 (24)</td>
<td>0.9</td>
</tr>
<tr>
<td>CRP (mg/L)(^d), median (IQR)</td>
<td>1 (1-1.5)</td>
<td>1 (1-2.2)</td>
<td>1 (1-2)</td>
<td>0.1</td>
</tr>
<tr>
<td>CD4 count (cells/mm(^3)), mean (SD)</td>
<td>551 (251)</td>
<td>643 (280)</td>
<td>465 (190)</td>
<td>0.04</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log(_{10}) copies/ml), mean (SD)</td>
<td>5.0 (1.2)</td>
<td>5.1 (1.2)</td>
<td>4.9 (1.3)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical markers relevant for bone metabolism</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium(^e), mean (SD)</td>
<td>2.2-2.6 mmol/L</td>
</tr>
<tr>
<td>Phosphate, mean (SD)</td>
<td>0.7-1.45 mmol/l</td>
</tr>
<tr>
<td>ALP, mean (SD)</td>
<td>40-120 U/L</td>
</tr>
<tr>
<td>25-hydroxyvitamin D, mean (SD)</td>
<td>19-126 nmol/L</td>
</tr>
<tr>
<td>1,25-dihydroxyvitamin D, median (IQR)</td>
<td>40-140 pmol/L</td>
</tr>
<tr>
<td>PTH, mean (SD)</td>
<td>0.6-6.7 pmol/L</td>
</tr>
<tr>
<td>TSH(^f), median (IQR)</td>
<td>0.5-5 mE/L</td>
</tr>
<tr>
<td>Testosterone, mean (SD)</td>
<td>11-35 nmol/L</td>
</tr>
<tr>
<td>SHBG, mean (SD)</td>
<td>12-75 nmol/L</td>
</tr>
<tr>
<td>FAI, median (IQR)</td>
<td>20-90</td>
</tr>
<tr>
<td>Osteocalcin, median (IQR)</td>
<td>2.4-23.5 µg/L</td>
</tr>
<tr>
<td>PINP, mean (SD)</td>
<td>22-87 µg/L</td>
</tr>
<tr>
<td>1CTP, mean (SD)</td>
<td>2.1-5.0 µg/L</td>
</tr>
<tr>
<td>CTX, median (IQR)</td>
<td>&lt;584 ng/L</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CRP, C-reactive protein; 1CTP, C-terminal telopeptide of type 1 collagen; CTX, C-telopeptide crosslink of type 1 collagen; FAI, free androgen index (FAI = (testosterone/SHBG) x 100); PINP, procollagen type I N-terminal propeptide; PHI, primary HIV-1 infection; PTH, parathyroid hormone; SHBG, sex hormone-binding globulin; TSH, thyroid stimulating hormone.

\(^a\) Reduced BMD defined as a T-score ≤ -1 in either the lumbar spine and/or the hip.

\(^b\) P-value based on independent T-test, Wilcoxon rank sum test or \(\chi^2\) test for proportions.

\(^c\) I: HIV-1 RNA positive and negative or low HIV-antibodies or indeterminate Western Blot; II: HIV-1 RNA, HIV-1 specific antibodies and Western Blot positive and a documented HIV-1 negative test in preceding 180 days.

\(^d\) 1 patient with missing result.

\(^e\) Serum calcium was corrected for serum albumin (g/L) using the equation: corrected calcium = calcium + ((40 – albumin) x 0.025).

\(^f\) 2 patients with missing results.
Factors associated with reduced BMD

Age was negatively associated with femoral neck ($\beta$-coefficient= -0.05 (95% CI -0.07 to -0.02); $P<0.001$) and total hip T-scores ($\beta$= -0.03 (-0.05 to -0.001); $P=0.04$). BMI was positively associated with lumbar spine ($\beta$=0.3 (0.2-0.4); $P<0.001$), femoral neck ($\beta$=0.2 (0.1-0.2); $P<0.001$) and total hip ($\beta$=0.2 (0.1-0.2); $P<0.001$) T-scores, and TSH levels with lumbar spine ($\beta$=0.5 (0.01-0.9); $P=0.045$) and femoral neck T-scores ($\beta$=0.4 (0.1-0.6); $P=0.005$). Increased levels of plasma HIV-1 RNA were associated with a lower total hip T-score ($\beta$= -0.2 (-0.4 to -0.04); $P=0.02$). In our study population of PHI-men, differences in BMI accounted for a substantial part of the observed differences in BMD: 43%, 45% and 56% for the lumbar spine, femoral neck and total hip T-scores, respectively, as patients with reduced BMD were on average 2.7 kg/m² lighter than patients with normal BMD. The three remaining covariates, although significantly associated with BMD, did not explain much of the observed differences in BMD.

Fracture risk

Ten-year probabilities of major osteoporotic fractures and hip fractures as predicted by the German FRAX algorithm are shown in table 2. Three men (9%) had a 10-year risk of a major osteoporotic fracture above 7.5%, the threshold at which in the UK antiresorptive treatment is considered to be cost effective at the age of 50 years [11, 12].

Table 2. Ten-year fracture risk assessment using the FRAX algorithm for Germany computed with the femoral neck T-score

<table>
<thead>
<tr>
<th></th>
<th>Total (N=33)</th>
<th>Normal BMD (N=16)</th>
<th>Reduced BMD* (N=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major osteoporotic fracture, median (IQR)</td>
<td>2.9 (2.7-5.7)</td>
<td>2.8 (2.4-4.6)</td>
<td>4.0 (2.9-6.8)</td>
</tr>
<tr>
<td>Hip fracture, median (IQR)</td>
<td>0.3 (0.1-0.9)</td>
<td>0.2 (0.1-0.3)</td>
<td>0.6 (0.3-2.0)</td>
</tr>
</tbody>
</table>

* Reduced BMD defined as a T-score ≤ -1 in either the lumbar spine and/or the hip.

Discussion

Reduced BMD is increasingly being recognized among HIV infected populations. It remains unknown whether this is caused by HIV infection itself, cART, traditional risk factors or a combination of all three [13]. The present study provides the first data on the frequency of osteopenia and osteoporosis in a cohort of untreated PHI-men. Half of them had reduced BMD, of whom 45% had osteopenia and 6% osteoporosis. These numbers are much higher than would be expected in a relatively young male population like ours, but are in agreement with frequency rates of 40-83% reported in patients with chronic HIV-1 infection [2].

In our cohort, reduced BMD was especially seen in the lumbar spine, which mostly consists of trabecular bone. Osteoporosis in men usually involves the cortical bone (e.g. femoral neck), which is less sensitive to acute changes in health and medication [14]. Studies describing the site of bone loss in HIV infected patients are limited and have been inconclusive [15, 16]. No differences were seen in biochemical markers of bone metabolism between patients with...
or without osteopenia/osteoporosis. None of our patients had low vitamin D metabolites, a finding that is frequently reported among chronic HIV-1 infected patients [17].

Not surprisingly, reduced BMD was independently associated with age and BMI at the measured bone sites, confirming findings obtained from the general population and studies evaluating bone disorders in HIV-1 infected populations [18, 19]. MSM have a lower body weight than heterosexual men [20] and might therefore be more at risk to develop a reduced BMD. Among our study population, the linear regression models showed that differences in BMI between patients with or without osteopenia/osteoporosis accounted for only 50% of the reduced BMD. TSH was positively associated with lumbar spine and femoral neck T-scores, as confirmed by the literature [21]. The total hip T-score was associated with the degree of HIV-1 replication. Previous studies have shown an association between bone loss and high plasma viral load levels [22]. As PHI is associated with high levels of HIV-1 viremia [23], this may suggest a direct role of HIV infection on osteoblast and osteoclast activity [24]. Reduced serum osteocalcin levels have been described in patients with acute viral hepatitis [25], suggesting that acute viral infections as such may affect bone turnover.

The study has several limitations. It is a cross-sectional analysis, which can not establish causal relationships between HIV infection and reduced BMD. Second, the majority of our patients have been identified as having PHI based on symptoms. Since ARS is known to be an independent prognostic factor of AIDS progression [26], the results may not be generalizable to those who are asymptomatic during PHI. Finally, it is remarkable that low BMD develops so quickly during PHI. Rapid bone loss can be seen during immobilization [27, 28]. On the other hand, a study from San Francisco among 209 healthy HIV seronegative MSM reported a high proportion of bone thinning, which was associated with popper and amphetamine use [29]. This raises the question whether it is actually the recent HIV-1 infection causing rapid bone loss shortly after transmission, or whether these bone disorders pre-date HIV infection and are caused by other risk factors. Of note, the reduced BMD present in our PHI-men was not associated with biochemical evidence of increased bone turnover or systemic inflammation as measured by CRP, which might have been expected in the context of the generalised inflammation associated with PHI. In patients with inflammatory bowel disease for example, bone turnover was found to be elevated in relation to the underlying disease activity and inversely related with BMD, suggesting that bone metabolism was affected by the underlying disease [30, 31].

In conclusion, this study shows a high rate of osteopenia and osteoporosis early in the course of HIV infection, before the possible influence of cART. Reduced BMD was associated with older age, lower BMI and TSH levels, and a higher degree of HIV-1 replication. Longitudinal studies are needed to evaluate changes over time. Studies involving HIV seronegative controls will be a key in understanding whether these findings relate to the presence of HIV or other risk factors affecting bone health among MSM.
Acknowledgments
The authors wish to thank the HIV research nurses and the nuclear medicine department of the Academic Medical Center (AMC), the laboratory staff of the endocrinology department of the VU Medical Center, the HIV Monitoring Foundation and in particular the study participants for helping to establish this cohort. We gratefully acknowledge the contribution of Prof. dr. B.L.F. van Eck-Smit (AMC) for the interpretation of the DXA-scans. Special thanks to S. Jurriaans and N. Back (AMC) for interpreting the HIV test results and Christoph Fux (Inselpital and University Hospital of Bern, Switzerland) for his useful input in developing the study questionnaire.

Contributors
MLG and JMP drafted the manuscript. MLG, FW and JMP conducted the statistical analysis. MLG, RS and JMP established the cohort. SMEV, RS, PL and PR provided valuable input into protocol development, interpretation of data and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
References


Low bone mineral density, regardless of HIV status, in men who have sex with men

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Low bone density in MSM regardless of HIV

Abstract
A high prevalence of low bone mineral density (BMD) has been reported among men with primary and chronic HIV infection. To gain further insight into the contribution of HIV infection, we compared the BMD of 41 men who have sex with men (MSM) with primary HIV infection, 106 MSM with chronic HIV infection, and a control group of 30 MSM without HIV infection. Low BMD, defined as a Z-score ≤ -2.0 SD at the lumbar spine or hip, was highly prevalent in all three groups. In the multivariate analyses, HIV infection was not associated with BMD, suggesting that low BMD previously reported in HIV infected MSM may pre-date HIV acquisition.
Background

Multiple studies have shown that HIV infected persons have a higher prevalence of low bone mineral density (BMD) compared to the general population [1]. In addition to traditional risk factors for low BMD, HIV infection alone and exposure to combination antiretroviral therapy (cART) have been suggested as contributing factors [1].

Recently we reported a high prevalence of low BMD in the absence of markers indicating increased bone turnover in a cohort of men with primary HIV infection. Low BMD was associated with older age, lower body mass index (BMI), decreased thyroid-stimulating hormone levels, and higher HIV viremia [2]. As these patients were cART-naive, our findings raised the question of whether low BMD was related to recent acquisition of HIV, whether it was related to other conventional risk factors affecting BMD that might be more prevalent among HIV infected persons, or alternatively, whether it predated HIV acquisition. To gain further insight into the contribution of HIV infection, we compared BMD and biochemical markers of bone metabolism among men who have sex with men (MSM), who had untreated primary or chronic HIV infection with those in a control group of MSM without HIV infection.

Methods

Between January 2008 and 2011, dual energy X-ray absorptiometry (DXA) scans were prospectively performed of all cART-naïve HIV positive patients presenting for care at the Academic Medical Center in Amsterdam, the Netherlands. In the present study we included all primary (n=41) and chronic (n=106) HIV infected MSM aged between 20 and 55 years in whom DXA scans were performed during this period. Primary HIV infection was defined as a negative or indeterminate Western Blot in combination with detectable plasma HIV-1-RNA, or, in case of a positive Western Blot, a negative HIV screening test result within the previous 180 days [3]. 30 of these primary HIV infected MSM were described in our previous paper [2], 11 additional primary HIV infected MSM who presented between November 2009 and December 2010 were added to this cohort. The chronic HIV infected MSM were cART-naïve patients who were not classified as having primary HIV infection. Controls were 30 HIV negative MSM who participate in the Amsterdam Cohort Studies and are at risk for HIV infection [4], had a follow-up visit at the Municipal Public Health Service of Amsterdam between March and July 2010, were aged 20-55 years, and tested HIV seronegative. We assumed that these HIV negative controls had lifestyles, which were comparable to the HIV infected MSM.

Exclusion criteria in all three cohorts were medical conditions known to affect bone metabolism (e.g. chronic renal disease, hypercalcemia), injecting drug use and corticosteroid therapy used 3 months or longer. The study was approved by the Ethics Committee of our hospital. All participants provided written informed consent.

Socio-demographic characteristics were obtained from all participants through a questionnaire. For the primary HIV infected MSM and HIV negative controls, we additionally assessed risk factors for low BMD (e.g. smoking, alcohol or drug use, dairy food intake, history of bone fracture) through a questionnaire and collected blood for biochemical markers relevant for bone metabolism, including bone formation markers (alkaline phosphatase and
procollagen type 1 N-terminal propeptide (PINP) and bone resorption markers (C-terminal telopeptide of type 1 collagen (ICTP) and C-telopeptide crosslink of type 1 collagen (CTX)) at enrolment. Calcium, phosphate, alkaline phosphatase, thyroid stimulating hormone and 25-hydroxyvitamin D were analysed by an immunoturbidimetric method (Roche Diagnostics GmbH, Manheim, Germany). Testosteron was analysed by an in-house radioimmunoassay and SHBG by an immunoradiometric assay (Orion Diagnostica, Espoo, Finland). PINP and ICTP were analysed by a radioimmunoassay (Orion Diagnostica, Espoo, Finland) and CTX was determined by an immunometric assay (Roche Diagnostics Corporation, Indianapolis, USA).

BMD (g/cm²) of the lumbar spine (L1-L4), femoral neck and total hip was measured by DXA using Hologic QDR 4500W densitometer, software version 12.4. The reference database of the National Health and Nutrition Examination Survey (NHANES IV) was used for calculating lumbar spine and hip T- and Z-scores. T-scores refer to the number of standard deviations (SDs) below the mean BMD of young, healthy adults matched for sex and ethnicity and Z-scores to the number of SDs below the mean BMD of an age, sex and ethnicity-matched reference population. The International Society for Clinical Densitometry defines low BMD in men aged younger than 50 years as a Z-score of -2.0 SD or lower at either the spine or hip and states that in this age group osteoporosis should not be diagnosed on BMD measurements alone [5]. We therefore did not further distinguish between osteopenia or osteoporosis.

Mean T- and Z-scores of lumbar spine, femoral neck and total hip of primary and chronically HIV infected MSM and HIV negative controls were compared to the reference population by one-sample T-tests. Patient characteristics and BMD were compared between the three groups using one-way Anova or independent sample T-tests for continuous data, and chi-square or Fisher’s exact tests for categorical data. Given a common standard deviation of the BMD Z-scores in the three groups of about 0.9, and sample sizes of 41 MSM with primary HIV infection, 106 with chronic HIV infection, and 30 HIV negative controls, we had 80% power to detect a difference in BMD Z-scores of +/- 0.68 when comparing primary HIV infection with HIV negative controls, +/- 0.58 comparing chronic HIV infection with controls, and +/- 0.52 comparing primary with chronic HIV infection. The association of HIV with T- and Z-scores was examined using multivariable linear regression, adjusted for BMI and in the case of T-scores also for age. Multivariable models were constructed separately for all three measured bone sites. Analyses were conducted using SPSS, version 18.0. P-values <0.05 were considered statistically significant.

Results

Forty-one primary and 106 chronically cART-naïve HIV infected MSM and 30 unmatched HIV negative controls were included in this study. Patient characteristics, bone markers and BMD are summarized in Table 1. Thirty primary HIV infected MSM (73%) had a negative or indeterminate Western blot (Fiebig stage I-IV), 33 (81%) had symptomatic primary HIV infection and all but one were cART-naïve (this patient had 9 days of cART exposure). The median number of days between diagnosis and the DXA-scan was 28 (IQR 20-43). For MSM with chronic HIV infection, the duration of infection was not known. The DXA-scan was performed at their initial visit to our hospital prior to starting cART. Among MSM with primary and chronic HIV infection, the
mean plasma HIV-1-RNA loads were 5.3 (SD 1.2) and 4.5 (0.9) log_{10} copies/ml, respectively, and the mean CD4 cell counts were 543 (253) and 438 (214) cells/mm³, respectively. All participants denied prior injection drug or corticosteroid use.

HIV infected MSM had a significantly lower mean body weight (P=0.009) and a lower BMI (P=0.04) than HIV negative MSM. Additional risk factors and bone markers were assessed in the primary HIV infected MSM and HIV negative controls only. Except for a higher percentage of smokers among primary HIV infected MSM, risk factors for low BMD were not significantly different between primary HIV infected MSM and HIV negative controls. Five primary HIV infected MSM (13%) and 3 HIV negative controls (10%) had low 25-hydroxyvitamin D (P=1.0) and none versus 2 (7%) had low testosterone (P=0.2), respectively. Serum phosphate and the bone formation marker PINP were lower, and alkaline phosphatase, sex hormone-binding globulin and the bone resorption marker CTX were significantly higher in primary HIV infected MSM. Yet, all values were within reference ranges (Table 1).

Mean T- and Z-scores of the primary and chronically HIV infected MSM and HIV negative controls were compared to the reference population. With the exception of the total hip Z-score in HIV negative controls, the lumbar spine, femoral neck and total hip T- and Z-scores of all three groups were statistically significantly lower than zero (which is the mean of the reference population), indicating that the average bone density of our study populations were lower than the average bone density of the NHANES IV reference population database. Lumbar spine BMD (g/cm²) and T- and Z-scores were slightly, but not significantly lower in the HIV infected MSM than in HIV negative controls. Femoral neck and total hip BMD, T- and Z-scores were not different for primary and chronically HIV infected and HIV negative MSM. BMD did not differ significantly between the primary and chronically HIV infected MSM.

Eight (20%) primary and 23 (22%) chronic HIV infected MSM, and four (13%) HIV negative controls had low BMD at one or more of the three bone sites (P=0.6).

In the multivariable analyses, primary and chronic HIV infection, compared to being HIV negative, were not associated with lumbar spine, femoral neck and total hip Z-scores. BMI was positively associated with Z-scores of all three bone sites (Table 2). The association of the various parameters with T-scores was comparable (data not shown).

**Discussion**

We found significantly decreased mean T- and Z-scores and a high prevalence of low BMD among MSM with untreated primary and chronic HIV infection, as well as among HIV negative MSM. Lumbar spine T- and Z-scores of the primary and chronic HIV infected MSM were slightly lower, though non-significantly, than those of the HIV negative controls, which could be explained by the significantly lower BMI of these patients. In the multivariable analysis, HIV infection was not associated with BMD, suggesting that at least part of the loss in BMD previously reported in HIV infected MSM pre-dates HIV acquisition.

Our findings are in agreement with recently presented preliminary data, which showed a comparable BMD in 43 untreated chronic HIV infected adults and 35 age-, sex- and race-matched HIV negative controls [6]. Moreover, two antiretroviral pre-exposure prophylaxis
Low bone density in MSM regardless of HIV

Table 1. Characteristics of men who have sex with men, by HIV status

<table>
<thead>
<tr>
<th></th>
<th>HIV-negative MSM (N=30)</th>
<th>Primary HIV-infected MSM (N=41)</th>
<th>Chronic HIV-infected MSM (N=106)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 (6)</td>
<td>38 (9)</td>
<td>36 (8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.0 (21.1)</td>
<td>73.6 (11.5)</td>
<td>73.7 (11.3)</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 (4.8)</td>
<td>22.7 (3.1)</td>
<td>22.7 (2.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Caucasian</td>
<td>24 (80)</td>
<td>34 (83)</td>
<td>85 (80)</td>
<td>0.9</td>
</tr>
<tr>
<td>Risk factors for low BMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>7 (23)</td>
<td>18 (44)</td>
<td>n.a.</td>
<td>0.07</td>
</tr>
<tr>
<td>Alcohol (≥ 3 units/day)</td>
<td>7 (23)</td>
<td>7 (17)</td>
<td>n.a.</td>
<td>0.5</td>
</tr>
<tr>
<td>Current recreational drug use</td>
<td>15 (50)</td>
<td>27 (66)</td>
<td>n.a.</td>
<td>0.2</td>
</tr>
<tr>
<td>Dairy food intake (≥ 3 products /week)</td>
<td>28 (93)</td>
<td>35 (90)b</td>
<td>n.a.</td>
<td>0.7</td>
</tr>
<tr>
<td>Multivitamin use</td>
<td>15 (50)</td>
<td>22 (56)b</td>
<td>n.a.</td>
<td>0.6</td>
</tr>
<tr>
<td>History of bone fracture</td>
<td></td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Strenuous physical activity (20 min ≥ 3x/week)</td>
<td>16 (53)</td>
<td>25 (64)b</td>
<td>n.a.</td>
<td>0.4</td>
</tr>
<tr>
<td>Biochemical markers relevant for bone metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium² [2.2-2.6 mmol/l]</td>
<td>2.22 (0.14)</td>
<td>2.17 (0.08)</td>
<td>n.a.</td>
<td>0.08</td>
</tr>
<tr>
<td>Phosphate [0.7-1.45 mmol/l]</td>
<td>1.32 (0.23)</td>
<td>0.93 (0.18)</td>
<td>n.a.</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alk Phos [40-120 U/l]</td>
<td>59 (18)</td>
<td>69 (21)</td>
<td>n.a.</td>
<td>0.04</td>
</tr>
<tr>
<td>25-hydroxi-vitamin D [28-107 nmol/l]</td>
<td>64 (27)</td>
<td>75 (37)a</td>
<td>n.a.</td>
<td>0.2</td>
</tr>
<tr>
<td>TSH [0.5-5 mE/l]</td>
<td>1.8 (0.8)</td>
<td>1.6 (0.8)*</td>
<td>n.a.</td>
<td>0.2</td>
</tr>
<tr>
<td>Testosterone [11-35 nmol/l]</td>
<td>19 (6)</td>
<td>21 (6)</td>
<td>n.a.</td>
<td>0.1</td>
</tr>
<tr>
<td>SHBG [12-75 nmol/l]</td>
<td>31 (11)</td>
<td>40 (16)</td>
<td>n.a.</td>
<td>0.01</td>
</tr>
<tr>
<td>FAI [20-90]</td>
<td>65 (18)</td>
<td>63 (38)</td>
<td>n.a.</td>
<td>0.8</td>
</tr>
<tr>
<td>PINP [22-87 µg/l]</td>
<td>52 (13)</td>
<td>42 (16)</td>
<td>n.a.</td>
<td>0.009</td>
</tr>
<tr>
<td>1CTP [2.1-5.0 µg/l]</td>
<td>3.2 (0.7)</td>
<td>3.3 (1.1)</td>
<td>n.a.</td>
<td>0.7</td>
</tr>
<tr>
<td>CTX [≤84 ng/l]</td>
<td>154 (93)</td>
<td>288 (196)b</td>
<td>n.a.</td>
<td>0.001</td>
</tr>
<tr>
<td>Bone mineral density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine (L1-L4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BMD (g/cm²)</td>
<td>1.0 (0.13)</td>
<td>0.96 (0.12)</td>
<td>0.93 (0.33)</td>
<td>0.4</td>
</tr>
<tr>
<td>- T-score</td>
<td>-0.9 (1.1)</td>
<td>-1.2 (1.1)</td>
<td>-1.1 (1.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>- Z-score</td>
<td>-0.8 (1.1)</td>
<td>-1.1 (1.1)</td>
<td>-1.0 (1.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Femoral neck</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BMD (g/cm²)</td>
<td>0.84 (0.1)</td>
<td>0.84 (0.12)</td>
<td>0.85 (0.13)</td>
<td>0.8</td>
</tr>
<tr>
<td>- T-score</td>
<td>-0.7 (0.7)</td>
<td>-0.7 (0.9)</td>
<td>-0.7 (0.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>- Z-score</td>
<td>-0.3 (0.7)</td>
<td>-0.3 (0.8)</td>
<td>-0.3 (0.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>Total hip</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BMD (g/cm²)</td>
<td>0.99 (0.11)</td>
<td>0.97 (0.12)</td>
<td>0.98 (0.14)</td>
<td>0.8</td>
</tr>
<tr>
<td>- T-score</td>
<td>-0.3 (0.7)</td>
<td>-0.4 (0.8)</td>
<td>-0.4 (0.8)</td>
<td>0.8</td>
</tr>
<tr>
<td>- Z-score</td>
<td>-0.1 (0.7)</td>
<td>-0.3 (0.8)</td>
<td>-0.3 (0.8)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Data are no. (%) of patients or means with standard deviations. * P-value based on one-way Anova or independent T-tests for continuous variables and χ² or Fisher’s exact tests for proportions. b 2 patients with missing results. c Reference values between brackets. * Calcium corrected for albumin. a 4 patients with missing results. 1 1 patient with missing results.

Alk Phos, alkaline phosphatase; 1CTP, C-terminal telopeptide of type 1 collagen; CTX, C-telopeptide cross-link of type 1 collagen; FAI, free androgen index = testosterone x 100/SHBG; n.a., not available; PINP, procollagen type 1 N-terminal propeptide; SHBG, sex hormone-binding globulin; TSH, thyroid stimulating hormone.
Low bone density in MSM regardless of HIV

Table 2. Multivariable linear regression analysis of variables associated with lumbar spine, femoral neck and total hip Z-scores among HIV infected men who have sex with men

<table>
<thead>
<tr>
<th></th>
<th>β-coefficient (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine Z-score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Primary HIV-infection(^b)</td>
<td>-0.2 (-0.7 to +0.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>- Chronic HIV-infection(^b)</td>
<td>-0.1 (-0.6 to +0.4)</td>
<td>0.7</td>
</tr>
<tr>
<td>- BMI (kg/m²)</td>
<td>0.08 (0.03 to 0.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Femoral neck Z-score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Primary HIV-infection(^b)</td>
<td>0.2 (-0.2 to +0.5)</td>
<td>0.4</td>
</tr>
<tr>
<td>- Chronic HIV-infection(^b)</td>
<td>0.2 (-0.2 to +0.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>- BMI (kg/m²)</td>
<td>0.1 (0.06 to 0.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total hip Z-score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Primary HIV-infection(^b)</td>
<td>0.04 (-0.3 to +0.4)</td>
<td>0.8</td>
</tr>
<tr>
<td>- Chronic HIV-infection(^b)</td>
<td>0.03 (-0.3 to +0.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>- BMI (kg/m²)</td>
<td>0.09 (0.06 to 0.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^a\) β-coefficients < 0.0 indicate an inverse association between the Z-scores and the parameter.
\(^b\) The reference group is the HIV-negative control group.

(PrEP) trials reported a 10-14% prevalence of low BMD (defined as Z-score ≤ -2.0) in healthy HIV seronegative MSM who were at risk for HIV infection [7, 8]. One of these studies observed that low BMD was associated with inhalants (i.e. poppers, amyl nitrates) and amphetamine use [7]. Numerous studies reported a high prevalence of low BMD among subjects with chronically HIV infection, which, in addition to traditional risk factors, is usually attributed to HIV infection itself and to HIV infection plus the use of cART. A meta-analysis revealed a 6.4-fold higher odds of low BMD (defined as T-score < -1.0) among HIV infected individuals, compared with HIV negative controls [9]. However, most of the studies included in this meta-analysis used as healthy controls individuals from the general population, who might have lifestyle risk factors related to low BMD that substantially differ from those of the HIV infected population. Indeed, (traditional) risk factors for low BMD, such as low body weight, smoking, alcohol use, and recreational drug use, are likely to be more prevalent among MSM than among heterosexual men [10-12]. Therefore, to precisely measure the effect of HIV infection on BMD among MSM, it is important to use adequate control groups composed of HIV negative MSM.

One might argue that the moment of BMD measurement among MSM with primary HIV infection was too early during infection MSM for significant HIV associated changes to have occurred. However, comparing the mean T- and Z-scores of the primary HIV infected MSM with those of the chronic HIV infected MSM in our study revealed similar, equally low BMD values, suggesting that more prolonged untreated HIV infection by itself did not contribute significantly to the observed low BMD.

Low levels of bone formation and high levels of bone resorption markers were measured in the primary HIV infected MSM compared to the HIV negative controls, suggesting an increase
in bone turnover as a result of primary HIV infection. This may well be explained by the acute inflammation associated with primary HIV infection, which might induce bone loss by promoting bone destruction by osteoclasts and by inhibiting bone formation by osteoblasts [13]. In patients with acute hepatitis A and B, bone formation was found to be transiently decreased, reflecting a state of low bone turnover during the acute illness [14]. However, the biochemical evidence for increased bone turnover in the primary HIV infected MSM may be transient and possibly may not result in significant bone loss, given the very similar BMD in the chronic HIV infected MSM. Nevertheless, prolonged HIV infection and the use of cART may still have an additional negative impact on BMD. Interestingly, serum phosphate levels were significantly lower in the primary HIV infected MSM, which may reflect renal tubular dysfunction as a result of HIV infection of the renal tubular epithelium, as has been reported previously in a primary HIV infected patient [15].

Our study has several limitations. Risk factors for low BMD and bone markers were not collected prospectively for the chronic HIV infected MSM. Second, the sample size of the primary HIV infected and HIV negative MSM cohorts was too small to detect significant associations between low BMD and conventional risk factors affecting bone health other than age and BMI. Third, 80% of participants were Caucasian men. Finally, because of the cross-sectional design, we cannot infer causal relationships.

In conclusion, we found significantly decreased T- and Z-scores and a similarly high prevalence of low BMD among MSM with untreated primary and chronic HIV infection, as well as among HIV negative MSM. This suggests that the low BMD found in acute and chronically untreated HIV infected MSM may pre-date HIV acquisition and that low BMD is not fully attributable to HIV infection alone or to HIV infection plus the use of cART. Our results also emphasize the need for adequate control groups with similar risk exposures in future studies that assess BMD and its changes over time in persons living with HIV.

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Contributors

MLG, PR and JMP drafted the manuscript. MLG and FWNMW conducted the statistical analysis. MLG and JMP established the Primo-SHM cohort, SMEV and JMP the chronically HIV infected cohort and IGS and MP the HIV negative cohort. All authors provided valuable input into protocol development and interpretation of data, and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.
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Low bone density in MSM regardless of HIV

References


PART V

Conclusion
chapter TWELVE

Summary & General discussion
The studies presented in this thesis focus on the treatment of primary HIV infection (PHI). The general discussion will provide a summary of the different studies, followed by clinical recommendations and suggestions for future research.

Part II Treatment during primary HIV infection

Combination antiretroviral therapy (cART) in chronically HIV infected persons has shown to be very effective in suppressing viral replication and preventing immunological deterioration and has remarkably altered the clinical course of HIV disease [1]. In current HIV research PHI has attracted tremendous attention, as studying transmission and events early in infection may aid the understanding of HIV pathogenesis and thereby contribute to future vaccine development [2, 3]. However, there is lack of consensus with regard to the clinical management of PHI. To date, it is the question whether or not temporary treatment during PHI offers unique long-term benefits, through preserving immune function otherwise lost, enhancing rapid viral control and limiting the viral reservoirs [1, 4]. Observational cohort studies have shown conflicting results with regard to the benefits of early treatment. Several research groups around the world have now published randomized studies that compared temporary early treatment during PHI with no treatment.

The first randomized controlled trial was published in 1995 and compared six months of zidovudine monotherapy with placebo in patients with PHI. The study reported a significant reduction of minor opportunistic infections after early treatment [5]. A second randomized trial with zidovudine monotherapy demonstrated similar immunological and virological benefits [6]. Subsequently, it lasted another seventeen years before the results of three randomized trials that were performed in the current cART era became available.

Between May 2003 and March 2010 we conducted the Primo-SHM trial, a multi-centre randomized trial comparing no treatment with 24 or 60 weeks of early cART (Chapter 3). The objective of the study was to assess the clinical benefit of temporary cART during PHI, measured by the time that patients could remain off therapy until subsequent (re)start of cART was indicated based on current treatment guidelines, and to assess the optimal duration of such early treatment. Patients with laboratory evidence of PHI were recruited in 13 HIV treatment centres in the Netherlands and randomly assigned to receive no treatment or 24 or 60 wk of early cART. In case therapy was clinically indicated, subjects were randomized over the two treatment arms only. Primary end points were the viral set point, defined as the plasma viral load (pVL) 36 wk after randomization in the no treatment arm and 36 wk after treatment interruption in the treatment arms, and the total time that patients were off therapy, defined as the time between randomization and start of cART in the no treatment arm, and the time between treatment interruption and restart of cART in the treatment arms. cART was (re)started in case of a confirmed CD4 cell count below 350 cells/mm³ or symptomatic HIV disease. The modified intention-to-treat-analysis comprised 168 patients: 115 were randomized over the three study arms and 53 were randomized over the two treatment arms only. Most patients randomized over the three study arms were men who have sex with men (MSM), had a negative or indeterminate Western blot and were symptomatic during PHI. Mean viral setpoint at week 36 was significantly lower in the 24- and 60-week treatment arms as compared to the no treatment arm, with a mean
The median total time off therapy was significantly longer in the 24- and 60-week treatment arms as compared to the no treatment arm: restart of cART during chronic HIV infection was deferred with approximately two years. Combining all treated patients, including the patients randomized over the two treatment arms only, the median total time off therapy was not different between the 24- and 60-week treatment arms. In the adjusted Cox analyses, temporary early cART was associated with time to (re)start of cART. Summarizing, the key findings of the Primo-SHM study were that temporary cART initiated during PHI transiently lowered the viral setpoint and deferred the need for restart of cART during chronic HIV infection. The effects of temporary early cART were not different for the two treatment arms, suggesting that 24 weeks of early cART would be sufficient.

Our findings are supported by two other recent randomized studies, the Short Pulse Anti-Retroviral Therapy at HIV Seroconversion (SPARTAC) trial, which compared no therapy with 12 or 48 weeks of cART during PHI [7], and the SETPOINT study, which compared no therapy with 36 weeks of cART in early HIV infection [8]. The primary endpoint in the SPARTAC trial was time to CD4 cell count below 350 cells/mm³ or initiation of long-term cART. A total of 366 participants were randomized: 123 in the no therapy group, 120 in the 12-week and 123 in the 48-week treatment groups. Preliminary results showed that the 48-week treatment group had a 0.44 log₁₀ copies/ml reduction in pVL 36 weeks after treatment interruption as compared to the no therapy group. 50% of the participants in the 48-week treatment group reached the primary endpoint, compared to 61% in both the no therapy and 12-week treatment groups. The median time to reach the primary endpoint was 65 weeks longer in the 48-week treatment group as compared to the no therapy group (average hazard ratio (HR) was 0.63 (95% CI: 0.45,0.90; p=0.01), whilst 12 weeks of treatment had no effect compared with no therapy. The SPARTAC trial demonstrated that 48 weeks of early cART modestly delayed disease progression, although not significantly longer than time already spent on treatment [7].

The aim of the SETPOINT study was to determine whether the viral setpoint could be altered after 36 weeks of early cART. However, the study was prematurely stopped by the Data Safety and Monitoring Board, because the untreated study arm experienced a higher rate of disease progression than expected compared to the treatment arm: the time to meeting eligibility criteria for initiating or reinitiating long-term cART was significantly shorter in the untreated arm than in the treated arm (log-rank, p=0.035) [8]. The viral setpoint endpoint could not be evaluated.

A major concern of temporary early cART during PHI is the potential negative impact on the health-related quality of life (HRQL), because of drug-related toxicity, pill burden and the need for strict adherence to cART, which may be more challenging during the acute stage of HIV, since patients are often physically and emotionally distressed. Another important concern is the risk of developing drug resistance mutations in non-adherent patients and/or after treatment interruption, which could compromise future treatment options [4]. To this end, we compared the HRQL and symptoms over 96 weeks in a cohort of untreated and early treated PHI-patients and found that early cART had a positive impact on patients’ HRQL as compared to no treatment, despite the initial, short-term occurrence of more physical symptoms that were related to drug toxicity (Chapter 4). Furthermore, we evaluated the effect of temporary early cART on the subsequent virologic response to long-term cART of the patients previously participating in the Primo-SHM
Summary & General discussion

TwelVe trial (Chapter 5). Temporary cART during PHI was not associated with a reduced virologic response after reinitiation of long-term cART during chronic HIV infection, when we compared early treated patients with untreated PHI-patients and when we compared the first and second treatment episodes in early treated patients. These results suggest that temporary early cART did not select for clinically relevant drug resistance mutations. Hence, potential disadvantages of early cART were not substantiated, which provides further support to initiate temporary cART during PHI.

A short course of cART during PHI deferred the need for subsequent restart of cART, which, according to findings of the Primo-SHM study, was most likely caused by the effects of the CD4 gain during treatment and the transient lowering of the viral setpoint (Chapter 3). After adding these two parameters to the Cox regression models, early cART was no longer significantly associated with time to restart of cART, suggesting that the increase of the CD4 cell count and the lowering of the viral setpoint during the early treatment period explained for the most part why early cART resulted in a clinical benefit. We examined whether the stage of PHI and the self-reported occurrence of an acute retroviral syndrome were possibly associated with time to (re)start of cART, but found no correlation.

It remains unclear how the lowering of the viral setpoint might be explained. Did early cART have a direct effect on the virus itself or did it affect the immunologic responses of the host? Therefore, in Chapter 6 we investigated whether the beneficial effect of early treatment was caused by the preservation of immunological responses. We compared 26 early treated with 13 untreated PHI-patients at viral setpoint (36 weeks after treatment interruption and randomization, respectively) and studied i) effector T-cell formation and function, ii) polyfunctionality of CD8+ T cells, by measuring the cytokines TNF, IFNγ, and IL-2, and the chemokine Mip1B, and iii) regulation of the cellular immune response by measuring various inhibitory and regulatory markers on T, B and NK cells and dendritic cells. We also assessed, by measurement of the gut homing marker α4β7, whether early treatment may prevent severe CD4+ T cell depletion in the GALT and thereby prevent excessive immune activation. Surprisingly few of the immunological parameters that we studied were affected by early treatment, although the sample size of our study was small. Treatment during PHI led to the preservation of a more polyfunctional HIV-gag specific T cell response, suggesting that early treatment may preserve important cytotoxic T-lymphocyte (CTL) functions, which are crucial in control of the HIV viraemia.

Another mechanism that independently influenced the clinical course of untreated PHI-patients is described in Chapter 7. Here we analyzed the effect of dual HIV infections (co- or superinfection) on disease progression in a cohort of untreated MSM with primary infection with HIV-1 subtype B. Between 2000 and 2009, 37 PHI-MSM were characterized with regard to dual infection or single infection and coreceptor use. Patients were followed to estimate the effect of these two parameters on clinical disease progression, as defined by the rate of CD4+ T-cell decline and the time to initiation of long-term cART. Four patients presented with a coinfection and six patients acquired a superinfection, on average 8.5 months from their primary infection. The other 27 patients remained infected with a single strain. The slopes of longitudinal CD4+ T-cell counts and time-weighted changes from baseline were significantly steeper for the patients with a dual infection than for patients with a single infection. Multivariate analysis showed that the most important parameter associated with CD4+ T-cell decline over time was dual infection.
(P = 0.001). Patients with a coinfection had a significantly earlier start of long-term cART (P < 0.0001). This study showed that HIV co- or superinfection was the main factor associated with CD4+ T cell count decline in a cohort of untreated PHI-MSM with subtype B virus. Thus, an additional benefit of temporary early cART may be the prevention of early HIV superinfection.

Several study groups suggested possible other viral or host factor(s) that might explain the lower viral setpoint after the initiation of temporary cART during PHI. Immunological events early in the acute stage are thought to fundamentally influence HIV outcome. Two crucial events in PHI are the massive destruction of CD4+ T cells in the gastrointestinal tract and the establishment of latent HIV reservoirs. Early treatment may result in viral suppression and immune restoration in gut-associated lymphoid tissue (GALT) [9]. A recent study revealed no viral evolution in the GALT during early cART, suggesting no or limited viral replication in this compartment during suppressive cART, which leads to less divergent viral populations over time, possibly contributing to the lower viral setpoint [10]. In addition, early cART may decrease the seeding of reservoirs of latent virus: on one hand by extinguishing viral production, and on the other hand by reducing the pool of latently infected CD4+ T cells, which will eventually lead to a smaller amount of virus production after treatment interruption. The prospective VISCONTI study (Virological and Immunological Studies in CONTrollers after Treatment Interruption) studied the distribution and magnitude of the HIV reservoir in 12 PHI-patients in whom treatment was initiated within 10 weeks post-infection during a period of 3 years and who controlled HIV for more than 6 years thereafter. Preliminary results suggest that early treatment lead to a limited HIV reservoir distributed mainly in short-lived memory CD4+ T cells which mimicked the distribution that is seen in elite controllers [11].

Another small study in early treated patients demonstrated that the fitness of viral strains present during PHI was higher than previously thought [12]. It is generally thought that the fitness of strains present during PHI is low relative to strains present during chronic HIV infection, due to the bottleneck imposed upon transmission. In patients with chronic HIV infection, rapidly replicating virus isolates have been associated with an increased disease progression [13]. The same study also observed that after early treatment the fitness of viral isolates reduced over time, whereas in the absence of treatment it increased over time, which could possibly affect the viral setpoint, even though the study did not find an association between viral fitness and the pVL [12]. Others have hypothesized that early treatment enables virus-specific CD8+ T cells to mature into fully differentiated effector cells, which might be important in viral control [14]. Another study observed a superior memory B-cell response to HIV and non-HIV antigens after one year of cART in early versus chronic HIV infected individuals, suggesting that temporary treatment during PHI is important for preserving overall immune competency [15].

The specific contribution of HIV specific CD4+ T cell responses in the control of viral replication in PHI is not clear. A recent cohort study observed that PHI-patients who were able to spontaneously control HIV replication in the absence of cART showed a significant expansion of HIV specific CD4+ T cell responses, which was characterized by robust cytolytic activity and expression of a distinct profile of perforin and granzymes, as compared to those who evolved to a higher viral setpoint [16]. The study revealed that the emergence of granzyme A(+) HIV specific CD4+ T cell responses at baseline was predictive of a slower disease progression.
Perhaps early cART supports the development of such HIV specific cytolytic CD4+ T cell responses and thereby causes a lower viral setpoint.

Part III Quadruple therapy in patients with primary and chronic HIV infection

In the Primo-SHM trial, patients initiated a triple-class quadruple regimen consisting of two nucleoside reverse transcriptase inhibitors (NRTIs), one non-nucleoside reverse transcriptase inhibitor (NNRTI) and one boosted protease inhibitor (PI). The reason we opted for this regimen is that we feared that standard-of-care triple therapy could possibly result in virological failure given the typically high pVL during PHI. In addition, at the time of treatment initiation genotypic resistance testing results are often not available, and since transmitted drug resistance is common [17], we favoured ‘overtreatment’.

Dual- or triple-class quadruple therapy has been suggested to augment the antiretroviral activity of cART compared to standard-of-care triple therapy. However, the randomized and non-randomized studies which support this contention are often out-dated, using quadruple or triple-class regimens containing an older generation unboosted PI as the supplementary drug or drug class, thereby questioning its relevance to current clinical practice [18-28]. As reported in many large HIV drug trials, a high baseline pVL is an independent predictor of virological failure. Therefore, adding an additional drug or drug-class to a standard-of-care regimen to increase its potency may in particular be of importance in patients with very high viraemia (≥ 500,000 c/ml).

Consequently, in Chapter 8 we investigated whether quadruple or triple-class therapy provides a more rapid pVL decline and an improved virologic response compared with standard dual-class triple therapy in treatment-naive chronic HIV infected patients with a pVL of 500,000 c/ml or more. Data were selected from the National observational HIV cohort in the Netherlands (ATHENA) [29]. 675 patients were included of whom 125 (19%) initiated quadruple and 550 (81%) triple therapy. The median pVL was similar in both groups (5.9 (IQR 5.8-6.1) log_{10} c/ml; P=0.49). The median time to viral suppression, defined as a pVL ≤ 50 c/ml, was 5.8 (IQR 4.6-7.9) and 6.0 (4.0-9.4) months in the patients on quadruple and triple therapy (log rank, P=0.42). In the adjusted Cox analysis, quadruple therapy was not associated with time to viral suppression (HR 1.07 (95% CI 0.86-1.33), P=0.53). Similar results were seen when comparing triple- versus dual-class therapy. The results of this study provide no evidence to add an extra drug or drug-class to standard triple therapy in patients with very high viraemia. Quadruple or triple-class therapy did not improve the antiretroviral activity of cART, but did expose patients to additional drug toxicity.

Similarly, studies comparing the virologic response to cART among individuals with primary versus chronic HIV infection have shown inconsistent results [30-34]. For PHI-patients a faster, similar and slower virologic response have all been reported, but most studies were small or did not adjust for differences between groups in baseline pVL. In Chapter 9 we compared the time to viral suppression between 70 PHI-patients and 80 naive chronic HIV infected patients, who were all treated with triple-class therapy and had an initial pVL above 100,000 copies/ml. The time to viral suppression after initiation of triple-class therapy was comparable for primary and chronic HIV infection, suggesting that the virologic response to therapy is not related to the stage of HIV infection.
Part IV Bone mineral density during primary HIV infection

Studies have shown an increased prevalence of low bone mineral density (BMD) among HIV infected individuals [35]. Low BMD in HIV infected individuals is caused by a multitude of factors, including traditional risk factors, which might be more prevalent among HIV infected individuals, HIV infection itself and cART [36]. In order to gain further insight into the contribution of HIV infection per se, we determined the BMD and the biochemical markers relevant for bone metabolism in a cohort of 33 untreated PHI-men, since they have limited exposure to HIV and no exposure to cART (Chapter 10). Osteopenia and osteoporosis were defined according to the WHO criteria as T-scores measured at lumbar spine, femoral neck and/or total hip between -1 and -2.5 SD and -2.5 SD or less, respectively. Mean lumbar spine T (P=0.001) and Z-scores (P=0.004) and femoral neck T-score (P=0.003) of the PHI-men were significantly lower than the average BMD of the NHANES IV reference population. Forty-five percent of the PHI-men had osteopenia and 6% had osteoporosis. The biochemical markers relevant for bone metabolism did not differ between patients with or without osteopenia/osteoporosis. In the multivariate linear regression analysis, age was negatively associated with femoral neck (β=-0.05, P=0.001) and total hip T-scores (β=-0.03, P=0.04). Body mass index (BMI) was associated with lumbar spine (β=0.3), femoral neck (β=0.2) and total hip (β=0.2) T-scores (P<0.001) and thyroid-stimulating hormone with lumbar spine (β=0.5, P=0.045) and femoral neck T-scores (β=0.4, P=0.005). Increased pVL was associated with lower total hip T-scores (β=-0.2, P=0.02). The current study presents the first data on the rate of osteopenia and osteoporosis in a cohort of untreated PHI-men, of whom more than half had osteopenia or osteoporosis. In accordance with the literature, low BMD was associated with an increased age and higher pVL, a lower body mass index and decreased thyroid-stimulating hormone levels.

Given that these PHI-men were cART-naive, the results of Chapter 10 raised the question whether the low BMD was related to the recent acquisition of HIV, to other conventional risk factors affecting BMD, or alternatively, that the low BMD previously reported in HIV infected populations pre-dates HIV acquisition. For this reason, in Chapter 11 we compared the BMD and biochemical markers relevant for bone metabolism of 41 untreated primary and 106 untreated chronically HIV infected MSM with that of a control group of 30 HIV negative MSM. Low BMD, which in this study was defined as a Z-score ≤ -2.0 SD at the lumbar spine or hip according to novel guidelines by the International Society for Clinical Densitometry, was highly prevalent in all three groups. In the multivariate analyses, HIV infection was not associated with BMD. The results of this study suggest that the low BMD found in acute and chronically HIV infected MSM may pre-date HIV acquisition and that low BMD is not fully attributable to HIV infection itself or the use of cART.

Clinical implications and Future research

The results of the studies presented in this thesis demonstrate a virological, immunological and clinical benefit of temporary early cART during PHI. Although extended follow-up studies are needed to evaluate the long-term benefits of early treatment, initiating cART for a duration of 24 weeks seems at present the most reasonable advice for patients with PHI (Chapter 3 and 4). The clinician should pay attention to the variability in pharmacokinetics when interrupting cART, as
Summary & General discussion

Stopping with a pharmacologically unbalanced regimen (i.e., a drug regimen comprising three drugs with different half-lives) may lead to ‘functional monotherapy’ and may thereby possibly induce drug resistance [37]. To date, there is no clear clinical guidance how to stop cART, as this also depends on the regimen prescribed (balanced or unbalanced) and whether the patients has a detectable or undetectable pVL at the time of treatment interruption. In general, a staggered stop, in which the long half-life drug (e.g. the NNRTI) is interrupted prior to the short half-life drugs (e.g. the NRTIs), and a switched stop, in which the long half-life drug is temporarily replaced by a boosted PI with a short half-life, are preferred over a simultaneous stop [38, 39]. However, in our large Primo-SHM cohort concerns for developing drug resistance mutations after treatment interruption in PHI have not been substantiated (Chapter 5, [40]). Of note, patients should be well informed by health care workers of the viral rebound dynamics shortly after TI [41] and the potential increased risk of sexual transmission during this period [42].

Decisions regarding treatment initiation should always be made on a patient-by-patient basis. For example, for some patients it may be better to postpone cART, especially in those patients who have difficulty in accepting their seropositive status or have barriers to treatment (young age, social background, MSM-related stigma). For every patient, potential advantages and disadvantages should be carefully reviewed before starting early cART [43]. We do not know whether our results are generalizable to asymptomatic seroconverters, since most patients were symptomatic during PHI, which is a known predictor of disease progression [44]. The timing of treatment initiation in PHI (Fiebig stage I-II, III-IV or V-VI [45]) did not seem to have an influence on the total time that patients were off therapy (Chapter 3). This might be related to the actual delay between the diagnosis of PHI and the start of early cART, which in our study was approximately five weeks, partly caused by the delay between start of symptoms and HIV diagnosis, and partly as a result of a clinical trial setting. Perhaps the golden hour, in which the greatest benefits of early cART could have been achieved, was already missed. The greatest effect might be achieved when initiating early cART at the time of symptom presentation. A posthoc analysis from the SPARTAC trial showed a non-significant trend towards benefit in total time off therapy when early cART was initiated nearer to the estimated seroconversion date (HR 0.48, P=0.09, [7]). Inevitably, also in clinical practice, some time is necessary to prepare the patient for treatment initiation. Nevertheless, even with a reasonable delay we observed a clear clinical benefit of initiating cART during PHI.

The next important question is what drugs to prescribe during PHI. In an unblinded, non-randomized prospective cohort study 90 PHI-patients were allocated to either triple or quadruple dual-class NNRTI-containing cART or a PI-based dual-class regimen. The study showed that of the three treatment regimens, quadruple dual-class cART enhanced the rate of pVL decline but at the cost of drug toxicity [46]. We found that quadruple triple-class therapy had no advantage over standard triple therapy in chronic HIV infection, and indeed patients were exposed to more drug-related adverse events (Chapter 8). In addition, the time to viral suppression after initiation of triple-class therapy was comparable for primary and chronic HIV infection, suggesting that the virologic response to therapy is not related to the stage of HIV infection (Chapter 9). Therefore, the addition of a fourth drug is only of significance in case the results of baseline genotypic resistance testing are not yet available at the moment of initiation of early cART.
However, according to the Department of Health and Human Services (DHHS) guidelines, the recommended first-line therapy in chronic HIV infection (two NRTIs plus one NNRTI [47]) may not be suitable during PHI, because of the increased risk of drug-related toxicity and its low genetic barrier to resistance. Most studies on early cART propose a regimen containing a boosted PI, for reasons that the level of transmitted drug resistance to this drug class is low [48] and the risk of virological failure is small [43, 49]. A recent cohort study nonetheless endorsed the use of a once-daily, co-formulated NNRTI-based regimen (Atripla®) during PHI, and reported a rapid and sustained viral response in most patients [30]. Another study compared the number of drug modifications between NNRTI- and boosted PI-based regimens initiated during PHI and observed that it was equally frequent for both drug classes and mostly related to minor drug toxicities [50]. The long-term use of boosted PIs may potentially be more toxic than NNRTIs [51], though this is probably less applicable to temporary treatment during PHI.

Temporary cART during PHI may not only be beneficial for the individual patient, but may also have a public health benefit with the potential to significantly impact further spread of HIV. PHI is associated with an extremely high viral load in both plasma and genital tract secretions, which makes this period hyper-infectious [52]. Studies have shown that PHI accounts for almost half of the onward transmission events, thereby fueling the epidemic [53-56]. A study estimated that every 1 log₁₀ increase in genital HIV RNA was correlated with a 1.79-fold increased risk of heterosexual transmission [57]. A recent study in coastal Kenya reported a HIV incidence of 8.6 per 100 person years among a large cohort of 449 MSM, and many of these seroconverters maintained a pVL of > 4.0 log₁₀ copies/ml for up to two years after infection [58]. Identifying individuals with PHI provides a crucial opportunity for the prevention of forward transmissions, either through behavioral prevention interventions to modify high-risk behaviors, frequent and targeted HIV testing, effective biomedical interventions such as pre-exposure prophylaxis (PrEP) and/or the initiation of early cART [30, 59, 60]. The downside of temporary early cART is however that patients will eventually be a longer period without therapy during chronic HIV infection, and will thus be a longer period at risk to transmit HIV to others.

Therefore, the final question is whether we should discontinue early cART at all. Given the concern that uncontrolled HIV replication and chronic immune activation carry an increased risk of morbidity and mortality [61], some clinicians will probably recommend their patients that once cART is initiated, it should be continued indefinitely. In addition, continuing cART might also favourably influence the epidemic (‘test and treat’) [62-64]. Recent studies showed that cART profoundly reduced the heterosexual transmission of HIV in discordant couples, presuming that the HIV positive patient is adherent to cART and has an undetectable pVL [65-67]. However, the costs and drug toxicity of such continuous long-term therapy have not been studied.

In conclusion, in this thesis we studied the treatment of PHI. Early cART transiently lowered the viral setpoint and deferred the need for restart of cART during chronic HIV infection, which was most likely caused by the effects of the CD4 gain during treatment and the transient lowering of the viral setpoint. Even though the exact mechanisms explaining the lowering of the viral setpoint after early cART remain unsolved, we observed a clear clinical benefit of temporary treatment during PHI. In case early cART is considered, it should be given for a duration of 24 weeks and contain a boosted PI, at least until resistance testing results are available.
References


ADDENDUM
Summary in Dutch | Nederlandse samenvatting
Een gedeelte van deze samenvatting is gepubliceerd in het HIV Bulletin 2013; 7(1).

HIV/AIDS
Het humaan immunodeficiëntie virus (hiv) is het virus dat AIDS (verworven immunodeficiëntie syndroom) veroorzaakt. HIV is een seksueel overdraagbare aandoening (SOA) en kan op verschillende manieren worden overgedragen: via onbeschermde seksuele contacten, bloed-op-bloed contacten (bijvoorbeeld via besmette naalden bij intraveneus drugs gebruikers) en moeder-op-kind transmissie, waaronder ook borstvoeding. Wereldwijd zijn er vanaf het begin van de pandemie naar schatting 60 miljoen mensen met hiv geïnfecteerd, waarbij Sub-Sahara Afrika het zwaarst getroffen is [1]. In Nederland leven naar schatting 25000 personen met hiv van wie er ongeveer 8500 (35%) hiervan niet op de hoogte zijn [2, 3].

AIDS is het gevolg van een sterk verzwakt afweersysteem doordat het virus een belangrijke groep witte bloedcellen, de CD4+ lymfocyten, infecteert en uitschakelt. De CD4+ lymfocyten beschermen het lichaam tegen bepaalde bacteriën en virussen. Bij een tekort aan CD4+ lymfocyten wordt het afweersysteem kwetsbaar en kunnen o.a. deze ziekteverwekkers, die onder normale omstandigheden geen problemen veroorzaken, opportunistische infecties en andere ernstige ziekten of kanker veroorzaken. In dit geval spreken we van AIDS. De hiv-infectie wordt onderverdeeld in vier opeenvolgende stadia: de acute fase, de asymptomatische chronische infectie, de symptomatische chronische infectie en uiteindelijk AIDS. De asymptomatische fase, waarbij de patiënt nog niet behandeld wordt, kan variëren tussen de 1 en 10 jaar. Sinds 1996 wordt hiv-infectie behandeld met een combinatie van tenminste 3 verschillende hiv-remmers, de zogenaamde antiretrovirale combinatie therapie (cART), waardoor het welzijn en de levensverwachting van hiv-geïnfecteerde patiënten spectaculair is verbeterd. De acute of de primaire hiv-infectie (PHI) is de fase van infectie die centraal staat in dit proefschrift. In deze samenvatting wordt een overzicht gegeven van de pathogenese, de klinische verschijnselen, de diagnostiek en de eventuele behandeling van een PHI. Daarna worden de verschillende hoofdstukken van de proefschrift apart behandeld.

Primaire hiv-infectie
Pathogenese
De PHI is het vroegste stadium van de hiv-infectie en verwijst naar de eerste zes maanden na de hiv- besmetting [4]. Overdracht van hiv vindt meestal plaats op de genitale slijmvlies. De eerste cellulaire doelwitten van hiv zijn de dendritische cellen, macrofagen en CD4+ T-lymfocyten, gevolgd door de verspreiding van hiv naar regionale lymfeklieren en uiteindelijk naar het bloed en de overige organen [5, 6]. Zodra hiv de CD4+ cel infecteert, wordt het hiv-DNA in het genetisch materiaal van de gastheercel geïntegreerd en worden er nieuwe virussen gemaakt die op hun beurt weer meer CD4+ cellen kunnen infecteren. Zodoende wordt de PHI gekenmerkt door een exponentiële stijging van virus replicatie: viruspopulaties kunnen zich elke zes tot tien uur verdubbelen, waardoor er gemiddeld drie tot vier weken na infectie een piek in de plasma virale load (pVL) ontstaat van miljoenen kopieen virussen per milliliter bloed.
In deze periode ontwikkelen zich meestal ook de lichamelijke klachten. Dit wordt ook wel het acuut retroviraal syndroom genoemd. De ernst en de duur van de klachten is geassocieerd aan een snellere ziekteprogressie [11-14]. Een deel van de geïnfecteerde CD4+ cellen zal inactief worden en vormt verspreid over het lichaam een reservoir van latent geïnfecteerde cellen welke in een latere fase weer actief kunnen worden [10].

Tijdens de PHI vinden verschillende kritische gebeurtenissen plaats, waaronder grote schade aan de CD4+ T-lymfocyten in het maagdarmkanaal [15-17], de vorming van virus reservoirs [18, 19] en de ontwikkeling van een immuunrespons tegen het virus. Een belangrijke immuunrespons is de activering van virus-specifieke CD8+ cytotoxische T-lymfocyten (CTLs), die samenvalt met een scherpe daling van de pVL tot een zogenaamde steady state is bereikt. Dit wordt het virale setpoint genoemd en ontstaat ongeveer zes tot negen maanden na infectie [20-22]. Verschillende studies hebben laten zien dat een lager viraal setpoint is gecorreleerd aan een langzamer ziektebeloop [23-26].

Symptomen
Een PHI verloopt meestal symptomatisch, maar deze klachten kunnen variëren in aard en ernst en ze zijn vaak weinig specifiek. Derhalve wordt de diagnose PHI vaak gemist. Dit blijkt ook uit eerder onderzoek waarbij werd vastgesteld dat meer dan 60% van de symptomatica PHI patiënten medische hulp zocht, maar dat de klachten slechts bij 5% van de patiënten werden herkend als passend bij een PHI [27, 28]. Een typische PHI verloopt als een Pfeiffer-achtig ziektebeeld met koorts en vermoeidheid. Overige veel voorkomende symptomen zijn huiduitslag, keelontsteking, gewichtsverlies, nachtwetten, lymfadenopathie, spierpijn, hoofdpijn, misselijkheid en diarree [29, 30]. Soms is het beloop veel heftiger en presenteert een patiënt zich met een Guillain-Barré syndroom of een aseptische meningitis. De aspecifieke klachten van een acuut retroviraal syndroom benadrukken het belang van een nauwkeurige seksuele anamnese naar mogelijk (hoog-)risico gedrag.

Diagnose
De diagnose van een PHI kan eenvoudig gemist worden, omdat de hiv-antilichamen meestal nog niet of nauwelijks ontstaan zijn op het moment dat iemand klachten ontwikkelt en getest wordt. Een standaard hiv screening test (ELISA, enzyme-linked immunosorbent assay) zal dus negatief zijn [31, 32]. Dit wordt ook wel de window-fase genoemd en duurt meestal 2 weken. Diagnostische testen om een PHI vast te stellen zijn een p24 antigeen test, een detecteerbare pVL en/of een dubieuze (indeterminate) Western blot, waarbij laatst genoemde gebaseerd is op de ontwikkeling van hiv- antilichamen [33, 34]. Het p24 antigeen is een viraal kerneiwit welke tijdelijk in het bloed te meten is tijdens de acute fase, gemiddeld 17 dagen na transmissie, nog voor de ontwikkeling van de hiv- antilichamen [35]. Het is belangrijk om een PHI tijdig te diagnosticeren, niet alleen voor de individuele patiënt, maar ook voor diens omgeving, aangezien patiënten met een PHI een hogere pVL hebben en hierdoor makkelijker het virus kunnen doorgeven. Verschillende studies hebben laten zien dat mensen die weten dat zij HIV-positief zijn bewuster met hun seksueel gedrag omgaan [36, 37]. Het tijdig diagnosticeren van een PHI kan zodoende besmetting van anderen voorkomen.
Waarom behandeling in de acute fase?

De PHI is geassocieerd met een enorme virus replicatie en de ontwikkeling van een eerste immuunrespons tegen het virus. Dit maakt deze fase heel anders dan de chronische hiv-infectie en biedt wellicht unieke mogelijkheden voor therapeutische en epidemiologische interventies [38]. Hoewel er tot nu toe geen consensus bestaat, lijkt het er steeds meer op dat (tijdelijke) antiretrovirale behandeling tijdens de PHI een gunstig effect heeft op de ziekte progressie [39-41]. Observationele studies hebben gesuggereerd dat vroegbehandeling de hiv-specifieke afweer kan beschermen [42-49] en de virale evolutie en het ontstaan van hiv reservoirs kan beperken [50, 51]. Een belangrijke immunologische studie observeerde dat tijdelijke vroegbehandeling zorgde voor een krachtige hiv-specifieke CD4+ en CD8+ T-cel respons, welke vergelijkbaar was met die van long-term nonprogressors (dit zijn personen die meer dan 10-20 jaar lang zonder medicatie kunnen) [46]. Daarnaast kan vroegbehandeling het virale setpoint verlagen, wat een positief effect heeft op de ziekte progressie [52-54]. Vroegbehandeling kan ook overwogen worden bij patiënten met ernstige klinische symptomen, zoals bijvoorbeeld een aseptische meningitis, waarbij afwachten eigenlijk niet verantwoord is. Het starten met cART resulteert in een snellere pVL daling en heeft daarmee een gunstig effect op de klinische verschijnselen [55]. Ten slotte kan de behandeling van een PHI belangrijke gevolgen hebben voor de volksgezondheid door de verspreiding van hiv transmissie, aangezien patiënten zeer besmettelijk zijn tijdens deze periode [56, 57]. Echter, niet alle studies suggereren een verlaging van het virale setpoint [58-61], en sommige cohort studies rapporteerden een vergelijkbare of zelfs snellere CD4-daling na onderbreking van de vroegbehandeling [62, 63].

Ondanks de voordelen, zijn er ook potentiële nadelen van vroegbehandeling. Een belangrijke zorg is de medicatie gerelateerde (lange termijn) toxiciteit, hoewel tegenwoordig de meeste cART regimes beter verdragen worden en minder bijwerkingen geven, en de mogelijke gevolgen voor de kwaliteit van leven. Een tweede nadeel is het risico op het ontwikkelen van resistentie door bijvoorbeeld therapieontrouw of na stopzetting van de vroegbehandeling. Dit risico is wellicht groter bij patiënten in de acute fase, aangezien zij overduisterd zullen zijn met emoties en veel nieuwe zorgen hebben. Dit zou de therapietrouw nadelig kunnen beïnvloeden. Anderzijds kan cART patiënten ook psychisch ondersteunen en het gevoel geven dat er actief iets gedaan wordt aan de ziekte, in plaats van af te wachten totdat het virus de overhand krijgt. Ten slotte is de kosteneffectiviteit van tijdelijke vroegbehandeling nooit onderzocht, ofschoon studies hebben aangetoond dat eerder starten met cART een kosteneffectieve strategie is omdat het meer AIDS events kan voorkomen [64, 65]. Bovendien zou vroegbehandeling kosteneffectief kunnen zijn door het voorkomen van nieuwe hiv transmissies.

Tot op heden bieden klinische richtlijnen geen definitief antwoord of tijdens de PHI wel of niet ingegrepen moet worden met (tijdelijke) cART, en zo ja, voor hoe lang [66, 67]. De eerste gerandomiseerde studie werd uitgevoerd begin 1990 en vergeleek zes maanden zidovudine (één van de eerste antiretrovirale middelen) monotherapie met placebo bij patiënten met een PHI, en rapporteerde een significante reductie van opportunistische infecties na vroegbehandeling [68]. Een tweede gerandomiseerde studie met zidovudine monotherapie observeerde soortgelijke immunologische en virologische voordelen [69]. Het duurde hierna
ruim tien jaar voordat de resultaten van gerandomiseerde studies die zijn uitgevoerd in het huidige cART-tijdperk beschikbaar kwamen.

Resultaten beschreven in dit proefschrift
Van mei 2003 tot en met maart 2010 hebben wij de Primo-SHM trial uitgevoerd, een multi-center studie waarbij patiënten met een bewezen PHI werden gerandomiseerd naar geen behandeling, 24 weken of 60 weken vroegbehandeling. Het grote cohort van PHI patiënten die in deze studieperiode is ontstaan, is de basis van de meeste studies beschreven in dit proefschrift.

Deel I Inleiding
Hoofdstuk 1 is de inleiding van dit proefschrift. Het geeft een overzicht over PHI en beschrijft de pro’s en contra’s van vroegbehandeling. Hoofdstuk 2 bevat een illustratieve casus van een patiënt die een Kaposi sarcoma ontwikkelde tijdens de PHI en laat zien dat een PHI een zeer progressief beloop kan hebben.

Deel II Behandeling van primaire hiv-infecties
Vroegbehandeling met cART tijdens de PHI kan mogelijk leiden tot een betere klinische uitkomst op de lange termijn. In hoofdstuk 3 worden de resultaten van de Primo-SHM trial beschreven. Het doel van de studie was om i) de klinische voordelen van tijdelijke vroegbehandeling te bepalen, gemeten als de totale netto tijd dat patiënten zonder therapie konden, totdat zij moesten gaan (her)starten met cART volgens de huidige richtlijnen: tweemaal achtereenvolgens een CD4-getal <350 x 10⁶/l, ernstige klachten of een AIDS diagnose (of wanneer een patiënt per se wilde (her)starten), en ii) de optimale duur van een dergelijke vroegbehandeling te bepalen. Patiënten met een bewezen PHI werden geïncludeerd in 13 hiv behandelcentra in Nederland en gerandomiseerd over drie studiearmen: geen behandeling, 24 weken cART of 60 weken cART. Indien arts of patiënt een sterke behandelwens hadden (bijvoorbeeld in geval van ernstige verschijnselen), werden patiënten over de 2 behandelarmen gerandomiseerd. In totaal werden 173 patiënten gerandomiseerd. De gemodificeerde intention-to-treat-analyse bestond uit 168 patiënten: 115 waren over de drie armen gerandomiseerd en 53 over de twee therapie armen. Het gemiddelde virale setpoint, gedefinieerd als de pVL 36 weken na randomisatie in de onbehandelde arm en 36 weken na stop therapie in de behandelde armen, was significant lager in de 24 en 60 weken behandelingsarmen dan in de onbehandelde arm met een verschil van 0.5-0.8 log_{10} kopieën/ml. De mediane totale tijd zonder therapie was ook significant langer in de 24 en 60 weken behandelingsarmen: het herstarten van therapie tijdens de chronische hiv-infectie werd uitgesteld met ongeveer twee jaar. Wij zagen geen verschil in de tijd zonder therapie tussen de 24 of 60 weken behandelingsarmen. In de gecorrigeerde Cox analyses was vroegbehandeling geassocieerd met de tijd tot (her)start therapie. Samenvattend, kortdurende vroegbehandeling van een PHI verlaagt het virale setpoint en stelt hiermee de start van chronische, levenslange therapie uit met een gemiddelde van twee jaar.
Zoals eerder beschreven zijn de twee belangrijkste aandachtspunten van vroegbehandeling de potentiële negatieve impact op de kwaliteit van leven, als gevolg van bijwerkingen van de medicijnen en de noodzaak tot therapietrouw, welke mogelijk in de acute fase in gedrang zou kunnen komen, en de kans op de selectie van resistentie mutaties tegen het vroegbehandelingsregiem. In hoofdstuk 4 hebben wij de kwaliteit van leven vergeleken over 96 weken tussen onbehandelde en vroegbehandelde (24 of 60 weken) PHI patiënten. Vroegbehandeling had een positief effect op de kwaliteit van leven, ondanks de kortdurende medicatie gerelateerde bijwerkingen na aanvang van therapie. Daarnaast wordt in hoofdstuk 5 beschreven dat vroegbehandeling niet is geassocieerd met een verminderde virologische respons na het herstarten met chronische therapie. Dit suggereert dat (het staken van) vroegbehandeling geen klinisch relevante resistentie heeft veroorzaakt. Kortom, beide studies ondersteunen het toepassen van vroegbehandeling gedurende de PHI.

In hoofdstuk 3 hebben we aangetoond dat vroegbehandeling het virale setpoint verlaagt. Hoe dit precies gebeurt is vooralsnog onduidelijk. Derhalve hebben wij in hoofdstuk 6 onderzocht of de gunstige effecten van vroegbehandeling mogelijk veroorzaakt worden door bescherming van de hiv-specifiche afweer. In totaal hebben wij deze afweer bij 26 vroegbehandelde patiënten en 13 onbehandelde patiënten op het moment van het virale setpoint (respectievelijk 36 weken na stop therapie en randomisatie) vergeleken. Vroegbehandeling zorgde voor behoud van een meer polyfunctionele hiv-gag specifieke T cel respons. Dit suggereert dat vroegbehandeling belangrijke CTL- (cytotoxische T-lymfocyten-) functies beschermt welke cruciaal zijn voor de controle van hiv. In hoofdstuk 7 wordt een mogelijke andere verklaring gegeven voor de snellere ziekteprogressie welke wij observeerden in de onbehandelde PHI patiënten. In deze studie werd onderzocht of dubbel-infecties (co - of superinfecties) een effect hebben op de ziekteprogressie in een groep van 37 onbehandelde PHI mannen die seks hebben met mannen (MSM) en geinfecteerd zijn met het subtype B virus. Na gemiddeld 8.5 maand bleken vier MSM een co-infectie te hebben en zes een superinfectie. De CD4-daling van deze tien MSM was significant sneller dan van diegenen met een monoinfectie, waardoor zij ook sneller moesten gaan starten met chronische cART. Concluderend, een bijkomend voordeel van vroegbehandeling is mogelijk dat het zulke dubbel-infecties voorkomt.

Deel III Quadruple therapie in primaire en chronisch hiv-geïnfecteerde patiënten
In de Primo-SHM studie werden patiënten met een triple-klasse quadruple regiem behandeld bestaande uit twee nucleoside reverse-transcriptaseremmers (NRTIs), een non-nucleoside reverse-transcriptaseremmer (NNRTI) en een ritonavir-gebooste protease remmer. Normaliter wordt hiv behandeld met een combinatie van drie antiretrovirale middelen, ofwel twee NRTIs plus een NNRTI of twee NRTIs en een gebooste protease remmer. In hoofdstuk 8 analyseren wij of quadruple therapie in vergelijking met triple therapie leidt tot een snellere pVL daling en verbeterde virologische respons bij chronische, therapie-naïeve hiv-patiënten met een zeer hoge pVL (≥500.000 k/ml). In totaal werden 675 patiënten geïncludeerd uit de database.
van de Stichting HIV Monitoring van wie 125 (19%) startten met quadruple en 550 (81%) met triple therapie. De mediane tijd tot virale suppressie, gedefinieerd als een pVL ≤ 50 k/ml, was niet verschillend tussen de twee groepen (quadruple: 5.8 (IQR 4.6-7.9) maanden, triple: 6.0 (4.0-9.4) maanden; log rank, P=0.42). In de gecorrigeerde Cox regressieanalyse was quadruple therapie niet geassocieerd met de tijd tot virale suppressie (hazard ratio 1.07, (95% betrouwbaarheidsinterval 0.86-1.33), P=0.53). Vergelijkbare resultaten werden gevonden wanneer triple- met duo-klasse therapie werd vergeleken. Initiële quadruple therapie gaf geen snellere pVL daling maar veroorzaakte wel meer bijwerkingen. Het lijkt dus geen meerwaarde te hebben om patiënten met een zeer hoge pVL te behandelen met quadruple therapie. In hoofdstuk 9 wordt ingegaan op de tijd tot virale suppressie bij 70 PHI patiënten en 80 chronische naïeve hiv-geïnfecteerde patiënten, die allen werden behandeld met triple-klasse therapie en een initiële pVL hadden van 100.000 k/ml of meer. De tijd tot virale suppressie na start therapie was vergelijkbaar voor de primaire en de chronische geïnfecteerde patiënten. Dit suggereert dat de virologische respons na start therapie niet gerelateerd is aan de fase van de hiv-infectie.

Deel IV De botmineraaldichtheid tijdens de primaire hiv-infectie

Hiv-geïnfecteerde patiënten hebben een verhoogd risico op een verminderde botmineraaldichtheid (BMD). De pathogenese is onduidelijk, maar hoogstwaarschijnlijk multifactorieel. Naast conventionele risicofactoren spelen hiv en het gebruik van cART een rol. Om het effect van de hiv-infectie zelf te bepalen, hebben wij in hoofdstuk 10 de BMD en de botstofwisselingsmarkers van 33 onbehandelde PHI-mannen bepaald, aangezien zij beperkte blootstelling hebben gehad aan hiv en geen blootstelling aan cART. De BMD werd gemeten op baseline met behulp van een DXA-scan van de lumbale wervelkolom, femurnek en totale heup. WHO criteria werden gebruikt om osteopenie en osteoporose te definiëren, respectievelijk een T-score tussen de -1 en -2.5 en ≤ -2.5 SD (standaard deviaties). De gemiddelde T (P=0.001) -en Z-scores (P=0.004) van de lumbale wervelkolom en de gemiddelde T-score (P=0.003) van de femurnek waren significant lager in de PHI-mannen dan bij mannen van de NHANES IV referentie populatie. Vijfenveertig procent van de PHI-mannen hadden osteopenie en 6% had osteoporose. De botstofwisselingsmarkers waren niet verschillend tussen diegenen met of zonder osteopenie en osteoporose. In de multivariate lineaire regressie analyse waren de body mass index (BMI) en het schildklier-stimulerend hormoon (TSH) positief geassocieerd met de BMD; leeftijd en de pVL waren negatief gecorreleerd met de BMD. Samenvattend komen osteopenie en osteoporose onverwacht veel voor bij mannen met een PHI. In overeenstemming met de literatuur was een verminderde BMD geassocieerd met een oudere leeftijd, een hogere pVL, een lagere BMI en een lager TSH niveau.

De in hoofdstuk 10 beschreven resultaten riepen vele nieuwe vragen op, met name of de verlaagde BMD gerelateerd was aan de recente hiv-infectie of met andere traditionele risicofactoren, of dat de verlaagde BMD reeds aanwezig was voordat deze mannen hiv-geïnfecteerd werden. Om dit vraagstuk te kunnen beantwoorden hebben wij in hoofdstuk 11 de BMD en botstofwisselingsmarkers van 41 primaire en 106 chronische, onbehandelde hiv-
geïnfecteerde MSM vergeleken met de BMD van een hiv-negatieve controle groep van 30 MSM. Een verlaagde BMD, welke in deze studie volgens de nieuwe richtlijnen van de *International Society for Clinical Densitometry* werden gedefinieerd als een Z-score ≤ -2.0 SD ter hoogte van de lumbale wervelkolom of de heup, kwam zeer veel voor in alle drie de groepen. In de multivariate analyses was hiv niet geassocieerd met een verlaagde BMD. De resultaten uit dit hoofdstuk suggereren dat de verlaagde BMD in acute en chronisch hiv-geïnfecteerde MSM reeds aanwezig is voor de hiv transmissie en dat hun verlaagde BMD niet volledig is toe te schrijven aan de hiv-infectie of het gebruik van cART.

**Deel V Conclusie**

In het laatste hoofdstuk, *hoofdstuk 12*, wordt een overzicht gegeven van de resultaten en bevindingen zoals beschreven in dit proefschrift. De verschillende studies worden vergeleken met recente bevindingen uit de literatuur. Tot slot worden aanbevelingen geformuleerd voor de huidige kliniek en suggesties gedaan voor toekomstig onderzoek. Samenvattend, wordt in dit proefschrift de behandeling van PHI beschreven. De resultaten van de gerandomiseerde Primo-SHM trial laten zien dat vroegbehandeling het virale setpunt (tijdelijk) verlaagt en de chronische, levenslange therapie uitstelt met een gemiddelde van twee jaar. Indien vroegbehandeling tijdens de PHI wordt overwogen, adviseren wij een cART regiem te starten met een gebooste protease remmer, iniedergeval totdat de resultaten van de resistentiebepaling bekend zijn, gedurende in totaal 24 weken.
Referenties


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LIST OF ABBREVIATIONS

AIDS  Acquired Immunodeficiency Syndrome  
AMC  Academic Medical Center  
ALP  Alkaline phosphatase  
ARS  Acute retroviral syndrome  
BMD  Bone mineral density  
BMI  Body mass index  
cART  Combination antiretroviral therapy  
CI  Confidence interval  
c/ml  Copies/ml  
CRP  C-reactive protein  
1CTP  C-terminal telopeptide of type 1 collagen  
CTX  C-telopeptide crosslink of type 1 collagen  
DXA  Dual energy X-ray absorptiometry  
FAI  Free androgen index  
HIV  Human Immunodeficiency Virus  
HLA  Human leucocyte antigen  
HR  Hazard ratio  
HRQL  Health-related quality of life  
IAS-USA  International Antiviral Society–USA  
IQR  Interquartile range  
mITT  Modified intention-to-treat  
MHS  Mental health summary score  
MOS-HIV  Medical Outcomes Study Health Survey for HIV  
MSM  Men who have sex with men  
NHANES IV  National Health and Nutrition Examination Survey  
NNRTI  Non-nucleoside reverse transcriptase inhibitor  
NRTI  Nucleoside reverse transcriptase inhibitor  
PHI  Primary HIV infection  
PHS  Physical health summary score  
PI  Protease inhibitor  
P1NP  Procollagen type 1 N-terminal propeptide  
PrEP  Pre-exposure prophylaxis  
PTH  Parathyroid hormone  
pVL  Plasma viral load  
RCT  Randomized controlled trial  
RNA  Ribonucleic acid  
SD  Standard deviation  
SHBG  Sex hormone-binding globulin  
TI  Treatment interruption  
TSH  Thyroid stimulating hormone  
WHO  World Health Organization
LIST OF PUBLICATIONS


List of Publications


CURRICULUM VITAE

From June 2012
Dermatology residency program, Leiden University Medical Center

2008-2012
PhD fellow Infectious Diseases, Academic Medical Center (AMC), University of Amsterdam
  – Nederlandse Internisten Vereniging Internistentdagen 2011 award for best scientific presentation
  – Clinical Epidemiology Course, Leiden University Medical Center
  – Masterclass on pathogenesis, immunology and clinical management of HIV/AIDS, Virology Education
  – AMC Graduate School PhD Course Program: Good Clinical Practice; clinical epidemiology; clinical data management; biostatistics; systematic reviews; scientific writing; oral presentation; infectious diseases

2007-2008
Resident Internal Medicine, Onze Lieve Vrouwe Gasthuis, Amsterdam

2006-2007
Post-graduate scholarship VSfonds: Research physician STI/HIV clinic, Kenya Medical Research Institute, Kilifi, Kenya. Research project: ‘Access to health care and establishing of high-risk HIV-1 negative cohorts in Kilifi District, Kenya’

2000-2006
Medical school, Maastricht University
  – Research elective, Federal University of Roraima, Boa Vista, Brazil. Research project: ‘Health service delivery for chronic respiratory disease in Brazilian primary care’
  – Rotations in psychiatry, paediatrics, otolaryngology & ophthalmology, University Hospital Brussel, Belgium
  – Internal Medicine rotation, RCSI Medical University, Manama, Bahrain
  – Hematology & Emergency medicine, University of Ferrara, Italy
  – Elective Internal Medicine, Mulago hospital, Makerere University, Kampala, Uganda
  – Elective Dermatology, Dr. Ram Manohar Lohia Hospital, New Delhi, India

Other relevant experiences:
  – Data manager of the integrated COPD management study in primary care in Bocholtz & Rotterdam, Maastricht University
  – Active member of the International Federation of Medical Students’ Association (IFMSA): National officer of reproductive health including HIV/AIDS

1998-2000
Health Sciences – Propaedeutic exam, Maastricht University

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