HIV: OPTIMIZING TREATMENT AND MONITORING BEYOND VIROLOGIC SUPPRESSION

PATRICK G.A. OOMEN

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HIV: optimizing treatment and monitoring beyond virologic suppression
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Prof. dr. S.E. Geerlings
Prof. dr. H.G.M. Heijerman (voorzitter)
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GENERAL INTRODUCTION AND OUTLINE OF THIS DISSERTATION

GENERAL INTRODUCTION

Human Immunodeficiency Virus: from past to present

The onset of the Human Immunodeficiency Virus (HIV) epidemic can be traced back to 1981 when five men who have sex with men (MSM) were diagnosed with *Pneumocystis carinii* pneumonia, marking the beginning of a devastating global health crisis unprecedented in history.[1] In 1983, French virologists successfully identified the causative pathogen: a T-lymphotropic retrovirus, now known as HIV.[2] HIV primarily infects $CD4^+$ lymphocytes, leading to their depletion and compromising the immune system's ability to function effectively.[3] The virus can be transmitted through various routes, including blood transfusions, sexual contact, and vertical transmission. The clinical picture of advanced HIV disease was characterized by a wide range of opportunistic infections or malignancies, which became known as the Acquired Immunodeficiency Syndrome (AIDS). Given the high mortality rate associated with AIDS[4], a quick search for effective treatment options became paramount (Fig. 1). However, developing effective antiretroviral therapy (ART) initially posed significant challenges due to virological resistance in the setting of mono or dual ART.[5,6] The introduction of combination antiretroviral therapy (cART) consisting of three antiretroviral drugs in 1996 revolutionized HIV treatment.[7–9] It led to an astounding increase in life expectancy of people with HIV-1 (PWH).[10–12] Despite the success, in the early days of cART, treatment for PWH remained challenging due to a high pill burden and serious, sometimes deadly side-effects.[13–15]

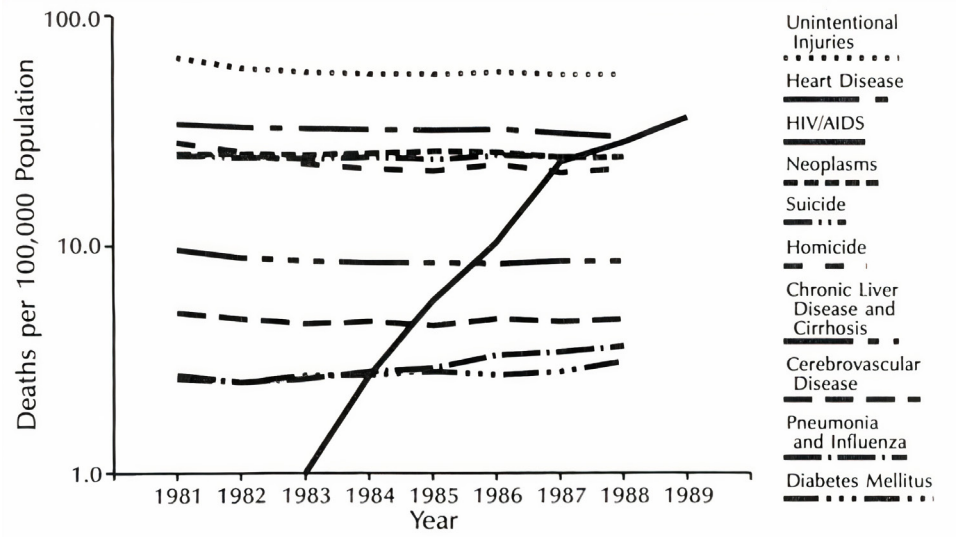

Figure 1. Leading causes of death among men 25-44 years of age – United States, 1981 – 1989. CDC Morbidity and Mortality Weekly Report, January 25, 1991.

Today, the landscape of HIV treatment has undergone a remarkable transformation compared to the early days of cART. Currently, approved ART drugs are divided into nine classes based on how each drug interferes with the HIV life cycle (Fig. 2). These nine classes include the nucleoside/nucleotide reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, CCR5 antagonists, attachment inhibitors, post-attachment inhibitors, capsid inhibitors, integrase strand transfer inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Most ART regimens recommended for use in PWH consist of a single pill per day with minimal side effects, composed of two or three highly effective ART agents with a high barrier to resistance.[16,17] Notably, the life expectancy of PWH has reached levels nearly comparable to that of the general population, though there exist large regional differences.[18–21] Although more people than ever are living with HIV – current estimations are 39.0 million globally in 2022[22] – the sentiment is more hopeful compared to the early days. This is evidenced by the UNAIDS target to reach '95-95-95' in 2025: the goals being that 95% of PWH know they're living with HIV, that 95% of these people have access to ART, and that 95% of those who have access ART are virologically suppressed.[23,24] In the Netherlands, this has almost been achieved, as in 2021, 94% of PWH had been diagnosed and linked to care, 94% of those diagnosed had started ART, and 96% of those treated were virologically suppressed.[25] Moreover, in conjunction with the strides made, a fourth major goal was proposed in 2016: that 90% of PWH with virologic suppression should have a good health-related quality of life.[26] Historically, virologic suppression was the overriding goal because that is what saved lives. However, thanks to these advances in ART, we now have the opportunity to look beyond virologic suppression.

The focus of this dissertation centers around optimizing treatment and monitoring of HIV. Although virologic suppression is widely achieved in the Western world today, important challenges remain. These include the substantial differences between virologic suppression in clinical trials versus the real world and the potential effects of contemporary ART beyond virologic suppression, such as HIV-associated immune activation, viral blips, residual viremia, and neurocognitive impairment. Finally, this dissertation focuses on investigating HIV-related co-morbidities, particularly *Pneumocystis jirovecii* pneumonia and hepatitis B virus co-infection, to improve their treatment and monitoring.

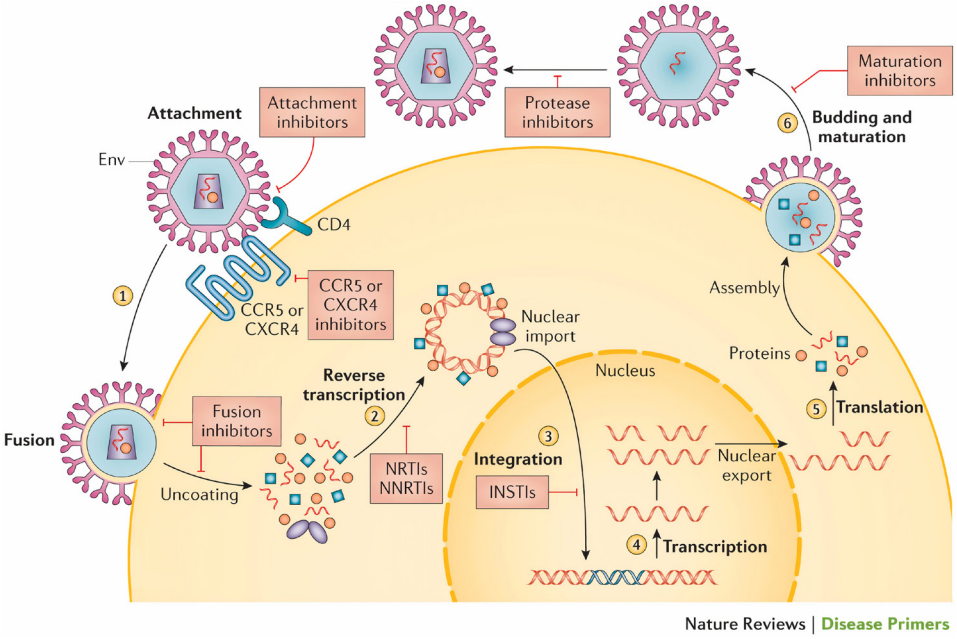

Figure 2. HIV enters its target cells via CD4 and either CC-chemokine receptor 5 (CCR5) or CXC-chemokine receptor 4 (CXCR4) through interaction with envelope (Env) glycoprotein (step 1). After fusion and uncoating, the viral RNA is then reverse transcribed into DNA (step 2). The ensuing pre-integration complex is imported into the nucleus, and the viral DNA is then integrated into the host genome (step 3). Mediated by host enzymes, HIV DNA is transcribed to viral mRNAs (step 4). These mRNAs are then exported to the cytoplasm where translation occurs (step 5) to make viral proteins and eventually mature virions (step 6). Each step — HIV entry, reverse transcription, integration and protein maturation — in the HIV life cycle is a potential target for antiretroviral drugs. INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor. Figure obtained from Deeks et al.[37], Springer Nature.

Optimizing treatment and monitoring of HIV

Since the introduction of cART in 1996, numerous new ART regimens have been approved for use in PWH. To gain market approval, new ART regimens must demonstrate through rigorously conducted registration trials that they result in non-inferior virologic efficacy compared to the prevailing ART regimens of that moment. Doravirine (DOR) is an non-nucleoside reverse transcriptase inhibitor (NNRTI) that has been approved for use as anchor drug in PWH since 2018.[27] Clinical trials have demonstrated non-inferiority regarding virological efficacy in both ART-naïve and ART-experienced individuals.[28–30] However, it is well known that findings observed in clinical trials are notoriously different from those observed in so-called “real-world” populations.[31–33] This discrepancy also extends to tolerability, with an important example being the substantial trial and real-world differences observed for the relatively new dolutegravir-based regimens, underscoring the need for real-world studies even for newer ART anchors.[34–36] Unfortunately, there is a lack of

real-world data comparing the effectiveness and tolerability of DOR-based ART with other contemporary ART regimens.

Even after achieving virologic suppression, challenges remain. One such challenge is the occurrence of viral blips in PWH on ART who are virologically suppressed.[38] Viral blips are temporary elevations of HIV plasma viral load above the detection limit of standard assays. The exact nature of viral blips remains unclear, and multiple hypotheses have been proposed, including intermittent release of virions from the latent reservoir, differences in assay accuracy, or ongoing viral replication.[39–47] Understanding the etiology of viral blips is critical due to the uncertainty they create for both PWH and caregivers.[38] Furthermore, viral blips have been associated with adverse clinical outcomes, including virologic failure.[48,49] It is currently unclear which factors are associated with the occurrence of viral blips, though the type of cART anchor appears to play a significant role.[38] One potential hypothesis is that viral blips may be attributed to residual viremia (RV): the detectable viremia below the commonly used assay threshold of 50 cop/mL (i.e., virologic suppression).[50] Therefore, it is important to investigate the factors associated with viral blips, including the potential role of RV. Subsequently, it becomes crucial to examine the factors associated with RV itself. However, to date, no studies have simultaneously explored both associations—the factors associated with viral blips and those associated with RV.

Besides RV, other challenges lie below the surface of virologic suppression. As ART has improved over the years with the emergence of potent drugs with a high genetic barrier to resistance, the question has arisen whether it might be possible to achieve virologic suppression with fewer ART drugs than the conventional triple therapy regimens, given the lifelong nature of ART and ART-associated toxicity. Although monotherapy ultimately seems inferior[51–53], the first dual therapy regimens were approved and included in guidelines in 2018.[16,17] Registration trials have demonstrated non-inferior virologic suppression compared with triple therapy regimens for both ART-naïve and ART-experienced PWH.[54,55] However, next to virologic suppression, it is crucial for ART to counteract HIV-associated immune activation: a hyperactive inflammatory state that ultimately leads to T-cell depletion and is associated with numerous comorbidities, including cardiovascular disease.[56] While several studies have investigated this aspect in various combinations of triple, dual, and monotherapy, no overview has been compiled to address the question of the impact of dual and mono versus triple therapy regimens on HIV-associated immune activation.

Although the effect of cART on HIV-associated immune activation has been clearly demonstrated, there are disorders in PWH where this has not yet been fully elucidated, such as in HIV-associated neurocognitive disorders (HAND). HAND is common among PWH and is comprised of three subtypes: HIV-associated dementia (HAD), mild neurocognitive disorder (MND), and asymptomatic neurocognitive impairment (ANI).[57] The incidence of

HAD has significantly decreased with the advent of cART, but MND and ANI continue to be prevalent.[58,59] HAND is relevant as it substantially impacts the quality of life of PWH.[60] Although the use of cART has, through neurotoxicity, been linked to the development of HAND, HAND's etiology remains a topic of debate.[61,62] Efavirenz (EFV), an NNRTI notorious for its neurocognitive side effects such as dizziness or insomnia[63], has also been linked to increased neurocognitive impairment[64–67], although there is ongoing debate in this regard[68,69]. Understanding the role of EFV in HAND is important as EFV is still recommended as an alternative first-line treatment in World Health Organization guidelines[70] and forecast analyses show approximately ten million PWH (25% of the global population with HIV) still using EFV in 2025.[71]

HAND is traditionally diagnosed using a neuropsychological assessment (NPA). An NPA evaluates various cognitive domains, and if an individual scores 1 or 2 standard deviations below the mean in one or more domains, and depending on functional complaints, a diagnosis of MND or ANI is made.[57] However, NPAs are time-consuming and expensive. Although not a validated clinical tool like NPA, blood oxygen level dependent (BOLD) functional MRI (fMRI) has also been widely used in the research setting to assess functional impairment.[72,73] It can detect localized changes in cerebral blood flow and oxygenation, providing insights into regional neuronal activity.[74] The use of BOLD fMRI can therefore help to shed light on neurocognitive impairment in PWH. Important neurocognitive systems are reward processing and response inhibition, as these have been shown to be disrupted in PWH[75,76] and are associated with depression and apathy[77] as well as gambling and substance abuse disorders[78,79]: comorbidities with an already high prevalence in PWH[80,81]. However, to our knowledge, no studies have investigated the impact of EFV on these neurocognitive systems using BOLD fMRI.

Optimizing treatment and monitoring of HIV-associated co-comorbidities

In light of the aforementioned target of 90% of PWH having a good health-related quality of life[26], it is important to optimize not only the treatment and monitoring of HIV, but also that of HIV-associated comorbidities, given their substantial impact[82]. The prevalence of HIV-associated comorbidities is increasing among PWH, partly due to an aging HIV population.[83] Co-morbidities such as cardiovascular or liver disease have become ubiquitous.[84] Thus, it is important that caregivers for PWH are not only knowledgeable, but also attentive regarding co-morbidities in PWH. This includes knowledge on current co-morbidities in PWH, as well as on potential residual sequelae from the past.

Possible pulmonary sequelae may be found in PWH who have previously had *Pneumocystis jirovecii* pneumonia (PJP). *Pneumocystis jirovecii*, a fungus, was a major cause of pneumonia in PWH with AIDS during the earlier stages of the HIV epidemic.[85] With the introduction of improved ART and PJP treatment strategies, mortality rates from PJP have since declined

significantly.[10,86] Although PJP is now relatively rare, it remains the most common AIDS-defining condition in the Western world.[87] As a result of improved treatment, a considerable number of PWH have survived PJP years ago. However, it is unclear whether having past PJP leads to long-term pulmonary dysfunction. This is particularly important given the already increased incidence of pulmonary morbidity in PWH.[88,89]

While PJP is relatively rare in PWH nowadays, the same cannot be said for co-infection with the hepatitis B virus (HBV). HBV co-infection is prevalent in PWH due to shared routes of transmission, with global estimates indicating that approximately 5-20% of PWH are affected.[90] The introduction of tenofovir has significantly facilitated treatment of HBV co-infection, leading to improved clinical outcomes.[91] However, there is concern that the convenience of tenofovir-containing regimens has led to reduced attention to the treatment and monitoring of HBV in PWH. This concern is even more relevant considering the emergence of newer ART regimens that do not contain tenofovir, such as dual or long-acting injectable therapy.[54,55,92] Previous studies have highlighted the need to improve various aspects of HBV care in PWH.[93–97] Despite these concerns, to date, there has been no comprehensive study of HBV care components. In addition, no study has retrospectively performed missing laboratory tests, providing valuable insight into missed clinical implications.

OUTLINE OF THIS DISSERTATION

Although the life expectancy is nearly similar to that of the general population and quality of life has improved markedly for PWH in recent decades, there are still several areas for significant improvement. In this dissertation, we focus on investigating and optimizing treatment and monitoring of HIV and HIV-associated comorbidities.

In the first part, we investigate the real-world virologic effectiveness and tolerability of switching to doravirine-based ART [**Chapter 2**]. Afterwards, in PWH that are virologically suppressed, we investigate viral blips and the potential role of residual viremia in its etiology, as well as their associated factors [**Chapter 3**]. Next, in a mini review, we elaborate on the effect of triple, dual and mono ART on HIV-associated immune activation [**Chapter 4**]. We then shift our focus towards neurocognitive impairment: a common condition significantly impacting the quality of life in PWH. In [**Chapter 5**], we use BOLD fMRI to evaluate whether efavirenz affects reward processing in PWH. Finally, in the same population, we also examine response inhibition and additionally explore potential neural mechanisms of cognitive improvement [**Chapter 6**].

In the second part of this dissertation, we focus on HIV-associated co-morbidities. We first investigate whether prior PJP is associated with long-term pulmonary impairment in PWH [**Chapter 7**]. Next, in [**Chapter 8**], we respond to a correspondence received after publication of our research on PJP and pulmonary impairment. Afterwards, in [**Chapter 9**], we present the results of a proof-of-concept quality improvement study in which we investigated and improved guideline-adherent HBV care in PWH.

Finally, in [**Chapter 10**], the main findings of this dissertation are discussed and perspectives for the future are presented.

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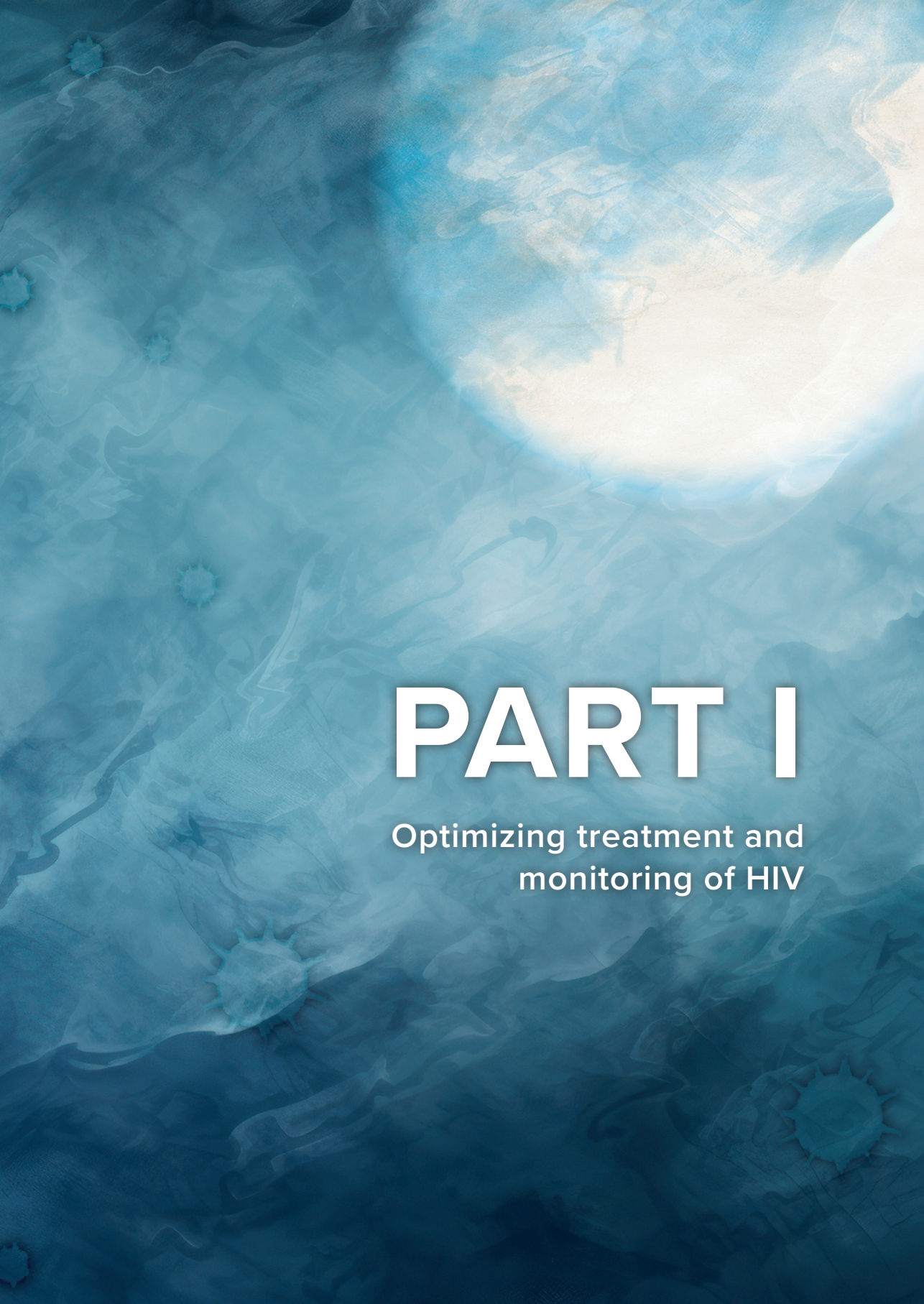


PART I

Optimizing treatment and monitoring of HIV

2

REAL-WORLD EFFECTIVENESS AND TOLERABILITY OF SWITCHING TO DORAVIRINE-BASED ANTIRETROVIRAL THERAPY IN PEOPLE WITH HIV: A NATIONWIDE MATCHED COHORT STUDY

Patrick G.A. Oomen*
Ferdinand W.N.M. Wit*
Kees Brinkman
Saskia M.E. Vrouenraets
Tania Mudrikova
Berend J. van Welzen
Marc van der Valk

*Contributed equally

Under review.

ABSTRACT

Background

We assessed real-world effectiveness and tolerability of switching to doravirine (DOR)-based triple antiretroviral therapy (ART) in people with HIV-1 (PWH).

Methods

We conducted a nationwide prospective cohort study of PWH without prior virological failure ≥12 months stable on non-DOR-containing triple or dual ART switching to DOR before September 1, 2020 (cases). Cases were matched 1:2 to individuals continuing stable non-DOR-containing ART, on age, sex, HIV acquisition category, time since ART initiation, calendar time, pre-ART CD4+ count, pre-ART plasma viral load (pVL) and anchor drug class before switching. The primary outcome was protocol-defined virological failure (PDVF) (pVL≥200 cop/mL) in the intention to treat (ITT) population at week 104, with participants modifying their regimen or becoming lost to follow-up (LTFU) considered as PDVF (non-inferiority margin +5%). In the on treatment (OT) population, those who modified their regimen or became LTFU were censored from that moment onwards. Tolerability was a secondary outcome.

Findings

In total, 590 cases and 1180 controls (of whom 55.3% used integrase strand transfer inhibitor-based regimens) were included. In the ITT analysis, PDVF occurred in 135 (22.9%) cases and in 295 (25.0%) controls (risk difference -2.12% (upper limit of the one-sided 95% confidence interval +1.40%). In the OT analysis, 10/455 (2.2%) non-censored cases and 26/885 (2.9%) non-censored controls experienced PDVF (risk difference -0.70% (upper limit of the one-sided 95% confidence interval +0.73%)). All cases with pVL≥200 cop/ml re-suppressed without regimen modification: no confirmed virological failure (two consecutive pVLs≥200 cop/mL) was observed. Hundred-and-four (17.6%) cases and 211 (17.9%) controls modified their regimen. Seventy-three (12.4%) cases discontinued DOR due to adverse events: abnormal dreams (1.7%) and insomnia (1.5%) were the most common.

Interpretation

Switching to doravirine in well-suppressed PWH without prior virological failure was non-inferior compared to continuing non-DOR-containing regimens after two years in a real-world setting.

RESEARCH IN CONTEXT

Evidence before this study

In 2018, the non-nucleoside reverse transcriptase inhibitor (NNRTI) doravirine (DOR) was approved for use in people with HIV-1 (PWH). Two large clinical trials conducted in ART-naïve PWH comparing DOR to either efavirenz or darunavir as anchor drug showed non-inferiority of DOR. The DRIVE-SHIFT trial demonstrated non-inferiority of DOR in well-suppressed, treatment-experienced individuals compared to participants predominantly treated with a protease inhibitor or an NNRTI as anchor drug. To date, these data have not been confirmed in a large real-world setting and no comparison has been made between DOR and integrase strand transfer inhibitors (INSTIs). We searched PubMed for articles published between January 1, 2014 and December 1, 2023, using the terms “HIV” and “doravirine” without language restrictions. We found eight published articles describing the real-world effectiveness and tolerability of DOR-based triple therapy regimens in treatment-experienced PWH in a single-arm design. Therefore, the real-world effectiveness and tolerability of DOR-based ART compared with non-DOR-containing ART regimens remains unclear.

Added value of this study

This study, using data from the ATHENA cohort, a Dutch nationwide cohort of PWH, compares the effectiveness and tolerability after 104 weeks of DOR treatment in well-suppressed PWH without prior virological failure to PWH continuing on a non-DOR-containing triple or dual therapy regimen. We matched 590 eligible cases who switched to DOR with 104 weeks follow-up to 1180 controls continuing a non-DOR-containing regimen. Specifically, 652 (55.3%) controls were treated with INSTIs. Our results confirm clinical trial data demonstrating that DOR is non-inferior to other standard-of-care ART regimens regarding effectiveness after 104 weeks. Long-term tolerability was also very similar, as both groups had comparable rates of and reasons for ART regimen modifications. Compared with discontinuation rates reported in clinical trials, we found a substantially higher rate of adverse event-related DOR discontinuations during the study period. The main adverse events leading to DOR discontinuation were of neuropsychiatric and gastrointestinal nature. This study is the first to compare DOR-based triple therapy regimens with INSTI-based regimens, which is important given that INSTIs currently are the preferred treatment option in HIV guidelines. Our study fills an important gap regarding the, until now, unclear real-world effectiveness and tolerability of DOR-based ART relative to other contemporary ART regimens.

Implications of all the available evidence

Switching to DOR in well-suppressed PWH without prior virological failure is a durable, efficacious, and well-tolerated treatment option in a real-world setting. Switching to DOR in well-suppressed PWH without prior virological failure is a durable, efficacious, and well-tolerated treatment option in a real-world setting.

INTRODUCTION

Doravirine (DOR) is a third-generation non-nucleoside reverse transcriptase inhibitor (NNRTI), approved for use in people with HIV-1 (PWH) since 2018.[1] It is dosed once daily, can be taken regardless of food intake, and has a favorable resistance profile among NNRTIs as well as low potential for drug-drug interactions.[2–5] DOR is available as part of a single tablet regimen in a fixed-dose combination with tenofovir disoproxil and lamivudine, and as a stand-alone tablet. Clinical trials in both treatment-naïve and well-suppressed PWH without prior virological failure showed non-inferior virological effectiveness and good tolerability of DOR compared to boosted protease inhibitor (PI)- or NNRTI-based regimens.[6–9] To date, no large-scale comparison has been made between DOR and integrase strand transfer inhibitors (INSTIs).[10,11]

Observational cohort studies play a crucial role in providing additional insights beyond those of registration studies, including on rare or late-onset adverse events.[12] Moreover, findings from real-life and clinical trials often differ, as the latter suffer from volunteer bias and lack external validity due to strict in- and exclusion criteria.[12–15] Several studies have reported real-world data on PWH switching to DOR[16–23], but all had short follow-up, small sample sizes, and, importantly, lacked a control arm. We conducted a nationwide matched prospective cohort study to investigate the effectiveness and tolerability of switching to DOR in well-suppressed PWH without prior virological failure, matched to PWH on non-DOR-containing triple or dual therapy regimens (including INSTIs) through week 104.

METHODS

Study design and population

We conducted a nationwide matched cohort study embedded within the prospective AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort. The structure and procedures of the ATHENA cohort are described in detail elsewhere.[24] Only data routinely collected by ATHENA were used for this analysis and therefore no additional review or consent was required. All available data were used until September 15, 2023.

We included PWH, aged ≥18 years, who switched to DOR-based triple therapy before September 1, 2020 to allow for a potential follow-up of 104 weeks (cases). The DOR-based regimen had to contain two nucleoside reverse transcriptase inhibitors (NRTIs): tenofovir disoproxil, tenofovir alafenamide or abacavir plus lamivudine or emtricitabine. Individuals had to be on ART for ≥12 months prior to inclusion, and ART prior to switching to DOR had to be composed of two NRTIs and either a boosted PI (atazanavir, darunavir or lopinavir), an NNRTI (efavirenz, nevirapine or rilpivirine) or an INSTI (bictegravir, dolutegravir, elvitegravir

or raltegravir). Those on dual therapy consisting of dolutegravir/lamivudine, dolutegravir/rilpivirine or dolutegravir/boosted-darunavir before switching to DOR were also eligible for inclusion as case. All individuals must have had ≥1 documented plasma viral load (pVL) measurement <200 cop/mL on ART prior to study entry. Individuals with prior virological failure (defined as being pre-treated with NRTI mono or dual therapy, 'virological failure' as recorded reason to switch prior ART according to the treating physician, pVL ≥1000 cop/mL immediately prior to discontinuation of a regimen, or observed drug-resistance associated mutations) were excluded. PWH with an isolated pVL ≥1000 cop/mL were eligible in case re-suppression occurred without any ART change. Previously planned or unplanned treatment interruptions were not considered as treatment failures and pVLs in these periods were ignored.

We matched cases 1:2 to PWH without prior virological failure (controls). Matching was performed on ART class of anchor drug (e.g., a case on an INSTI-based regimen prior to switching to DOR was matched to two controls on INSTI-based regimens), on age, sex at birth, HIV acquisition category, time since ART initiation, lowest pre-ART $CD4^+$ count, highest pre-ART pVL, and calendar time. Regarding calendar time, controls were eligible in case of a routine clinical visit with pVL in a time window of maximum three months before or after the DOR switch date of the associated case. Follow-up for the matched controls started at the date of their clinic visit that was closest to the date the associated case switched to DOR. As region of origin was collinear with HIV acquisition category, matching was only performed on the latter characteristic. Pre-ART $CD4^+$ count was categorized into three categories (0-199, 200-499 or ≥500 cells/mm^3) and, in case of missing pre-ART $CD4^+$ counts, matching was conducted on $CD4^+$ count measured at study entry. Pre-ART pVL was categorized in two categories (< or ≥100,000 cop/mL) and, in cases with missing pre-ART pVLs, this criterion was dropped. Nearest neighbor matching was used for continuous variables and participants were exact matched on categorical variables. Follow-up for cases started on the DOR start date and for controls on the clinic visit date closest to the DOR start date of the associated case.

Measurements and outcomes

All data used for this analysis were recorded as part of routine prospective data collection for the ATHENA cohort. We extracted demographic and clinical data of included participants at the time of study entry (i.e., age, sex at birth, region of origin, HIV acquisition category, time since HIV diagnosis and ART initiation, prior acquired immunodeficiency syndrome (AIDS) diagnosis, chronic hepatitis B virus (HBV) infection (defined as two consecutive hepatitis B surface antigen-positive and/or HBV-DNA detectable results during a period of ≥6 months), prior or active hepatitis C virus (HCV) infection (defined as a positive HCV RNA polymerase chain reaction or antibody test result), smoking behavior (categorized as never, past and current), obesity (defined as a BMI >30 kg/m^2), diabetes mellitus type 2,

hypertension, estimated glomerular filtration rate, and history of cardiovascular disease, stroke and non-AIDS-defining malignancies). Laboratory data were the $CD4^+$ count and pVL at study entry, and pre-ART $CD4^+$ count and pVL. ART-related data were the number of prior ART regimens and ART class, anchor drug, NRTI backbone or dual therapy regimen used before and during the study. Finally, for cases the reason for switching to DOR and the reason for discontinuing DOR during the study (including adverse events) were collected.

Our primary outcome was the proportion of individuals with protocol-defined virological failure (PDVF) (pVL ≥200 cop/mL) in an 'intention to treat' (ITT) analysis comparing cases to controls. A time window of ±12 weeks was used and, if there was no measurement within this window, we used the first available pVL >12 weeks after week 104 as end of study pVL. Participants were also considered as PDVF in case the week 104 pVL was missing, the regimen was modified, or the participant was lost to follow-up before week 104. Effectiveness was also assessed in an 'on treatment' (OT) analysis in which participants were censored if week 104 pVL was missing, or from the moment regimen was modified, or the moment they were considered lost to follow-up. A subgroup analysis, restricted to cases previously and controls currently on INSTI-based regimens, was performed. We also performed sensitivity analyses defining PDVF as a pVL ≥50 cop/mL. The change in $CD4^+$ count from baseline to week 104 was compared between cases and controls. In individuals with PDVF, we report the pVL at the time of PDVF, genotypic resistance testing, and rate of and regimen used for re-suppression.

The proportion of participants who modified their regimen without PDVF was assessed as a measure of regimen tolerability. Regimen modification was defined as a change in one or more of the drugs included in the regimen. Simplification to a fixed-drug combination containing the same drugs, breakup of a single tablet regimen into generic components and switches of the pharmacologic booster were not considered a regimen modification. In individuals discontinuing DOR, we report the reasons (i.e., adverse events, simplification, patient decision, pregnancy, and other reasons). Discontinuations due to adverse events were categorized as neuropsychiatric, gastrointestinal, dermatological, renal, systemic, liver, cardiovascular, ear-nose-throat, headache, musculoskeletal, or other. Laboratory abnormalities leading to discontinuation were reported and graded according to Division of AIDS Table for Grading the Severity of Adult and Pediatric AEs (Version 2.1, 2017).[25]

Statistical analysis

Categorical data were expressed as numbers with percentages, and continuous data as means with standard deviations or medians and interquartile ranges. Baseline categorical data were compared using Fisher's exact test or χ^2; and continuous variables were compared using the independent samples *t*-test, Wilcoxon rank-sum test or Kruskal-Wallis test, with two-sided p-values <0.05 were considered statistically significant.

Non-inferiority in both ITT and OT analyses was declared when the upper limit of the one-sided 95% confidence interval (CI) of the risk difference (RD) in the occurrence of PDVF between cases and controls was below 5.0%. Formal sample size calculation for our primary outcome, assuming a virological suppression rate of 95%, a control-to-case ratio of 2:1, and a non-inferiority margin δ=0.05, resulted in a required sample size for the active arm of ≥390 cases to have 90% power to detect non-inferiority of switching to DOR versus continuing non-DOR-containing regimens.

Kaplan-Meier analyses were used to estimate the week 104 PDVF and regimen modification rate. In participants experiencing PDVF, exploratory multivariable logistic regression was conducted to examine associations of PDVF with characteristics, with odds ratios and 95% Cis reported. All statistical analyses and data visualization were performed using SAS (version 9.4, Cary, NC, USA).

Role of the funding source

The authors received no funding for this work. The ATHENA database is maintained by the HIV Monitoring Foundation and is financed by the Dutch Ministry of Health, Welfare and Sport through the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment.[24]

RESULTS

Participant characteristics

In the year DOR became available in the Netherlands (2019), the ATHENA cohort consisted of 21,642 adult PWH on ART. Appendix Table 1 provides cross-sectional descriptive statistics of the cohort in 2019, 2020, 2021 and 2022, including the annual number of PWH on DOR-based triple therapy. A total of 590 PWH without prior virological failure were switched to a DOR-based triple therapy regimen before September 1, 2020 and were thus included as cases and matched 1:2 to 1,180 controls (Table 1). Baseline characteristics used in matching were similar between groups, but significant differences were found for non-matched characteristics including region of origin and number of prior ART regimens. The main reasons for switching to DOR were (presumed) toxicity of previous regimen (37.5%), simplification (30.8%), and cost reduction (10.2%) (Table 1). The majority of cases used an INSTI-based triple drug regimen before switching to DOR (55.3%).

Table 1. Characteristics of cases and controls at baseline

	Cases (n= 590)	Controls (n= 1180)	p-value
Age, years	49.8 (40.8 – 57.3)	49.5 (41.1 – 56.2)	0.476°
Male sex (at birth)	524 (88.8)	1048 (88.8)	>0.999~
Region of origin*			0.009~
- Netherlands	343 (58.3)	759 (64.5)	
- Western	90 (15.3)	128 (10.9)	
- Sub-Saharan Africa	31 (5.3)	89 (7.6)	
- Caribbean / Latin America	76 (12.9)	126 (10.7)	
- South Asia	27 (4.6)	43 (3.7)	
- Other	21 (3.6)	31 (2.6)	
HIV acquisition category*			>0.999^
- MSM	472 (80.0)	944 (80.0)	
- Heterosexual	96 (16.3)	193 (16.4)	
- IDU	4 (0.7)	8 (0.7)	
- Blood contact	5 (0.8)	9 (0.8)	
- Pediatric	3 (0.5)	6 (0.5)	
Time since HIV diagnosis, years	11.0 (6.8 – 15.2)	10.8 (7.0 - 15.2)	0.861°
Time since ART initiation, years	8.9 (5.6 – 12.3)	8.8 (5.7 – 12.3)	0.955°
Pre-ART pVL (cop/mL)*	114000 (38800 – 357770)	100000 (37500 -300000)	0.374°
Pre-ART CD4$^+$ count (cells/mm$^{3)*}$	300 (180 - 420)	291 (190 - 410)	0.899°
Pre-ART CD4$^+$ count < 200 cells/mm^{3*}	195 (33.1)	322 (27.3)	0.012
pVL at study entry (cop/mL)			0.933~
- Detectable, below 200 cop/mL	25 (4.2)	49 (4.2)	
- Undetectable	565 (95.8)	1131 (95.8)	
CD4$^+$ count at study entry (cells/mm^3)	747.0 (568.0 – 959.0)	720.0 (549.0 – 940.0)	0.075°
Chronic HBV infection*	26 (4.4)	64 (5.4)	0.486~
Prior HCV infection**	93 (15.8)	123 (10.4)	0.005~
Prior AIDS diagnosis	97 (16.4)	203 (17.2)	0.687~
History of non-AIDS-defining malignancy*	21 (3.6)	47 (4.0)	0.306^
History of cardiovascular disease*	20 (3.4)	52 (4.4)	0.198^
History of stroke*	7 (1.2)	15 (1.3)	0.371^
Smoking status*			0.086~
- Never	241 (40.8)	455 (38.6)	
- Past	137 (23.2)	293 (24.8)	
- Current	177 (30.0)	325 (27.5)	
Obese (BMI >30 kg/m^2)*	63 (10.7)	116 (9.8)	0.279^
Diabetes mellitus type 2*	18 (3.1)	41 (3.5)	0.305^
Hypertension*	159 (26.9)	300 (25.4)	0.262^
eGFR (ml/min)*			0.002^
- ≥ 90	297 (50.3)	536 (45.8)	
- 60-90	276 (46.8)	552 (47.2)	
- 30-60	15 (2.5)	77 (6.6)	
- 15-30	1 (0.2)	2 (0.2)	
- 0-15	1 (0.2)	3 (0.3)	
Number of prior ART regimens			<0.001~
0-2	219 (37.1)	839 (71.1)	
3-5	309 (52.4)	289 (24.5)	
>5	62 (10.5)	52 (4.4)	
ART class before study inclusion			>0.999~
- Dual therapy	6 (1.0)	12 (1.0)	
- INSTI-based triple therapy	326 (55.3)	652 (55.3)	
- NNRTI-based triple therapy	202 (34.2)	404 (34.2)	
- PI-based triple therapy	56 (9.5)	112 (9.5)	

Table 1. Continued.

	Cases (n= 590)	Controls (n= 1180)	p-value
NRTI backbone before study inclusion			<0.001^
- ABC/3TC	67 (11.4)	222 (19.0)	
- TAF/3TC	0 (0)	0 (0.0)	
- TAF/FTC	337 (57.1)	576 (49.3)	
- TDF/3TC	5 (0.9)	7 (0.6)	
- TDF/FTC	175 (29.7)	363 (31.1)	
- 3TC	5 (0.8)	6 (0.5)	
- No NRTI	1 (0.2)	6 (0.5)	
Anchor drug during study			<0.001~
- ATV/r	0 (0.0)	23 (2.0)	
- BIC	0 (0.0)	114 (9.8)	
- DOR	590 (100.0%)	0 (0.0)	
- DRV/b	0 (0.0)	89 (7.6)	
- DTG	0 (0.0)	249 (21.3)	
- EFV	0 (0.0)	117 (10.0)	
- EVG/COB	0 (0.0)	267 (22.9)	
- NVP	0 (0.0)	150 (12.8)	
- RAL	0 (0.0)	22 (1.9)	
- RPV	0 (0.0)	137 (11.7)	
NRTI backbone during study			<0.001^
- ABC/3TC	7 (1.2)	222 (19.0)	
- TAF/3TC	1 (0.2)	0 (0.0)	
- TAF/FTC	20 (3.4)	576 (49.3)	
- TDF/3TC	559 (94.7)	7 (0.6)	
- TDF/FTC	3 (0.5)	363 (31.1)	
Dual therapy regimen during study			
- 3TC/DTG	0 (0.0)	6 (50.0)	
- DRV/DTG/COB	0 (0.0)	2 (16.7)	
- DRV/DTG/RTV	0 (0.0)	1 (8.3)	
- RPV/DTG	0 (0.0)	3 (25.0)	
Reasons for switching to DOR			
- Simplification	182 (30.8)	NA	
- Cost saving	60 (10.2)		
- Toxicity	221 (37.5)		
- To prevent toxicity	60 (10.2)		
- New treatment option available	3 (0.5)		
- Pharmacological interaction	33 (5.6)		
- Study participation	8 (1.4)		
- Other	15 (2.5)		
- Unknown	8 (1.4)		

All categorical data are expressed as number (percentage of total population) and all continuous data are expressed as median (interquartile range).

~ Chi-square test; ^ Fisher`s Exact test; ° Wilcoxon test; # Students` T test

* Missing data: region of origin 2 (0.4%) cases and 4 (0.4%) controls; HIV acquisition category 10 (1.7%) cases and 20 (1.7%) controls; pre-ART pVL 55 (9.3%) cases and 19 (3.2%) controls; pre-ART CD4$^+$ count 54 (9.2%) cases and 23 (1.9%) controls; chronic HBV infection 9 (1.5%) cases and 24 (2.0%) controls; prior HCV infection 3 (0.5%) cases and 7 (0.6%) controls; history of cardiovascular disease 5 (0.4%) controls; history of non-AIDS-defining malignancy 5 (0.4%) controls; smoking status 35 (5.9%) cases and 107 (9.1%) controls; obese 5 (0.4%) controls; diabetes mellitus type two 5 (0.4%) controls; hypertension 5 (0.4%) controls; stroke 5 (0.4%) controls; eGFR 10 (0.8%) controls.

** None of the cases and controls had an active HCV infection.

Abbreviations: 3TC, lamivudine; ABC, abacavir; ATV/r, ritonavir-boosted atazanavir; AIDS, acquired immunodeficiency syndrome; ART, antiretroviral therapy; BIC, bictegravir; BMI, body mass index; COB, cobicistat; cop, copies; DOR, doravirine; DRV/b, ritonavir- or cobicistat-boosted darunavir; DTG, dolutegravir; EFV, efavirenz; eGFR, estimated glomerular filtration rate; EVG/COB, cobicistat-boosted elvitegravir; FTC, emtricitabine; HBV, hepatitis B virus; HCV, hepatitis C virus; IDU, intravenous drug use; INSTI, integrase strand transfer inhibitor, MSM, men who have sex with men; NVP, nevirapine; no., number; NRTI, nucleoside reverse transcriptase inhibitor, NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor, pVL, plasma viral load; PWH, people with HIV; RAL, raltegravir; RPV, rilpivirine; RTV, ritonavir; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Effectiveness

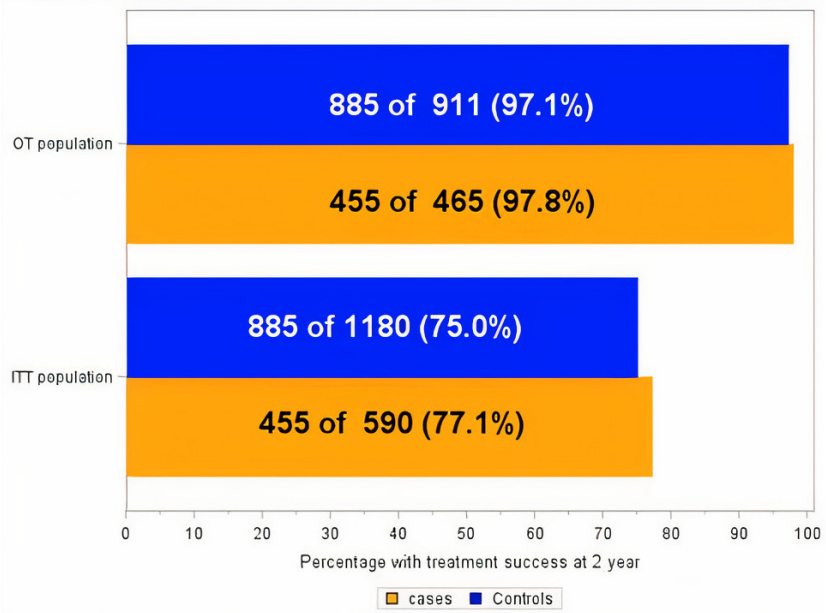
In the ITT analysis, PDVF occurred in 135 (22.9%) cases and in 295 (25.0%) controls (Fig. 1). Reasons for reaching the PDVF criterion are listed in Table 2. The RD was -2.12% (upper limit of the one-sided 95% CI +1.40%), indicating non-inferiority of DOR (Fig. 2). In the OT analysis, respectively 10/455 (2.2%) non-censored cases and 26/885 (2.9%) non-censored controls experienced PDVF (Fig. 1)**.** The OT RD was -0.70% (upper limit of the one-sided 95% CI +0.73%), also indicating non-inferiority of DOR (Fig. 2). The mean change in $CD4^+$ count from baseline to week 104 in participants who were still on the study medication on week 104 did not differ between cases and controls: +19 cells/mm^3 (interquartile range (IQR) -119–126) in cases and +25 cells/mm^3 (IQR -90–150) in controls (Kruskal-Wallis test, p=0.16).

Table 2. Outcomes in the ITT and OT population after 104 weeks, stratified by cases and controls

	Cases (n= 590)	Controls (n= 1180)
ITT population: week 104 outcome		
- Failure: insufficient or lost to follow-up, pVL<200 at last contact	21 (3.6)	58 (4.9)
- Failure: switched ART regimen, all pVL<200 before switch	104 (17.6)	211 (17.9)
- Failure: pVL>=200	10 (1.7)	26 (2.2)
- Success: all pVL<200	455 (77.1)	885 (75.0)
ITT population: outcome (dichotomized)		
- Treatment failure	135 (22.9)	295 (25.0)
- Treatment success	455 (77.1)	885 (75.0)
OT population: week 104 outcome		
- Censored: insufficient or LTFU, pVL<200 until last contact	21 (3.6)	58 (4.9)
- Censored: switched ART regimen, all pVL<200 before switch	104 (17.6)	211 (17.9)
- Failure: pVL>=200	10 (1.7)	26 (2.2)
- Success: all pVL<200	455 (77.1)	885 (75.0)
OT population: outcome (dichotomized, excluding censored individuals)	10 (2.2)	26 (2.9)
- Treatment failure	455 (97.8)	885 (97.1)
- Treatment success	125	269
Reason for insufficient or lost to follow-up		
- Death	8 (38.1)	16 (27.1)
- LTFU	2 (9.5)	5 (8.5)
- Moved abroad	7 (33.3)	19 (32.2)
- Withdrew from the ATHENA cohort	0 (0.0)	2 (3.4)
- No week 104 visit, but remaining in care	2 (9.5)	11 (18.6)
- No pVL measured at week 104 visit	2 (9.5)	6 (10.2)

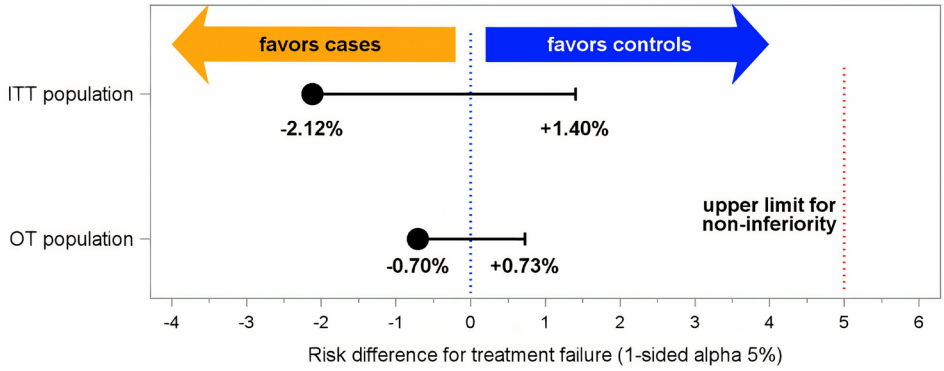
All categorical data are expressed as number (percentage).
Abbreviations: cop, copies; ITT, intention to treat; LTFU, lost to follow-up; OT, on treatment; pVL, plasma viral load.

Figure 1. Treatment success between cases and controls after 104 weeks in the ITT and OT population



* In the ITT analysis, participants without a pVL ≥200 cop/mL, with a week 104 pVL, who did not switch ART or were not lost to follow-up were considered 'a treatment success' at week 104.
** In the OT analysis, participants without a pVL ≥200 cop/mL were considered 'a treatment success' at week 104. Participants without a week 104 pVL, who switched ART or were lost to follow-up were censored from that moment onwards.
Abbreviations: cop, copies; ITT, intention to treat; OT, on treatment; pVL, plasma viral load.

Figure 2. Protocol-defined virological failure risk difference between cases and controls after 104 weeks in the ITT and OT population



Non-inferiority was declared when the upper limit of the one-sided 95% CI of the risk difference between cases and controls was below 5.0%.
* In the ITT analysis, participants with a pVL ≥200 cop/mL, without a week 104 pVL, who switched ART or were lost to follow-up were considered as protocol-defined virological failure.
** In the OT analysis, participants with a pVL ≥200 cop/mL were considered protocol-defined virological failure. Participants without a week 104 pVL, who switched ART or were lost to follow-up were censored from that moment onwards.
Abbreviations: cop, copies; CI, confidence interval DOR, doravirine; ITT, intention to treat; OT, on treatment; pVL, plasma viral load.

The Kaplan-Meier plot showed similar curves for cases and controls regarding PDVF over time, with a linear increase in the proportion of virological failure over 104 weeks of follow-up (Fig. 3). The median pVL at the time of PDVF was 361 cop/mL (IQR 256–2,520) for cases and 498 cop/mL (IQR 293–3,946) for controls. All cases re-suppressed to <200 cop/mL without regimen modification. Twenty-two controls re-suppressed to <200 cop/mL without regimen modification, with 3 controls modifying their regimen before a second pVL and 1 control having no pVL after a virological failure of 3800 cop/mL. No genotypic resistance testing was recorded. Exploratory univariable and multivariable logistic regression between characteristics and PDVF showed a significant association only for pre-ART $CD4^+$ count <200 (vs. ≥200) cells/mm^3 in cases (odds ratio 10.0, 95% CI 2.14–47.0, p=0.0035). In the OT analysis, 6.2% of PWH with a pre-ART $CD4^+$ count <200 cells/mm^3 experienced PDVF, as opposed to 0.7% of PWH with ≥200 cells/mm^3.

When limiting the ITT analysis to 326 cases previously and 652 controls currently on INSTI-based regimens, 71 (21.8%) cases and 166 (25.5%) controls had PDVF at week 104. In the OT analysis, 4 (1.5%) cases and 15 (3.0%) controls had PDVF (Appendix Table 2).

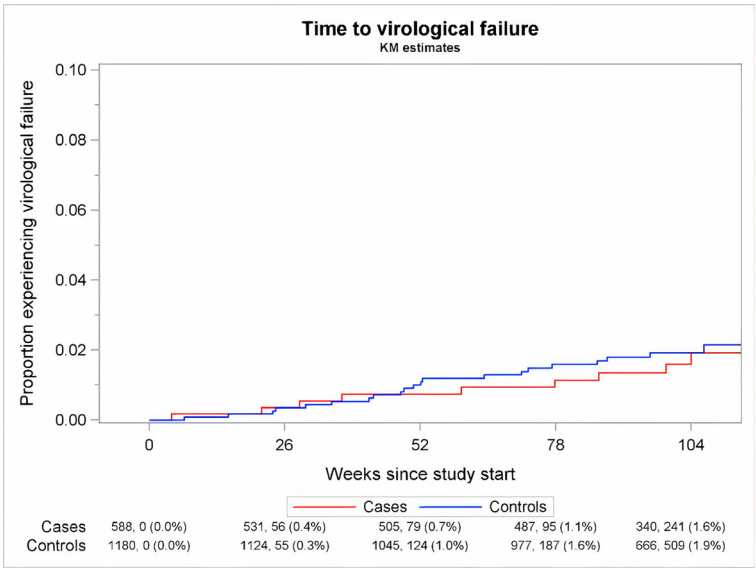
Sensitivity analyses, where the threshold for declaring PDVF was 50 cop/ml ($PDVF_{modified}$), showed that at week 104 in the ITT population 155 (26.3%) cases and 331 (28.1%) controls had experienced $PDVF_{modified}$ (RD of -1.8% (upper limit of the one-sided 95% CI +1.9%)), and that in the OT population 34 (7.2%) cases and 73 (7.9%) controls had experienced $PDVF_{modified}$ (RD of -0.67% (upper limit of the one-sided 95% CI +1.8)), indicating non-inferiority in both sensitivity analyses (Appendix Table 3).

Tolerability

Regimen modification with a pVL <200 cop/mL was observed in 104 (17.6%) cases and 211 (17.9%) controls. The Kaplan-Meier plot showed a higher rate of regimen modification for cases in the first year, but similar rates were observed after 104 weeks follow-up (Fig. 4).

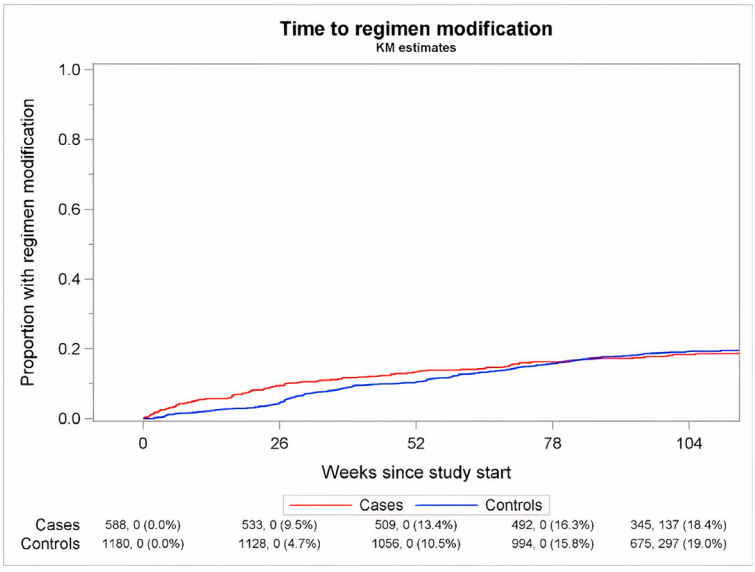
In the 104 cases who discontinued DOR, recorded reasons for discontinuation were toxicity in 73 (70.2%), simplification in 10 (9.6%), patient decision in 6 (5.8%), pregnancy in 3 (2.9%), and other reasons in 12 (11.5%). The most common adverse events resulting in discontinuation of DOR were neuropsychiatric (mainly abnormal dreams and insomnia) (5.4% of cases) and gastrointestinal (2.7%) (Table 3). Most common laboratory abnormalities leading to DOR discontinuation were elevated transaminases (1.0%) and elevated creatinine (0.8%). All adverse events were mild and no grade III/IV adverse events were recorded.

Figure 3. Kaplan-Meier estimates of protocol-defined virological failure (pVL ≥200 cop/mL), stratified by cases and controls



The table below the figure shows the number of individuals per study group remaining in the risk set, followed by the number of censored individuals and the Kaplan-Meier estimate of the percentage of individuals with protocol-defined virological failure.

Figure 4. Kaplan-Meier estimates of week 104 regimen modification, stratified by cases and controls



The table below the figure shows the number of individuals per study group remaining in the risk set, followed by the number of censored individuals and the Kaplan-Meier estimate of the percentage of individuals with regimen modification in parentheses.

Table 3. Adverse events of the 73 participants discontinuing DOR due to toxicity at week 104

	n = 108
Neuropsychiatric	Total = 32 (29.6)
- insomnia	9 (8.3)
- abnormal dreams / nightmares	10 (9.3)
- mood changes	3 (2.8)
- depression	2 (1.9)
- dizziness	2 (1.9)
- paresthesia	2 (1.9)
- other neuropsychiatric	4 (3.7)
Gastrointestinal	Total = 16 (14.8)
- nausea	7 (6.5)
- flatulence	1 (0.9)
- vomiting	1 (0.9)
- loss of appetite	2 (1.9)
- other gastrointestinal	5 (4.6)
Systemic	Total = 14 (13.0)
- fatigue	5 (4.6)
- malaise	1 (0.9)
- fever	1 (0.9)
- weight gain	2 (1.9)
- change in taste	1 (0.9)
- anemia	2 (1.9)
- other systemic	2 (1.9)
Dermatological	Total = 11 (10.2)
- itching	3 (2.8)
- rash	2 (1.9)
- dry skin	1 (0.9)
- alopecia	4 (3.7)
- other dermatological	1 (0.9)
Hepatic	Total = 9 (8.3)
- elevated transaminases	6 (5.6)
- toxic hepatitis	2 (1.9)
- other liver	1 (0.9)
Renal	Total = 5 (4.6)
- elevated creatinine	5 (4.6)
Musculoskeletal	Total = 6 (5.6)
- myalgia	2 (1.9)
- osteoporosis	1 (0.9
- arthralgia	1 (0.9)
- other musculoskeletal	2 (1.9)
Headache	Total = 7 (6.5)
- headache	7 (6.5)
Ear, nose and throat	0
Cardiovascular	0
Other	Total = 8 (7.4)
- unspecified toxicity	7 (6.5)
- other sexual	1 (0.9)

All categorical data are expressed as number (percentage).

DISCUSSION

In this nationwide real-world matched cohort study of 590 virologically well-suppressed adults with HIV-1 without prior virological failure, the effectiveness and tolerability of switching to DOR was non-inferior to continuing standard-of-care non-DOR-containing triple or dual ART at 104 weeks of follow-up. Virological failure was rare in both groups (1.7% vs. 2.2%) and all DOR cases who experienced virological failure re-suppressed to <200 cop/mL without modifying their regimen.

In ITT analyses, the DRIVE-SHIFT trial reported 83.7% efficacy after 120 weeks, compared to 77.1% in our real-world data.[9] The DRIVE-SHIFT trial also assessed virological efficacy using the observed failure approach, reporting efficacy of 94.6% at week 120, whereas our OT analysis showed that 97.8% of PWH who were still on DOR at week 104 had a pVL <200 cop/mL. Moreover, only 5 DRIVE-SHIFT participants (0.8%) had a pVL ≥200 cop/mL, which is substantially lower than the 1.7% we observed. In addition to the different virological cut-off (50 versus 200 cop/mL), we believe these differences are likely due to volunteer bias, better adherence support for trial participants and, in case of the ITT analyses, a higher likelihood that PWH in an observational cohort will change their ART compared to participants of a randomized clinical trial who are actively encouraged to continue their study medication until the end of the trial.

Another driver of the higher rate of protocol defined virological failure in our study may lie in the relatively large proportion of PWH with pre-ART $CD4^+$ counts <200 cells/mm^3. We observed a strong association between a pre-ART $CD4^+$ count <200 cells/mm^3 and protocol defined virological failure in participants on DOR, with those individuals having a 10 times higher odds of experiencing protocol defined virological failure than participants with ≥200 cells/mm^3. Our study population consisted of 33.1% PWH with a pre-ART $CD4^+$ count <200 cells/mm^3, versus 2.5% in the DRIVE-SHIFT trial. Additionally, as no such association was observed in the controls (of whom 27.3% had a pre-ART $CD4^+$ count <200 cells/mm^3), and all participants on DOR who experienced virological failure re-suppressed without modifying their regimen, it seems both the anchor drug type and temporary non-adherence could also play a role. INSTI-based regimens (accounting for 55% of the control group) have been shown to be relatively forgiving regarding missed doses[26], possibly more so than DOR. Moreover, lower nadir $CD4^+$ counts have been linked with larger viral reservoirs[27], which may predispose to more rapid or increased viremia in case of missed doses. Consequently, the observed correlation might result from a combination of a large viral reservoir (reflected by a nadir $CD4^+$ count <200 cells/mm^3), temporary non-adherence, and DOR being less forgiving than INSTIs regarding missed doses. Given the nature of our study, we were unable to measure treatment adherence or DOR plasma levels at time of virological failure. Interestingly, the same association was found in two clinical trials in treatment-naïve PWH

with rilpivirine that showed higher rates of virological failure in individuals with a pre-treatment CD4$^+$ count <200 cells/mm^3 [28], resulting in the recommendation to not prescribe rilpivirine in this sub-group.[10,11] This association has not been found in rilpivirine-switch studies nor in real-world cohorts. We believe this warrants further investigation, to uncover the underlying factors (including a potential pathophysiological mechanism) contributing to this association.

We performed a subgroup analysis to more directly compare DOR and INSTI-based regimens, as INSTIs are currently the primary guideline-recommended ART class and guidelines state that DOR and INSTIs have never been compared in previous research.[10,11] We found that DOR is non-inferior to INSTI-based regimens in PWH switching ART. Although these results are reassuring for PWH on DOR, this finding should be reconfirmed in randomized clinical trials.

A significant proportion of our participants discontinued DOR, with overall and adverse event-related discontinuation rates of 17.6% and 12.4% after 104 weeks, respectively. These rates are markedly higher than the 3.4% drug-related discontinuation rate after 144 weeks reported in the DRIVE-SHIFT trial.[9] Abnormal dreams and insomnia were the most frequently reported adverse events leading to discontinuation (1.7% and 1.5% of DOR cases). In line with the DRIVE-SHIFT trial, 1.0% of our participants on DOR discontinued because of (asymptomatic) elevated transaminases.[9]

The rate of DOR discontinuation varied over time. Kaplan-Meier estimates showed that a higher percentage of cases discontinued DOR shortly after switching, while similar rates between arms were observed after 104 weeks. The higher rate observed in the first months is not unexpected since controls consisted of participants already ≥12 months stable on their regimen. Additionally, cases may have already been more inclined to switch regimens due to adverse events, given the higher number of prior regimens prescribed in a similar time compared to controls. At 104 weeks of follow-up similar regimen modification rates were observed, confirming that, although DOR was less well tolerated in the real-world setting of our cohort study compared to the DRIVE-SHIFT trial, long-term tolerability was similar to that of other commonly used regimens.

We provide the most extensive and robust description of the effectiveness and tolerability of DOR to date. We used a comprehensive nationwide database, containing validated demographic, clinical, and laboratory data of PWH. We included a well-matched group of non-DOR users and had a long follow-up time. Given the large sample size in the ATHENA cohort, we were able to exact match participants on a large number of confounders, including calendar time, so that any calendar effects (e.g., the COVID-19 pandemic) were comparable between groups.

Our study has several limitations. First, only treatment-limiting adverse events, and not adverse events reported by participants that did not lead to regimen modification, were recorded and available for analysis, precluding an in-depth assessment of DOR's tolerability. Second, since matching can only be performed on known confounders, there may still be between-group differences due to unmeasured confounding. Third, matching occurred on ART class and not on anchor drugs, which may have limited comparability due to potential intra-class differences in effectiveness and adverse events. However, we expect the impact of this potential bias to be small because participants were matched on duration of prior ART use, had already been on ART for many years, and prior treatment failure was an exclusion criterion. Fourth, PWH from sub-Saharan origin and women were not well-represented in our data. Lastly, there is likely some confounding by indication in this observational cohort. This is illustrated by the difference in the number of prior ART regimens, suggesting that cases are more prone to switch – possibly due to adverse events experienced while on earlier regimens. In this sense, the comparable regimen modification rate after 104 weeks is reassuring.

Although our study has shed light on DOR's effectiveness and tolerability when used in a real-world setting, knowledge gaps remain. It remains unclear how DOR compares with INSTI-based regimens in treatment-naïve individuals. We excluded PWH with prior virologic failure, leaving DOR's effectiveness and tolerability in this group unclear.

In conclusion, this large nationwide matched cohort study showed that switching to DOR-based ART in well-suppressed PWH without prior virological failure was non-inferior after two years regarding effectiveness and tolerability compared with matched PWH continuing non-DOR-containing regimens in a real-world setting. Further research is needed on the observation of a possible higher virologic failure risk in those with low pre-ART $CD4^+$ counts on DOR.

Statements

Author contributions

PGAO, FWNMW, BvW, and MvdV designed the study. FWNMW statistically analyzed the data. PGAO drafted the manuscript in close collaboration with FWNMW, BvW, and MvdV. All authors contributed to the interpretation of the results, critically reviewed and approved the final manuscript. All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of interests

FWNMW has received consultancy fees from ViiV Healthcare. BvW has received a research grant and speaker fees from Gilead Health Sciences as well as speaker and advisory board fees from ViiV Healthcare: all fees were paid to his institution. MvdV has received grants and consultancy fees from ViiV Healthcare, Gilead Health Sciences, and MSD: all paid to his institution**.** For the remaining authors none were declared.

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Appendix table 1. Characteristics of adult ATHENA participants on ART in 2019, 2020, 2021, 2022

	2019 (n=21,642)	2020 (n=21,901)	2021 (n=22,073)	2022 (n=22,269)
Age, years	50.4 (40.7 – 58.3)	51.1 (41.3 – 59.0)	51.8 (41.8 – 59.8)	52.3 (42.1 – 60.4)
Male sex (at birth)	17,699 (81.8)	17,919 (81.8)	18,055 (81.8)	18,158 (81.5)
Region of origin				
- Netherlands	12,485 (57.7)	12,563 (57.4)	12,571 (57.0)	12,490 (56.1)
- Western	1416 (6.5)	1417 (6.5)	1407 (6.4)	1375 (6.2)
- Sub-Saharan Africa	2601 (12.0)	2628 (12.0)	2626 (11.9)	2635 (11.8)
- Caribbean / Latin America	2769 (12.8)	2805 (12.8)	2856 (12.9)	2871 (12.9)
- South Asia	807 (3.7)	822 (3.8)	832 (3.8)	849 (3.8)
- Other	1564 (7.2)	1666 (7.6)	1781 (8.1)	2049 (9.2)
HIV acquisition category				
- MSM	13,704 (63.3)	13,882 (63.4)	13,966 (63.3)	14,005 (62.9)
- Heterosexual	6168 (28.5)	6228 (28.4)	6258 (28.4)	6319 (28.4)
- IDU	312 (1.4)	294 (1.3)	288 (1.3)	309 (1.4)
- Blood contact	272 (1.3)	276 (1.3)	283 (1.3)	279 (1.3)
- Pediatric	236 (1.1)	252 (1.2)	273 (1.2)	288 (1.3)
- Unknown	950 (4.4)	969 (4.4)	1005 (4.6)	1069 (4.8)
Time since HIV diagnosis, years	11.8 (6.5 – 17.9)	12.5 (7.2 – 18.6)	13.3 (7.8 – 19.5)	13.8 (8.2 – 20.1)
Time since ART initiation, years	9.5 (5.3 – 15.5)	10.3 (6.0 – 16.3)	11.0 (6.6 – 17.0)	11.6 (7.2 – 17.6)
Pre-ART CD4$^+$ count (cells/mm^3)	242.0 (115.0 – 380.0)	250.0 (120.0 – 385.0)	250.0 (120.0 – 390.0)	250.0 (120.0 – 400.0)
CD4$^+$ count (cells/mm^3)	681.0 (507.0 – 896.0)	700.0 (510.0 – 910.0)	700.0 (512.0 – 910.0)	710.0 (520.0 – 927.0)
pVL (cop/mL)				
- ≤50	20,129 (94.3)	20,642 (95.3)	20,870 (95.6)	20,950 (95.3)
- 51-199	574 (2.7)	499 (2.3)	492 (2.3)	548 (2.5)
- 200 – 999	232 (1.1)	212 (1.0)	175 (0.8)	162 (0.7)
- ≥1000	408 (1.9)	315 (1.5)	292 (1.3)	331 (1.5)
- Not recorded	299	233	244	278
Prior HBV infection	1119 (5.2)	1125 (5.1)	1130 (5.1)	1147 (5.2)
Prior HCV infection	2148 (9.9)	2151 (9.8)	2156 (9.8)	2191 (9.8)
Prior AIDS diagnosis	4839 (22.4)	4880 (22.3)	4898 (22.2)	4871 (21.9)

Appendix table 1. Continued.

	2019 (n=21,642)	2020 (n=21,901)	2021 (n=22,073)	2022 (n=22,269)
Smoking status				
- Never	8414 (38.9)	8547 (39.0)	8610 (39.0)	8733 (39.2)
- Past	5108 (23.6)	5401 (24.7)	5619 (25.5)	5723 (25.7)
- Current	6041 (27.9)	5688 (26.0)	5379 (24.4)	5117 (23.0)
- Missing	2079 (9.6)	2265 (10.3)	2465 (11.2)	2696 (12.1)
ART class				
- DOR-based triple therapy	178 (0.8)	934 (4.3)	1479 (6.7)	1692 (7.6)
- Other NNRTI-based triple therapy	6011 (27.8)	5357 (24.5)	4845 (21.9)	4406 (19.8)
- INSTI-based triple therapy	10,528 (48.6)	10,169 (46.4)	9867 (44.7)	9737 (43.7)
- PI-based triple therapy	2469 (11.4)	2276 (10.4)	2081 (9.4)	1945 (8.7)
- Other regimen	1704 (7.9)	2480 (11.3)	3145 (14.2)	3888 (17.5)
- No ART (interrupted)	752 (3.5)	685 (3.1)	656 (3.0)	601 (2.7)
Use of DOR in ART regimen	184 (0.9)	961 (4.4)	1533 (6.9)	1759 (7.9)

All categorical data are expressed as number (percentage of total population) and all continuous data are expressed as median (interquartile range).
Abbreviations: AIDS, acquired immunodeficiency syndrome; ART, antiretroviral therapy; cop, copies; DOR, doravirine; HBV, hepatitis B virus; HCV, hepatitis C virus; IDU, intravenous drug use; INSTI, integrase strand transfer inhibitor, MSM, men who have sex with men; no., number; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor, pVL, plasma viral load; PWH, people with HIV.

Appendix Table 2. Outcomes of the sensitivity analyses in the ITT and OT population consisting of cases previously on and controls currently on INSTI-based regimens after 104 weeks

	Cases (n= 326)	Controls (n= 652)
ITT population: week 104 outcome		
- Failure: insufficient or lost to follow-up, pVL<200 at last contact	10 (3.1)	30 (4.6)
- Failure: switched ART regimen, all pVL<200 before switch	57 (17.5)	121 (18.6)
- Failure: pVL>=200	4 (1.2)	15 (2.3)
- Success: all pVL<200	255 (78.2)	486 (74.5)
ITT population: outcome (dichotomized)		
- Treatment failure	71 (21.8)	166 (25.5)
- Treatment success	255 (78.2)	486 (74.5)
OT population: week 104 outcome		
- Censored: insufficient or LTFU, pVL<200 until last contact	10 (3.1)	30 (4.6)
- Censored: switched ART regimen, all pVL<200 before switch	57 (17.5)	121 (18.6)
- Failure: pVL>=200	4 (1.2)	15 (2.3)
- Success: all pVL<200	255 (78.2)	486 (74.5)
OT population: outcome (dichotomized, excluding censored individuals)	4 (1.5)	15 (3.0)
- Treatment failure	255 (98.5)	486 (97.0)
- Treatment success	67	151
Reason for insufficient or lost to follow-up		
- Death	3 (30.0)	4 (13.3)
- LTFU	1 (10.0)	3 (10.0)
- Moved abroad	4 (40.0)	13 (43.4)
- Withdrew from ATHENA cohort	0 (0.0)	1 (3.3)
- No week 104 visit, but remaining in care	1 (10.0)	6 (20.0)
- No pVL measured at week 104 visit	1 (10.0)	3 (10.0)

All categorical data are expressed as number (percentage).
Abbreviations: cop, copies; ITT, intention to treat; LTFU, lost to follow-up; OT, on treatment; pVL, plasma viral load.

Appendix Table 3. Outcomes of the sensitivity analyses with protocol-defined virological failure as pVL ≥50 cop/mL in the ITT and OT population after 104 weeks

	Cases (n= 590)	Controls (n= 1180)
ITT population: week 104 outcome		
- Failure: insufficient or lost to follow-up, pVL<50 at last contact	21 (3.6)	55 (4.7)
- Failure: switched ART regimen, all pVL<50 before switch	100 (16.9)	203 (17.2)
- Failure: pVL ≥50	34 (5.8)	73 (6.2)
- Success: all pVL <50	435 (73.7)	849 (71.9)
ITT population: outcome (dichotomized)		
- Treatment failure	155 (26.3)	331 (28.1)
- Treatment success	435 (73.7)	849 (71.9)
OT population: week 104 outcome		
- Censored: insufficient or LTFU, pVL<50 until last contact	21 (3.6)	55 (4.7)
- Censored: switched ART regimen, all pVL<50 before switch	100 (16.9)	203 (17.2)
- Failure: pVL ≥50	34 (5.8)	73 (6.2)
- Success: all pVL <50	435 (73.7)	849 (71.9)
OT population: outcome (dichotomized, excluding censored individuals)	34 (7.2)	73 (7.9)
- Treatment failure	435 (92.8)	849 (92.1)
- Treatment success	121	258
Reason for insufficient or lost to follow-up		
- Death	8 (38.1)	16 (27.1)
- LTFU	2 (9.5)	5 (8.5)
- Moved abroad	7 (33.3)	19 (32.2)
- Withdrew from the ATHENA cohort	0 (0.0)	2 (3.4)
- No week 104 visit, but remaining in care	2 (9.5)	11 (18.6)
- No pVL measured at week 104 visit	2 (9.5)	6 (10.2)

All categorical data are expressed as number (percentage).
Abbreviations: cop, copies; ITT, intention to treat; LTFU, lost to follow-up; OT, on treatment; pVL, plasma viral load.

3

DUAL ANTIRETROVIRAL THERAPY— ALL QUIET BENEATH THE SURFACE?

Berend J. van Welzen
Patrick G.A. Oomen
Andy I.M. Hoepelman

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ABSTRACT

Infection with the human immunodeficiency virus (HIV) is characterized by progressive depletion of CD4+ lymphocytes cells as a result of chronic immune activation. Next to the decreases in the number of CD4+ cells which leads to opportunistic infections, HIV-related immune activation is associated with several prevalent comorbidities in the HIV-positive population such as cardiovascular and bone disease. Traditionally, combination antiretroviral therapy (cART) consists of three drugs with activity against HIV and is highly effective in diminishing the degree of immune activation. Over the years, questions were raised whether virological suppression could also be achieved with fewer antiretroviral drugs, i.e., dual- or even monotherapy. This is an intriguing question considering the fact that antiretroviral drugs should be used lifelong, and their use could also induce cardiovascular and bone disease. Therefore, the equilibrium between drug-induced toxicity and immune activation related comorbidity is delicate. Recently, two large clinical trials evaluating two-drug cART showed non-inferiority with respect to virological outcomes when compared to triple-drug regimens. This led to adoption of dual antiretroviral therapy in current HIV treatment guidelines. However, it is largely unknown whether dual therapy is also able to suppress immune activation to the same degree as triple therapy. This poses a risk for an imbalance in the delicate equilibrium. This mini review gives an overview of the current available evidence concerning immune activation in the setting of cART with less than three antiretroviral drugs.

INTRODUCTION

In 1983, a group of French virologists identified a T-lymphotropic retrovirus - now called the human immunodeficiency virus (HIV) - as causative agent of the acquired immunodeficiency syndrome (AIDS) [1]. The clinical picture of AIDS is characterized by opportunistic infections such as pneumocystis jirovecii pneumonia and candida esophagitis [2]. These opportunistic infections are the result of a severe depletion of CD4+ lymphocytes, which are central mediators of immune response, coordinating both cellular and humoral responses against infections [3].

Although HIV uses the CD4 receptor to gain access to target cells, the depletion of CD4+ lymphocytes is only partly due to a direct cytolytic effect of HIV [4]. The current leading hypothesis states that chronic HIV infection is accompanied by a hyperactive inflammatory state in which there is an increased turnover of activated naïve T-cells, eventually leading to T-cell depletion by means of apoptosis [5,6]. Immune activation is driven by both the HIV viremia and bacterial translocation from the gut [7,8] and is associated with numerous comorbidities in HIV-positive patients [9–11]. Therefore, immune activation is not only considered to be a predictor for the risk for progression to AIDS but also an important cause of HIV-related comorbidity [12,13].

Till the end of 1995, the nucleoside reverse transcriptase inhibitors (NRTIs) were the only available antiretroviral agents – targeting reverse transcriptase, an enzyme essential for HIV replication [14]. Unfortunately, NRTI mono- or dual therapy had only temporary effects due to rapid resistance development and virological failure [15]. However, the perspective for people living with HIV changed dramatically as result of the introduction of a new class of drugs: the protease inhibitors (PIs) combined with a pharmacological booster[16]. Combination antiretroviral therapy (cART) – drug regimens consisting of multiple antiretroviral classes – diminished the risk of resistance development and led to a spectacular increase in life expectancy [17]. Over the years, the development of antiretroviral drugs took off and several other third drug ('anchors') classes - such as the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and integrase strand transfer inhibitors (INSTIs) – were introduced [18,19]. Nowadays, triple antiretroviral therapy is highly successful with most patients reaching the main treatment goal of an 'undetectable' viral load – defined as <50 copies/ml of HIV RNA when measured by polymerase chain reaction – and with the mortality risk declining [20,21]. Current immunoassays however, due to improvement of sensitivity, are able to detect viral loads that are below 50 copies/ml but can still be quantified: so-called 'residual viremia'. A small group of patients - 'elite controllers' - are able to maintain an undetectable viral load in absence of antiretroviral drugs [22]. However, these patients display significant immune activation when compared to HIV-negative controls [23,24] and this is linked to an increased risk for cardiovascular disease in these patients [25]. These findings emphasize

the importance of immune activation in the pathophysiology of HIV-related comorbidity. In the modern antiretroviral era, there is no role for in depth monitoring of immune activation as these markers are generally considered to reduce simultaneously with the viral load, albeit they do not show complete normalization [26].

In the recent years, questions were raised whether there is a need to hold on to the mantra that cART should always consist of three antiretroviral drugs [27]. Indeed, the current available agents have high genetic barriers for resistance and the life-long use of multiple drugs could lead to long-term toxicity. Numerous studies evaluated the efficacy of mono- or dual antiretroviral therapy [28–36] and some of these two-drug regimens gained ground in the current treatment guidelines [37,38]. However, there are concerns as to whether the two-drug regimens suppress the degree of HIV-related immune activation enough [39]. A rebound in immune activation which occurs beneath the surface despite virological suppression could be harmful. In the end, the development of comorbidity in HIV is the net result of potential harmful effects of antiretroviral drugs versus the degree in which these drugs suppress the virus and the related immune activation [Figure 1]. Therefore, any change in the current standard of care might lead to disruption of this equilibrium. In this mini review, we will discuss the best current available data on immune activation in non-traditional cART regimens.

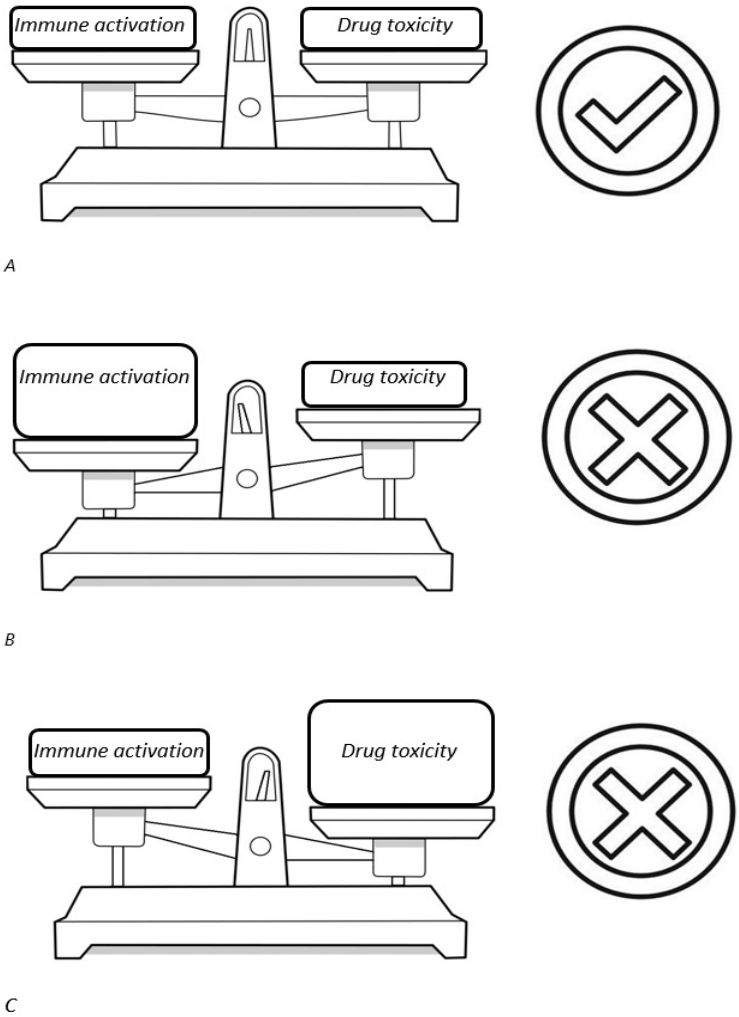
IMMUNOLOGICAL MARKERS IN HIV-INFECTION

The test battery for HIV-related immune activation is extending ever since the recognition of the hyperactive inflammatory status. The available markers can be divided into soluble and cellular markers for inflammation and immune activation, with some being more readily available than others [40] (Table 1).

The soluble markers are easy to measure in a large number of test facilities and can subdivided into markers of inflammation, coagulation and microbial translocation. The most commonly used inflammation markers include high-sensitivity c-reactive protein (hs-CRP) and plasma interleukin-6 (IL-6), both considered to be extremely sensitive for systemic inflammation [41,42] and associated with HIV-related mortality [43–46]. Other soluble markers include tumor necrosis factor alpha (TNF-α), interferon-γ, neopterin, mitochondrial DNA (mtDNA), β2-microglobulin, soluble CD27 and soluble CD40 ligand [47–54]. The latter two are markers of T-cell activation. The main example for coagulation markers is D-dimer, which levels increase in several pro-inflammatory states and high levels being associated with cardiovascular disease [55,56]. The last group of soluble markers are surrogates of microbial translocation. These include bacterial lipopolysaccharide (LPS) – present in gram-negative bacteria - and bacterial DNA (16s ribosomal RNA subunit) [57]. In addition, plasma

soluble CD14 (sCD14) and soluble CD163 (sCD163) – products of monocyte activation - are also considered to be markers for impaired mucosal integrity [58]. None of these markers are exclusively found in the setting of HIV-infection [59].

Figure 1. Three possible scenarios in the equilibrium between drug toxicity and damage from HIV-related immune activation.



Three scenario's: (A) A perfect balance between these factors with the smallest possible risk for comorbidity. (B) The reduction in the number of antiretroviral drugs diminishes the risk for drug toxicity but a flare in immune activation could lead to HIV-associated comorbidity. (C) Multiple antiretroviral drugs are able to fully suppress the virus but this poses a significant risk for cART-associated toxicity.

Table 1. An overview of the most important soluble and cellular markers for HIV-associated immune activation that are reported in current literature

Markers	Biological and clinical characteristics	Ref.
Soluble markers		
Tumor necrosis factor α	- Produced by macrophages and T-cells - Used for cell signaling and cytokine stimulation - Associated with disease progression	47
Interferon-γ (IFN-γ)	- Produced by T-helper cells, CD8+ lymphocytes and NK cells - Induction of several pro-inflammatory cytokines and anti-viral characteristics - Especially active during acute HIV infection	52
Interleukin-6	- Released by monocytes and macrophages - Elevated during chronic stage of infection - Associated with disease progression, especially CVD	46
D-dimer	- Fibrin degradation product -Associated with disease progression, especially CVD	56
Soluble CD14	- Marker of monocyte activation and indirect marker of microbial translocation - Associated with disease progression	58
LPS	- Endotoxin, a marker for microbial translocation - Associated with disease progression	50
Bacterial 16s DNA	- Marker for microbial translocation - Prognostic value in HIV is unknown	50
Soluble CD27	- Marker of T-cell activation - Rapid increase in case of viral rebound	53
Soluble CD40 ligand	- Marker for platelet activation - Implicated to contribute to innate and adaptive immune dysfunction - Prognostic value in HIV is unknown	54
Cellular markers		
HLA-DR+	- MHC class II receptor on CD4+ and CD8+ lymphocytes - Upregulated in response to signaling and being a marker for T-cell activation	60
CD38+	- Glycoprotein expressed on lymphocytes and macrophages - Upregulation mediated by IFN- γ and LPS - Considered as a T-cell activation marker	61
Ki67+	- Nuclear antigen being a marker for cell proliferation. Present in all cells during mitosis, including T lymphocytes	7
PD-1 co-stimulatory receptor	- Regulating T-cell response - High levels are considered to be result of T-cell exhaustion	63
Annexin-V+	- Marker for apoptosis	62

For parameters predictive of disease progression, it is not further specified whether this includes declining CD4+ cell counts or clinical AIDS-defining events. CVD, cardiovascular disease; LPS, lipopolysaccharide; Ref, reference; PD-1, programmed death-1.

Although the soluble markers can be assessed relatively easy, their reflection of inflammation and immune activation is considered to be less specific than the cellular activation markers in the setting of HIV [40]. Assessing cellular markers is more labor-intensive, requiring the isolation of peripheral blood mononuclear cells and performing flow cytometry. For the cellular activity, some well-defined markers are available: CD38+/HLA-DR+ expression on lymphocytes for T-cell activation [60,61], Ki-67 positivity for proliferation [7], annexin-V for apoptosis [62] and programmed-death-1 co-stimulatory receptor for T-cell exhaustion [63]. The CD4+ lymphocyte counts and CD4/CD8 ratio are more readily available, but these

changes occur more slowly and are therefore kept out of this review [64].

RESIDUAL IMMUNE ACTIVATION DURING TRIPLE-DRUG THERAPY

The initiation of cART results in fast virological suppression and significant reduction in immune activation in most patients, subsequently leading to CD4+ cell recovery [65]. However, antiretroviral therapy does not normalize the HIV-induced inflammatory response with some residual immune activation persisting [66]. Studies describing the effect of cART on the soluble markers report inconsistent outcomes [43,67,68], but especially the degree of T-cell activation rarely normalizes [69].

The clinical impact of this residual immune activation is largely unknown but, for example, the higher incidence of cardiovascular disease among HIV-positive individuals despite cART and the elite controllers implies clinical significance. The reason for residual immune activation in the setting of virological suppression has not been fully elucidated, but it is suggested that low-grade HIV replication in certain anatomical or cellular compartments is the main driver [70]. These 'sanctuary sites' are compartments, such as the central nervous system (CNS), gastrointestinal tract and lymph nodes, where cART reaches insufficient drug levels to completely suppress local viral replication and subsequent low-grade inflammation. The variable – and often suboptimal – drug penetration in lymph nodes [71], mucosal tissues [72] and the CNS [73] have been demonstrated in several papers. Besides these sites, persisting microbial translocation and the presence of viral coinfections are associated with persistent immune activation [74,75]. There is no consistent evidence that favors one anchor over another with respect to the degree of immune activation [76–78]. Studies evaluating whether therapy intensification with additional anchors results in further suppression of immune activation, are conflicting [79–81].

IMMUNE ACTIVATION AND VIROLOGICAL EFFICACY IN MONOTHERAPY

After the introduction of cART in the mid-nineties, monotherapy for HIV-infection was abandoned because of virological inferiority. However, the idea of antiretroviral monotherapy made a comeback after the introduction of agents with a high antiviral potency and a high genetic barrier for resistance. Such a mono-drug regimen would have significant advantages, including less side-effects and pill burden. The hypothesis that one powerful antiretroviral drug would be sufficient to maintain virological suppression, led to several trials comparing the virological efficacy of PI or INSTI monotherapy to traditional three-drug regimens

[28,30–33]. Unfortunately, monotherapy with these drugs seem to result in higher rates of virological rebound when compared to cART. Therefore, current guidelines recommend against monotherapy as maintenance therapy in treatment-experienced patients with an undetectable viral load [37,38]. However, from a pathophysiological viewpoint it is interesting to have a closer look at the impact of monotherapy on immune activation markers.

One study that provides an insight in the mechanisms of immune activation rebound was published by BenMarzoek-Hidalgo *et al.* [82]. In their paper, the authors describe the relationship between microbial translocation and viremia with immune activation in 71 patients receiving boosted darunavir monotherapy. In this cohort, only 26% of the patients maintained a viral load below 20 copies/ml, while 16 patients displayed virological failure (2 consecutive HIV-RNA levels exceeding 200 copies/mL). The remaining patients had (transitory) episodes of a detectable viral load during follow-up yet without meeting the criteria for virological failure. Although separate analysis per outcome group found that only patients with virological failure showed an increase in T-cell activation, it became clear that time with viral suppression was inversely correlated with T-cell activation (percentage HLA-DR+-CD38+ lymphocytes in both CD8+ and CD4+ lymphocyte subsets) at a follow-up of 24 months. In this study, there was a clear correlation between the viral load and the percentage of activated CD4+ and CD8+ lymphocytes. In addition, another study showed that intensification with INSTI (raltegravir) to PI monotherapy (either darunavir/ritonavir or lopinavir/ritonavir), resulted in a decline in the degree of residual viremia and a decrease in the percentage of activated CD8+ lymphocytes [83].

There are several studies that evaluated the non-specific soluble markers in highly selected populations [84–86], while other studies evaluated the cellular markers. The smallest study of Merlini *et al.* did not find a difference in T-cell activation between baseline and after 96 weeks for both patients receiving PI monotherapy with atazanavir (n=18) and those receiving atazanavir-based cART (n=22 [87]). However, patients on monotherapy were more likely to display increased T-cell apoptosis than patients receiving three drugs. Torres *et al.* evaluated the markers for monocyte activation in 40 patients receiving PI monotherapy (either lopinavir/ritonavir or darunavir/ritonavir) and 20 patients on PI-based cART for at least 48 weeks and an undetectable viral load [88]. This cross-sectional analysis showed that patients on monotherapy display higher levels of monocyte activation – CD14+CD16-CD163+ cells and sCD14 levels - when compared to those receiving standard therapy. The last, most well-designed, study of Petrara *et al.* described the dynamics of the HIV-1 viral reservoir and T- and B-cell activation markers at 48 and 96 weeks of therapy in patients switched to PI mono-therapy (n=32) and patients continuing PI-based triple therapy (n=32) [89]. It should be noted that ten percent of the patients in the monotherapy group experienced virological failure compared to zero patients receiving cART. Furthermore, the authors observed a significant increase of T- and B-cell activation in patients receiving one drug, while these markers remained low in patients on cART.

So the best available evidence suggests that a switch to monotherapy is associated with an increase of T-cell activation and apoptosis markers, while soluble markers data are more inconsistent. These observations seem to be the result of (low-grade) viral rebound. The increased risk for virological failure and the suggestion of a rebound in immune activation, disqualify monotherapy as maintenance therapy.

IMMUNE ACTIVATION IN DUAL THERAPY

Antiretroviral monotherapy is not likely to play a role in the near future, so the current focus is on the effectiveness of dual therapy. In fact, two-drug regimens have already gained a position in current HIV treatment guidelines; in 2018 a single-tablet regimen (STR) consisting of dolutegravir (INSTI) and rilpivirine (NNRTI) was introduced and in 2020 a STR with dolutegravir and lamivudine (NRTI) was registered as a first-line treatment option. Currently, there are several large trials that support the use of these two STRs in clinical practice: SWORD-1&2 [36], GEMINI-1&2 [35] and TANGO study [29].

The SWORD-1&2 studies evaluated the efficacy, safety and tolerability of dolutegravir/rilpivirine as maintenance therapy in patients with an undetectable viral load. Patients were randomized to either dual therapy (n=512) versus continuing triple-drug therapy (n=516). After 148 weeks, the data showed that dolutegravir/rilpivirine was non-inferior with respect to virological outcomes to triple therapy [90]. In the first paper evaluating this regimen, there was a brief mention on the dynamics of the inflammatory and cardiovascular markers in both groups. The authors state there was no consistent pattern of change from baseline to week 48 or differentiation between both groups in the following markers: IL-6, CRP, sCD14, sCD163 and D-dimer. Exact data were not shown and specific T-cell markers were not evaluated. The use of STR dolutegravir/lamivudine for treatment-experienced patients is supported by the TANGO study [29]. In this study, 743 patients with an undetectable viral load were enrolled and were randomized to either dolutegravir/lamivudine or a triple drug regimen (two NRTIs as backbone and an anchor from one of major groups). In this study, dual therapy was also found to be non-inferior in maintaining virological suppression compared to triple therapy. In the study cohort, the authors describe a significantly smaller decrease in serum IL-6 levels in patients on dual therapy, but for sCD14 there was an exact opposite trend. The dynamics of D-dimer, hs-CRP and sCD163 were comparable for both groups. In the GEMINI-1&2 studies, it was shown that dolutegravir/lamivudine was virologically non-inferior to INSTI-based cART in treatment-naïve patients, but there were no data on immune activation [35].

As mentioned above, the registration trials briefly addressed the concerns regarding HIV-related immune activation in dual therapy. In general, the results were inconsistent and focused on soluble markers. Fortunately, a few other studies described this issue more extensively although

not for the registered treatment regimens. In the study of Concepción Romero-Sánchez *et al.* 58 patients, having an undetectable viral load for at least six months, were switched to a two-drug regimen consisting of a boosted PI and Maraviroc, a HIV entry inhibitor; there was no control group in this study [91]. The authors observed no change in β2-microglobuline, sCD40L, sCD14, hs-CRP, D-dimer and mtDNA at 24 (±12) weeks of follow-up when compared to baseline. However, for patients with high baseline levels of β2-microglobuline, sCD40L and hs-CRP there was marked decrease at final follow-up. Two other papers evaluated the differences between patients on dual antiretroviral therapy versus those on triple therapy. Belmonti *et al.* describe the dynamics of IL-6, CRP, sCD14 and D-dimer from baseline to 48 weeks [92]. A switch to dual therapy (n=70 boosted atazanavir plus lamivudine) did not result in a significant changes in the markers mentioned above and did not differ from the markers in patients continuing triple therapy (n=69). In addition, Vallejo *et al.* published a cross-sectional pilot study evaluating a broad spectrum of inflammation and immune activation biomarkers (interferon-gamma-induced protein 10, hs-CRP, sCD14, D-dimer, interferon-γ, TNF-α and IL-4) in patients on dual therapy versus those continuing triple therapy[93]. The dual therapy group consisted of 13 patients that were evaluated at 24 weeks after switch and 36 patients at 48 weeks, the control group included 26 patients. The authors found the lowest IL-6 and sCD14 levels in the patients on dual therapy for 48 weeks; the other markers were not different from the triple-therapy groups. Other studies worth to mention were performed by Quiros-Roldan *et al.* and Mussini *et al.* but these papers reported less commonly used parameters such as CD4/CD8 ratio, platelet-to-lymphocyte and neutrophil-to-lymphocyte ratio [94,95].

In the studies presented above, the switch from triple to dual therapy is not accompanied with a consistent increase in the soluble inflammatory markers. However, in contrast to the monotherapy studies none of the papers assessed T-cell activation, proliferation or apoptosis markers. At this moment, there is sufficient evidence to support certain two drug regimens as treatment options for HIV in terms of virological efficacy but robust data on effects on immune activation are lacking.

CONCLUSIONS

In this review we presented the current best available evidence on the dynamics in immune activation in non-traditional antiretroviral therapy. We found that the most well-designed studies show that monotherapy is associated with insufficient suppression of T-cell activation when compared to traditional triple therapy; there might be an association with a detectable viral load. Furthermore, we observed that the dynamics of T-cell activation, proliferation and apoptosis do not necessarily follow the trends observed in the soluble markers, confirming earlier observations.

Especially the last finding is of great importance when we have a look at the data presented for the two-drug regimens, which now have become a reasonable option in modern antiretroviral therapy. The fact that the large registration trials for treatment-experienced patients included inflammatory makers as secondary outcomes is laudable; it emphasizes the recognition of the importance of this outcome. In contrast, the founders of these studies missed an excellent opportunity for a thorough assessment of the immune activation markers in dual therapy. In SWORD-1&2 and TANGO, the soluble markers are only briefly mentioned, or the authors stay away from firm statements. Furthermore, the studies only included soluble markers but there are no data on T-cell activation. As we learned from the monotherapy data, especially those markers might display abnormalities. The fact that T-cell activation is correlated with a detectable viremia and that the two-drug regimens show virological non-inferiority with the 50 copies/ml threshold, is reassuring. However, as we are not aware of the degree of residual viremia in the two-drug regimens, a negative impact of dual drug therapy cannot be excluded at this moment.

Based on the presented studies, we believe there is insufficient evidence that mono- and dual therapy are non-inferior to triple therapy when it comes to the suppression of HIV-related immune activation. Although dual therapy is an attractive option as it diminishes the life-time exposure to antiretroviral drugs with potential toxicity, the impact of a rebound in immune activation are currently unknown. We need to keep the potential negative impact of cART in an equilibrium with the degree of immune activation, as a misbalance could lead to HIV or cART-related comorbidity. There is a need for well-designed, longitudinal studies with a proper, unbiased patients selection evaluating both the soluble and the cellular immune activation markers. Only such studies can tell us whether everything is quiet beneath the surface in dual therapy.

Statements

Author contributions

BW and PO: draft of the manuscript and editing. AH: critical review and editing. All authors contributed to the article and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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4

INTEGRATED ANALYSIS OF VIRAL BLIPS, RESIDUAL VIREMIA, AND ASSOCIATED FACTORS IN PEOPLE WITH HIV: RESULTS FROM A RETROSPECTIVE COHORT STUDY

Patrick G.A. Oomen*
Suzan Dijkstra*
L. Marije Hofstra
Monique M. Nijhuis
Annelies Verbon
Tania Mudrikova
Annemarie M.J. Wensing
Andy I.M. Hoepelman
Berend J. van Welzen

*Contributed equally

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ABSTRACT

Background

The etiology of viral blips is not yet fully elucidated. One of the hypotheses is that blips reflect variations in residual viremia (RV) near the detectability threshold. In this study, we evaluated whether RV is associated with viral blips and which factors are associated with RV.

Methods

All treatment regimens in 2010-2020 consisting of 2 nucleos(-t)ide reverse transcriptase inhibitors and 1 anchor (integrase strand transfer inhibitor (INSTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or protease inhibitor (PI)) in people with HIV (PWH) were evaluated for RV [detectable viremia <50 cp/mL] and blips [isolated viral loads (VLs) 50–499 cp/mL between measurements <50 cp/mL]. All medical records were reviewed and regimens in which a VL ≥50 cp/mL was deemed to result from non-adherence (based on the documented conclusion by the treating physician) were excluded. Factors associated with blips and RV were identified using generalized linear mixed models.

Results

In total, 24,518 VLs from 1658 PWH were analyzed. VLs were measured during INSTI- (n=5119; 20.9%), PI- (n=8935; 36.4%), and NNRTI-use (n=10,464; 42.7%). VLs were categorized as blips in 1.4% (n=332). The 24,186 non-blip VLs were RNA^{neg} (no RV) (n=15,326; 63.4%), 1-19 cp/mL (n=6318; 26.1%), 20-49 cp/mL (n=1620; 6.7%), or <50 cp/mL with an unknown RV level (n=922; 3.8%). In 193/1658 PWH (11.6%), the RV level was RNA^{neg} in all VLs assessed. RV 1-19 cp/mL and 20-49 cp/mL (*vs.* RNA^{neg}) were significantly associated with subsequent viral blips (respective odds ratio 2.66 and 4.90 (95% confidence intervals 1.98-3.58 and 3.41-7.04)). Zenith VL and use of PIs (*vs.* INSTIs/NNRTIs) were associated with higher RV and blip odds.

Conclusions

This large cohort study showed that blips were associated with higher preceding RV. Both the anchor type and factors previously linked to the latent viral reservoir were associated with RV, suggesting blips having a multifactorial origin.

INTRODUCTION

Most people with HIV (PWH) on combination antiretroviral therapy (cART) receive a treatment regimen consisting of two nucleos(-t)ide reverse transcriptase inhibitors (NRTIs) and a third (anchor) drug: either a non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase strand transfer inhibitor (INSTI). The main goals of antiretroviral treatment are to achieve and maintain suppression of viral replication with resulting immunological recovery, as well as prevention of drug-resistant variant selection and HIV transmission.[1] Nevertheless, even in PWH with optimal treatment adherence and well-suppressed viral replication, temporary elevations of plasma HIV viral load (VL) above the detection limit, referred to as viral blips, are frequently observed.[2] To explain the etiology of this phenomenon, multiple hypotheses have been proposed, mostly related to intermittent virion release from the latent reservoir, differences in assay accuracy, or ongoing viral replication.[3–11]

The occurrence of blips generates uncertainty for both PWH and healthcare providers and has been linked to adverse clinical outcomes, including virologic failure.[12,13] Blip occurrences are associated with non-modifiable factors linked to the viral latent reservoir (*e.g.,* time since ART initiation, zenith (highest VL before cART start) VL and nadir $CD4^+$ count). Previously, we demonstrated that blips resulted in increased clinical burden, with fewer blips observed during INSTI-based regimens (*vs.* PIs and NNRTIs).[2] However, the question remains by what mechanism cART anchors influence the occurrence of blips. An intuitive explanation may be found in residual viremia (RV): detectable viremia below the commonly used threshold of 50 cp/mL.[14,15] It is conceivable that the RV level may (partly) contribute to the occurrence of blips, since at low RV levels a relatively larger increase in viral replication is required to reach the 50-copy threshold, resulting in a blip. A follow-up question that arises in this context is which factors, both modifiable and non-modifiable, are associated with the magnitude of RV.

To date, few studies have been performed on the relationship between RV and blips, and only for PI- and NNRTI-based regimens, rendering their findings less relevant in the current INSTI era.[16–18] Studies aimed at identifying factors associated with RV, reported conflicting data regarding the effect of different cART anchors. Moreover, all these studies lacked information on treatment adherence[19–22], a factor that could have majorly impacted RV results. Given the clinical implications of blips, including increased healthcare burden and suggested link with virological failure, it is key to better understand the mechanism behind blips, which may partly lie in higher RV levels. Subsequently, it is important to comprehensively investigate factors influencing RV. Therefore, the aim of this cohort study was twofold: first, to assess whether higher RV levels, below the threshold of 50 cp/mL, were associated with the subsequent occurrence of viral blips. Second, to examine which factors (including cART anchor and factors potentially related to the latent reservoir such as the zenith VL) were associated with RV.

METHODS

Study population and follow-up

A retrospective assessment was conducted of VL measurements in adult PWH treated in the University Medical Center Utrecht, the Netherlands, between April first 2010 and 2020. PWH were eligible for the study when treated for HIV-1 with cART consisting of two NRTIs plus one anchor (NNRTI/ PI/ INSTI, booster allowed) and had achieved virologic suppression, defined as a plasma VL <50 cp/mL, on this regimen. cART composed of only these antiretrovirals was chosen to optimize comparability of the treatment regimens. PWH with a registered objection to data research were excluded. All VLs after the first suppressed VL were analyzed until the ending of the study period. We chose not to include the first suppressed VL after start of cART with the rationale that the presumed RV steady state during that treatment regimen may not have been reached yet. A graphical overview of the study is presented in Supplemental Figure S1. All VLs ≥50 cp/mL not meeting the blip definition (definition below) were excluded from analysis, as viral rebound was considered to be a different clinical phenomenon that was not part of the objectives of this study. When the last VL before ending of the study period was ≥50 cp/mL, subsequent VLs up to 2020-09-01 were reviewed to determine if a blip had occurred. When PWH had a VL ≥50 cp/mL and were deemed non-adherent to treatment by their treating physician, as based on the conclusion documented in the medical records, the VLs during that treatment regimen were excluded from analysis to reduce the influence of adherence variability on RV levels and blips**.** Physicians' documentation that was assessed included statements regarding number of missed doses, timing of doses, and adherence to food instructions. Ultimately, the documented conclusion regarding the cause of the VL ≥50 cp/mL was followed. When non-adherence was documented to have existed only in a specific and well-defined time period, only VLs during this specific time period were excluded. The institutional ethical review board judged that the study met the criteria for exemption from formal review.

Measurements and study outcomes

Demographic, laboratory, and adherence data were extracted from the medical records. VLs were assayed with the Roche COBAS® TaqMan® v2.0 during the entire study period. All VL results <50 cp/mL were reported to the treating clinician as "<50 cp/mL", without specifying the level of residual viremia.

The main study outcomes were 1) the odds of viral blip occurrences when comparing different RV levels and 2) the odds of higher RV levels when comparing different cART anchors and multiple virologic factors. VLs <50 cp/mL were categorized as "RNA^{neg}" (no HIV RNA detected), "RV 1-19 cp/mL", and "RV 20-49 cp/mL", consistent with the detection and quantification limits of the assay used (*i.e.,* able to detect but not quantify 1-19 cp/mL and able to quantify 20-49 cp/mL). A blip was defined as a VL of 50-499 cp/mL, preceded and

followed by a VL <50 cp/mL without an anchor change. Multiple, consecutive measurements 50-499 cp/mL within 30 days were considered a single blip if preceded and followed by VLs <50 cp/mL without an anchor change.[23] Single VLs 50-499 cp/mL immediately before loss to follow-up were censored, as it was uncertain what the subsequent VL would have been (and thus whether the VL 50-499 cp/mL would have met the blip definition). Similarly, single VLs 50-499 cp/mL immediately before anchor switch were censored, as it was uncertain whether the VL would have been a blip if the original cART had been continued.

Statistical analysis

Categorical data were compared using Fisher's exact test or χ^2; and continuous variables were compared using the independent samples *t*-test or Mann-Whitney U test. Correlates of viral blips and the RV level were examined using multivariable generalized linear mixed effect models (GLMMs) for repeated measures with a random intercept and slope per individual. A GLMM logistic regression was used to assess the binomial outcome blips and a backwards continuation ratio model was used for the ordinal outcome RV level (RNA^{neg}/1-19 / 20-49 cp/mL).[24] For each included VL, both outcomes were assessed, meaning that for the outcome RV level, we assessed the RV level of the *current* VL and for the outcome blip occurrence, we examined whether the individual's *next* VL was a blip. This approach ensured that the analysis of both outcomes used all information from the PWH present up to that point in time but not future information (which is the case when summarizing the RV level of a given cART regimen or time period). The following time-varying (*i.e.,* per VL) covariates, chosen based on existing literature, were included in the analysis for both study outcomes: age, time since study inclusion and ART initiation, and cART anchor. Additionally, for the outcome blip occurrence, the preceding RV level (*i.e.,* the RV level in the VL prior to the VL assessed for blip occurrences) was used as a time-varying determinant of interest. The time-independent covariates for both outcomes were sex assigned at birth, lowest recorded $CD4^+$ count, Zenith VL, and Fiebig stage at ART initiation. In case of convergence issues, continuous variables were centered to improve convergence. Model diagnostics were performed, including assessment for multicollinearity and the proportion odds assumption.

To minimize potential bias resulting from missing variables (including missing RV values), multi-level multiple imputation (MI) was conducted. Level-1 (*i.e.,* varying within one person) and level-2 (*i.e.,* constant within one person) predictors were imputed. Because of collinearity between time since study inclusion, ART initiation, and age, resulting in non-convergence, only time since study inclusion was included as a predictor for MI, as this covariate was deemed the most important to include as it describes the random slope for time for GLMM regressions. Five imputed datasets were constructed using ten iterations and trace line plots were used to assess MI convergence. Results from MI were pooled using Rubin's Rules.[25] Several sensitivity analyses were performed to assess the robustness of observed associations: analysis including the NRTI backbone as a covariate, complete case

analysis (*i.e.*, no multiple imputation, only participants without missing data), analysis within each anchor group including the specific anchor drug as a covariate, and analysis excluding PWH with specific periods of non-adherence completely. Results were expressed as odds ratios (ORs) and their 95% confidence interval (CI) with two-sided p-values <0.05 being considered statistically significant. All analyses were conducted using RStudio (v1.3.1093).

RESULTS

Study population and viral load measurements

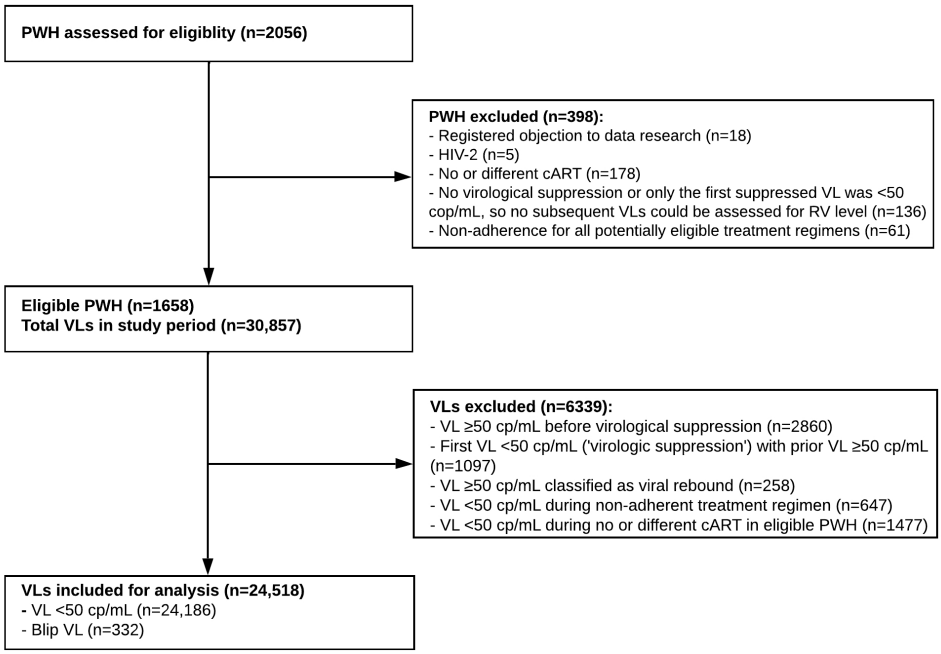
A total of 2056 PWH with VL measurements in the study period were assessed, of whom 1658 had VL data eligible for analysis (Fig. 1). At baseline, the mean age was 43.6 years and 317 (19.1%) were female (Table 1). A median of 14 VLs (range 1-35) per individual were included in the study. In total, 30,857 individual VLs were assessed for eligibility, of which 6339 were excluded for the reasons listed in Fig. 1. Adherence was documented at least once in all but four PWH with VLs stable <50 cp/mL (99.8%). A total of 74/1658 PWH (4.5%) had specific and well-defined periods of non-adherence, resulting in excluded VLs during that non-adherent period. After assessing the physicians' documented conclusion on treatment adherence, VLs <50 cp/mL during PI-based treatment regimens were more frequently excluded based on documented non-adherence than VLs during other regimens (5.4% *vs.* 1.1% excluded; p<0.001). No difference in documented non-adherence was found between NNRTIs and INSTIs (1.1% *vs.* 1.0% excluded; p=0.89). Ultimately, a total of 24,518 VLs were analyzed, of which 5119 (20.9%), 8935 (36.4%) and 10,464 (42.7%) were during INSTI-, PI-, and NNRTI-use, respectively (Supplemental Table S1 for the anchor, backbone and booster specification).

Viral blips

Of the 24,518 VLs included, 332 (1.4%) were categorized as blips, occurring in 254 PWH (15.3% of the study population). In 2/332 blips (0.6%), the NRTI backbone was changed after the blip. Of the 332 blips, 52 were found immediately after the first suppressed VL, rendering them ineligible for the analysis of the association with RV, as the RV level of the first suppressed VL was not included. This left 280 blips eligible for regression analysis. The 24,186 non-blip VLs were categorized as RNA^{neg} (n=15,326; 63.4%), RV 1-19 cp/mL (n=6318; 26.1%), and RV 20-49 cp/mL (n=1620; 6.7%). Additionally, 922 VLs (3.8%) were known to be <50 cp/mL, but no RV level was reported and thus considered missing. In 193/1658 PWH (11.6%), the RV level was RNA^{neg} in all VLs assessed. Multivariable GLMM logistic regression (including all 24,518 VLs after multiple imputation) showed that preceding RV 1-19 cp/mL and 20-49 cp/mL (*vs.* RNA^{neg}) were associated with a 2.66 (95% CI 1.98–3.58) and 4.90 (95% CI 3.41–7.04) higher odds of the next VL being a blip (Table 2). Regarding cART anchor, both PIs and NNRTIs were found to have higher odds of blips than INSTIs (OR 2.28; 95% CI 1.47–3.54 and OR 1.79; 95% CI 1.15–2.79, respectively). Additionally, a zenith VL ≥1,000,000

cp/mL (*vs.* <10,000) was associated with significantly higher odds of viral blips. Longer times since ART initiation and study inclusion were associated with lower odds of blips.

Figure 1. Study flow chart.



Viral rebound was defined as a VL ≥50 cp/mL after virological suppression not meeting the blip criteria.
Abbreviations: cART, combination antiretroviral therapy; cp, copies; n, number; PWH, people with HIV; VL, viral load

Table 1. Characteristics of PWH at baseline.

	1658 PWH
Demographics	
Age – years	43.6 (± 11.9)
Female sex	317 (19.1)
Region of origin	
- Europe / North America	1185 (71.5)
- Other	473 (28.5)
Clinical characteristics	
Time since HIV diagnosis – years[a]	4.5 (1.7 – 9.5)
Time since ART initiation – years[a]	2.3 (1.2 – 7.5)
Mode of transmission	
- MSM	937 (56.5)
- Heterosexual	337 (20.3)
- IVD	33 (2.0)
- Other / unknown	351 (21.2)
Co-infections	
- HBsAg^{pos} at any point during follow-up	60 (3.6)
- HCV RNA^{pos} at any point during follow-up	69 (4.2)

Table 1. Continued.

	1658 PWH
Fiebig stage at ART initiation[a]	
- Stage I-V	54 (3.3)
- Stage VI	1567 (94.5)
Biochemical characteristics	
Lowest recorded $CD4^+$ count - cells/mm^3	240.0 (113.0 – 360.0)
Zenith VL cp/mL[a]	
- <10,000	103 (6.2)
- 10,000-99,999	472 (28.5)
- 100,000-999,999	773 (46.6)
- ≥1,000,000	72 (4.3)
Study characteristics	
Follow-up duration per individual – years	5.5 (2.5 – 9.1)
cART regimens per individual during follow-up	2 (1 – 3)
VLs per individual included in study	14 (7 – 23)

All categorical data are expressed as number (percentage of total population) and all continuous data are expressed as median (interquartile range) or mean (standard deviation (±)).
[a] Missing data: Time since HIV diagnosis (n=15, 0.9%); Time since ART initiation (n=14, 0.8%); Fiebig Stage at ART initiation (n=44, 2.7%); Zenith VL (n=238, 14.4%).
Abbreviations: ART, antiretroviral therapy; cp, copies; IVD, intravenous drug use; MSM, men who have sex with men; no., number; PWH, people with HIV; VL, viral load.

Table 2. Generalized linear mixed model logistic regression results: associations with viral blip occurrences.

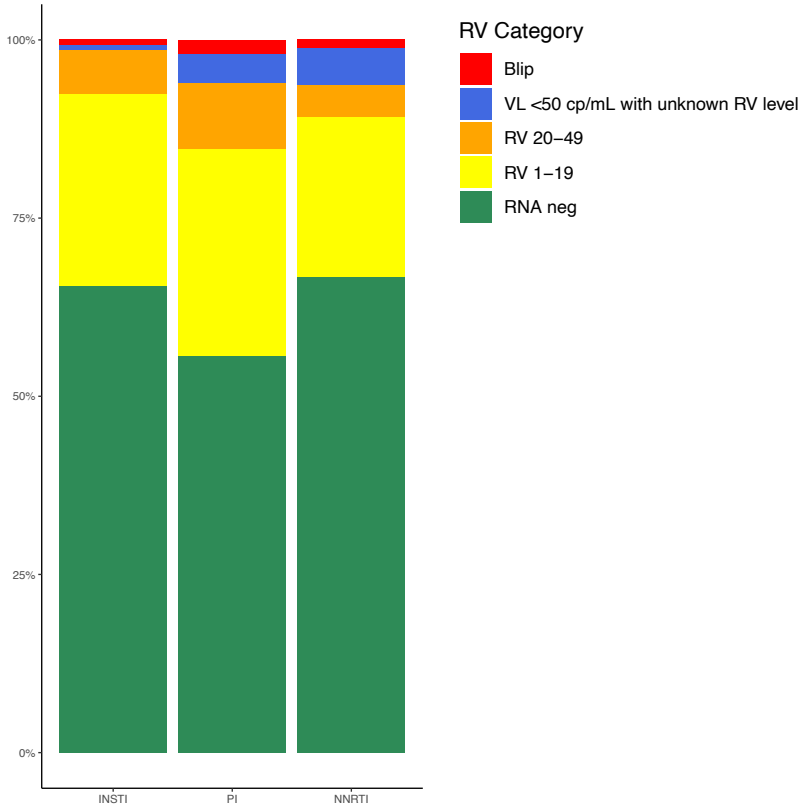
	Occurrence of blips			
	Univariable analysis		**Multivariable analysis**	
Variables	***OR (95% CI)***	***p-value***	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.79 (0.69 – 0.90)	**<0.001**	0.87 (0.78 – 0.98)	**0.03**
Female sex (*vs.* male sex)	0.76 (0.51 – 1.13)	0.17	0.94 (0.62 – 1.42)	0.77
Age (per year increase)	1.03 (0.88 – 1.20)	0.75	1.00 (0.86 – 1.16)	0.99
European / North American region of origin (*vs.* other)	0.66 (0.46 – 0.94)	**0.021**	1.33 (0.91 – 1.93)	0.14
cART anchor				
- INSTI	1	-	1	-
- PI	2.41 (1.55 – 3.78)	**<0.001**	2.28 (1.47 – 3.54)	**<0.001**
- NNRTI	1.58 (1.01 – 2.48)	**0.047**	1.79 (1.15 – 2.79)	**0.01**
Time since ART initiation (per year increase)	0.93 (0.90 – 0.97)	**<0.001**	0.97 (0.93 - 1.00)	**0.04**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.27 (0.39 – 4.18)	0.70	1.78 (0.59 – 5.35)	0.31
Lowest recorded $CD4^+$ count (per 10 cell/mm^3 increase)	1.00 (0.99 – 1.01)	0.44	1.00 (0.99 – 1.01)	0.47
Zenith VL cp/mL				
- <10,000	1	-	1	-
- 10,000-99,999	2.77 (0.90 – 8.58)	0.08	1.97 (0.64 – 6.00)	0.24
- 100,000-999,999	3.97 (1.34 – 11.94)	**0.01**	2.42 (0.80 – 7.30)	0.12
- ≥1,000,000	9.30 (2.83 – 30.57)	**<0.001**	4.64 (1.43 - 15.05)	**0.01**
Residual viremia level				
- RNA^{neg}	1	-	1	**-**
- RV 1-19 cp/mL	3.06 (2.27 – 4.10)	**<0.001**	2.66 (1.98 – 3.58)	**<0.001**
- RV 20-49 cp/mL	6.11 (4.26 –8.67)	**<0.001**	4.90 (3.41 – 7.04)	**<0.001**

Pooled associations were obtained from the five datasets after multiple imputation of all 24,518 VLs.
Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; RV, residual viremia; VL, viral load; *vs.*, versus.

Residual viremia

For the 24,186 non-blip VLs, RV levels were evaluated by anchor type and virologic factors. RNA^{neg} was observed in 66.6% of VLs during INSTI-use, 59.5% during PI-use and 70.9% during NNRTI-use (Fig. 2). Multivariable GLMM ordinal regression (including all 24,186 non-blip VLs after multiple imputation) showed that, compared with INSTIs, PIs had significantly higher odds of higher RV levels (OR 1.28; 95% CI 1.14–1.44) whereas NNRTIs had lower odds (OR 0.76; 95% CI 0.68–0.86) (Table 3). Moreover, shorter time since ART initiation or study inclusion, lower $CD4^+$ counts, Fiebig stage VI (*vs.* stage I-V), and a higher zenith VL were associated with higher RV levels.

Figure 2. Stacked bar plot showing the percentage of VLs per observed VL category by cART anchor group.



Distribution of the 24,518 included VLs is shown. Of these, 5119 VLs were during INSTI-use: RNA^{neg} (n=3358; 65.6%), RV 1-19 (n=1369; 26.7%), RV 20-49 (n= 315; 6.2%), blip (n=40; 0.8%) and VL <50 cp/mL with unknown RV level (n=37; 0.7%). A total of 8935 VLs were during PI-use: RNA^{neg} (n=5003; 56.0%), RV 1-19 (n=2596; 29.1%), RV 20-49 (n=806; 9.0%), blip (n=163; 1.8%) and VL <50 cp/mL with unknown RV level (n=367; 4.1%).
A total of 10,464 VLs were during NNRTI-use: RNA^{neg} (n=6965; 66.6%), RV 1-19 (n=2353; 22.5%), RV 20-49 (n= 499; 4.8%), blip (n=129; 1.2%) and VL <50 cp/mL with unknown RV level (n=518; 5.0%).
Abbreviations: cART, combination antiretroviral therapy; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RV, residual viremia; VL, viral load.

Table 3. Generalized linear mixed model ordinal regression results: associations with the level of residual viremia.

	Level of residual viremia			
	Univariable analysis		Multivariable analysis	
Variables	***OR (95% CI)***	***p-value***	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.85 (0.83 - 0.87)	**<0.001**	0.88 (0.86 - 0.91)	**<0.001**
Female sex (*vs.* male sex)	0.81 (0.69 – 0.96)	**0.01**	0.88 (0.74 - 1.04)	0.13
Age (per year increase)	1.01 (1.00 – 1.01)	**0.02**	1.00 (1.00 - 1.01)	0.18
European / North American region of origin (*vs.* other)	0.94 (0.82 – 1.09)	0.42	0.91 (0.78 – 1.06)	0.25
cART anchor				
- INSTI	1	-	1	-
- PI	1.36 (1.22 - 1.53)	**<0.001**	1.28 (1.14 - 1.44)	**<0.001**
- NNRTI	0.79 (0.70 – 0.89)	**<0.001**	0.76 (0.68 – 0.86)	**<0.001**
Time since ART initiation (per year increase)	0.96 (0.95 - 0.97)	**<0.001**	0.95 (0.94 - 0.96)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.17 (0.79 – 1.72)	0.44	1.51 (1.02 - 2.25)	**0.04**
Lowest recorded CD4$^+$ count (per 10 cell/mm^3 increase)	0.99 (0.98 – 0.99)	**<0.001**	0.99 (0.99 – 1.00)	**0.03**
Zenith VL cp/mL				
- <10,000	1	-	1	-
- 10,000-99,999	2.21 (1.67 – 2.94)	**<0.001**	2.06 (1.56 - 2.71)	**<0.001**
- 100,000-999,999	3.86 (2.90 – 5.14)	**<0.001**	3.38 (2.54 - 4.49)	**<0.001**
- ≥1,000,000	6.20 (4.24 – 9.06)	**<0.001**	5.30 (3.58 - 7.86)	**<0.001**

Pooled associations were obtained from the five datasets after multiple imputation of the 24,186 non-blip VLs.
RV level, categorized as an ordinal outcome (RNA^{neg} , 1-19 cp/mL, and 20-49 cp/mL), was analyzed using a backwards continuation ratio model for ordinal regression. The odds of 1-19 cp/mL *vs.* RNA^{neg} and 20-49 cp/mL *vs.* 1-19 cp/mL + RNA^{neg} were investigated.
Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; VL, viral load; *vs.*, versus.

Sensitivity analyses

The sensitivity analysis including the NRTI backbone as covariate and complete case analysis showed regression coefficients similar to the main analysis (Table S2-S3). The within-anchor group comparisons for the RV level showed no significant within-anchor associations for INSTIs and NNRTIs, but “other” PIs (*vs.* darunavir) were independently associated with less RV (Table S4-S6). Finally, sensitivity analyses excluding the PWH with periods of non-adherence yielded similar results as the main analyses (Table S7-S8).

DISCUSSION

In the present study, we analyzed viral blips, subclinical RV, and the associated modifiable and non-modifiable risk factors. In more than 24,000 VLs from adherent PWH treated with contemporary cART regimens, we found that the probability of a blip increased with higher RV levels. The magnitude of RV was strongly associated with the type of cART anchor: the lowest RV levels were seen during NNRTI-use, followed by INSTI- and then PI-use.

Moreover, lower RV levels were observed in PWH with low zenith VLs, acute infection at ART initiation, and longer time since ART initiation.

The strong, positive correlation between different RV levels and blips persisted even after adjusting for potential confounders, confirming observations from a small case-control study where RV was assessed up to one year before the blip[16] and two observational studies where PWH were stratified as RNA^{neg} or RNA^{pos} based on their baseline VLs[17,18]. In the last two studies, it should be noted that classifying PWH into an RV group on this basis (rather than on individual VL results) reduced the sensitivity of the findings, as a single RNA^{pos} result can already change the classification. The present study majorly extends these previous data by the assessment of three different, ordinal levels of RV, taking repeated measurements over time into account, and including a group of INSTI recipients. Additionally, we showed that the previously demonstrated difference in blip rates for different cART anchors still held true when accounting for RV level [2]. This implies that blips have a multifactorial origin, with cART exerting its effect on blips not solely by altering the RV level.

RV is driven by both modifiable, cART-related factors, as well as non-modifiable, virological characteristics. Regarding the impact of cART anchor type, conflicting results have been reported. One study found that INSTIs, but not NNRTI or PIs, were significantly associated with RNA^{neg} [20], whereas another reported both NNRTI- and INSTI-based regimens to show significantly lower time spent with RV than PI-based regimens.[21] Yet another study reported no significant differences regarding RV rates between individual drugs within ART classes in ART-naïve PWH.[22] In the largest prior analysis comparing anchor types, 11,045 VLs 1-19 cp/mL were assessed and categorized as either “detectable” or “undetectable”, finding that the probability to have a detectable VL was lower during NNRTI- and INSTI-use than during PI-use.[19] However, no difference was found between NNRTIs and INSTIs. In our analysis, that included a time-varying model, an additional RV level, adherence restrictions, and considerably more VLs, significantly lower RV levels were seen for NNRTI- and INSTI-based cART compared to PI-based cART. Moreover, significantly less RV was seen for NNRTIs compared to INSTIs. PIs were found to be associated with both higher RV levels and blips, even in the context of an adherent group of PWH. As PIs, in contrast to NNRTIs and INSTIs, exert their antiretroviral effect after HIV-1 integration and proviral transcription[26], these steps in the HIV-1 replication cycle could have an influence on blip occurrences. Interestingly, when comparing NNRTIs and INSTIs, less RV but more blips are observed for NNRTIs, despite the strong relationship between RV and blips. Thus, cART appears to influence blips not solely by affecting the RV level. The opposite correlations for RV and blips during NNRTI-use reinforce this idea, as they imply that there are different pathways at play: though the lower RV level ‘protects’ NNRTI-recipients against blips, PWH using NNRTIs experience a higher blip frequency through another, yet unidentified, pathway. Although we cannot exclude that certain unaccounted person-specific characteristics or

pharmacokinetic properties of the anchor groups (*e.g.*, half-life, penetration into anatomical compartments, time above 90% inhibitory concentration) influenced the results, one would not expect a complete reversal in the effect direction between NNRTIs and INSTIs regarding RV and blips.

In addition to cART anchor, several non-modifiable factors were associated with the magnitude of RV, such as Fiebig stage at ART initiation, zenith VL, time since ART initiation, and lowest $CD4^+$ count. Interestingly, these factors have previously been linked to the size of the viral reservoir: treatment initiation during acute infection (Fiebig I-V) reduces viral reservoir seeding[27], pre-treatment VL is related to the reservoir size, even years after treatment initiation[28], and the reservoir decays slowly over time on ART[29]. Moreover, an inverse relationship between $CD4^+$ nadir and HIV-1 proviral DNA in circulating $CD4^+$ T cells on ART has previously been suggested to either reflect the repopulation of the $CD4^+$ compartment after ART initiation by the relatively highly frequent infected $CD4^+$ T cells harboring HIV-1 (proviral) DNA (resulting in a larger reservoir) or the incomplete suppression of HIV-1 replication in PWH with low $CD4^+$ nadirs.[30] Indeed, some studies into reservoir measures like cell-associated HIV RNA and DNA have shown a correlation between these measures and RV, suggesting that RV is at least partially indicative of the reservoir size.[28,31] In contrast to the association between Fiebig stage and RV, no statistically significant impact of Fiebig stage on blips was found in this study, but both blips and ART initiation in the acute phase of infection were relatively rare, precluding a rigorous assessment of their potential relationship.

The clinical relevance of transient detectable viremia at these very low levels remains a topic of discussion. Though most clinicians agree that isolated blips should not prompt treatment changes[32], multiple studies have found an association with the emergence of drug resistance[33,34] or virologic failure[12,13], especially if blips with a higher VL were encountered[35]. Moreover, blips have been shown to lead to extra outpatient visits and laboratory tests.[2] Similar to the much debated relevance of blips, no consensus has been reached on the nature and virologic consequences of RV. It is unclear whether RV represents virus released from latently infected cells, ongoing viral replication (with the subsequent risk of selection of resistance), or both. Although studies are highly heterogeneous, several studies have found a higher risk of viral rebound in PWH with RV.[16–18,36,37] Additionally, RV has been associated with low-grade immune activation and chronic inflammation.[38,39] HIV persistence has been suggested to both cause inflammation and be 'fueled' by it when new target cells are generated[19] and to replenish the HIV reservoir[6]. However, much is still unclear, and the long-term clinically relevant health effects of RV and immune activation remain to be elucidated.

To our knowledge, this is the first study that uses an integrated approach to comprehensively study the relationship between RV, blips, all contemporary cART anchors, and virological

factors in a large, real-world cohort of PWH. A major strength is the long follow-up period of up to 10 years and the substantial amount of repeated samples allowing us to model RV levels and subsequent blip occurrences over time: the relationship between RV and blips was assessed using only information available at the time of each assessed VL, in contrast to other studies incorporating information from the future on predictors not available at the time of the outcome (such as time spent with RV in general).[21] The results are further strengthened by the comprehensive assessment of medical records for information on adherence, as this reduced the influence of adherence variability on RV levels and blips[40] and allowed us to focus on underlying virological factors as a mechanism for RV and blips. Nevertheless, as with any observational study, the potential for confounding by indication existed. As PIs (compared with NNRTIs) traditionally had the highest barrier to resistance before the arrival of the newer INSTIs[41], physicians may have preferentially prescribed PIs when suspecting poor adherence. Moreover, different anchors (and associated boosters) are associated with different adverse effects and may thus present different tolerability issues. Indeed, VLs <50 cp/mL during PI-based regimens were more likely to be excluded based on documented non-adherence than VLs during other regimens. However, excluding VLs from periods with non-adherence has strengthened our analyses, as the higher rate of non-adherence in PI recipients could potentially have skewed the data toward even more RV and blips during PI-based therapy. Such skewing of results would not be expected for comparisons between NNRTIs and INSTIs, as adherence levels were observed to be similar. Moreover, though residual confounding can never be completely excluded, the risk of confounding bias generally present in observational studies was minimized by controlling for a large number of factors known to be associated with the virological response to cART.

In conclusion, in this large cohort of adherent PWH, we demonstrated that viral blips were strongly associated with higher preceding RV. Rates of both RV and blips were correlated with the type of cART anchor used and several virologic parameters linked to the latent viral reservoir. These findings indicate that blips observed in the clinic have a multifactorial origin, with underlying RV attributable to cART anchor type partially contributing to this phenomenon. Future studies should explore other pathways in which anchor types may lead to higher blip rates and, since the viral reservoir appears to play a role in them, study blips in the context of reservoir measures such as cell-associated HIV RNA and DNA.

Statements

Author contributions

P.O., S.D., and B.W. designed the study. B.W. wrote the study protocol. P.O. and S.D. collected and analyzed the data. P.O. and S.D. drafted the manuscript in close collaboration with B.W. All authors contributed to the interpretation of the results, critically reviewed the manuscript and approved the final manuscript.

Conflicts of interest

B.W.: research grant and speaker fees from Gilead Sciences as well as speaker and advisory board fees from ViiV Healthcare: all fees were paid to the institution. T.M.: research grant from Gilead Sciences, as well as advisory board (consultancy) fees from Gilead sciences, MSD, ViiV healthcare: all paid to institution. A.W.: investigator-initiated research grant from Gilead sciences, and consultancy fees from ViiV Healthcare/GSK and Gilead sciences: all paid to the institution. M.N.: consultancy fees from Gilead sciences. For the remaining authors none were declared.

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APPENDIX

Supplemental Table S1. Frequencies of specific antiretroviral drugs at the time of VLs

Antiretroviral therapy	Samples – n = 24,518[a]
cART Anchor	**n (%)**
INSTI	n = 5,119
- dolutegravir	2756 (11.2)
- raltegravir	698 (2.8)
- elvitegravir	1061 (4.3)
- bictegravir	210 (0.9)
NNRTI	n = 10,464
- efavirenz	7322 (29.9)
- nevirapine	1820 (7.4)
- rilpivirine	1307 (5.3)
- other NNRTI	15 (0.1)
PI	n = 8,935
- atazanavir	5250 (21.4)
- darunavir	2756 (11.2)
- other PI	929 (3.8)
Booster	n = 9,909
- ritonavir	7974 (32.5)
- cobicistat	1935 (7.9)
Dual NRTI backbone	
- emtricitabine	19,126 (78.0)
- tenofovir disoproxil fumarate	16,243 (66.2)
- lamivudine	5280 (21.5)
- tenofovir alafenamide	3032 (12.4)
- abacavir	4037 (16.5)
- other NRTI	1318 (5.4)

[a] As PWH could have had different treatment regimens during follow-up and cART was assessed for each VL, frequencies of cART regimens are shown per VL.

Abbreviations: cART, combination antiretroviral therapy; INSTI, integrase strand transfer inhibitor; n, number; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleos(-t)ide reverse transcriptase inhibitor; PI, protease inhibitor.

Supplemental Table S2. Results of generalized linear mixed model regression: multivariable associations with the level of residual viremia including NRTI backbone as covariate.

	Level of residual viremia	
Variables	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.88 (0.85 – 0.91)	**<0.001**
Female sex (*vs.* male sex)	0.88 (0.74 – 1.04)	0.15
Age (per year increase)	1.03 (0.94 – 1.13)	0.44
European / North American region of origin (*vs.* other)	1.08 (0.93 – 1.27)	0.32
cART anchor		
- INSTI	1	**-**
- PI	1.29 (1.14 – 1.46)	**<0.001**
- NNRTI	0.76 (0.66 – 0.87)	**<0.001**
Time since ART initiation (per year increase)	0.95 (0.94 – 0.96)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.53 (1.03 – 2.27)	0.03
Lowest recorded CD4^{+} count (per 10 cell/mm^{3} increase)	0.95 (0.85 – 1.05)	0.36
Zenith VL cp/mL		
- <10,000	1	-
- 10,000-99,999	2.06 (1.57 – 2.71)	**<0.001**
- 100,000-999,999	3.39 (2.55 – 4.51)	**<0.001**
- ≥1,000,000	5.31 (3.59 – 7.87)	**<0.001**
NRTI 1		
- TDF	1	-
- TAF	1.03 (0.91 – 1.17)	0.63
- ABC	1.50 (0.72 – 3.12)	0.27
- Other	1.32 (0.63 – 2.78)	0.46
NRTI 2		
- FTC	1	-
- 3TC	0.66 (0.32 – 1.36)	0.26
- Other	0.40 (0.11 – 1.40)	0.15

Pooled associations were obtained from the five datasets after multiple imputation of the 24,186 non-blip VLs.
RV level, categorized as an ordinal outcome (RNA^{neg}, 1-19 cp/mL, and 20-49 cp/mL), was analyzed using a backwards continuation ratio model for ordinal regression. The odds of 1-19 cp/mL *vs.* RNA^{neg} and 20-49 cp/mL *vs.* 1-19cp/mL + RNA^{neg} were investigated.
Abbreviations: ABC, abacavir; ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleos(-t)ide reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; VL, viral load; *vs.*, versus; 3TC, lamivudine.

Supplemental Table S3. Complete Case Analysis including only PWH without missing data. Generalized linear mixed model regression results: multivariable associations with viral blip occurrences and level of residual viremia.

	Occurrence of blips		Level of residual viremia[a]	
Variables	***OR (95% CI)***	***p-value***	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.85 (0.75 – 0.97)	**0.02**	0.87 (0.85 – 0.89)	**<0.001**
Female sex (*vs.* male sex)	1.14 (0.74 – 1.75)	0.56	0.80 (0.66 – 0.98)	**0.03**
Age (per year increase)	1.01 (0.87 – 1.19)	0.86	1.00 (1.00 – 1.01)	0.22
European / North American region of origin (*vs.* other)	1.47 (0.97 – 2.23)	0.07	0.94 (0.79 – 1.12)	0.49
cART anchor				
- INSTI	1	-	1	-
- PI	2.64 (1.64 – 4.25)	**<0.001**	1.25 (1.09 – 1.43)	**0.002**
- NNRTI	2.00 (1.23 – 3.25)	**0.004**	0.77 (0.67 – 0.89)	**<0.001**
Time since ART initiation (per year increase)	0.99 (0.95 – 1.03)	0.62	0.95 (0.93 – 0.96)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	2.07 (0.58 – 7.33)	0.26	1.62 (1.04 – 2.53)	**0.03**
Lowest recorded $CD4^+$ count (per 10 cell/mm^3 increase)	1.00 (0.99 – 1.01)	0.41	0.99 (0.99 – 1.00)	**<0.001**
Zenith VL cp/mL				
- <10,000	1	-	1	-
- 10,000-99,999	2.69 (0.81 – 8.93)	0.11	2.41 (1.76 – 3.31)	**<0.001**
- 100,000-999,999	3.27 (0.99 – 10.78)	0.05	4.05 (2.96 – 5.55)	**<0.001**
- ≥1,000,000	6.11 (1.70 – 21.96)	**0.005**	6.12 (3.99 – 9.37)	**<0.001**
Residual viremia level				
- RNA^{neg}	1	-	-	-
- RV 1-19 cp/mL	3.02 (2.20 – 4.15)	**<0.001**	-	-
- RV 20-49 cp/mL	5.52 (3.78 –8.09)	**<0.001**	-	-

The complete case analysis was conducted on 17,563 VLs from 1344 PWH without missing data.

[a] RV level, categorized as an ordinal outcome (RNA^{neg}, 1-19 cp/mL, and 20-49 cp/mL), was analyzed using a backwards continuation ratio model for ordinal regression. The odds of 1-19 cp/mL *vs.* RNA^{neg} and 20-49 cp/mL *vs.* 1-19 cp/mL + RNA^{neg} were investigated.

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; VL, viral load; *vs.*, versus.

Supplemental Table S4. Results of generalized linear mixed model regression: multivariable associations for the within-anchor group comparisons of INSTIs and the level of residual viremia.

	Level of residual viremia	
Variables	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.87 (0.83 - 0.91)	**<0.001**
Female sex (*vs.* male sex)	0.69 (0.51 – 0.96)	**0.03**
Age (per year increase)	1.01 (1.00 - 1.02)	**0.03**
European / North American region of origin (*vs.* other)	1.06 (0.81 – 1.38)	0.69
Time since ART initiation (per year increase)	0.96 (0.93 - 0.98)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.66 (0.96 - 2.85)	0.07
Lowest recorded CD4⁺ count (per 10 cell/mm³ increase)	0.99 (0.99 – 1.00)	**0.03**
Zenith VL cp/mL		
- <10,000	1	-
- 10,000-99,999	1.85 (1.18 – 2.88)	**0.007**
- 100,000-999,999	3.14 (1.99 – 4.95)	**<0.001**
- ≥1,000,000	5.43 (2.62 – 11.27)	**<0.001**
INSTIs		
- DTG	1	-
- RTG	0.93 (0.69 – 1.24)	0.61
- EVG	0.87 (0.67 – 1.13)	0.29
- BTG	0.68 (0.45 – 1.03)	0.07

GLMM ordinal regression was performed on 5079 non-blip VLs obtained during INSTI -use, of which 3118 were during DTG, 696 during RTG, 1057 during EVG, and 208 during BTG. Pooled associations were obtained from the five datasets after multiple imputation.

Abbreviations: ART, antiretroviral therapy; BTG, bictegravir; CI, confidence interval; cp, copies; DTG, dolutegravir; EVG, elvitegravir; INSTI, integrase strand transfer inhibitor; OR, odds ratio; RTG, raltegravir; VL, viral load; *vs*., versus.

Supplemental Table S5. Results of generalized linear mixed model regression: multivariable associations for the within-anchor group comparisons of PIs and the level of residual viremia.

	Level of residual viremia	
Variables	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.87 (0.83 - 0.91)	**<0.001**
Female sex (*vs.* male sex)	0.92 (0.70 – 1.19)	0.52
Age (per year increase)	1.00 (0.99 - 1.01)	0.82
European / North American region of origin (*vs.* other)	1.23 (0.96 – 1.59)	0.11
Time since ART initiation (per year increase)	0.95 (0.93 - 0.97)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.55 (0.79 – 3.06)	0.21
Lowest recorded CD4$^+$ count (per 10 cell/mm^3 increase)	0.99 (0.99 – 1.00)	0.10
Zenith VL cp/mL		
- <10,000	1	-
- 10,000-99,999	2.86 (1.69 – 4.84)	**<0.001**
- 100,000-999,999	4.36 (2.58 – 7.36)	**<0.001**
- ≥1,000,000	7.87 (4.11 – 15.06)	**<0.001**
PIs		
- DRV	1	-
- ATV	0.90 (0.76 – 1.06)	0.21
- Other PIs	0.72 (0.54 – 0.97)	**0.03**

GLMM ordinal regression was performed on 8772 non-blip VLs obtained during PI-use, of which 2706 were during DRV, 5151 during ATV, and 1796 during other PI. Pooled associations were obtained from the five datasets after multiple imputation.

Abbreviations: ART, antiretroviral therapy; ATV, atazanavir; CI, confidence interval; cp, copies; DRV, darunavir; OR, odds ratio; PI, protease inhibitor; VL, viral load; *vs*., versus.

Supplemental Table S6. Results of generalized linear mixed model regression: multivariable associations for the within-anchor group comparisons of NNRTIs and the level of residual viremia.

	Level of residual viremia	
Variables	***OR (95% CI)***	***p-value***
Time since study inclusion (per year increase)	0.89 (0.86 - 0.93)	**<0.001**
Female sex (*vs.* male sex)	0.89 (0.69 – 1.15)	0.36
Age (per year increase)	1.05 (0.93 - 1.19)	0.40
European / North American region of origin (*vs.* other)	1.02 (0.82 – 1.27)	0.87
Time since ART initiation (per year increase)	0.95 (0.93 - 0.97)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.80 (0.76 – 4.31)	0.18
Lowest recorded $CD4^+$ count (per 10 cell/mm^3 increase)	1.00 (0.99 – 1.01)	0.82
Zenith VL cp/mL		
- <10,000	1	**-**
- 10,000-99,999	1.76 (1.16 – 2.66)	**0.008**
- 100,000-999,999	2.88 (1.86 – 4.45)	**<0.001**
- ≥1,000,000	3.79 (2.13 – 6.72)	**<0.001**
NNRTIs[a]		
- RPV	1	**-**
- EFV	0.89 (0.73 – 1.10)	0.29
- NVP	0.84 (0.62 – 1.10)	0.19

GLMM ordinal regression was performed on non-blip 10,320 VLs obtained during NNRTI-use, of which 1290 were during RPV, 7232 during EFV, and 1796 during NVP. Pooled associations were obtained from the five datasets after multiple imputation.

[a] For the purpose of these analysis, the NNRTI-based VLs with NNRTIs other than nevirapine, efavirenz, or rilpivirine where excluded, as this group existed of only 15 VLs.

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; cp, copies; EFV, efavirenz; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; OR, odds ratio; PI, protease inhibitor; RPV, rilpivirine; VL, viral load; *vs*., versus.

Supplemental Table S7. Results of generalized linear mixed model logistic regression: multivariable associations with viral blip occurrences excluding the 74 PWH with periods of non-adherence.

	Occurrence of blips	
	OR (95% CI)	***p-value***
Time since study inclusion (per year increase)	0.89 (0.79 – 1.00)	**0.05**
Female sex (*vs.* male sex)	0.86 (0.56 – 1.32)	0.49
Age (per year increase)	0.99 (0.85 – 1.15)	0.89
European / North American region of origin (*vs.* other)	0.74 (0.51 – 1.09)	0.13
cART anchor		
- INSTI	1	-
- PI	2.28 (1.46 – 3.56)	**<0.001**
- NNRTI	1.77 (1.13 – 2.77)	**0.01**
Time since ART initiation (per year increase)	0.97 (0.94 - 1.00)	**0.05**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	2.43 (0.69 – 8.57)	0.17
Lowest recorded $CD4^+$ count (per 10 cell/mm^3 increase)	1.00 (0.99 – 1.01)	0.41
Zenith VL cp/mL		
- <10,000	1	-
- 10,000-99,999	2.00 (0.69 – 5.80)	0.20
- 100,000-999,999	2.33 (0.79 – 6.93)	0.13
- ≥1,000,000	4.50 (1.35 - 15.04)	**0.02**
Residual viremia level		
- RNA^{neg}	1	-
- RV 1-19 cp/mL	2.65 (1.95 – 3.61)	**<0.001**
- RV 20-49 cp/mL	5.16 (3.54 – 7.51)	**<0.001**

Pooled associations were obtained from the five datasets after multiple imputation of all 24,518 VLs. For this analysis, 74/1658 (4.5%) PWH with periods of non-adherence were excluded.

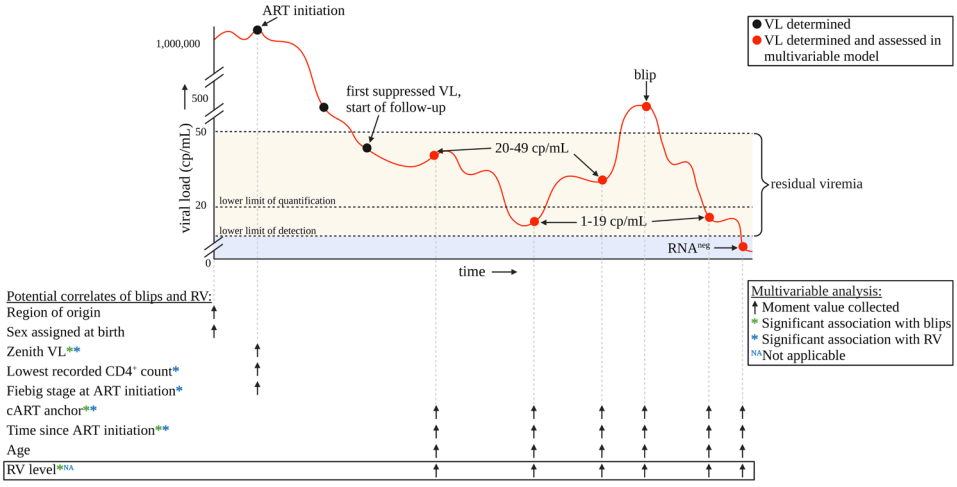
Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; RV, residual viremia; VL, viral load; *vs.*, versus.

Supplemental Table S8. Results of generalized linear mixed model ordinal regression: multivariable associations with the level of residual viremia excluding the 74 PWH with periods of non-adherence.

	Level of residual viremia	
	OR (95% CI)	***p-value***
Time since study inclusion (per year increase)	0.88 (0.86 - 0.90)	**<0.001**
Female sex (*vs.* male sex)	0.85 (0.71 - 1.01)	0.07
Age (per year increase)	1.00 (1.00 - 1.01)	0.26
European / North American region of origin (*vs.* other)	1.12 (0.96 – 1.31)	0.16
cART anchor		
- INSTI	1	-
- PI	1.28 (1.14 - 1.45)	**<0.001**
- NNRTI	0.77 (0.68 – 0.87)	**<0.001**
Time since ART initiation (per year increase)	0.95 (0.94 - 0.96)	**<0.001**
Fiebig stage VI at ART initiation (*vs.* stage I-V)	1.50 (1.00 – 2.24)	**0.05**
Lowest recorded CD4^{+} count (per 10 cell/mm^{3} increase)	0.99 (0.99 – 1.00)	**0.02**
Zenith VL cp/mL		
- <10,000	1	-
- 10,000-99,999	2.09 (1.56 - 2.79)	**<0.001**
- 100,000-999,999	3.51 (2.66 – 4.64)	**<0.001**
- ≥1,000,000	5.43 (3.55 – 8.33)	**<0.001**

Pooled associations were obtained from the five datasets after multiple imputation of the 24,186 non-blip VLs. For this analysis, 74/1658 (4.5%) PWH with periods of non-adherence were excluded. RV level, categorized as an ordinal outcome (RNA^{neg} , 1-19 cp/mL, and 20-49 cp/mL), was analyzed using a backwards continuation ratio model for ordinal regression. The odds of 1-19 cp/mL *vs.* RNA^{neg} and 20-49 cp/mL *vs.* 1-19 cp/mL + RNA^{neg} were investigated.
Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; VL, viral load; *vs.*, versus.

Supplemental Figure S1. Graphical abstract



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THE EFFECT OF EFAVIRENZ ON REWARD PROCESSING IN ASYMPTOMATIC PEOPLE LIVING WITH HIV: A RANDOMIZED CONTROLLED TRIAL

Patrick G.A. Oomen
Charlotte S. Hakkers
Joop E. Arends
Guido E.L. van der Berk
Pascal Pas
Andy I.M. Hoepelman
Berend J. van Welzen
Stefan du Plessis

AIDS Research and Human Retroviruses.
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ABSTRACT

Functional MRI studies have demonstrated that HIV-infection affects the fronto-striatal network. It has not been examined what impact efavirenz, an antiretroviral drug notorious for its neurocognitive effects, has on the reward system: a key subcomponent involved in depressive and apathy symptoms. Therefore, this study aims to investigate the effect of efavirenz on reward processing using a monetary incentive delay task. In this multicenter randomized controlled trial, asymptomatic adult participants stable on emtricitabine/tenofovirdisoproxil/efavirenz (FTC/TDF/EFV) were randomly assigned in a 2:1 ratio to switch to emtricitabine/tenofovirdisoproxil/rilpivirine (FTC/TDF/RPV) (n=30) or continue taking FTC/TDF/EFV (n=13). At baseline and twelve weeks after therapy switch, both groups performed a monetary incentive delay task. Behavior and functional brain activity related to reward anticipation and reward outcome were assessed with blood oxygen level dependent functional MRI. Both groups were matched for age, education-level and time since HIV-diagnosis and on EFV. At the behavioral level, both groups had faster response times and better response accuracy during rewarding versus non-rewarding trials, with no improvement resulting from switching FTC/TDF/EFV to FTC/TDF/RPV. No significant change in activation related to reward anticipation in the ventral striatum was found after switching therapy. Both groups had significantly higher activation levels over time, consistent with a potential learning effect. Similar activity related to reward outcome in the orbitofrontal cortex was found. Discontinuing FTC/TDF/EFV was not found to improve activity related to reward anticipation in asymptomatic people living with HIV, with similar cortical functioning during reward outcome processing. It is therefore likely that EFV does not affect motivational control. Further research is needed to determine whether EFV affects motivational control in HIV populations with different characteristics.

INTRODUCTION

The introduction of combination antiretroviral therapy (cART) has dramatically increased the life expectancy of people living with HIV (PLWH)[1]. As a result of the improved life expectancy, the focus of HIV-related care has shifted to treatment and prevention of comorbidities due to HIV and cART itself. A common but relatively little studied comorbidity with a major impact on quality of life in PLWH, is the presence of HIV-associated neurocognitive disorders (HAND)[2,3]. HAND is characterized by neurocognitive functional impairments in memory, concentration, attention and motor skills, which are traditionally assessed with a neuropsychological assessment (NPA)[3]. Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI), although not a validated clinical tool like NPA, is also widely used in the research setting to assess functional impairment[4]. Since BOLD fMRI can detect early changes in the brain in the absence of symptomatic neurocognitive functional impairment, it is considered to be more sensitive in assessing the impact of cART on the brain than NPA alone[5].

Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), is an important and frequently used antiretroviral anchor drug worldwide. It is part of a single tablet regimen composed of emtricitabine/tenofovirdisoproxil fumarate/efavirenz (FTC/TDF/EFV) and was the preferred first-line therapy for over 15 years until 2018 according to the World Health Organization[6]. Although use has slowly declined since then, EFV is still recommended as an alternative first-line regimen anchor and remains widely used in low- and middle-income countries, with forecast analyses predicting 10 million PLWH (i.e., 25% of the estimated total population) will still be using EFV-based regimens by 2025[7,8]. Moreover, even in high-income countries EFV continues to be used, as 7% of PLWH in the Netherlands used FTC/TDF/EFV in 2020[9].

EFV is notorious for its neurocognitive side effects such as dizziness or insomnia[10] and is also associated with neurocognitive functional impairment[11–14], though this is still debated as it is not confirmed in all studies[15–17]. In previous work, we showed that discontinuing EFV in asymptomatic PLWH resulted in an improvement in the cognitive domains attention and speed of information processing, as assessed by NPA[18]. The question is what dysfunction in the neurocognitive network is underlying this.

To date, little is known about exactly which neurocognitive systems are affected by EFV. One possibility is the fronto-striatal reward system: a key subcomponent involved in depressive and apathy symptoms and responsible for reward processing[19]. Reward processing consists of several neurocognitive processes such as processing the outcome of a reward and anticipating future rewards and is crucial for decision-making and goal-directed[20]. These ‘reward anticipation’ and ‘reward outcome’ processes are modulated

by the subcortical ventral striatum and the orbitofrontal cortex (OFC) and are indicators of respectively subcortical and cortical functioning[21–23]. Previous fMRI research has suggested that HIV-infection impairs subcortical functioning, including reward anticipation, but spares cortical functioning[24,25]. Additionally, there is evidence that EFV negatively impacts executive functioning and, although in an adolescent population, even cortical functioning[26,27]. It is therefore essential to investigate whether and what part of reward processing is affected by EFV.

The aim of this study is to determine the effect of EFV on reward processing using BOLD fMRI. We conducted a randomized controlled trial (RCT) and randomly assigned asymptomatic PLWH stable on FTC/TDF/EFV to switch to emtricitabine/tenofovirdisoproxil fumarate/rilpivirine (FTC/TDF/RPV) or continue FTC/TDF/EFV. Since our entire study population used EFV at the onset of the trial, we specifically studied the effect of EFV by discontinuation in one group. We hypothesized that, due to HIV-infection impairing subcortical functioning and potentially rendering it susceptible to neurotoxic damage of EFV, switching from FTC/TDF/EFV to FTC/TDF/RPV would result in relatively improved ventral striatal responses, while cortical functioning would remain stable.

MATERIALS AND METHODS

Participants

The current study is a sub-analysis of the ESCAPE (Effect of SwitChing AtriPla to Eviplera on neurocognitive and emotional functioning) trial, which was conducted from 2015 to 2017 at two large HIV treatment centers in the Netherlands (OLVG (Amsterdam) and University Medical Centre Utrecht (Utrecht)[18]. We chose to create a homogenous study population by way of strict in- and exclusion criteria as fMRI can easily be influenced by confounding factors and PLWH already exhibit greater variability with respect to fMRI measurements[28,29]. In short, we included asymptomatic male PLWH aged 25-50 years stable on FTC/TDF/EFV for at least 6 months. Potential participants were excluded if they had an active or past central nervous system infection, an active psychiatric or neurologic disorder, a history or evidence of alcohol or drug abuse as assessed by the Drug Abuse Screening Test (DAST-10)[30]. During the study, participants with a viral load (VL) of >200 copies/mL were excluded from analysis, as we believed this could confound fMRI results. For the full list of in- and exclusion criteria, see the published study[18].

The trial was performed in accordance with the Declaration of Helsinki, was approved by the Medical Research Ethics Committee of the UMC Utrecht and was registered at Clinicaltrials.gov under number NCT02308332. Findings were reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) guideline[31]. The trial

was funded by Gilead Sciences. The funder had no role in trial design, data collection or analysis, or drafting of the manuscript. All participants provided written informed consent.

Trial design and procedures

Participants taking FTC/TDF/EFV were randomized (2:1), using computer-generated block randomization with a variable block size (range 3-9), to switch to FTC/TDF/RPV or continue FTC/TDF/EFV. A study nurse, not involved in the trial, generated the random allocation sequence and assigned participants. FTC/TDF/RPV was chosen because it is a single tablet regimen composed of the same backbone and a similar NNRTI anchor drug as FTC/TDF/EFV. They were instructed to take one tablet daily and, in case of FTC/TDF/RPV, with a substantial amount of food. The NPA was conducted by researchers who were unaware of the participant's allocated treatment. Researchers performing the fMRI-scan and participants were not blinded, since we believed that their knowledge of the allocated treatment would not affect our objective outcome of fMRI brain activity.

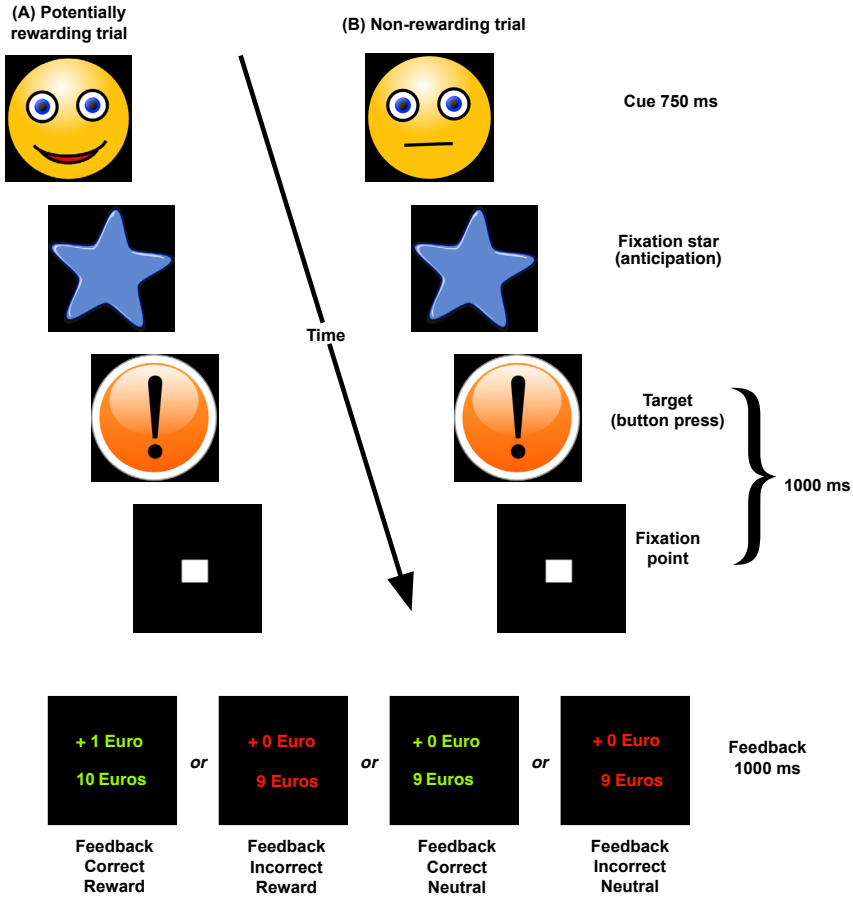
At baseline and after twelve weeks, participants underwent fMRI scanning. All MRI-scans were examined by a radiologist for intracranial pathology. Cognition was assessed using a NPA and it was determined whether the distribution of potentially confounding asymptomatic neurocognitive impairment (ANI) as defined according to the Frascati criteria was similar between groups[3]. Routine safety blood samples were obtained to assess laboratory abnormalities, disease progression and virologic suppression.

Participants switching to FTC/TDF/RPV had two additional outpatient visits after two and four weeks to monitor for side effects and obtain blood samples, which was standard clinical procedure at the time. A follow-up time of twelve weeks was chosen as previous research showed that neurocognitive changes after initiation or therapy switch were observed within this time frame[32–34].

Monetary incentive delay task

The reward task used in our study is based on the original monetary incentive delay (MID) task by Knutson et al. (Fig.1)[23,35–38]. HIV-associated neuropathological changes mainly occur in subcortical regions, such as the striatum, and in the white matter tracts connecting to the cortex[39,40]. As these regions are presumed more susceptible to possible neurotoxic effects of EFV, we selected the MID-task as it reliably activates the ventral striatum during reward anticipation and the OFC during reward outcome[24,36,37].

Figure 1. Schematic representation of the reward task, based on the Monetary Incentive Delay task[35,49].



There were two types of trials: potentially rewarding (A) and non-rewarding (B) trials as indicated by the cue (a smiling face for a potentially rewarding trial and a neutral face for a non-rewarding trial). Participants were instructed to press a button as fast as possible when the target stimulus (i.e., exclamation mark) appeared, irrespective of cue type. The fixation time between cue and target (indicated with the star) varied between trials. Feedback was given after the response and indicated via color if the response was given within the time limit (green) or not (red). The amount of money won in that trial (either +1 or +0) and their cumulative total at that moment were also presented. Target duration was individually adjusted to ensure that each participant could succeed in 50% of the trials.

The task consists of 60 separate trials of which 30 are potentially rewarding and 30 non-rewarding. The rewarding trials are indicated with a smiling face as reward cue and the non-rewarding trials with a non-smiling face, at the onset of each trial. A fixation star, which acts as an anticipation cue, appears after the reward cue. Following the fixation star, a target (exclamation mark) is presented requiring participants to react as fast as possible by pressing a button with their dominant index finger, irrespective of trial type. Participants were instructed they were able to win €1.00 if they responded within the time limit (i.e., duration of the target being

presented on the screen) in rewarding trials. Feedback after each trial notified participants of their performance indicating if they had earned money, as well as their cumulative total at that moment. We told participants that they would receive the cumulative total of the reward won from the actual experiment. During a practice session prior to the actual task, the participant's quickest response time was recorded to act as a baseline to adjust the task to individual performance levels. In 50% of trials, the target was presented for the duration of the individual's quickest response time plus 200ms, enabling participants to be successful in these trials. In the other trials, the time limit was decreased with 150ms, so that participants could not respond in time. This resulted in sufficient power for analysis of successful versus unsuccessful trials and ensured all participants received an equal reward amount (target €15.00). The task was designed so that maximum statistical power for the fMRI analyses could be achieved in a relatively short time: only one level of reward was used, and no loss trials were included.

To reduce the collinearity between reward anticipation and reward outcome, the anticipation cue time and the inter-trial interval time were varied (mean duration 3535ms, range 779-6729ms; mean duration 3535ms, range 1029-6979ms, respectively). The BOLD signal in response to reward anticipation could in this way be modelled independently of that of the reward outcome. Individual trials had an average duration of 9571ms (range 4946–16107ms), resulting in a total task duration of 9 min 35 s.

Behavioral analysis

Repeated measures ANOVA was performed to test for effects of trial (rewarding and non-rewarding trials), group (FTC/TDF/RPV versus FTC/TDF/EFV) and time (baseline versus week twelve) on the response time, response accuracy and reward amount. The Cook's distance was used to check for possible influential outliers (>1.0) and homogeneity of variances was tested using Levene's test[41]. A two-sided alpha level of 0.05 was used and statistical analyses were conducted using SPSS version 25.0 (IBM Corp. Armonk. NY).

Functional MRI

For an explanation of the procedures for fMRI image acquisition, pre-processing and initial individual analyses, see supplementary text 1 (Supplemental Digital Content 1).

Region-of-interest analyses

We performed primary analyses in one region of interest (ROI) per contrast: the combined bilateral ventral striatum for reward anticipation and the combined OFC for reward outcome, based on the previous findings[23,35]. These regions were defined using the Automated Anatomical Labeling-Atlas for the OFC and the Oxford-GSK-Imanova Striatal Connectivity Atlas for the ventral striatum[42,43]. For each participant, the mean activation level (expressed as percentage signal change) during the contrasts of interest (reward anticipation, neutral anticipation, reward outcome and neutral correct outcome) was

calculated over all the voxels of each ROI.

These values were used in a repeated measures ANOVA, testing for main within- and between-subject effects in activation levels between rewarding and non-rewarding trials with respect to reward anticipation and reward outcome.

Confirmatory analysis

In case of a negative finding, to ensure that we did not miss activation over time or between groups in regions neighboring the ventral striatum and known to be active during reward anticipation, we confirmed our findings by repeating the repeated measures ANOVA on the caudate nucleus.

Multi-region analysis across the reward network

To verify that there were no missed between-group differences in the change in activation levels over time in regions other than the ROI involved in reward processing, we conducted a multivariate analysis of variance (MANOVA). See supplementary figure 1 for the list of analyzed regions other than the ROI (Supplemental Digital Content 2).

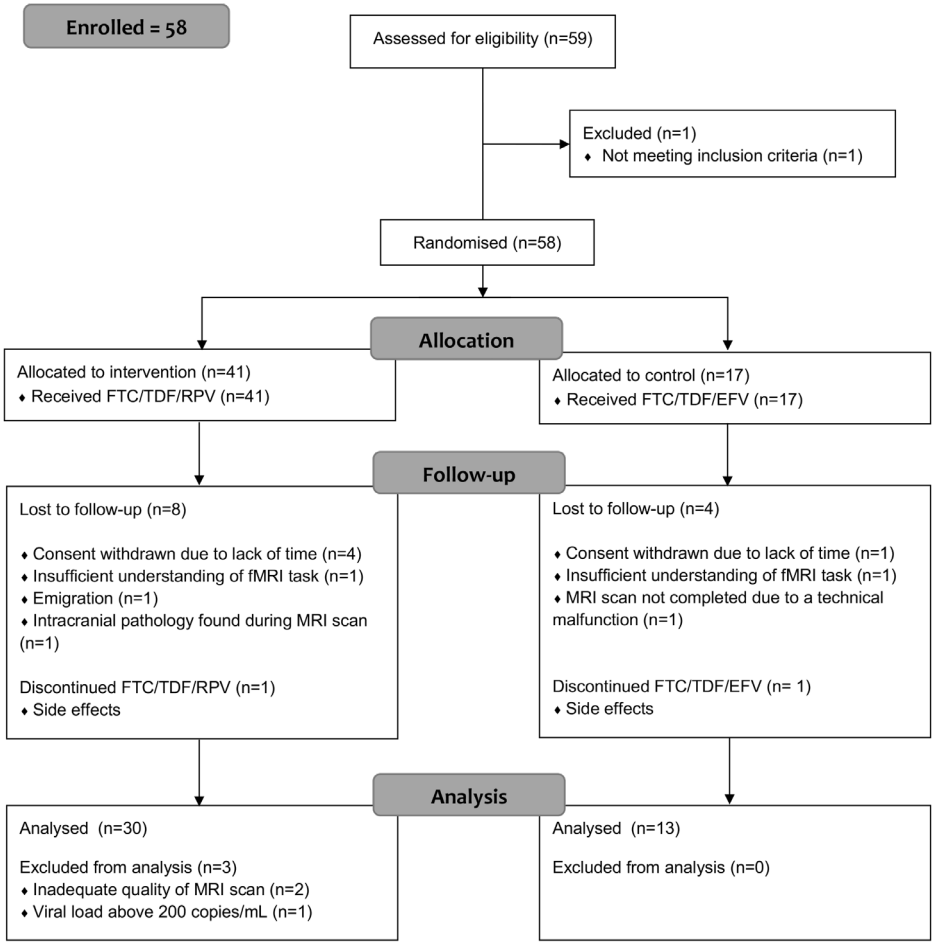
RESULTS

Demographics

From July 9 2015 to May 11 2017, a total of 59 potential participants were screened, one of them not meeting the eligibility criteria (Fig.2). Of the 58 participants randomized (2:1), 41 were assigned to the intervention group and 17 to the control group. A total of eleven intervention participants were lost-to-follow-up or excluded from analysis, because they withdrew their consent due to lack of time (4), side effects of FTC/TDF/RPV (1), emigration abroad (1) or because the investigator withdrew consent because the participant did not understand the fMRI task sufficiently (1), intracranial pathology was found during the MRI-scan (1), the quality of the MRI-scan was inadequate (2), or due to a VL of more than 200 copies/mL (1). Of the 17 controls who continued FTC/TDF/EFV, four participants were lost-to-follow-up or excluded from analysis because of withdrawal of consent due to lack of time (1), discontinuation of FTC/TDF/EFV during the study due to side effects (1), insufficient understanding of the fMRI task (1), or failure to complete an MRI-scan due to technical error (1). This resulted in a total of 30 intervention participants and 13 controls in the final analysis.

The characteristics of these 43 participants were similar (Table 1). At baseline, one intervention participant and one control had a viral blip, with a VL of 96 and 166 copies/mL, respectively. No blips were observed at week twelve and one intervention participant had persistent low level viremia (VLs of 165 and 168 copies/mL at baseline and week twelve).

Figure 2. Trial flow chart of participants enrolled and included in our analysis.



Abbreviations: EFV, efavirenz; fMRI, functional magnetic resonance imaging; FTC, emtricitabine; RPV, rilpivirine; TDF, tenofovirdisoproxil fumarate.

Behavioral results

Assumptions

First, we checked assumptions and assessed the Cook's distance on our outcomes of interest. The maximum Cook's distance was <1.0, which indicated no influential outliers. The Levene's tests yielded non-significant results, reflecting homogeneity of variances.

Table 1. Characteristics of the study participants according to study arm.

	FTC/TDF/RPV (intervention)		FTC/TDF/EFV (control)	
Demographics	**n = 30**	**(IQR) / (%)**	**n = 13**	**(IQR) / (%)**
Age (years)	41.19	35.19 – 47.34	42.17	34.55 – 46.48
Gender (male)	30	100	13	100
BMI (kg/m2)	24.53	21.81 – 27.04	24.72	21.74 – 26.55
Education (years)	16.00	16.00 – 17.00	17.00	16.00 – 17.50
Clinical characteristics				
Time since HIV diagnosis (months)	93.47	46.92 – 117.38	102.00	69.72 – 145.63
Time on EFV (months)[a]	56.29	32.16 – 77.73	54.00	29.03 – 80.25
Time on cART (months)[a]	56.29	33.91 – 77.73	54.00	29.03 – 80.25
ANI at baseline	7	23.3	2	15.4
Co-medication				
0	19	63.3	7	53.8
1	9	30.0	5	38.5
2 or more	2	6.7	1	7.7
Biochemical characteristics				
Nadir CD4 (cells/mm^3)[a]	299.50	209.25 – 343.75	267.00	150.50 – 380.00
Baseline CD4	619.50	471.50 – 816.75	695.00	536.00 – 695.00
Baseline VL <50 (copies/mL)	28	93.3	12	92.3
Baseline VL 50-200	2	6.7	1	7.7
CD4 at week 12[a]	656.50	522.50 – 768.00	600.00	546.50 – 808.50
VL<50 at week 12	30	100	12	92.3
VL 50-200 at week 12	0	0	1	7.7
Task outcome	**Mean**	**(SD)**	**Mean**	**(SD)**
Baseline reward amount won	14.03	1.59	14.54	1.20
Reward amount won at week 12	14.10	1.49	14.38	2.00

All categorical data are expressed as frequency (percentage) and all continuous data are expressed as median (interquartile range) unless indicated otherwise.

[a.] Missing data: time on EFV (1 control (2.3%)), time on cART (1 control (2.3%)), nadir CD4 (2 intervention participants (4.7%)), CD4 at week 12 (2 intervention participants (4.7%)).

Abbreviations: ANI, asymptomatic neurocognitive impairment (according to the Frascati criteria[3]); BMI, body mass index; cART, combination antiretroviral therapy; EFV, efavirenz; FTC, emtricitabine; RPV, rilpivirine; TDF, tenofovirdisoproxil fumarate; VL, viral load.

Baseline

Next, we evaluated the task performance of participants. At baseline, we found an expected main effect of trial on response time, with both groups reacting significantly faster on potentially rewarding trials than non-rewarding trials [$F(1,41)= 25.07$, $p<0.001$]. There was no group-by-trial interaction effect during reward task performance [$F(1,41)= 0.00$, $p=0.98$], indicating that both groups had a similar longer response time in non-rewarding trials. This shows that both groups performed as expected. Similar results were found with regard to response accuracy. Twelve weeks after therapy switch, response time and accuracy were similar to baseline results.

Prospective analysis after twelve weeks

Afterwards, the effect of discontinuing EFV on task performance was examined. When comparing the difference in response time of rewarding and non-rewarding trials between baseline and twelve weeks after therapy switch, we found no main effect of time [$F(1,41)= 0.22$, $p=0.64$], showing that response time differences remained similar over time. In addition, there was no group-by-time interaction effect [$F(1,41)= 0.21$, $p=0.65$] and no main group effect [$F(1,41)= 0.25$, $p=0.62$], indicating that both groups had similar response time differences and that switching to FTC/TDF/RPV did not lead to a significant different response time difference compared to continuing FTC/TDF/EFV. We found similar results for the combined response accuracy when comparing baseline and twelve weeks after therapy switch.

Finally, to confirm that the task successfully controlled for the amount of money won, we compared the reward amount between groups and over time. A similar reward amount at baseline versus after twelve weeks [$F(1,41)= 0.03$, $p=0.87$] was found. There was no group-by-time effect [$F(1,41)= 0.18$, $p=0.68$] nor main group effect [$F(1,41)= 1.03$, $p=0.32$], indicating both groups won a similar amount of money at both time points. This was expected as task difficulty was individually adjusted to ensure an equal reward amount for all participants.

Imaging results

Task validation

First, we started with the validation of the fMRI task. We found significant increases in activation in the ventral striatum during reward anticipation ($F(1,41)= 17.20$, $p<0.001$; $F(1,41)= 41.64$, $p<0.001$) and during reward feedback in the OFC for both time points ($F(1,41)= 17.20$, $p<0.001$; $F(1,41)= 41.64$, $p<0.001$), demonstrating that the task functioned as expected at both time points.

Reward processing in the ventral striatum

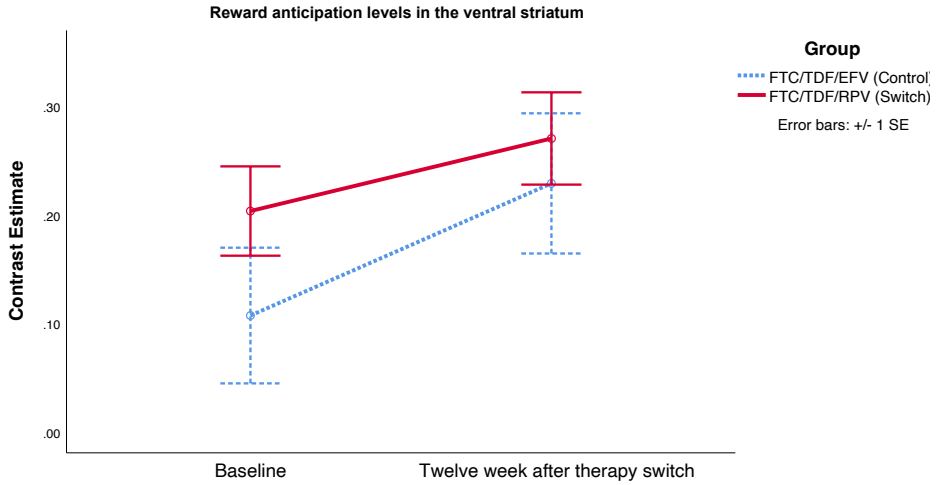
We then investigated the effect of discontinuing EFV on reward anticipation. We found a main effect of time in the combined ventral striatum [$F(1,41)= 6.69$, $p=0.01$], indicating that the ventral striatal responses of anticipation between rewarding and non-rewarding cues significantly increased in both groups over time (Fig.3). There was no group-by-time interaction [$F(1,41)= 0.57$, $p=0.46$] demonstrating that these responses did not differ between the two groups over time. Finally, there was no significant main group effect [$F(1,41)= 1.06$, $p=0.31$], indicating both groups showing similar responses.

Reward processing in the orbitofrontal cortex

Subsequently, we assessed the effect of discontinuing EFV on reward outcome processing. We found no significant main effect of time in the combined OFC [$F(1,41)= 0.67$, $p=0.42$], indicating similar OFC responses in reward outcome over time. As expected, we found no group-by-time interaction [$F(1,41)= 0.20$, $p=0.66$] nor a significant main group effect [$F(1,41)=$

0.95, p=0.34], showing that OFC responses between groups were similar and remained similar over time.

Figure 3. Reward anticipation levels in the ventral striatum



Line graph showing the repeated measures analysis of activation levels in the ventral striatum at baseline and twelve weeks after therapy switch for the control group (FTC/TDF/EFV) and the intervention group (FTC/TDF/RPV). Abbreviations: EFV, efavirenz; FTC, emtricitabine; RPV, rilpivirine; TDF, tenofovirdisoproxil fumarate.

Confirmatory analysis

Similar to what we did with the ventral striatum during reward anticipation, we proceeded to investigate the caudate nucleus, which neighbors the ROI. We found comparable results, with increased activation levels over time [F(1,41)= 5.57, p=0.02] and no significant group-by-time interaction or between-group differences, thus confirming our prior findings.

Multi-region analysis across the reward network

Finally, we assessed whether discontinuing EFV resulted in changes in activation levels in regions other than the ROI involved in reward processing (supplementary fig. 1 in Supplemental Digital Content 2). No between-group changes were found [F(10,32)= 1.80, p=0.10], indicating no additional results. See Supplemental Digital Content 3 for the uncorrected voxel-wise whole-brain activation (p<0.001) during reward anticipation of all participants at baseline and both study groups twelve weeks after switch.

DISCUSSION

To our knowledge, this multicenter RCT was the first investigating the effect of EFV on reward processing using BOLD fMRI. At the behavioral level, apart from the expected improvement in response time and accuracy between rewarding and non-rewarding trials, no differences were found between groups or over time. Secondly, we found that discontinuing EFV did not result in improved ventral striatal responses. Both groups did have a similar significant increase in ventral striatal activation at follow-up assessment. Finally, we established that cortical functioning was not affected by discontinuing EFV.

Multiple *in vitro* studies have demonstrated that EFV is neurotoxic[44,45] and previous BOLD fMRI studies have also reported a negative effect[26,27]. We were therefore surprised that our study found no improvement in behavioral responses after switching from EFV to RPV. Behavioral analyses did show that both groups had faster response times and accuracy during rewarding trials at both assessments. This is consistent with prior studies reporting similar results in both PLWH and healthy controls and ruled out task incomprehension as a possible explanation for this negative result[24,38]. One explanation may be that EFV's neurotoxic effect only affects certain PLWH, depending on their specific characteristics. Our study population consisted of a homogeneous group of asymptomatic men with a relatively high level of education and a median age of 42 years, which may have been characteristics protecting them from EFV's neurotoxic effect. Indeed, the first fMRI study finding a negative effect of EFV was conducted in young adolescents undergoing active neurodevelopment, which may have made them more vulnerable to neurotoxicity[26]. The other was conducted in an older population with longer-diagnosed HIV, both factors which may have also made them more susceptible to neurotoxicity[27]. Additionally, as age itself has a major influence on fMRI brain function, results might not be entirely comparable[36,37,46].

With respect to functional brain activity, we found a significant increase in activation levels related to reward anticipation in its commonly associated subcortical regions (i.e., the ventral striatum) over time in both groups. As this was observed across groups, we believe this can be attributed to a learning effect due to repetition of the fMRI task. However, contrary to our hypothesis, no effect of discontinuing EFV was found. This again was surprising as the aforementioned fMRI studies did find EFV-related functional differences in subcortical regions associated with proactive inhibition and with response inhibition, although the results from the second study were not significant after correction for multiple comparisons[26,27].

It may therefore be that EFV affects specific parts of the fronto-striatal network, as these fMRI studies examined executive functioning, rather than reward processing[26,27]. Previous studies have shown that HIV-infection impairs the fronto-striatal-parietal network, which is

involved in visual attention, working memory and motor control[5,25,47]. These areas may thus be susceptible to EFV in contrast to the reward system, which is located in the ventral fronto-striatal network. Nevertheless, not all studies support this hypothesis, as shown by Payne et al. who assessed attentional processing, which is located in fronto-striatal-parietal network, but found no difference in brain activation after discontinuing EFV[15]. Finally, although our sample size was large for a fMRI study, especially compared to other prospective fMRI studies[15,27], we cannot rule out the possibility that our negative finding was due to too small a sample size.

We found that discontinuing EFV did not affect cortical functioning. This was in line with our hypothesis and previous research showing that HIV-infection primarily affects subcortical functioning[25,37]. Even though chronic antiretroviral therapy has been associated with greater cortical activation, since both study groups had a comparable time on antiretroviral therapy, this should not have confounded our results[48].

The present study has several strengths. The main strength is our design, as conducting a RCT ensured all known and unknown confounders were similar across groups. Other prospective fMRI studies were single-arm and compared participants before and after therapy switch[15,27]. Our control group and longitudinal design allowed us to distinguish learning effects and thus to properly compare the effect of switching to FTC/TDF/RPV versus continuing FTC/TDF/EFV. Moreover, as mentioned, our sample size was large for a prospective bold fMRI study. Lastly, strict inclusion and exclusion criteria meant that known fMRI confounders such as age, gender, drug use and psychiatric disorders were either homogenous in our population or excluded, enabling us to adequately assess EFV with BOLD fMRI.

Certain limitations apply to our study. First, the power calculation was not performed for this sub-analysis, which may have resulted in insufficient power. Additionally, a considerable number of participants were lost to follow-up or excluded from analysis. However, except for one participant from each study group withdrawing due to side effects, the reasons for exclusion or lost to follow-up were unrelated to our determinant or outcome. We therefore believe this did not result in bias.

CONCLUSIONS

This study showed that discontinuing EFV did not lead to improved activity related to reward anticipation in asymptomatic PLWH. It is therefore likely that EFV does not affect motivational control. Further research is needed to determine whether EFV affects motivational control in HIV populations with different characteristics.

Statements

Author contributions

J.A. designed the study. C.H. wrote the study protocol under supervision of J.A.. C.H. and P.P. were responsible for the site work including the recruitment, follow-up and data collection. All authors had access to data. P.O. performed the analysis, interpreted results and drafted the manuscript in close collaboration with S.D.P and B.W. All authors contributed to the interpretation of the data, critically reviewed the manuscript and approved the final manuscript.

Conflicts of interest

J.A. has received advisory board fees from ViiV Healthcare. B.W. has received a research grant and speaker fees from Gilead Health Sciences, has received speaker and advisory board fees from ViiV Healthcare: all fees were paid to the institution. For the remaining authors none were declared.

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SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1. Text explaining the procedures for fMRI image acquisition, pre-processing and initial individual analyses.

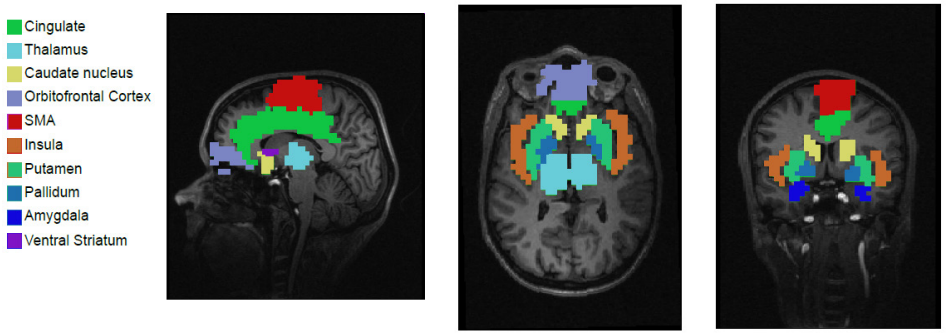
Image acquisition
MRI-scans were acquired using a 3.0T Philips Achieva MRI-scanner (Philips Medical Systems, Best, the Netherlands) in the UMC Utrecht. Head motion was restricted using a vacuum cushion and foam wedges. An eight-channel sensitivity-encoding (SENSE) parallel-imaging head coil was used to acquire the images. Whole-brain T2-weighted echo planar images with BOLD contrast, oriented in a transverse plane tilted 20° over the left-right axis, were acquired in a single run (622 volumes; 30 slices per volume; repetition time 1600ms; echo time 23.5ms; field of view: 208x120x256mm; flip angle = 72.5◦; 64x64 matrix; 4x4mm in-plane resolution; 4mm slice thickness SENSE-factor 2.4 (anterior-posterior). A whole-brain three-dimensional fast field echo T1-weighted scan (185 slices; repetition time 8.4ms; echo time 3.8ms; flip angle 8°; field of view 252x288x185mm; voxel size 1 mm isotropic) was acquired for within-subject registration purposes.

Image pre-processing
Image data were analyzed with SPM (https://www.fil.ion.ucl.ac.uk/spm/). Pre-processing and first-level statistical analyses were performed as described previously[36]. In short, pre-processing included correction for differences in slice timing, realignment to correct for head motion, spatial normalization according to the Montreal Neurological Institute template brain and spatial smoothing to account for inter-individual differences in neuro-anatomy. In addition, head motion parameters were analyzed to ensure that the maximum motion did not exceed a predefined threshold (scan-to-scan >3mm). If this threshold was exceeded, the MRI scan was deemed of insufficient quality and the participant was excluded from the analysis.

Individual analyses
The pre-processed time series data of each individual was analyzed using a general linear model analysis. The model consisted of six factors of interest, representing hemodynamic changes temporally associated to anticipation during and after the presentation of the reward cue (reward anticipation), anticipation during and after a neutral cue (neutral anticipation), feedback indicating a positive monetary reward outcome (reward outcome), feedback indicating no reward, feedback indicating a correct response in a neutral trial (neutral correct outcome) and feedback indicating an incorrect response in a neutral trial (Fig.1). The onset of the factors modelling anticipation was at the presentation of the cue, whereas the onset of the factors modelling feedback (duration 2000ms) was at the presentation of the target, including the button press and the subsequent feedback. Motion parameters from the realignment procedure were included as factors of no interest. Low-frequency drifts were removed from the signal by applying a high-pass filter with a cut-off frequency of 128Hz.

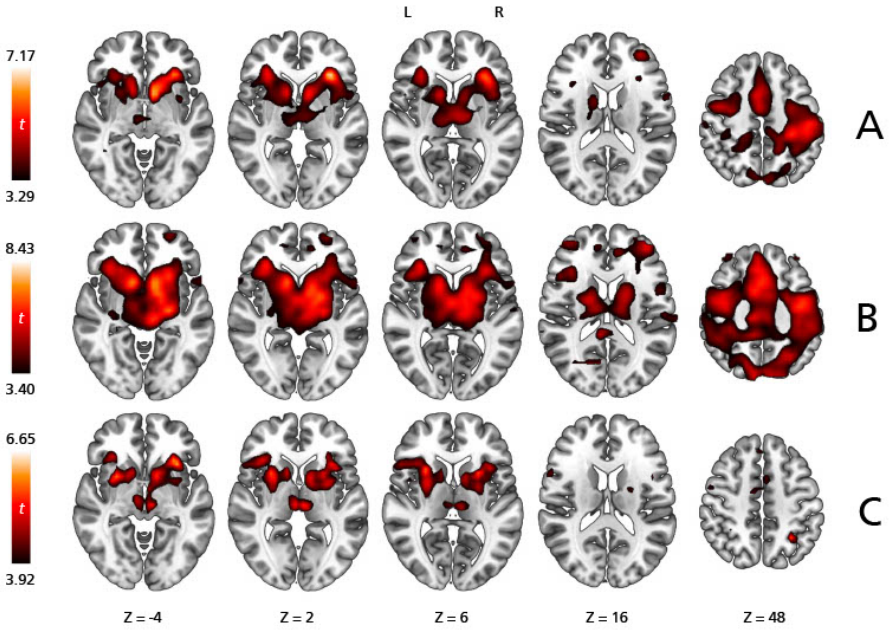
For each participant, we generated statistical maps for each of the conditions, as well as the following contrasts: reward anticipation versus neutral anticipation and reward outcome versus neutral correct outcome.

Supplemental Digital Content 2. Supplementary Figure 1 showing analyzed regions other than the region of interest



Analyzed regions were (1) right cingulate; (2) left cingulate; (3) right thalamus; (4) left thalamus; (5) right caudate nucleus; (6) left caudate nucleus; (7) right orbitofrontal cortex (ROI); (8) left orbitofrontal cortex (ROI); (9) right supplementary motor area (SMA); (10) left supplementary motor area (SMA); (11) right insula; (12) left insula; (13) right putamen; (14) left putamen; (15) right pallidum; (16) left pallidum; (17) right amygdala; (18) left amygdala; (19) right ventral striatum (ROI); (20) left ventral striatum (ROI)

Supplemental Digital Content 3. Whole-brain activation during reward anticipation of participants (all on FTC/TDF/EFV) at baseline **(A)**, participants on FTC/TDF/RPV twelve weeks after switch **(B)** and participants continuing FTC/TDF/EFV twelve weeks after switch **(C),** while performing the Monetary Incentive Delay task. Paired-samples *t*-tests of reward anticipation versus neutral anticipation are displayed on the normalized and skull-stripped group-average brain (neurological orientation) at an activation threshold of p<0.001 (uncorrected voxel-wise).



Abbreviations: L, left; R, right.

6

UNDERLYING NEURAL MECHANISMS OF COGNITIVE IMPROVEMENT IN FRONTO-STRIATAL RESPONSE INHIBITION IN PEOPLE LIVING WITH HIV SWITCHING OFF EFAVIRENZ: A RANDOMIZED CONTROLLED BOLD FMRI TRIAL

Patrick G.A. Oomen
Charlotte S. Hakkers
Joop E. Arends
Guido E.L. van der Berk
Pascal Pas
Andy I.M. Hoepelman
Berend J. van Welzen
Stefan du Plessis

Under review.

ABSTRACT

It is unclear whether neurotoxicity due to the antiretroviral drug efavirenz (EFV) results in neurocognitive impairment in people living with HIV (PLWH). Previously, we found that discontinuing EFV was associated with improved processing speed and attention on neuropsychological assessment. In this study, we investigate potential neural mechanisms underlying this cognitive improvement using a BOLD fMRI task assessing cortical and subcortical functioning. Asymptomatic adult PLWH stable on emtricitabine/tenofovirdisoproxil/efavirenz were randomly (1:2) assigned to continue their regimen (n=12) or to switch to emtricitabine/tenofovirdisoproxil/rilpivirine (n=28). At baseline and after twelve weeks, both groups performed the Stop-Signal Anticipation Task, which tests reactive and proactive inhibition, involving executive functioning, working memory and attention. Behavior and activation levels related to processing speed and attention Z-scores were assessed. Both groups had comparable patient and clinical characteristics. Reactive inhibition behavioral responses improved for both groups on week twelve, with other responses unchanged. For reactive inhibition activation, significant interactions between discontinuing EFV and Z-scores for processing speed and attention were found in six and nine of 17 regions assessed, respectively, the left precuneus remaining significant after multiple comparison correction (p=0.001). Between-group activation was unaffected. Participants switching off EFV showed a relative increase in activation in all interaction regions when improving Z-scores, whereas those continuing EFV showed the opposite effect. Potential neural mechanisms underlying cognitive improvement after discontinuing EFV in PLWH were found in subcortical functioning, with our findings suggesting that EFV's effect on attention and processing speed is, at least partially, mediated by reactive inhibition.

INTRODUCTION

Although the advent of combination antiretroviral therapy (cART) has resulted in a significant increase in the life expectancy of people living with HIV (PLWH), the burden of disease due to comorbidities remains substantial.[1,2] A common comorbidity with a major impact on quality of life is the presence of HIV-associated neurocognitive disorders (HAND).[3,4] The etiology of HAND has not been fully elucidated, but one suggested mechanism is neurotoxicity due to antiretroviral therapy itself.[5] One of the antiretroviral agents most often implicated is efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI).[6] EFV is currently recommended in World Health Organization guidelines as alternative first-line treatment, is still one of the most prescribed antiretroviral drugs globally and is expected to continue to be widely used, with forecast analyses showing ten million PLWH (i.e., 25% of the total HIV-positive population) using EFV in 2025.[7,8] Despite its widespread use, EFV is notorious for neurocognitive side effects such as dizziness or insomnia and has frequently been associated with neurocognitive impairment, although the latter remains a topic of debate as studies report conflicting findings.[9–15]

Neurocognitive impairment due to HAND is traditionally investigated by neuropsychological assessment (NPA). In the ESCAPE-trial, we showed that discontinuing EFV in asymptomatic PLWH resulted in an improvement in the cognitive domains attention and processing speed, as assessed by NPA.[16] However, the NPA findings reflect an overall effect in cognitive domains and it therefore remains unclear which neural mechanisms in the brain underlie the cognitive improvement. One way to assess this is blood oxygenated level dependent (BOLD) functional magnetic resonance imaging (fMRI). BOLD fMRI can detect local changes in cerebral blood flow and oxygenation that reflect regional neuronal activity. It has been shown to be more sensitive in assessing the impact of cART on neurocognition than NPA alone, as it can reliably detect early changes in the brain in the absence of symptomatic neurocognitive impairment.[17–19] Therefore, by using both diagnostic modalities, it is possible to link cognitive changes assessed by NPA to more localized BOLD fMRI findings and investigate potential underlying neural mechanisms of neurocognitive improvement.

Previous research has shown that HIV-infection primarily impairs the fronto-striatal network and, more specifically, subcortical functioning.[17,20] Combined with EFV's propensity for neurocognitive side effects and demonstrated *in vitro* neurotoxicity, the fronto-striatal network may thus be at increased risk for additional neurotoxic damage.[21,22] Prior studies support this hypothesis, showing altered fronto-striatal activation after discontinuing EFV in both adult and adolescent PLWH.[23,24] The Stop-Signal Anticipation Task (SSAT) is an event-related fMRI task that has been shown to reliably test executive functioning, working memory and attention.[25,26] It tests response inhibition, one of the main functions of the fronto-striatal network, which reflects the ability to suppress irrelevant or

interfering information or impulses. [25,27] It consists of several sub-processes, such as motor execution, outright stopping as an immediate reaction to a STOP-signal (i.e., reactive inhibition) and proactive anticipation of stopping (i.e., proactive inhibition), with reactive and proactive inhibition indicative of subcortical and cortical functioning, respectively.[25,27]

We hypothesized that, due to HIV-infection impairing subcortical functioning and rendering it potentially susceptible to neurotoxic damage of EFV, subcortical and not cortical functioning would be affected. A potential neural mechanism underlying the cognitive improvement observed for attention and processing speed after discontinuing EFV might therefore be found in improved reactive inhibition. To investigate this, we performed a sub-analysis of the ESCAPE trial and combined task-based BOLD fMRI, in the form of the SSAT, with NPA findings. Participants stable on the single-tablet regimen emtricitabine/tenofovirdisoproxil fumarate/efavirenz (FTC/TDF/EFV) were randomly allocated to continue FTC/TDF/EFV or to switch to emtricitabine/tenofovirdisoproxil fumarate/rilpivirine (FTC/TDF/RPV). As our entire study population used EFV at the onset of the trial, we specifically studied the effect of EFV by discontinuation in one group. NPA and BOLD fMRI scans were performed at baseline and after twelve weeks.

MATERIALS AND METHODS

Participants

The present study is a sub-analysis of the ESCAPE (Effect of SwitChing AtriPla to Eviplera on neurocognitive and emotional functioning) trial, which was conducted at two major HIV treatment centers in the Netherlands (OLVG (Amsterdam) and Universitair Medisch Centrum Utrecht (Utrecht)) from 2015 until its completion in 2017**.**[16] Strict in- and exclusion criteria were chosen to ensure a homogenous study population as PLWH exhibit greater variability with respect to fMRI measurements and fMRI results can be readily influenced by confounding factors.[28,29] To summarize, asymptomatic male PLWH aged 25-50 years stable on FTC/TDF/EFV for over six months were included. Prospective participants were excluded in case of an active psychiatric or neurological disorder, an active or past central nervous system infection, or a history or evidence of alcohol or drug abuse as assessed by the Drug Abuse Screening Test.[30] During the trial, participants with a viral load (VL) of >200 copies/mL were excluded from analysis, as we judged this might interfere with fMRI results. For the full list of in- and exclusion criteria, see the published study.[16]

The trial was reviewed and approved by the Medical Research Ethics Committee of the UMC Utrecht, performed in accordance with the Declaration of Helsinki and registered at Clinicaltrials.gov [NCT02308332]. Findings were reported in accordance with the CONSORT guideline.[31] The trial was funded by Gilead Sciences. The funder had no role

in trial design, data collection or analysis, or in the preparation of the manuscript. Data were collected by the investigators with the use of case-report forms. All participants provided written informed consent. The data and corresponding analysis code that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Study design and procedures

Participants on FTC/TDF/EFV were randomly assigned in a 2:1 ratio, using computer-generated block randomization with a variable block size (range 3-9), to switch to FTC/TDF/RPV or to continue taking FTC/TDF/EFV. A study nurse, not involved in the study, generated the random assignment sequence and allocated participants. FTC/TDF/RPV was chosen for the switch group as it is a single-tablet regimen comprised of the same backbone and a similar NNRTI anchor drug as FTC/TDF/EFV. Participants were instructed to take one tablet daily and, in the case of FTC/TDF/RPV, with a significant amount of food. The NPA was performed by neuropsychologists who were unaware of the assigned treatment. Researchers performing the fMRI-scan and participants were not blinded, as we believed that their knowledge of the treatment would not affect our objective outcome of fMRI brain activity.

All participants had fMRI-scans at baseline and after twelve weeks. The MRI-scans were reviewed by a radiologist for intracranial pathology. Cognition was examined by way of NPA and it was ascertained whether the distribution of potentially confounding asymptomatic neurocognitive impairment, as defined by the Frascati-criteria, was comparable between groups.[3] Routine blood samples were obtained to assess laboratory abnormalities and confirm virologic suppression. Participants switching to FTC/TDF/RPV had two additional routine outpatient visits after two and four weeks to monitor for side effects and obtain blood samples. Lastly, participants completed multiple questionnaires at baseline and week twelve, including the Hospital Anxiety and Depression Scale (HADS) and Patient Reported Outcome Measurement Information System (PROMIS) questionnaires testing depression, anxiety and sleep disorders. The HADS questionnaire consisted of a 7-item scale with a maximum of 21 points, with score of 11 points or more indicating a probable mood disorder. The raw PROMIS questionnaire scores for depression, anxiety and sleep disorders were transformed into T-scores with a mean of 50 and a SD of 10. For full information on these and other study questionnaires used, see the published study.[16]

NPA

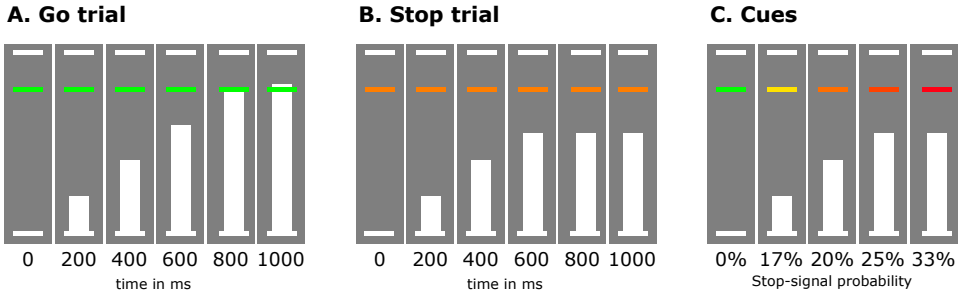
The NPA consisted of 16 subtests and tested for seven cognitive domains.[16] The tests were specifically selected to detect minimal changes in neurocognitive performance, as our study population was asymptomatic. For attention and processing speed, the Letter-Number-Sequencing WAIS-IV NL, Paced Auditory Serial Addition Test, Digit Symbol WAIS-IV NL, Symbol Search WAIS-IV NL and Trailmaking Test part A were used.[32–34]

Stop-Signal Anticipation Task

Participants performed the SSAT, a task based on the theory by Logan and Cowan.[25,35] They postulated that a response, either starting or stopping, is the result of a race between the 'GO' and 'STOP' brain processes. If the STOP process is finished before the GO process reaches the execution threshold, the GO response is stopped.

The task and experimental procedures are the same as previously described by Zandbelt & Vink [25]. The experiment was performed using Presentation® software (Version 14.6, www.neurobs.com). In short, participants were presented with three background lines (Fig. 1). On each trial, a bar moved at a constant speed from the bottom towards the top bar, reaching the middle line in 800ms. On GO-trials, participants were asked to stop the bar as close as possible to the middle line, by pressing a button. If the bar passed the top line after 1000ms, the GO-trial was considered a failure. STOP-trials were identical to GO-trials, except that the bar stopped moving automatically before the middle bar, indicating a STOP-signal. Participants were then required to withhold the button press (i.e., reactive response inhibition). To measure proactive response inhibition, the probability that a STOP-signal would appear was manipulated across trials and could be anticipated on the basis of the color of the middle line. There were five STOP-signal probability levels: 0% (green), 17% (yellow), 20% (amber), 25% (orange), and 33% (red). The interval between start of a trial and the STOP-signal, the stop-signal delay (SSD), was initially 550ms and varied for each STOP-signal according to the participant's performance. In case of a successful STOP-trial, the trial difficulty was increased as the SSD was raised by 25ms. If the STOP-trial was unsuccessful, the SSD was reduced with the same time limit, ensuring an equal amount of successful and unsuccessful STOP-trials. The inter-trial interval was kept at 1000 ms. In total, 414 GO-trials (0%, n = 234; 17%, n = 30; 20%, n = 48; 25%, n = 54; 33%, n = 48) and 60 STOP-trials (17%, n = 6; 20%, n = 12; 25%, n = 18; 33%, n = 24) were presented in a single run-in pseudorandom order.

Figure 1. Schematic representation of the Stop-Signal Anticipation task



Three horizontal lines were displayed during the task. A bar moved from the bottom line to the top in 1000 ms. At 800 ms the bar reached the middle colored line and had to be stopped (GO-trials: A). In a small proportion of trials, the bar stopped moving on its own before reaching the middle colored line, requiring the stop response to be withheld (STOP-trials, B). The color of the middle line indicated the stop-signal probability (C).[25]

All participants received standardized training in task performance before scanning. They were instructed that the GO- and STOP-trials were equally important and that it would not always be possible to suppress a response when a STOP-signal occurred. We informed them that a STOP-signal would never occur on a trial with a green cue and that they were more likely in the context of, in consecutive order, yellow, amber, orange and red cues. The total task duration was 16 m 36 s.

Behavioral data analysis

Motor execution was studied using the response time and accuracy of GO-trials with no possibility of a STOP-signal (0%). Reactive inhibition was analyzed using the stop signal reaction time (SSRT), which was computed according to the integration method and calculated across all STOP-signal probability levels (17–33%).[35] The SSRT reflects the latency of the inhibition process and better reactive inhibition is indicated by a smaller SSRT.

Proactive inhibition is the anticipation of stopping based on the STOP-signal probability and was measured as the slope of the mean response time to increasing STOP-signal probability (0-33%). In general, participants slow their response as the STOP probability increases, resulting in larger response times. When proactive inhibition is impaired, participants thus show a reduced effect of the STOP-signal probability on their response times, reflected by a less steep slope.[25] Repeated measures ANOVA were conducted on the mean response times, response accuracy and on the slope of the response time to stop-signal probability, with the STOP-signal probabilities, group (FTC/TDF/RPV versus FTC/TDF/EFV) and time (baseline versus twelve weeks) as factors.

Functional MRI

Image acquisition

MRI-scans were acquired using a 3.0T Philips Achieva MRI-scanner (Philips Medical Systems, Best, the Netherlands) in the UMC Utrecht. An eight-channel sensitivity-encoding (SENSE) parallel-imaging head coil was used to acquire the images. Head motion was restricted using a vacuum cushion and foam wedges. Whole-brain T2-weighted echo planar images with BOLD contrast, oriented in a transverse plane tilted 20° over the left-right axis, were acquired in a single run (622 volumes; 30 slices per volume; repetition time 1600ms; echo time 23.5ms; field of view: 256x208mm x256mm; flip angle=72.5◦; 64x64 matrix; 4x4mm in-plane resolution; 4mm slice thickness SENSE-factor 2.4 (anterior-posterior)). We discarded the first six images to allow for T1 equilibration effects. A whole-brain three-dimensional fast field echo T1-weighted scan (185 slices; repetition time 8.4ms; echo time 3.8ms; flip angle 8°; field of view 288x252x185mm; voxel size 1mm isotropic) was acquired for within-subject registration purposes.

Image pre-processing

Image data were analyzed with SPM (https://www.fil.ion.ucl.ac.uk/spm/). Pre-processing and first-level statistical analyses were performed as described previously.[25] In short, pre-processing included correction for differences in slice timing, realignment to correct for head motion, spatial normalization according to the Montreal Neurological Institute template brain and spatial (8x8x8mm) smoothing to account for inter-individual differences in neuro-anatomy. Head motion parameters were analyzed to ensure that the maximum motion did not exceed a predefined threshold (scan-to-scan >3 mm).[36] If this threshold was exceeded, the MRI-scan was considered of insufficient quality and the participant was excluded from the analysis.

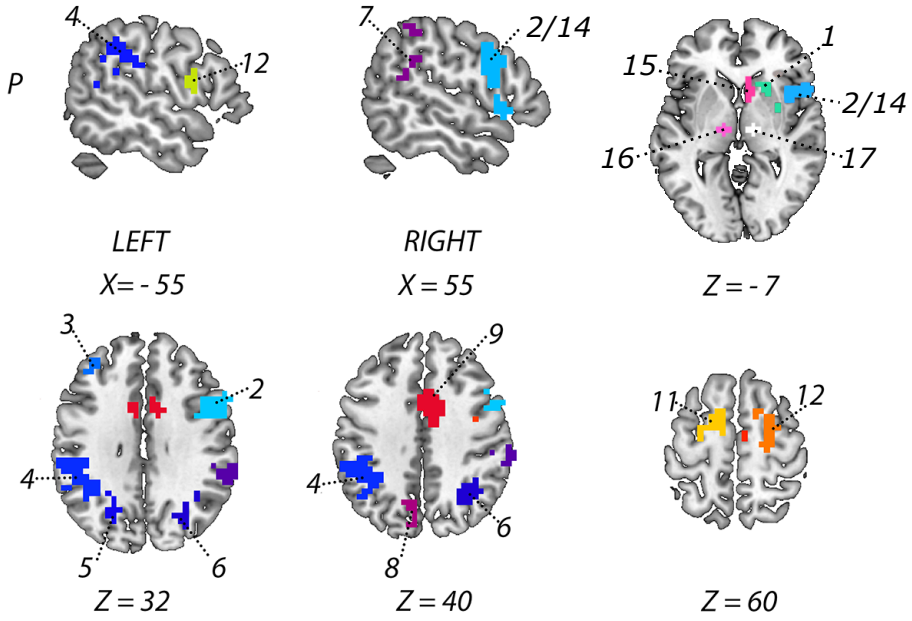
Individual analyses

Each participant's pre-processed fMRI data were high-pass filtered (cut-off 128 Hz) to remove low-frequency drifts and were modelled voxel-wise using a general linear model. The following events were included as regressors: Timed GO-trials with STOP-signal probability above 0%, successful STOP-signal trials and unsuccessful STOP-signal trials. For the GO-trials with a STOP-signal probability above 0%, we included a parametric regressor modelling the STOP-signal probability level and variation in response time. In addition, GO-trials with 0% STOP-signal probability and activity were also modelled. We computed two contrast images for each participant: activation during successful STOP-trials versus unsuccessful STOP-trials (to assess reactive inhibition) and the parametric effect of STOP-signal probability on GO-trial activation (to assess proactive inhibition).

Region-of-interest analyses

Differences in activation between groups were assessed in pre-defined regions-of-interest (ROIs), using mask-based activation maps acquired in a previous experiment in healthy controls performing the same task (Fig. 2).[25] These 17 ROIs were defined using a cluster-level threshold (cluster-defining threshold of $p<0.001$, cluster probability of $p<0.05$, family-wise error corrected for multiple comparisons). Mean activation levels during reactive and proactive inhibition were calculated over the ROIs as defined by the a-priori masks. For each ROI, activation levels for the interaction between discontinuing EFV and processing speed and attention NPA Z-scores was assessed using an analysis of variance (ANOVA). Group-wise differences between baseline and twelve weeks after therapy switch were also assessed. All statistical tests were corrected for multiple comparisons using the Bonferroni method. A two-sided alpha level of 0.05 was used and statistical analyses were conducted using SPSS version 25.0 (IBM Corp. Armonk. NY).

Figure 2. Regions used to assess activation levels related to reactive and proactive inhibition after discontinuing EFV



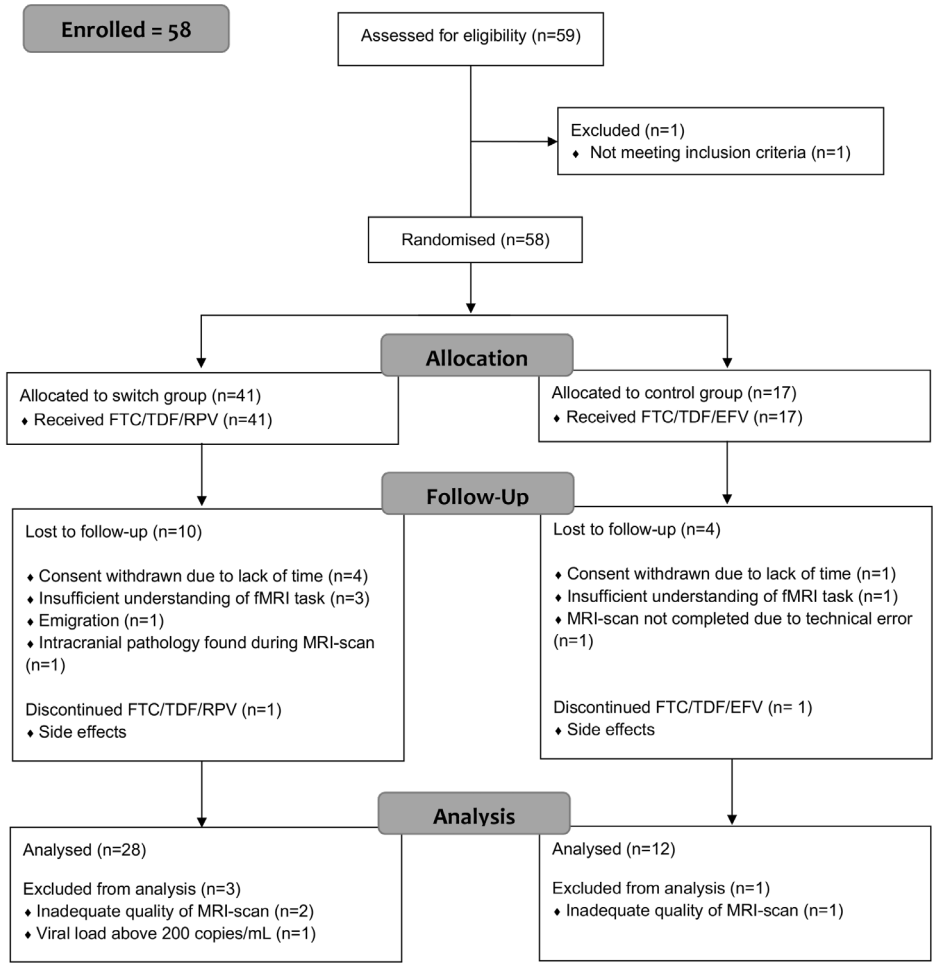
Regions were (1) right striatum; (2) right inferior frontal cortex ventral; (3) left middle frontal gyrus; (4) left temporoparietal junction; (5) left superior parietal gyrus; (6) right superior parietal gyrus; (7) right temporoparietal junction; (8) left precuneus; (9) anterior cingulate gyrus; (10) right superior frontal gyrus; (11) left superior frontal gyrus; (12) left inferior frontal gyrus; (13) right anterior insula; (14) right inferior frontal cortex dorsal; (15) right caudate; (16) left subthalamic nucleus; (17) right subthalamic nucleus.

RESULTS

Demographics

A total of 59 potential participants were screened for inclusion, of which one participant was a screen failure due to not meeting eligibility criteria (Fig. 3). The remaining 58 participants were randomized (2:1), with 41 assigned to the switch group and 17 to the control group. Of these, 18 participants were lost-to-follow-up or excluded from analysis for reasons indicated in the flowchart, leaving 28 and 12 participants in the switch and control group for analysis. These 40 participants had a median age of 43.37 (IQR 35.47 – 47.23) years, were all male and had 16.50 (IQR 16.00 – 17.00) median years of education (Table 1). The median time since HIV and on EFV was, respectively, 96.80 (IQR 62.02 – 121.15) and 62.00 (IQR 32.53 – 78.00) months, with 38 (95%) and 39 (97.5%) of participants virologically suppressed at baseline and after twelve weeks.

Figure 3. Trial flow chart of participants enrolled and included in our analysis



Abbreviations: EFV, efavirenz; fMRI, functional magnetic resonance imaging; FTC, emtricitabine; RPV, rilpivirine; TDF, tenofovirdisoproxil fumarate.

Table 1. Characteristics of the study participants according to study arm.

	FTC/TDF/RPV (switch)		FTC/TDF/EFV (control)	
Demographics	**n = 28**	**(IQR) / (%)**	**n = 12**	**(IQR) / (%)**
Age (years)	42.68	35.47 – 47.23	44.19	36.64 – 48.20
Gender (male)	28	100	12	100
BMI (kg/m2)	24.90	22.05 – 27.13	25.25	22.31 – 26.68
Education (years)	16.00	16.00 – 17.00	17.00	16.00 – 17.75
Clinical characteristics				
Time since HIV diagnosis (months)	95.95	49.95 – 118.02	102.50	74.45 – 137.94
Time on EFV (months)[a]	64.00	33.93 – 77.90	55.00	22.60 – 86.00
Time on cART (months)[a]	64.00	38.82 – 77.90	55.00	22.60 – 86.00
ANI at baseline	7	25.00	3	25.00
Co-medication				
0	17	60.7	7	58.3
1	8	28.6	4	33.3
2 or more	3	10.7	1	8.3
Biochemical characteristics				
Nadir CD4 (cells/mm^3)[a]	299.50	220.50 – 341.25	241.00	145.25 – 367.50
Baseline CD4	619.50	470.50 – 804.25	688.00	547.00 – 783.75
Baseline VL <50 (copies/mL)	27	96.4	11	91.7
Baseline VL 50-200	1	3.6	1	8.3
CD4 at week 12[a]	650.50	520.50 – 740.00	638.00	566.00 – 820.25
VL<50 at week 12	28	100	11	91.7
VL 50-200 at week 12	0	0	1	8.3
Baseline questionnaire results		**(SD)**		**(SD)**
HADS – anxiety [a]	2.92	2.63	3.27	3.35
HADS – depression [a]	1.92	2.78	2.36	3.11
PROMIS – anxiety [a]	46.70	7.35	48.25	6.97
PROMIS – depression [a]	45.70	8.24	45.73	9.99
PROMIS – sleep disorder [a]	47.63	8.16	45.59	5.78

All categorical data are expressed as frequency (percentage) and continuous data are expressed as median (interquartile range) or mean (standard deviation).

[a.] Missing data: time on EFV (1 control (2.5%)), time on cART (1 control (2.5%)), nadir CD4 (2 switch participants (5.0%)), CD4 at week 12 (2 switch participants (5.0%)), HADS questionnaire (1 control (2.5%) and 3 switch participants (7.5%)), PROMIS questionnaire (1 control (2.5%) and 2 switch participants (5.0%)).

Abbreviations: ANI, asymptomatic neurocognitive impairment (according to the Frascati criteria(Antinori et al., 2007)); BMI, body mass index; cART, combination antiretroviral therapy; FTC/TDF/EFV, emtricitabine/ tenofovirdisoproxil fumarate/ efavirenz; FTC/TDF/RPV, emtricitabine/ tenofovirdisoproxil fumarate/ rilpivirine; HADS , Hospital Anxiety and Depression Scale; PROMIS, Patient Reported Outcome Measurement Information System; VL, viral load.

Behavioral analyses

Motor execution

In order to assess the effect of discontinuing EFV on response inhibition, we first evaluated motor execution in the two groups. Response time for baseline GO-trials (with a STOP-signal probability of 0%) was similar between groups at both time points [$F(1,38)=0.65$, $p=0.43$], with no group-by-time interaction effect [$F(1,38)=0.44$, $p=0.51$] nor main group effect [$F(1,38)=0.38$, $p=0.54$]. Similar results were found for response accuracy.

Reactive inhibition

Next, we evaluated reactive inhibition behavioral outcomes after discontinuing EFV. Both groups responded significantly faster during incorrect STOP-trials compared to successful GO-trials at both time points [F(1,38)=126.33, p<0.001]; [F(1,38)=103.34, p<0.001], indicating that the underlying assumption of the SSAT task (i.e., the race between the 'STOP' and 'GO' brain processes model) was valid.[35] Surprisingly, we found that the speed of reactive inhibition (SSRT) improved in both groups over time [F(1,38)=6.84, p=0.01], with the mean SSRT of the switch and control groups decreasing by 8.61ms and 6.80ms, respectively. There was no group-by-time interaction [F(1,38)=0.09, p=0.76] nor main group effect [F(1,38)=1.30, p=0.26]. Response accuracy of pooled STOP-trials was also found to improve for both groups [F(1,38)=4.28, p=0.05], but no group-by-time interaction effect [F(1,38)=0.17, p=0.68] nor main group effect [F(1,38)=0.04, p=0.84] was observed. The latter result was expected as we manipulated the stop-signal delay according to individual performance to ensure a similar number of successful trials.

Proactive inhibition

We then examined proactive inhibition behavioral outcomes. A significant main effect of STOP probability was found at both time points [F(2.39,93.19)=12.26, p<0.001]; [F(1.69,65.82)=6.10, p=0.01], indicating participants adequately performed the task by slowing their response with increased probability for a STOP-signal. No main effect on proactive inhibition was found, as the slopes of the mean response times in STOP-trials with an increasing STOP probability were similar over time [F(1,38)=0.079, p=0.78]. Additionally, there was no group-by-time interaction effect [F(1,38)=0.12, p=0.73] nor main group effect [F(1,38)=0.95, p=0.34].

Functional ROI analyses

Reactive inhibition

Afterwards, we assessed reactive inhibition activation in the 17 ROIs for the interaction between discontinuing EFV and attention Z-score changes using ANOVA. A significant interaction was found in the left precuneus after Bonferonni correction (p=0.001). Several additional regions also showed a significant interaction effect when left uncorrected, including the right inferior frontal ventral and dorsal cortex, left middle frontal gyrus, left and right temporoparietal junction, left and right superior parietal gyrus and right anterior insula (Table 2). The relationship between group and attention Z-score was consistent for all interaction regions: an increase in activation levels in participants continuing EFV was associated with a decrease in Z-score, i.e., cognitive outcome, whereas those switched to RPV showed the opposite effect (Fig. 4).

For processing speed Z-scores, ANOVA analyses revealed interaction effects in the right superior frontal gyrus, right temporoparietal junction, anterior cingulate gyrus, right anterior insula, right inferior frontal gyrus and right subthalamic nucleus, but none remained after

Bonferonni correction. Between-group activation differences were found in the right anterior insula, but these also did not survive correction for multiple testing.

Proactive inhibition

Finally, we assessed proactive inhibition activation after discontinuing EFV in the two groups. ANOVA analyses revealed no interactions with attention or processing speed NPA Z-score changes over time nor between-group differences after discontinuing EFV.

Table 2. Results of ANOVA analysis for reactive inhibition activation per ROI for the interaction between discontinuing EFV and change in attention or processing speed NPA Z-scores.

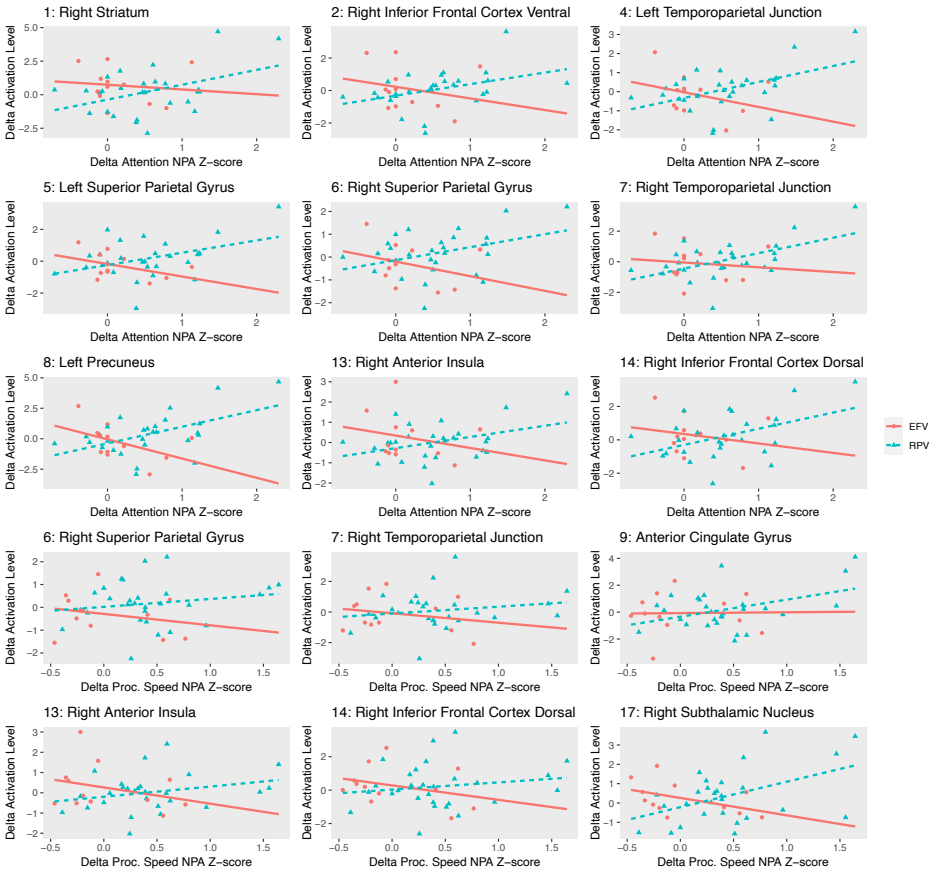
	Group * delta attention		Group * delta processing speed	
ROIs	***F***	***p-value***	***F***	***p-value***
(1) Right striatum	.297	.590	2.577	.118
(2) Right inferior frontal cortex ventral	4.503	.042*	3.922	.056
(3) Left middle frontal gyrus	5.146	.030*	1.712	.200
(4) Left temporoparietal junction	9.679	.004*	1.848	.184
(5) Left superior parietal gyrus	6.041	.020*	2.753	.107
(6) Right superior parietal gyrus	7.375	.011*	4.320	.046*
(7) Right temporoparietal junction	6.646	.015*	5.461	.026*
(8) Left precuneus	12.865	.001**	1.128	.296
(9) Anterior cingulate gyrus	1.635	.210	4.184	.049*
(10) Right superior frontal gyrus	.280	.600	.564	.458
(11) Left superior frontal gyrus	.366	.549	.020	.888
(12) Left inferior frontal gyrus	.651	.426	1.349	.254
(13) Right anterior insula	4.449	.043*	5.089	.031*
(14) Right inferior frontal cortex dorsal	4.986	.033*	4.199	.049*
(15) Right caudate	3.032	.091	3.671	.064
(16) Left subthalamic nucleus	.002	.961	1.953	.172
(17) Right subthalamic nucleus	.001	.970	4.837	.035*

*Results significant at a p=0.05 value

**Result significant after Bonferonni correction

Significant interactions (at p <0.05 level) were found in 9/17 and 6/17 ROIs between discontinuing EFV and difference in attention and processing speed Z-score between baseline and after twelve weeks (delta), respectively. After Bonferonni correction, a significant interaction was found in the left precuneus between discontinuing EFV and the delta attention of Z-score.

Figure 4. Scatter plots of regions with significant interations showing the difference (delta) in activation levels and attention and processing speed NPA Z-scores between baseline and after twelve weeks by study group



Trend lines by study group show the switch group (who discontinued EFV) having a relative increase in activation when improving Z-scores, whereas those continuing EFV show the opposite effect. No significant differences were found in other regions.
Abbreviations: EFV, efavirenz; NPA, neuropsychological assessment; PS, processing speed;; RPV, rilpivirine.

DISCUSSION

This multicenter BOLD fMRI RCT was, to our knowledge, the first to investigate underlying neural mechanisms of cognitive improvement after discontinuing EFV in fronto-striatal response inhibition by combining NPA and BOLD fMRI findings. Potential neural mechanisms were found in subcortical functioning, with functional imaging revealing altered reactive inhibition activation upon improving attention and processing speed in multiple regions after discontinuing EFV. Participants switching off EFV showed a relative increase in activation in all interactions regions when improving Z-scores, while those who continued EFV showed

the opposite effect. Reactive inhibition responses improved for both groups, whereas other responses did not change. No group-wise differences were found after discontinuing EFV and, for proactive inhibition, no interactions were found.

Consistent with our hypothesis that EFV would affect subcortical functioning, no difference in motor execution and proactive inhibition behavioral outcomes was found after stopping EFV. However, to our surprise, we found that for reactive inhibition both groups improved, in the form of improved SSRTs and response accuracy. Although response accuracy can be practiced, thus seeming a logical explanation, the SSRT cannot be improved through repetition. A previous BOLD fMRI-study in PWLH switching from EFV to RPV also observed improved SSRTs and suggested this might reflect a detrimental effect of EFV.[23] However, since this was a before-after study without a control group continuing EFV, our results raise the question of whether the improvement can truly be attributed to EFV, as both our study groups – regardless of EFV switch - improved over time. Another BOLD fMRI-study, though cross-sectional in nature and conducted in adolescent PLWH, showed similar SSRTs for those on EFV versus other antiretroviral agents, providing further evidence of EFV most likely not affecting subcortical functioning behavioral outcomes.[24] Finally, it is possible that the SSRT improvement is unrelated to HIV, but since our study did not include a HIV-negative population, we were unable to investigate this. Although we are unsure why the SSRT improved, we believe it is not due to discontinuing EFV and therefore unrelated to our research question.

In line with our hypothesis, we found significant interactions for reactive inhibition activation between discontinuing EFV and attention NPA Z-score changes, with over half of ROIs showing significant results and the left precuneus remaining significant after Bonferonni correction. For processing speed, significant interactions were found in six of the 17 regions, but none survived correction for multiple testing. Interestingly, all participants who continued EFV and improved in Z-scores showed decreasing activation levels in interaction ROIs compared to increasing activation levels after discontinuing EFV. These findings suggest that the neurocognitive changes observed in NPA after discontinuing EFV are, at least in part, mediated by reactive inhibition. Previous research had already shown that HIV-infection and cART in general impair neurocognitive systems related to attention and processing speed and here we show that reactive inhibition is involved in this process. [37–39] However, not all ROIs showed significant interactions, suggesting that either EFV selectively impacts fronto-striatal regions involved in attention or processing speed or, importantly, that our sample size was ultimately not large enough to detect all differences. As our power calculation was performed for another outcome, this may have led to lack of power for detecting all differences in activation levels. This could also be the case for group-wise differences, of which we found none except the right anterior insula that did not survive correction for multiple testing. Nevertheless, the fact that we do find interactions

clearly demonstrate EFV-induced changes in reactive inhibition, but additional research with larger sample sizes is needed to further explore whether these neural mechanisms may serve as markers for neurocognitive impairment.

Proactive inhibition activation was unaffected after discontinuing EFV. Although the aforementioned study evaluating EFV found altered proactive inhibition, it was conducted in adolescent PLWH undergoing active neurodevelopment.[24] Our population consisted of adult asymptomatic men with a longer time since HIV diagnosis and higher level of education and we therefore hypothesized that not cortical but subcortical functioning – since studies already demonstrated this to be impaired by HIV-infection – would be affected, which our findings confirmed.[20,40]

The main strength of our study lies in its design, as our RCT design ensured that all known and unknown confounders were similar across groups. Moreover, our control group and longitudinal design allowed us to distinguish practice effects and adequately compare the effect of switching off EFV versus continuing EFV. Furthermore, we used strict in- and exclusion criteria which ensured that known fMRI confounding due to variability in gender, age, psychiatric disorders and drug use was homogenous across participants or reason for exclusion.

Our study has several limitations besides the aforementioned sample size, although ours was still relatively large for a fMRI study, particularly compared to other prospective fMRI studies[13,23]. A substantial number of participants were lost to follow-up or excluded from analysis, which may have contributed to any lack of power. However, only two participants withdrew due to side effects and other reasons for loss to follow-up or exclusion were not related to our determinant or outcome, leading us to believe this did not result in bias.

CONCLUSIONS

This study found evidence of potential neural mechanisms underlying cognitive improvement after discontinuing EFV in PLWH in subcortical functioning. Our findings suggest that EFV's effect on attention and processing speed is, at least partially, mediated by reactive inhibition and thus affects these key subcortical areas involved in executive functioning, working memory and attention.

Statements

Author contributions

J.A. designed the study. C.H. wrote the study protocol under supervision of J.A.. C.H. and P.P. were responsible for the site work including the recruitment, follow-up and data collection. All authors had access to data. P.O. performed the analysis, interpreted results and drafted the manuscript in close collaboration with S.D.P and B.W. All authors contributed to the interpretation of the data, critically reviewed the manuscript and approved the final manuscript.

Conflicts of interest

J.A. has received advisory board fees from ViiV Healthcare. B.W. has received a research grant and speaker fees from Gilead Sciences, has received speaker and advisory board fees from ViiV Healthcare: all fees were paid to the institution. For the remaining authors none were declared.

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PART II

Optimizing treatment and monitoring of HIV-associated co-morbidities

7

NO LONG-TERM EFFECT OF PAST *PNEUMOCYSTIS JIROVECII* PNEUMONIA ON PULMONARY FUNCTION IN PEOPLE WITH HIV

Patrick G.A. Oomen
Inez Bronsveld
Andy I.M. Hoepelman
Berend J. van Welzen
Tania Mudrikova

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ABSTRACT

Objective

To assess the impact of past *Pneumocystis jirovecii* pneumonia (PJP) on the pulmonary diffusion capacity in people with HIV (PWH) with a history of advanced immunodeficiency.

Design

Prospective cross-sectional study.

Methods

Adult PWH with past PJP >1 year ago were included as the study group. The control group consisted of PWH with a nadir $CD4^+$ lymphocyte count <200 cells/mm^3, matched by age, sex, smoking status and time since HIV diagnosis. All PWH completed a pulmonary function test (PFT) consisting of pre-bronchodilation spirometry, body plethysmography and single-breath carbon monoxide transfer factor (TLCO) measurement. TLCO, diffusion impairment (defined as a TLCO Z-score <-1.645), total lung capacity (TLC) and forced expiratory volume in one second/forced vital capacity (FEV1/FVC) Z-scores were assessed. Multivariable regression analyses were conducted with Z-scores and odds of diffusion impairment as outcomes.

Results

PFTs of 102 participants were analyzed, 51 of whom had past PJP with a median of ten years since PJP. Mean TLCO Z-score and diffusion impairment rate did not differ significantly between groups (p=0.790; p=0.650). Past PJP was not independently associated with TLCO Z-score (β 0.14; 95% confidence interval (CI) -0.30 – 0.57), diffusion impairment (odds ratio 1.00; 95% CI 0.36 – 2.75) nor TLC or FEV1/FVC Z-scores, whereas current (*vs.* never) smoking was associated with more diffusion impairment and lower TLCO Z-scores.

Conclusion

In our study, past PJP was not associated with long-term diffusion impairment. Our findings suggest that smoking plays a more important role in persistent pulmonary function impairment whereas PJP-related changes seem to be reversible.

INTRODUCTION

Pneumocystis jirovecii pneumonia (PJP) is one of the most common opportunistic infections in people with HIV (PWH) and advanced immunodeficiency[1], leading to severe hypoxemia due to diffusion capacity impairment.[2] The introduction of combination antiretroviral therapy and improved PJP treatment strategies have led to a significant decline in mortality from PJP, but it is unclear whether pulmonary function abnormalities completely resolve in this population.[3,4] Older, short-term data suggest that diffusion impairment persists in a significant percentage of PWH[5–8], but the long-term consequences of past PJP remain largely unknown.

Establishing the potential long-term impact of PJP on pulmonary function, and more specifically the diffusion capacity, is clinically relevant, as the background prevalence of diffusion impairment in PWH is high, likely due to prevalent smoking and HIV infection itself.[9–13] In this study, we evaluated the pulmonary function in PWH with previous advanced immunodeficiency with and without past PJP.

METHODS

Study design and population

We performed a prospective, single-center, cross-sectional analysis of pulmonary function in adult PWH with a history of advanced immunodeficiency – defined as a nadir $CD4^+$ lymphocyte count <200 cells/mm^3, all under follow-up at the University Medical Center Utrecht (UMCU), the Netherlands. We chose to include only PWH with a history of advanced immunodeficiency as control group as earlier research identified lower $CD4^+$ counts as predictors for diffusion impairment, possibly due to damage caused by HIV-associated immune activation or not clinically apparent opportunistic pulmonary infections.[10,13] The study group consisted of PWH with a history of PJP >1 year ago; PWH in the control group had no previous PJP. Both groups were matched regarding age, sex at birth, smoking status and time since HIV diagnosis to limit confounding. All participants underwent standardized pulmonary function testing. To ensure we did not miss pulmonary impairment from PJP that was misclassified as COPD due to smoking, COPD was deliberately not chosen as an exclusion criterion. Exclusion occurred in case of active pulmonary infection, cardiac decompensation, onset of unexplained dyspnea, tachypnea or other respiratory complaints within three weeks before PFT and pre-existent conditions unrelated to PJP but known to impact pulmonary function impairment (i.e., interstitial pulmonary disease, pulmonary hypertension or prior tuberculosis). Demographic, clinical and biochemical data at the time of PFT were collected from electronic medical records, with certain parameters (e.g., smoking habits) specifically asked.

The study was approved by the UMCU ethical review board and informed consent was obtained from all participants. The study was funded by Gilead Sciences, which had no role in trial design, data collection, analysis or manuscript preparation.

Pulmonary function testing

Each participant performed one PFT consisting of pre-bronchodilation spirometry, body plethysmography and single-breath transfer factor for carbon monoxide (TLCO) measurement - i.e., measurement of diffusion capacity - according to European Respiratory Society/American Thoracic Society (ERS/ATS) standards using a Geratherm spirometer.[14–17] If no ERS/ATS-qualifying effort was recorded, the PFT was excluded.

The TLCO Z-score was chosen as primary outcome. As secondary outcome we assessed diffusion impairment defined as a TLCO Z-score <-1.645 (i.e., belonging to the <5th percentile), with a Z-score of -1.65 – -2.50, -2.51 – -4.00, <-4.10 defined as mild, moderate and severe, respectively.[17] Other secondary outcomes - obstructive and restrictive impairment - were defined as a forced expiratory volume in one second/forced vital capacity (FEV1/FVC) and total lung capacity (TLC) Z-score <-1.645. Global Lung Initiative reference value Z-scores based on age, sex at birth, height and, for spirometry, ethnicity were used and TLCO values were corrected for hemoglobin.[14–16] All PFTs were reviewed by a pulmonologist and participants with a successful TLCO measurement were included in the analysis.

Statistical analysis

Continuous data were expressed as mean ± standard deviation or median and interquartile range for parametric and nonparametric data. A chi-square and Mann-Whitney U or independent samples t-tests were used to compare categorical and continuous variables. Multivariable linear regression and logistic regression using Firth's bias correction for small samples were used to assess mean Z-scores and pulmonary impairment.[18] Additionally, in PWH with PJP the association between time since PJP, adjunctive steroid-use during PJP and TLCO Z-score was investigated. Multicollinearity and assumptions of linearity, homoscedasticity and normality of residuals were assessed. Two sensitivity analyses were performed using only PWH with an undetectable viral load at the time of PFT and without previous COVID-19**.** A two-sided alpha level of 0.05 was used and all statistical analyses were conducted using RStudio (v1.3.1093).

Sample size calculation

We hypothesized that past PJP would result in lower TLCO Z-scores and calculated that a sample of 100 participants would provide our study with 80% power to show a significant effect of PJP, with an estimated TLCO Z-score difference between study groups of 0.57.

RESULTS

Demographics

A total of 105 PWH underwent the PFT evaluation between 2016 and 2022. Three of them were excluded as their PFT did not meet ERS/ATS quality standards. This left 102 participants available for analysis, including 51 with past PJP with median ten years since PJP (interquartile range (IQR) 5.00 – 16.00). Six of all participants had a mild COVID-19 infection more than 6 months before PFT; none of them required specific treatment or hospital admission. Both groups were comparable regarding age (median 54.00 *vs.* 53.00 years, p=0.453) and sex at birth (44/51 (86.27%) *vs.* 45/51 (88.24%), p=0.767). Compared to those without PJP (PJP-), PWH with PJP (PJP+) had lower median nadir CD4$^+$ lymphocyte counts (28.00 *vs.* 78.00 cells/mm^3, p<0.001) (Table 1).

Table 1. Characteristics of PWH according to PJP status at time of PFT

	PJP+		PJP-		
Demographics	**n = 51**	**(IQR) / (%)**	**n = 51**	**(IQR) / (%)**	***p-value***
Age (years)	54.00	50.00 - 58.00	53.00	46.00 - 59.00	0.453
Sex at birth (male)	44	86.27	45	88.24	0.767
Clinical characteristics					
Time since HIV diagnosis (years)	10.00	6.00 – 17.00	11.00	7.00 – 17.00	0.245
Time since PJP (years)	10.00	5.00 – 16.00	-	-	-
Time since start antiretroviral therapy (years)	10.00	6.00 – 16.00	10.00	7.00 – 16.00	0.341
Smoking					0.834
- current	27	52.94	24	47.06	
- former	13	25.49	15	29.41	
- never	11	21.57	12	23.53	
Mode of transmission					0.207
- MSM	25	49.02	31	60.78	
- heterosexual	8	15.69	10	19.61	
- other / unknown	18	35.29	10	19.61	
History of pneumothorax [a]	5	9.80	-	-	
Admission to ICU during PJP [a]	9	17.64	-	-	
Adjunctive steroids during PJP [a]	33	64.71	-	-	
Biochemical characteristics					
Nadir CD4$^+$ lymphocyte count (cells/mm^3)	28.00	10.00 – 50.00	78.00	41.00 – 152.00	**<0.001**
CD4$^+$ lymphocyte count at PFT [a]	478.00	372.75 – 559.75	537.00	392.50 – 691.75	0.120
VL <400 at PFT (cop/mL)*	51	100	51	100	-

All categorical data are expressed as frequency (percentage) and all continuous data are expressed as median (interquartile range).

[a.] Missing data: CD4$^+$ lymphocyte count at PFT (1 PJP- (1.96%) / 1 PJP+ (1.96%)), History of pneumothorax (3 PJP+ 5.88%), Admission to ICU during PJP (3 PJP+ 5.88%), Adjunctive steroids during PJP (2 PJP+ 3.92%).

* Detectable viral loads at the time of PFT were observed for three PJP+ (VLs of 319, 83, 75 copies/mL) and one PJP- (VL of 179 copies/mL).

Abbreviations: ICU, intensive care unit; IQR, interquartile range; MSM, men who have sex with men; PFT, pulmonary function test; PJP, *Pneumocystis jirovecii* Pneumonia; PWH, people with HIV; VL, viral load.

Diffusion impairment

TLCO Z-score as diffusion parameter was found to be similar between PJP+ and PJP- (-0.98 (1.11) *vs*. -0.92 (1.04), p=0.790). Multivariable linear regression showed that only current (*vs.* never) smoking was associated with lower TLCO Z-scores (β -1.10; 95% CI -1.61 – -0.59), while no association was observed with past PJP (β 0.14; 95% CI -0.30 – 0.57) (Table 2).

Table 2. Multivariable logistic and linear regression analysis for TLCO Z-scores and diffusion impairment (defined as TLCO Z-score <-1.645).

	TLCO Z-score			Diffusion impairment		
	β	**95% CI**	***p-value***	**OR**	**95% CI**	***p-value***
Past PJP (*vs.* no past PJP)	0.14	-0.30 – 0.57	0.545	1.00	0.36 – 2.75	0.997
Age at PFT (per year increase)	-0.02	-0.04 – 0.01	0.164	1.00	0.95 – 1.06	0.971
Male sex (*vs.* female sex)	0.21	-0.40 – 0.81	0.504	0.46	0.13 – 1.65	0.235
Time since HIV diagnosis (per year increase)	0.01	-0.02 – 0.04	0.454	1.02	0.95 – 1.09	0.648
Smoking						
- never	1		-	1		-
- former	-0.14	-0.63 – 0.36	0.597	1.68	0.51 – 5.74	0.396
- current	-1.10	-1.61 – -0.59	**<0.001**	6.02	1.94 – 18.72	**0.002**
Nadir CD4⁺ lymphocyte count (per 5 cell/mm³ increase)	0.02	0.00 – 0.04	0.061	0.97	0.93 – 1.02	0.195

Abbreviations: CI, confidence interval; OR, odds ratio; PFT, pulmonary function test; PJP, *Pneumocystis jirovecii* Pneumonia; TLCO, transfer factor for carbon monoxide.

When diffusion impairment was defined dichotomously as TLCO Z-score <-1.645, comparable rates were found in PJP+ and PJP- (14/51 (27.45%) *vs.* 12/51 (24.53%), p=0.650). Rates of mild and moderate diffusion impairment were also similar between groups and no severe diffusion impairment was observed. Current (*vs.* never) smokers had higher odds of diffusion impairment (odds ratio (OR) 6.02; 95% CI: 1.94 – 18.72), whereas similar odds were found for past PJP *vs.* no past PJP (OR 1.00; 95% CI: 0.36 - 2.75). See Table S1, Supplemental Digital Content 1, for all PFT Z-scores, % predicted and exact rates of diffusion impairment severity by group.

The association between time since PJP, steroid-use during PJP and TLCO Z-score was evaluated in the PJP+ group. Only current smoking was independently associated with lower TLCO Z-scores (β -1.36; 95% CI -2.10 – -0.63) and no association was found with time since PJP (β 0.03; 95% CI -0.02 – 0.08) nor steroid-use during PJP (β -0.60; 95% CI -1.21 – 0.00).

Obstructive and restrictive impairment

FEV1/FVC Z-scores were similar for PJP+ and PJP- (-0.31 (1.08) *vs*. -0.28 (1.10), p=0.894). No independent association for past PJP was found (β 0.03; 95% CI -0.44 – 0.50), whereas current smoking was associated with lower FEV1/FVC Z-scores (β -0.69; 95% CI -1.24 –

-0.14). Past PJP was not associated with obstructive impairment (4/51 (7.84%) *vs.* 6/51 (12.00%), p=0.484). See Table S2, Supplemental Digital Content 2, for linear regression results.

Similar TLC Z-scores were found for both groups (-0.09 (1.04) *vs*. –0.01 (1.13), p=0.705). Current smoking was independently associated with higher TLC Z-scores (β 0.58; 95% CI 0.04 – 1.12). No association was found for past PJP (β 0.09; 95% CI -0.36 – 0.55). Restrictive impairment was not associated with past PJP (4/51 (7.84%) *vs*. 2/51 (3.92%), p=0.400). Given the low number of obstructive and restrictive impairment, multivariable logistic regression was not performed for these outcomes.

Sensitivity analyses

Sensitivity analyses were conducted using 98/102 PWH with an undetectable viral load at the time of PFT (excluding 3/51 PJP+ and 1/51 PJP-) and 96 without previous COVID-19 (excluding 6/51 PJP+). All results were similar to those of the main analyses.

DISCUSSION

In this cross-sectional study on the potential long-term pulmonary sequelae of PJP in PWH, we found no effect of past PJP on the diffusion capacity, evaluated either as a continuous TLCO Z-score or dichotomously as Z-score <-1.645. Current (*vs.* never) smoking was found independently associated with lower TLCO Z-scores and diffusion impairment. Notably, more than 25% of PWH in our study had diffusion impairment, as defined by the latest ERS/ATS guidelines.[14–17]

Previous PJP does not seem to put PWH at greater risk of long-term diffusion impairment. Although their diffusion capacity is substantially more often diminished compared to the general population, this seems to be driven by other factors, such as HIV infection and smoking.[9–13] Though the effect of HIV infection itself could not be investigated with our study design, the presented findings confirm the detrimental effect of smoking. Other authors who specifically investigated PJP's effect on diffusion impairment reported conflicting results, but their studies had short follow-up times or did not specify the time since PJP – the latter being particularly relevant given the improvement of pulmonary function over time described in some studies.[5–8] To our knowledge, this is the first study which examined long-term sequelae of past PJP, with a median of ten years since PJP.

We hypothesize that PJP-related damage either recovers in the long-term or its contribution is negligible in the presence of persistent pulmonary impairment from smoking and HIV infection. From a pathophysiological perspective, the diffusion impairment is the result of a different process in the acute and post-acute phase of PJP. It is assumed that high influx

of inflammatory cells is responsible for hypoxemia in the acute stage of PJP[2], while post-acute diffusion impairment is suggested to be due to lasting alveolar surfactant alterations resulting from the inflammatory response.[19,20] Our data suggest that the latter is probably reversible over time. A gradual improvement of the pulmonary function after PJP is supported by the findings of a longitudinal study of 84 PJP patients using HR-CT scans[21], in which complete resolution of the radiological abnormalities was seen in 90% of participants 302 days after PJP. It should be noted, however, that this study did not include PWH, but people on immunosuppressive therapy.

Our study has several strengths. Next to the aforementioned long-term PJP-related outcomes, the PFT measurement was performed systematically in accordance with the latest ERS/ATS quality standards and a matched group of PWH with advanced immunodeficiency without past PJP serving as control, as well as multivariate adjustment were used to minimize confounding bias.

Certain limitations also apply to our study. We could not account for previous bacterial pneumonias or underlying undiagnosed pulmonary vascular and interstitial lung disease, all of which can result in diffusion impairment, but given the relatively homogeneous study population, we do not expect these factors to differ between groups.[22] Additionally, the observed association between PJP and pulmonary function impairment may be an underestimation of the true association, since PWH who died due to severe PJP-sequelae with potentially severe impairment were not included in our study. Future research should preferably be longitudinal in nature and include the above factors, to ultimately disentangle the effects of specific risk factors on the course of pulmonary impairment in PWH.

In conclusion, our study did not show an association between past PJP and persistent diffusion impairment in PWH. Our findings suggest that PJP-related pulmonary damage recovers in the long-term or that its contribution, in the presence of pulmonary impairment from smoking or HIV infection, is marginal.

Statements

Author contributions

I.B., B.W. and T.M. designed the study. B.W. wrote the study protocol. P.O. was responsible for the site work including the recruitment and data collection. All authors had access to data. P.O. performed the analysis, interpreted results and drafted the manuscript. All authors contributed to the interpretation of the data, critically reviewed the manuscript and approved the final manuscript.

Conflicts of interests

The authors declare that they have no conflicts of interest.

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SUPPLEMENT DIGITAL CONTENT

Supplemental Digital Content 1

Table S1. Pulmonary function test results according to PJP-status

	PJP+		PJP-		
Diffusion indices *	**n = 51**	**(SD) (%)**	**n = 51**	**(SD) / (%)**	***p-value***
KCO (Z-score)	-0.90	1.12	-0.80	1.20	0.642
KCO (% predicted)	87.56	15.66	89.06	16.73	0.641
TLCO (Z-score)	-0.98	1.11	-0.92	1.04	0.790
TLCO (% predicted)	86.08	15.71	86.08	15.71	0.772
Diffusion Impairment (TLCO Z-score <-1.645)	14	27.45	12	24.53	0.650
Severity of diffusion impairment					0.619
- **mild (Z-score -1.65 – -2.50)**	8	57.14	8	66.66	
- **moderate (Z-score -2.51 – 4.00)**	6	42.86	4	33.33	
- **severe (Z-score <-4.10)**	0	0	0	0	
Obstruction indices [a]					
FVC (Z-score)	0.07	1.01	-0.02	1.05	0.675
FVC (% predicted)	100.97	13.91	99.95	14.67	0.721
FEV1 (Z-score)	-0.13	1.07	-0.12	1.30	0.961
FEV1 (% predicted)	98.01	14.70	97.23	17.26	0.807
FEV1/FVC (Z-score)	-0.31	1.08	-0.28	1.10	0.894
FEV1/FVC (% predicted)	97.35	8.88	96.96	10.32	0.838
Obstructive Impairment (FEV1/FVC Z-score <-1.645)	4	7.84	6	12.00	0.484
Restriction indices					
TLC (Z-score)	-0.09	1.04	-0.01	1.13	0.705
TLC (% predicted)	98.92	12.43	100.13	13.61	0.641
Restrictive Impairment (TLC Z-score <-1.645)	4	7.84	2	3.92	0.400

All categorical data are expressed as frequency (percentage) and all continuous data are expressed as mean (standard deviation).

[a.] Spirometry data of one PJP- is missing due to not meeting ERS/ATS-qualifying standards.

***** Diffusion values are corrected for hemoglobin.

Abbreviations: FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; KCO, transfer coefficient for carbon monoxide; SD, standard deviation; TLC, total lung capacity; TLCO**,** transfer factor for carbon monoxide**.**

Supplemental Digital Content 2

Table S2. Multivariable linear regression analysis for FEV1/FVC and TLC Z-scores

	FEV1/FVC Z-score			TLC Z-score		
	β	**95% CI**	***p-value***	***β***	**95% CI**	***p-value***
Past PJP (*vs.* no past PJP)	0.03	-0.44 – 0.50	0.904	0.09	-0.36 – 0.55	0.689
Age at PFT (per year increase)	0.00	-0.02 – 0.02	0.964	0.01	-0.02 – 0.03	0.545
Male sex (*vs.* female sex)	0.11	-0.54 – 0.75	0.751	0.15	-0.48 – 0.77	0.651
Time since HIV diagnosis (per year increase)	0.00	-0.03 – 0.04	0.786	0.01	-0.02 – 0.04	0.528
Smoking						
- never	1		-	1		-
- former	0.11	-0.44 – 0.65	0.704	-0.01	-0.54 – 0.51	0.963
- current	-0.69	-1.24 – -0.14	**0.016**	0.58	0.04 – 1.12	**0.037**
Nadir $CD4^+$ lymphocyte count (per 5 cell/mm^3 increase)	0.01	-0.01 – 0.03	0.515	0.02	0.00 – 0.04	0.092

Abbreviations: FEV1/FVC, forced expiratory volume in one second/ forced vital capacity; PJP, *Pneumocystis jirovecii* Pneumonia; PFT, pulmonary function test, TLC, total lung capacity.

8

RESPONSE TO CORRESPONDENCE TO "NO LONG-TERM EFFECT OF PAST *PNEUMOCYSTIS JIROVECII* PNEUMONIA ON PULMONARY FUNCTION IN PEOPLE WITH HIV"

Patrick G.A. Oomen
Berend J. van Welzen
Inez Bronsveld
Andy I.M. Hoepelman
Tania Mudrikova

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RESPONSE

We thank Byanova and Huang[1] for their insightful comments regarding our publication titled “No long-term effect of past *Pneumocystis jirovecii* pneumonia on pulmonary function in people with HIV”.[2] In this response, we would like to address their points.

As the authors point out, research on the topic of pulmonary function impairment in people with HIV (PWH) and *Pneumocystis jirovecii* pneumonia (PJP) is notably scarce. Moreover, due to the decreasing incidence of PJP, most of these studies have been conducted in the era predating combination antiretroviral therapy (cART). The most comprehensive study of that time identifies past PJP as an independent contributing factor for impaired single-breath transfer factor for carbon monoxide (TLCO), but since none of the participants started cART, there was no immunological recovery in this population.[3] This raises the critical question: do the findings from these earlier investigations still hold relevance in the contemporary cART era, where improved HIV management (more) effectively mitigates HIV-related immune activation and consequently preserves pulmonary function, in turn potentially attenuating the impact of PJP?

Indeed, when examining studies conducted in the cART era that have investigated risk factors (including PJP) for impaired TLCO in PWH, none provide evidence that after successful treatment for PJP and subsequent initiation of optimal cART, prior PJP alone – not combined with prior bacterial pneumonias – has a significant effect on long-term TLCO.[4–7] These findings align with results from our study: a history of PJP seems to consistently lose significance as an independent contributing factor in the presence of other risk factors, including those mentioned in the correspondence.[4–7] Advanced immunodeficiency, detectable viremia, current or past smoking, and a history of bacterial pneumonia all emerge as significant contributors to TLCO impairment and seem to attenuate the effects of past PJP in multivariable analyses.

In their correspondence, Byanova and Huang argue that our study most likely underestimates the long-term effects of past PJP on diffusion capacity as a result of survival bias and unaddressed confounders. As stated in our manuscript, we fully acknowledge that our study was inherently limited by its design and that underestimation of the effect of PJP cannot be completely excluded. Our approach, involving two groups of similarly immunocompromised PWH with a nadir $CD4^+$ count of <200 cells/mm^3, aimed to isolate the specific impact of PJP and explore its independent effect on diffusion impairment, disentangled from other HIV-related factors which might influence pulmonary function. Although one cannot correct for survival bias in cross-sectional studies such as ours, given our findings in combination with the previously mentioned studies[4–7], we believe that the impact of past PJP on TLCO is limited in the modern cART era.

In conclusion, there is currently no convincing evidence indicating a significant impact of past PJP alone on diffusion impairment in the context of optimally treated HIV. Nevertheless, we fully agree with the need for prospective, longitudinal studies of diffusion impairment in PWH, including those with a history of PJP, to overcome potential survival bias. Furthermore, given that PWH today can reach an advanced age even after a history of opportunistic infection, we believe that the focus should also be on healthy aging and that these studies should evaluate patient reported outcomes, such as respiratory symptoms and health-related quality of life.

Statements

Author contributions

P.O. – Conceptualization, Writing - Original Draft & Editing. B.W. – Conceptualization, Writing - Review & Editing. I.B. – Writing - Review & Editing. A.H. – Writing - Review & Editing. T.M. – Writing - Review & Editing.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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9

ASSESSING AND IMPROVING THE QUALITY OF GUIDELINE-ADHERENT HEPATITIS B VIRUS CARE IN PEOPLE WITH HIV: A CROSS-SECTIONAL STUDY

Patrick G.A. Oomen
Vincent J.P. van Kraaij
Anna M. Gerritsma
Frans M. Verduyn Lunel
Greet J. Boland
Andy I.M. Hoepelman
Berend J. van Welzen

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ABSTRACT

The increasing use of non-tenofovir containing antiretroviral regimens calls for renewed attention to the prevention and management of hepatitis B virus (HBV) in people with HIV (PWH). We retrospectively assessed adherence to HBV guidelines, including complete HBV screening in PWH. In people with HIV/HBV co-infection, this included HBV therapy, screening for hepatitis delta virus (HDV) and on-therapy virologic response monitoring. HIV/HBV co-infection in PWH was defined as the presence of hepatitis B surface antigen (HBsAg) at the last measurement before study entry or detectable HBV-DNA for ≥6 months. After assessment, missing laboratory tests were performed to optimize HBV monitoring and screening for co-infections. Of all PWH under follow-up, 1484/1633 (90.9%) were adequately screened for HBV. After performing missing screening tests, 466 of 1618 PWH with complete screening results (28.8%) were non-immune for HBV infection. Fifty-one (3.2%) with HIV/HBV co-infection were identified. HBV treatment was adequate in 51/51 (100%). Screening for hepatitis A, C and delta virus antibodies and fibrosis was performed in 51/51 (100%), 49/51 (96.1%), 17/51 (35.3%) and 38/51 (74.5%). Annual HBV-DNA or HBsAg monitoring was done in 18/51 (35.3%) and hepatocellular carcinoma (HCC) surveillance in 2/9 (22.2%) of those indicated. Additional testing in those with missing data identified 4/34 (11.8%) persons with HDV antibodies and 3/30 (10%) with HBsAg seroclearance. Our study demonstrates the feasibility and added value of evaluating HBV care components and performing missing laboratory tests, identifying a large number of HBV vaccination candidates and HDV antibody screening, HBsAg monitoring and HCC surveillance as key areas for improvement.

INTRODUCTION

The advent of tenofovir-containing antiretroviral therapy (ART) has facilitated treatment of hepatitis B virus (HBV) co-infection in people with HIV (PWH), and has been associated with improved clinical outcomes.[1] Tenofovir also protects against the acquisition of HBV infection.[2] However, the increasing use of non-tenofovir containing ART such as dual or long-acting injectable therapy requires renewed attention to the prevention and management of HBV in PWH.[3] This includes recurrent evaluation of multiple care areas, such as HBV screening in PWH (including for those non-immune and eligible for vaccination). In PWH with HBV co-infection (HIV/HBV), additional screening for hepatitis A, C and delta virus (HAV/HCV/HDV) and hepatic fibrosis is advised. Furthermore, evaluation of anti-HBV treatment and on-therapy HBV-DNA or hepatitis B surface antigen (HBsAg) virological response monitoring and hepatocellular carcinoma (HCC) surveillance is recommended.[3,4]

The quality of guideline-adherent HBV care in people with HIV/HBV has previously been investigated by Lee et al.[5] Despite being essential HBV care components[3], HBV screening in PWH and virologic response monitoring in people with HIV/HBV were not included in this study[5]. Moreover, no follow-up action was taken regarding the missing laboratory tests found during the study.

Therefore, the aim of our proof-of-concept quality improvement study was to first comprehensively evaluate all aforementioned care areas and then perform missing laboratory tests and inform caregivers of all results, with the goal of exploring the feasibility and added value of this approach for improving HBV care in PWH.

METHODS

Study design, population and data collection

A retrospective cross-sectional assessment was conducted in adult PWH between March 2022 and March 2023 in the University Medical Center Utrecht, the Netherlands. PWH were eligible for inclusion if they had an outpatient visit during the study period. Those who objected against participation in research and audits were excluded. Demographic, clinical and laboratory data from the most recent outpatient visit were collected from electronic medical records. This project was undertaken as an audit and as such no ethical approval was required. Data were anonymized at the point of data collection with no patient identifiers retained.

Study definitions

The following definitions were used:

- HBV co-infection as hepatitis B surface antigen (HBsAg) presence at the last

measurement before study entry or detectable HBV-DNA ≥6 months;
- complete HBV screening as assessment of serum HBsAg, HB core antibodies (anti-HBc) and HB surface antibodies (anti-HBs);
- non-immune for HBV infection as HBsAg, anti-HBs and anti-HBc negative;
- HBV/HIV therapy as tenofovir-based cART or entecavir combined with cART;
- HAV, HCV and HDV screening as antibody laboratory testing;
- fibrosis screening as transient elastography (TE) or liver biopsy;
- HBV monitoring as HBV-DNA or, in case of undetectable HBV-DNA, quantitative HBsAg in the year prior to assessment;
- HCC screening indication as people with HIV/HBV and advanced fibrosis or cirrhosis (defined as Metavir stage F3/F4 on liver biopsy or liver stiffness ≥8.1 kPa on TE);[6]
- HCC surveillance as the performance of two hepatic ultrasounds ≤6.5 months apart in the year prior to assessment.[3,4]

Outcomes

In PWH, we assessed HBV screening results to identify those non-immune for HBV infection and thus eligible for vaccination, and those with HBV co-infection. In those non-immune for HBV infection, we specifically assessed whether they were on tenofovir (as tenofovir has been shown to have a protective effect against acquisition of HBV infection).[2]

In people with HIV/HBV, we evaluated seven guideline-recommended HBV quality measures: **1)** HBV therapy **2)** HAV antibody screening and vaccination status **3)** HCV antibody screening **4)** HDV antibody screening **5)** fibrosis screening **6)** HBV-DNA or HBsAg monitoring **7)** HCC surveillance in case of advanced fibrosis/cirrhosis.[3,4]

Missing laboratory tests

Following the above assessment, we investigated whether those with missing laboratory tests were under follow-up on March 1, 2023. In individuals still in care, the caregiver's consent was obtained and missing co-infection screening and HBV monitoring tests were determined retrospectively in blood samples.

Statistical analysis

Continuous data were expressed as mean (standard deviation) or median (interquartile range) for parametric and nonparametric data. Categorical data were compared using Fisher's exact test or $\chi 2$. Continuous variables were compared using the independent samples t-test or Mann-Whitney U test. Two-sided p-values <0.05 were considered statistically significant. All analyses were conducted using SPSS version 27.0.

RESULTS

HBV screening and laboratory tests in PWH

Between March 2022 and March 2023, 1633 PWH were included (Table 1). In total, 1484 (90.9%) were completely screened for HBV. On March 1, 2023, 134/149 PWH with missing screening tests were still in care. Performing missing tests resulted in 1618 PWH with complete HBV serology, of whom 466 (28.8%) were non-immune for HBV infection, 604 (37.3%) vaccinated, 385 (23.8%) with a resolved HBV infection, 112 (6.9%) only anti-HBc positive, and 51 (3.2%) with HIV/HBV (Figure 1).

Table 1. Characteristics of PWH and people with HIV/HBV at the time of the outpatient visit.

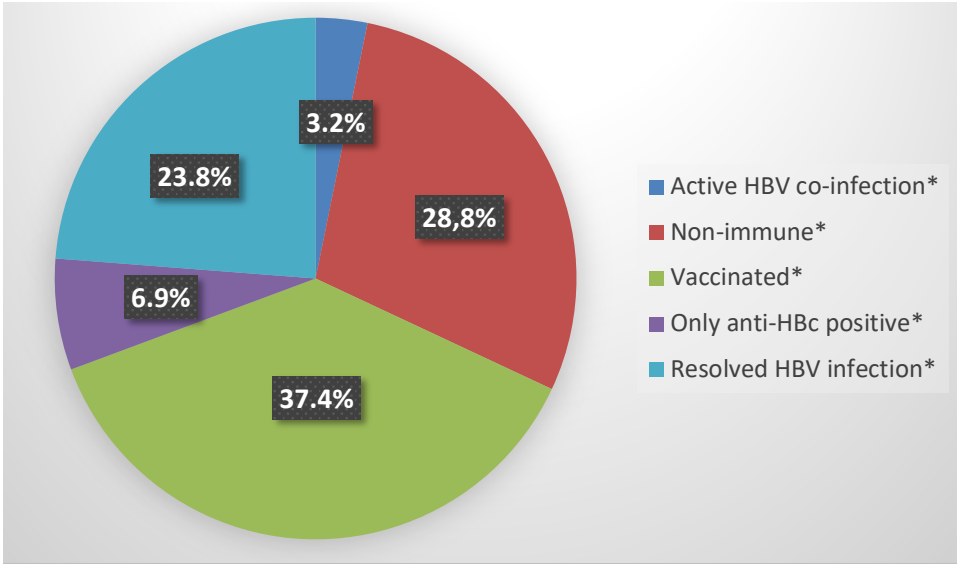
	1582 PWH	**51 PWH / HBV**	**p-value**
Demographics			
Age – years	50.69 (40.65 – 58.86)	46.89 (40.73 – 56.00)	0.21
Female sex (%)	315 (19.9)	15 (29.4)	0.10
Region of origin (%)			**<0.001**
- Europe / North-America / Australia	1139 (72.0)	19 (37.3)	
- Sub-Saharan Africa	224 (14.2)	21 (41.2)	
- Asian	61 (3.9)	7 (13.7)	
- South American / Caribbean	88 (5.6)	1 (2.0)	
- North Africa / Middle East	53 (3.4)	3 (5.9)	
- Unknown	17 (1.1)	0 (0)	
Clinical characteristics			
Time since HIV diagnosis – years[a]	12.03 (6.42 – 18.05)	14.94 (8.43 – 21.65)	**0.03**
Time since ART initiation – years[a]	9.89 (5.73 – 16.09)	12.66 (7.51 – 18.44)	**0.04**
Type of cART anchor (%)[b]			0.52
- PI-based	417 (26.4)	14 (27.5)	
- NNRTI-based	363 (22.9)	16 (31.4)	
- INSTI-based	767 (48.5)	21 (41.2)	
Mode of transmission (%)			**0.05**
- MSM	898 (56.8)	18 (35.3)	
- Heterosexual	350 (22.1)	16 (31.4)	
- Vertical transmission	20 (1.3)	0 (0)	
- IVD	15 (0.9)	1 (2.0)	
- Blood products	19 (1.2)	1 (2.0)	
- Other / unknown	280 (17.7)	15 (29.4)	
Biochemical characteristics			
Nadir CD4$^+$ count - cells/mm^3[a]	248.00 (103.00 – 370.00)	189.00 (115.00 – 359.50)	0.35
Current CD4$^+$ count - cells/mm^3[a]	615.50 (461.00 – 796.00)	528.50 (400.75 – 743.00)	0.08
Current VL <50 copies/mL (%)[a]	1401 (94.5)	46 (95.8)	0.91
Creatinine – µmol/L[a]	80.00 (70.00 – 93.00)	80.00 (66.00 – 96.00)	0.89
ALAT – U/L[a]	24.00 (18.00 – 33.00)	24.00 (18.00 – 34.00)	0.67
HBV co-infection characteristics			
Time since HBV diagnosis – years	NA	13.39 (8.47 – 20.21)	NA
HBeAg at last follow-up (%)[a]			
- Positive	NA	13 (25.5)	
- Negative		37 (72.5)	

Table 1. Continued.

	1582 PWH	51 PWH / HBV	p-value
HBV-DNA at last follow-up[c] (%)			NA
- Detectable	NA	17 (33.3)	
- Undetectable		34 (66.7)	

All categorical data are expressed as number (percentage of total population) and all continuous data are expressed as median (interquartile range) or mean (standard deviation (±)).
a. Missing data: Time Since HIV diagnosis (2 PWH; 0.1%), time since ART initiation (6 PWH; 0.0%), Creatinine (97 PWH; 6.1%, 2 PWH/HBV; 3.9%), ALAT (22 PWH; 1.4%), current $CD4^+$ count (54 PWH; 3.4%, 3 PWH/HBV; 5.9%), nadir $CD4^+$ count (167 PWH; 10.6%, 6 PWH/HBV; 11.8%), current VL <50 copies/mL (100 PWH 6.3%, 3 PWH HBV 5.9%), HBeAg (1 PWH/HBV; 2.0%),
[b] Excluding 15 elite controllers or long-term non-progressors not on cART and 20 PWH on cART consisting of multiple anchors (1.3%).
[c] Undetectable HBV-DNA was defined as <7 IU/mL.
Abbreviations: ALAT, Alanine transaminase; ART, antiretroviral therapy; cART, combination antiretroviral therapy; cop, copies; HBV, Hepatitis B Virus; HIV, Human Immunodeficiency Virus; INSTI, integrase strand transfer inhibitor; IVD, intravenous drug use; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; no., number; PI, protease inhibitor; PWH, people with HIV; VL, viral load.

Figure 1. Pie chart showing distribution of HBV serology status in 1618 PWH



Abbreviations: anti-HBc, HB core antibodies; anti-HBs, HB surface antibodies; HBsAg, hepatitis B surface antigen; HBV, Hepatitis B Virus; PWH, people with HIV.
*Active HBV co-infection: HBsAg pos, anti-HBs neg, anti-HBc pos; non-immune: HBsAg neg, anti-HBs neg, anti-HBc neg; vaccinated: HBsAg neg, anti-HBs pos, anti-HBc neg; only anti-HBc positive: HBsAg neg, anti-HBs neg, anti-HBc pos; resolved HBV infection: HBsAg neg, anti-HBs pos, anti-HBc neg.

Of the 916 (56.2%) men who have sex with men and therefore considered at high risk for HBV infection, 174 (10.8%) were non-immune and eligible for vaccination. Moreover, 27 (3.0%) of this group were not prescribed a tenofovir-based regimen, placing them at highest risk of HBV infection (given tenofovir's protective effect against acquisition of HBV

infection). Following this assessment, caregivers were informed of their patients' serologic status and potential vaccination candidates.

Quality measures and laboratory tests in people with HIV/HBV

HBV treatment was adequate, as 51/51 (100%) were on tenofovir-based cART or entecavir combined with cART. Screening for HAV was conducted in 51/51 (100%), five of whom had no HAV antibodies making them eligible for vaccination. Screening for HCV and HDV was performed in 49/51 (96.1%) and 17/51 (33.3%). In 38/51 (74.5%) fibrosis had been screened by TE (31/51) or biopsy (7/51). Annual HBV-DNA or HBsAg monitoring was adequate in 18/51 (35.3%). Inadequate monitoring occurred in 33/51 (64.7%), the most recent HBV-DNA was undetectable in 30/33 individuals, rendering them eligible for HBsAg assessment. Lastly, 9/51 (17.6%) had an HCC indication due to significant fibrosis/cirrhosis. HCC surveillance was adequately conducted in 2/9 (22.2%).

After assessing these quality measures, we investigated whether those with HIV/HBV were in care on March 1, 2023. All were still in care and missing laboratory tests were performed, revealing 2/2 (100%) individuals without HCV antibodies, 4/34 (11.8%) with HDV antibodies but without HDV RNA and 3/30 (10%) with HBsAg seroclearance. None of those with HBsAg seroclearance had anti-HBsAg antibodies. Following this assessment, caregivers were informed of all results, particularly regarding inadequate quality measures and abnormal laboratory tests.

DISCUSSION

In this cross-sectional study, we aimed to assess and improve guideline-adherent HBV care in PWH. Our study demonstrates the feasibility and added value of evaluating HBV care components and performing missing laboratory tests. Main findings include the large number of HBV vaccination candidates as well as HDV antibody screening, HBsAg monitoring and HCC surveillance being key areas for improvement in people with HIV/HBV.

A recent study by Lee et al. that examined guideline-adherent HBV care in people with HIV/HBV found that although HBV treatment and HCV screening were adequate, HAV screening and HCC surveillance were substandard.[5] Importantly, there was no mention of HBV screening and virologic response monitoring and missing laboratory tests were not performed retrospectively, so that any clinical implications remained unknown. We assessed all aforementioned care components and performing missing tests, thus revealing important key improvement areas.

HDV antibody screening had only been performed in 33% of individuals and missing tests revealed 4 (11.8%) with antibodies. In 2021, 5% of Dutch people with HIV/HBV tested for HDV had an active HDV infection, while only 12% of the total population had been tested, suggesting a large group of undetected active HDV infections.[7] Our numbers are consistent with this and underscore the need for increased awareness of HDV in caregivers, particularly in light of new treatment options.[8] HBV monitoring was identified as second improvement area: only 35% received guideline-adherent monitoring. After performing missing tests, we observed 10% HBsAg seroclearance. HBsAg seroclearance is relevant as it is associated with low risk of developing HCC and improved survival, and it allows caregivers to consider discontinuing anti-HBV treatment, limiting tenofovir-based toxicity and greater ART choice.[3,9] Importantly, HBsAg seroclearance seems more prevalent in people with HIV/HBV than those with HBV mono-infection, presumably due to an immune reconstitution inflammatory syndrome type-effect with rapid expansion of $CD4^+$ T-cells and anti-HBV immune response.[9] European HIV guidelines recommend annual HBsAg testing until loss of HBsAg and that in case of HBsAg loss with anti-HBsAg antibody acquisition, anti-HBV therapy may be stopped.[10] In 2021, <10% of Dutch people with HIV/HBV were tested for HBsAg seroclearance, underscoring the limited attention among caregivers and suggesting that a large group nationwide may also have HBsAg seroclearance.[7] Finally, the rate of guideline-adherent HCC surveillance was 22%. Although strikingly low, these numbers are in line with previous studies reporting rates of 7-55%.[5,11–13] It seems awareness of HCC surveillance is low among caregivers, and our study reinforces this notion and identifies this as an important area of improvement.

The next step in improving HBV care in PWH would be implementation of targeted healthcare interventions in areas found to be substandard in this study. For example, in the case of HDV, reflex testing could be used, where a positive HBsAg result immediately triggers HDV antibody screening in PWH. In Spain, the implementation of this has led to five times more HDV diagnoses, albeit in people with HBV mono-infection.[14] Given this result and our research findings, our center has chosen to implement this step and is currently evaluating its impact. In addition, interventions for HCC surveillance could include automated checklists for every newly diagnosed person with HIV/HBV to assess their HCC surveillance indication, as well as automated reminders or educational programs for both caregivers and those with HIV/HBV.[15]

Our study has several strengths. To our knowledge, our study has the most comprehensive evaluation of HBV quality measures, unlike previous studies often focusing on a limited number of measures[5,11–13,16], and was thus able to identify multiple HBV care improvement areas. Additionally, our quality improvement study is the first to subsequently perform missing laboratory tests and, as a proof-of-concept, demonstrates that our approach is feasible and results in additional important clinical findings.

Our study is limited by the single-center design and small number of individuals with HIV/HBV, which may render our results not fully generalizable to other centers.

In conclusion, our study demonstrates the feasibility and added value of systematically evaluating HBV care components and performing missing laboratory tests. The key areas for improvement are the large number of HBV vaccination candidates and the substandard HDV antibody screening, HBsAg monitoring and HCC surveillance in people with HIV/HBV.

Statements

Author contributions

B.W. designed the study and wrote the study protocol. P.O., V.K. and A.G. collected and analyzed the data. P.O. drafted the manuscript in close collaboration with B.W. All authors contributed to the interpretation of the results, critically reviewed the manuscript and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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PART III

Discussion

10

GENERAL DISCUSSION AND SUMMARY

GENERAL DISCUSSION

Over forty years ago, the world witnessed the beginning of a global health crisis unprecedented in history: the Human Immunodeficiency Virus (HIV) epidemic.[1] Initially, the mortality rate among people with HIV (PWH) was staggering[2] and developing effective antiretroviral therapy (ART) posed significant challenges due to virological resistance in the setting of mono or dual ART.[3,4] The introduction of combination antiretroviral therapy (cART) in 1996 revolutionized HIV treatment[5–7], and dramatically improved life expectancy for PWH[8–10]. In the early years, however, cART was characterized by low virologic efficacy, high pill burden and poor tolerability, often accompanied by severe adverse effects.[11–13] cART traditionally consisted of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) and a third anchor drug, either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI).[14] Later, integrase strand transfer inhibitors (INSTIs) were approved for use as anchor agents in PWH and quickly became first-line therapy in treatment guidelines.[15,16]

Today, the life expectancy of PWH on cART is almost comparable to that of the general population.[17–20] Treatment is relatively simple and effective, and virologic suppression is achieved in the vast majority of those who receive treatment. There are multiple options for first-line cART regimens for ART-naïve PWH, all of which are highly potent, well-tolerated and have a high barrier to resistance.[15,16] The recent guideline approval of dual therapy (consisting of two rather than the three antiretroviral agents required in earlier years) for use in PWH is clear evidence of the effectiveness of current cART options.[21,22] Historically, virologic suppression was the overriding goal, as that is what saved lives. Thanks to these advances in ART, however, we now have the opportunity to look beyond virologic suppression.

In this dissertation, I focus on optimizing treatment and monitoring of HIV. I delve into the issue of the substantial difference between virologic suppression observed in clinical trials versus the real world, as well as the potential effects of contemporary ART beyond virologic suppression, such as HIV-associated immune activation, viral blips, residual viremia and neurocognitive impairment. Additionally, I investigate HIV-associated co-morbidities, particularly *Pneumocystis jirovecii* pneumonia (PJP) and hepatitis B virus (HBV) co-infection, to improve their treatment and monitoring.

Below, I discuss the findings of the studies performed for this dissertation, future perspectives and make recommendations regarding further research.

Part I: Optimizing treatment and monitoring of HIV

Newly developed ART regimens are evaluated through randomized controlled trials (RCTs)

to establish their non-inferiority in terms of virologic efficacy compared to the prevailing ART regimens at that time.[23] If these regimens are shown to be non-inferior, they may be approved for use in PWH and included in treatment guidelines. However, it is well known that due to volunteer bias and strict in- and exclusion criteria in RCTs, findings observed in clinical trials are notoriously different from those observed in real-world populations.[24–26] This discrepancy also extends to tolerability, with newer ART regimens often being less tolerated outside of controlled trial environments. Doravirine (DOR) is an NNRTI that has been approved as anchor drug for PWH since 2018.[27] Registration trials have demonstrated non-inferior virologic efficacy and good tolerability in both ART-naïve and ART-experienced individuals.[28–30] In **Chapter 2**, we investigated the real-world effectiveness and tolerability of switching to DOR-based triple therapy regimens in ART-experienced PWH. Using data from the Dutch nationwide ATHENA cohort[31], we found that switching to DOR-based ART was non-inferior in terms of virologic effectiveness compared to well-matched controls who continued guideline-approved or other common ART regimens after 104 weeks. Although more PWH who had switched to DOR-based ART modified their regimen within the first year, tolerability of the regimens was ultimately similar between study groups at the end of the study period. Of note, the percentage of individuals who discontinued DOR-based ART due to toxicity in our study was five times greater than that of the DRIVE-SHIFT trial: a registration trial involving ART-experienced PWH switching to DOR-based ART (12.4% after 104 weeks versus 2.7% after 144 weeks).[30] Nevertheless, considering the non-inferior effectiveness and similar tolerability observed between study groups, we concluded that DOR-based triple therapy is an effective and well-tolerated ART option for treatment-experienced PWH considering a regimen switch.

There are two noteworthy findings in our study that deserve attention. First, our results further emphasize the disparity between trial and real-world outcomes, as both effectiveness and tolerability were worse compared to the findings of the DRIVE-SHIFT trial. This trend has also been observed in newer INSTI-based regimens such as dolutegravir (DTG), particularly concerning tolerability.[32–34] This is likely due to differences in characteristics of both populations[35], resulting from volunteer bias and strict trial in- and exclusion criteria[26]. Consequently, real-world data can reveal intolerability not previously observed in trials, complementing information obtained from these studies, and, in turn, can help caregivers and their patients in making well-informed decisions regarding adverse effects of ART regimens. A second intriguing observation came from individuals on DOR-based ART with a pre-ART $CD4^+$ count <200 cells/mm^3, as this $CD4^+$ count was strongly associated with virological failure (VF). In contrast, no such association was observed in controls experiencing VF. Although all individuals on DOR eventually achieved virologic suppression without changing ART, suggesting temporary non-adherence, this does not fully explain the observed association. One possible explanation may lie in the combination of a varying degree of “forgiveness” of ART regimens with respect to missed doses and the magnitude

of the latent viral reservoir. Lower nadir $CD4^+$ counts have been linked with larger latent viral reservoirs[36], which in turn may predispose to more rapid or increased viremia in case of missed doses. However, this would explain why specifically this factor is associated with VF, but does not explain why this association is only observed in the DOR group. INSTI-based regimens (accounting for 55% of the matched control group) have been shown to be relatively forgiving regarding missed doses[37], potentially more so than DOR. Consequently, the observed correlation might result from a combination of a large viral reservoir (reflected by a nadir $CD4^+$ count <200 cells/mm^3) with subsequent quicker or increased viremia in case of missed doses, and a "lower forgiveness" of DOR versus INSTIs. Another factor of interest might that a $CD4^+$ count <200 cells/mm^3 reflects slightly less self-care compared to ≥200 cells/mm^3, with the rationale being that the latter group is more likely to have sought medical attention due to symptoms (and thus be more attentive to their health). This lesser self-care may also be reflected in their adherence to cART, resulting in more missed doses in PWH with $CD4^+$ counts <200 cells/mm^3. It is important to note that while some studies observe an association between lower nadir $CD4^+$ counts and reduced adherence[38,39], not every study support this relationship[40,41]. In my opinion, the latter explanation seems less likely than the explanation of a larger viral reservoir predisposing to more and faster viremia. Further investigation is warranted to better understand this association.

In the coming years, research efforts should continue to focus on investigating the performance and tolerability of ART regimens in real-world settings. However, these studies need to be well-designed to account for confounding bias and should include an inter-cohort control group consisting of PWH on other contemporary ART regimens, as the substantial differences between cohorts make comparisons challenging. Regarding DOR specifically, the question at hand is to what extent DOR has a place in the current INSTI era. The registration studies in ART-naive PWH have compared DOR only with a PI or NNRTI[28,29], not with INSTI-based anchor agents. It should be noted that, although rare, instances of VF with emergent DOR-associated genotypic resistance were observed, unlike the INSTI registration studies[21,42,43]. Moreover, the virologic response rates of DOR-based regimens were lower than those of INSTIs, though this may be partly attributed to the stricter criteria used in the DOR trials. However, an important argument in favor of DOR is cost, as DOR-based regimens are considerably more affordable than the prevailing bictegravir (BIC)-based and DTG-based regimens. In addition, BIC and DTG have both been associated with weight gain, as has tenofovir alafenamide (TAF) (which is a main component of the single tablet regimen containing BIC ('Biktarvy')).[44] The single tablet regimen containing DOR ('Delstrigo') does not contain TAF and may therefore be a good alternative for PWH who have gained excessive weight on an INSTI-based regimen. Thus, the question arises to what extent are we willing to tolerate the small but present risk of emerging genotypic DOR-associated resistance in case of VF and reduced response rate versus the better virological outcomes of INSTIs, because of more favorable costs

and less potential for excessive weight gain. Although RCTs comparing BIC- and DTG-based regimens versus DOR in both ART-naïve and ART-experienced PWH would clarify this issue, to my knowledge such trials are not ongoing. Personally, I think they will never be conducted given the current place of DOR as recommended first-line regimen (in certain clinical situations) in Western guidelines[15,16] and the seemingly worse efficacy of DOR when comparing outcomes across DOR and INSTI trials. Because no head-to-head comparison was made, though, this latter statement should be interpreted with caution. Given the findings of our study, I believe there is a role for DOR, particularly as a switch regimen. However, the benefits and risks should be clearly discussed by caregivers to individuals looking to switch to DOR-based ART. For ART-naïve individuals, I presently see a less pronounced role for DOR, given the lower response rate and instances of VF with DOR-associated genotypic resistance compared to INSTIs.

As advances in ART allow us to look beyond virologic suppression, there is increasing attention to HIV-associated immune activation. HIV-associated immune activation is a hyperactive inflammatory state that ultimately leads to depletion of CD4-positive lymphocytes and is associated with numerous comorbidities as well as increased mortality.[45–49] Triple therapy regimens provide adequate virologic suppression, while also decreasing HIV-associated immune activation. However, studies show that even under triple therapy immune activation does not fully normalize.[50] Given the lifelong nature of ART (with associated drug toxicity), the question has been raised what the effect of dual or monotherapy compared to triple therapy is regarding HIV-associated immune activation. **Chapter 3** is a mini review on HIV-associated immune activation in triple, dual and mono therapy regimens in PWH. Herein, we first address the different markers of HIV-associated immune activation, focusing on soluble and T-cell activation and apoptosis markers pertaining to HIV-associated immune activation. Subsequently, we delve into studies assessing these markers in PWH on ART. Studies regarding monotherapy show that a switch to monotherapy is associated with increased T-cell markers and that the pattern in terms of soluble markers is conflicting. Importantly, given the virologic inferiority of monotherapy compared to triple therapy, this will not be an issue of importance in the years to come. Dual ART regimens have been on the market since 2018: studies in PWH switching to dual therapy show no consistent increase in soluble markers compared to triple therapy. However, there are no studies that specifically examined T-cell activation and apoptosis markers. Therefore, we conclude that there is insufficient evidence that dual therapy is non-inferior to triple therapy in terms of suppressing HIV-associated immune activation. Moreover, we emphasize the need for well-designed, longitudinal studies with proper, unbiased participant selection that evaluate both soluble and T-cell activation and apoptosis markers in PWH on dual therapy.

Following the publication of our mini-review in February 2021, an interesting systematic literature review regarding DTG/lamivudine (3TC) appeared in 2022.[51] It encompasses

two RCTs and six real-world studies, with a primary focus on soluble and atherogenesis markers.[52–59] Additionally, the review reports changes in the $CD4^{+/}CD8^{+}$ ratio, a T-cellular marker. We chose not to focus on the $CD4^{+/}CD8^{+}$ ratio given its relatively slow change over time, making it less relevant in the predominantly short-term studies. However, considering this review, it is important to address it. A low $CD4^{+/}CD8^{+}$ ratio indicates increased immune activation and is associated with a higher risk of severe non-acquired immunodeficiency syndrome (AIDS) events (SNAEs).[60] In fact, the European AIDS Clinical Society recommends measuring the $CD4^{+/}CD8^{+}$ ratio as a stronger predictor of SNAEs than $CD4^{+}$ counts.[15] Conversely, a high ratio during ART suggests restored immune function and reduced chronic inflammation. The review concludes that no consistent pattern was observed in inflammatory or atherogenesis markers, with most markers showing comparability between groups, apart from sCD14 (favoring the DTG/3TC group in both trials [58,59]) and IL-6 (favoring the triple therapy group in one trial[59]). As for the $CD4^{+/}CD8^{+}$ ratio, though consistent increases were noted following the switch to DTG/3TC in all real-world studies [52–57], the TANGO trial reported no significant differences between the dual and triple therapy groups[59]. These findings are in line with our observations regarding the heterogeneous findings in soluble markers. The fact that no between-group differences were observed regarding the $CD4^{+/}CD8^{+}$ ratio in the TANGO RCT, suggests no differences in HIV-associated immune activation between dual and triple therapy. However, it's important to note that the T-cell activation and apoptosis markers mentioned in our review were not explored in these studies, leaving the picture regarding dual and triple therapy and HIV-associated immune activation still incomplete in my view.

An important question surrounding HIV-associated immune activation biomarker research revolves around the clinical implications associated with biomarker alterations. An intriguing study in this regard is a recent modeling study, which reported that PWH on triple therapy were projected to spend less time in higher IL-6 quartiles over a span of 3 years compared to those on dual therapy.[61] This finding is significant considering that IL-6 has been identified as the most influential inflammatory soluble biomarker predictor of mortality in PWH.[49] Moreover, the modeling indicated that for every 81 PWH receiving a triple therapy regimen instead of a dual therapy regimen for 3 years, one additional SNAE or death could be prevented. However, several limitations must be acknowledged. First, only IL-6 and D-dimer were modeled, without considering other markers such as sCD14, which exhibited conflicting trends to IL-6 in the TANGO RCT[59]. Incorporating multiple markers, especially those showing conflicting trends, would have strengthened their results. However, the model utilized is not capable of accounting for conflicting trends, as well as changes in inflammation over time. Moreover, participant demographics and lifestyle factors, which are substantial confounders regarding inflammatory biomarkers, were not accounted for and only specific dual and triple therapy regimens were investigated, limiting generalizability. Given these limitations, it is difficult to translate these findings to clinical

practice. A potentially more applicable study is the one conducted by Greenberg et al.[62]: a prospective multicenter cohort study encompassing 9,791 PWH initiating dual or triple therapy. Their investigation focused on a composite endpoint of clinical events, including AIDS, non-AIDS defining malignancies, cardiovascular disease, end-stage liver and renal disease, and death. The results showed a similar incidence of these events across study groups. Although this study provides encouraging evidence for dual therapy, caution should be exercised in its interpretation. Baseline characteristics differed between study groups in this observational study, and it therefore has a large risk of confounding by indication and residual confounding. Moreover, interpretability is further limited by the composite end point and composite determinant of dual or triple therapy regimens. Looking ahead, I believe HIV-associated immune activation will continue to be a central topic in the coming years. It is already currently in the spotlight, with multiple recently published intervention studies aimed at reducing inflammation and HIV-associated immune activation. Notable examples include a study of ruxolitinib[63], a JAK 1/2 inhibitor, which was found to affect both soluble and T-cellular biomarkers, as well as the REPRIEVE study[64], which observed reduced CVD outcomes in PWH on a statin and hypothesized that this was partly due to its anti-inflammatory effect. The book is not yet closed when it comes to inflammation and HIV-associated immune activation, including on the question of dual versus triple therapy regimens. I look forward to seeing what the next years will bring.

Although achieving virologic suppression is now commonplace for PWH initiating ART, challenges remain after virologic suppression, such as the occurrence of viral blips.[65] Viral blips are temporary elevations of HIV plasma viral load (pVL) above the detection limit of standard assays. The exact cause of viral blips is unclear, and several hypotheses have been proposed, including the intermittent release of virions from the latent reservoir, assay accuracy differences, or ongoing viral replication.[66–74] Understanding the underlying cause of viral blips is crucial as they create uncertainty for both PWH and caregivers (resulting in an increased clinical burden[65]), and have been associated with adverse clinical outcomes, most notably VF[75,76]. However, it is currently unclear which factors contribute to the occurrence of viral blips, although the type of cART anchor appears to play a significant role.[65] One hypothesis is that viral blips may be attributed to residual viremia (RV), which refers to detectable viremia below the commonly used threshold of 50 cop/mL.[77] In **Chapter 4**, we investigated factors associated with viral blips, including RV (as a determinant), and additionally examined factors associated with RV itself (as an outcome). Using a single-center cohort design, we observed that RV was indeed associated with subsequent viral blips in virologically suppressed PWH on triple therapy regimens. Importantly, higher preceding RV was associated with higher odds of subsequent blips during follow-up. When investigating factors associated with RV as an outcome, we found that PI-based regimens, compared to NNRTI- and INSTI-based regimens, were associated with higher odds of RV. In addition, certain factors previously linked to the viral reservoir,

such as Fiebig stage at ART initiation, zenith pVL, lowest recorded CD4$^+$ count, and time since ART initiation, were also associated with RV. Of note, while PI-based regimens were associated with the highest odds of subsequent viral blips (consistent with the observed association between PI-based regimens with the highest odds of RV), INSTI-based regimens were associated with the lowest odds of blips, in contrast to NNRTI-based regimens, which were associated with the lowest odds of RV. These findings suggest that viral blips have a multifactorial origin and that the effect of cART on blips is not solely determined by changes in RV. If that were the case, we would have expected the same cART anchor to be associated with the lowest odds of RV and lowest odds of blips.

Our study has several findings that warrant further discussion. Contrary to our hypothesis, the cART anchor associated with the lowest RV (NNRTIs) did not correspond to the fewest occurrences of blips (INSTIs), indicating that viral blips are influenced by multiple factors. An interesting study on this topic is the work by Alvarez et al.[78] In this large multicenter multinational prospective cohort study, the association between various virological outcomes and baseline characteristics in therapy-naive PWH starting ART was investigated. These outcomes included virologic suppression, viral blips, and RV. They found that a high baseline (zenith) pVL and low (nadir) CD4$^+$ count were associated with lower virologic suppression rates and higher rates of viral blips and RV. These associations remained significant in sub-analyses restricted to participants on INSTI-based and DTG-based regimens, suggesting that the zenith pVL and nadir CD4$^+$ count are associated with virological non-suppression outcomes, regardless of the regimen used. This points to underlying mechanisms established before starting ART, likely related to the HIV reservoir size. Our study reinforces these findings, as we observed strong associations between viral blips, RV, and several non-cART anchor factors, which have all been linked to the viral reservoir: high zenith pVL[79], low nadir CD4$^+$ count[36], but also Fiebig stage at ART initiation[80], and time since ART initiation[81]. Thus, our results, combined with those of Alvarez et al., suggest a large role of the viral reservoir in the incidence of virological non-suppression outcomes in PWH.

It would be interesting to explore this further in future research. One option to investigate this could be an observational cohort study of individuals with a new HIV infection. These individuals would undergo a baseline measurement of their viral reservoir size, such as cell-associated HIV RNA and DNA[82,83], before starting ART. They would then be followed over time to monitor the occurrence of blips, or the level of RV. This approach would provide insight into the association between the actual baseline reservoir size (and not proxies thereof) and RV and blips. Finally, there is the question of how the findings of our current study can be translated into clinical practice. Our study shows that the highest occurrence of both RV and blips is observed in PI-based regimens. It's important to note that RV levels are unknown for both caregivers and PWH in clinical practice. However,

both parties are aware of any blips, and since they generate uncertainty and result in an increased clinical burden[65], it may be worth considering switching from PIs to INSTIs when encountering (multiple) blips, given the lower blip rates associated with INSTIs. From a viral reservoir perspective, we also observed that several factors associated with a larger reservoir were linked to higher rates of RV and blips. Therefore, when clinicians are faced with a new PWH presenting with a high zenith pVL, low nadir $CD4^+$ count, and Fiebig stage VI, besides the obvious reasons to start with INSTIs, starting INSTIs may also help mitigate any increased risk of blips. Nevertheless, it's important to emphasize that our observational study investigated associations rather than causality. Definitive conclusions cannot be drawn at this stage and the above are only conjectures. More research is needed first, and in my opinion, the above-mentioned study design would a good next step towards elucidating these pathways.

Neurocognitive impairment, collectively known as HIV-associated neurocognitive disorders (HAND) in PWH, is a prevalent condition and significantly impacts quality of life.[84,85] It is hypothesized that cART itself contributes to the development of HAND by exerting a neurotoxic effect.[86,87] The characteristic features of HAND include neurocognitive dysfunction affecting memory, concentration, attention, and motor skills. Clinical diagnosis of HAND involves the use of a neuropsychological assessment (NPA), whereas in the research setting blood oxygen level dependent (BOLD) functional MRI (fMRI) is also used to assess neurocognition.[88,89] BOLD fMRI detects localized changes in cerebral blood flow and oxygenation, providing insight into regional neuronal activity and may therefore potentially facilitate a better understanding of the role of cART in the etiology of HAND.[90] Of particular interest in this context is efavirenz (EFV), an NNRTI notorious for its neurocognitive side effects such as dizziness or insomnia.[91]

From 2015 to 2017, the ESCAPE (Effect of Switching AtriPla to Eviplera on neurocognitive and emotional functioning) RCT was conducted to evaluate the impact of discontinuing EFV on neurocognition in asymptomatic PWH. Both NPA and BOLD fMRI were utilized to assess neurocognitive outcomes.[92] The NPA results showed improvement in the cognitive domains attention and processing speed 12 weeks after discontinuing EFV. In **Chapter 5**, using data from the ESCAPE trial, we investigated the effect of EFV on reward processing using BOLD fMRI. Reward processing consists of several neurocognitive processes such as processing the outcome of a reward and anticipating future rewards, and is crucial for decision-making and goal-directed behavior.[93] Our findings indicated that discontinuing EFV did not result in any significant alterations in reward processing. This is particularly reassuring considering the elevated prevalence of depression and apathy in PWH[94], which are associated with reward processing abnormalities.[82] In **Chapter 6,** we focused on another neurocognitive process in PWH on EFV: response inhibition. Response inhibition reflects the ability to suppress irrelevant or interfering information or impulses.

[95] Assessing response inhibition is important due to its association with other prevalent disorders in PWH[96], namely gambling and substance abuse[97,98]. By combining NPA and BOLD fMRI findings, we examined potential neural mechanisms underlying the observed cognitive improvements in attention and processing speed during NPA. Although no statistically significant differences were observed in response inhibition after discontinuing EFV, which is comforting, we did find noteworthy interactions between changes in attention and processing NPA Z-scores and neuronal activity in multiple regions. These interactions suggest that EFV's effect on attention and processing speed is, at least partially, mediated by reactive inhibition and thus affects these key subcortical areas involved in executive functioning, working memory and attention. It is important to note that due to limited sample size in both analyses, the lack of significant findings regarding reward processing and response inhibition may be attributed to insufficient statistical power and thus prohibits us from drawing definitive conclusions regarding EFV's potential neurotoxic effect.

Although EFV is no longer recommended as first-line therapy in guidelines, it has been widely used as such for over 15 years until 2018.[15,16,99] It remains recommended as an alternative first-line therapy and continues to be extensively used in low- and middle-income countries.[100] Forecast analyses suggest that by 2025, approximately 10 million PWH, constituting 25% of the estimated total population, will still be using EFV-based regimens.[101] Furthermore, even in high-income countries like the Netherlands, EFV continues to be employed, as evidenced by the 6% of PWH who used EFV-based regimens in 2021.[102] In light of this, it is encouraging that our findings did not indicate any significant alterations in reward processing or response inhibition associated with EFV. However, with the advent of the newer INSTI-based regimens, which offer superior virological efficacy and tolerability, the global use of EFV is ultimately expected to gradually decline in the coming years. Nonetheless, milder forms of HAND, asymptomatic neurocognitive impairment and mild neurocognitive disorders, continue to be prevalent in the cART era, even in the setting of optimal virologic suppression and immunological recovery.[103–105] Consequently, I believe that both HIV caregivers and researchers should maintain their focus on HAND in the upcoming years. It is important to note that INSTI-based regimens, particularly those containing DTG, have been associated with neurocognitive adverse effects.[34,106–109] Additionally, they have been linked to neurocognitive impairment[110], though not all studies have found this association[111,112]. Moreover, in our real-life study on DOR, 3.9% of PWH on DOR-based regimens discontinued to neuropsychiatric adverse effects. Given the recommendation of DTG, BIC and DOR (in certain clinical situations) as first-line therapy, it is crucial to evaluate their role in HAND among PWH. In my opinion, the best way to adequately investigate this are large-scale RCTs incorporating both NPA and BOLD fMRI as diagnostic modalities. NPA can then be used to examine potential DTG- and BIC-associated changes in neurocognitive performance in cognitive domains, while BOLD fMRI can be used to investigate localized region-specific neuronal changes and explore

neuronal mechanisms underlying any ART-related neurocognitive impairment. Such studies could shed light on the hypothesis that HAND is (partially) attributed to cART, and more specifically DTG, BIC or DOR, and, if this proves to be the case, would provide more insight into the specific brain regions affected by cART-related damage.

Part II: Optimizing treatment and monitoring of HIV-associated comorbidities

In addition to HIV itself, co-morbidities associated with HIV are a large driver of health-related quality of life for PWH.[113] In fact, they are becoming increasingly important as their prevalence continues to rise over the years, partly due to an ageing population.[114] Co-morbidities such as cardiovascular or liver disease, for example, are already widespread among PWH.[115] To achieve good health-related quality of life, it is crucial to optimize not only the treatment and monitoring of HIV, but also that of HIV-associated comorbidities. Therefore, caregivers must be knowledgeable about and attentive to co-morbidities that may affect PWH. This includes knowledge about current comorbidities, but also about possible residual sequelae from the past. In this second part of the discussion, we focus on optimizing the treatment and monitoring of two specific HIV-associated comorbidities: PJP and HBV co-infection.

Pneumocystis jirovecii, a yeast-like fungus, used to be a significant cause of pneumonia in PWH with AIDS during the early stages of the HIV epidemic.[116] However, with the advent of improved ART and PJP treatment strategies, mortality due PJP has significantly decreased.[8,117] Although PJP is nowadays relatively uncommon, it remains the most prevalent AIDS-defining condition in the Western world.[118] Consequently, there is currently a significant number of aging PWH who previously had PJP. It is unclear whether past PJP leads to long-term pulmonary dysfunction in this group, which is of particular concern considering the already elevated and even increasing incidence of pulmonary morbidity in PWH.[119,120] In **Chapter 7,** we investigated the impact of past PJP on long-term pulmonary dysfunction in PWH. Employing a cross-sectional design, we enrolled two study groups: one comprised 51 PWH who were infected with PJP more than a year ago, and the other composed of 51 well-matched controls without a history of PJP. We assessed pulmonary dysfunction using one pulmonary function test (PFT), with a focus on diffusion impairment as the primary outcome, as well as evaluating obstructive and restrictive impairment. Our findings revealed that past PJP was not associated with diffusion impairment, nor with obstructive and restrictive impairment. However, we did find that current (compared to never) smoking was independently associated with lower transfer factor of the lung for carbon monoxide (TLCO) Z-scores and diffusion impairment (defined as a TLCO Z-score <1.645). Notably, over 25% of PWH in our study had diffusion impairment, as defined by the latest European Respiratory Society/American Thoracic Society guidelines.[121,122]

Our study provided several important insights. Contrary to our initial expectations, past PJP

was not associated with pulmonary dysfunction. However, as we found that a substantial proportion of our study population had diffusion impairment, this confirmed the previously observed high prevalence of pulmonary morbidity in PWH.[119,120] The question remains which factors then contribute to this impairment, since PJP seems less likely. While our study could not directly investigate the effect of HIV itself on pulmonary dysfunction, other studies have indicated that HIV itself has a substantial impact on pulmonary function.[123–125] This is likely due to HIV-associated immune activation and systemic inflammation resulting in pulmonary damage. A study by Thudium et al. supports this notion, as they investigated the association between different inflammatory biomarkers and pulmonary indices in PWH and controls without HIV, reporting that elevated levels of IL-6 in well-treated PWH were independently associated with low dynamic lung function and airflow limitation.[126] Together with our finding that current (versus never) smoking was significantly associated with pulmonary dysfunction, it appears that persistent pulmonary damage (from various factors such HIV-associated immune activation, inflammation and smoking) plays a greater role in pulmonary dysfunction than a single episode of pulmonary damage from PJP. Nevertheless, it is important to note that our study had several limitations, making it premature to draw this conclusion in full.

In **Chapter 8**, we responded to a correspondence we received after publication of our study, which mentioned several unaddressed confounders (i.e., history of bacterial pneumonias, inhaled or intravenous drug abuse and hepatitis C virus (HCV) infection). Moreover, the authors pointed out the possibility of survival bias due to our study's cross-sectional design. Because those with severe PJP sequelae may have died before they could participate in our study, this may have led to an underestimation of the association between PJP and diffusion impairment. In our response, we acknowledged these limitations (which were mentioned already in the original study) and elaborated on the effect of PJP in the modern cART era. The question is whether the improved HIV management in the current era (more) effectively mitigates HIV-related immune activation and consequently preserves pulmonary function, in turn potentially attenuating the impact of PJP? We emphasized that in reviewing studies conducted in the cART era that examined risk factors (including PJP) for diffusion impairment, none provided evidence that past PJP alone is independently associated with diffusion impairment.[123,125,127,128] These findings aligned with results from our study: a history of PJP seems to consistently lose significance as an independent contributing factor in the presence of other risk factors, including those mentioned in the correspondence. We therefore concluded that there is currently no convincing evidence indicating a significant impact of past PJP alone on diffusion impairment in the context of optimally treated HIV.

Given the potential survival bias, we believe that more longitudinal research on this topic is needed, as it would provide a better understanding of the progression of pulmonary dysfunction over time and allow for the examination of specific underlying risk factors. An

interesting study in this regard is the study by Verboeket et al.[129] In this prospective cohort study, longitudinal changes in spirometry indices were examined in PWH and matched controls without HIV, considering confounding factors like smoking. They also investigated the effect of previous PJP solely in PWH, which occurred in 48 individuals (10% of the study population) and found no association with forced expiratory volume in one second (FEV1) or forced vital capacity (FVC) decline. It would have been interesting if the researchers of this cohort had assessed the effect of PJP on diffusion impairment (TLCO Z-score) rather than FEV1 and FVC, which are measures of obstructive and restrictive impairment, because one would expect diffusion capacity to be most affected based on the pathophysiological mechanism of PJP. In the future, to provide a more definitive answer on the question of PJP and diffusion impairment, ideally, a large-scale prospective cohort study should be conducted in people with advanced HIV infection (i.e., nadir $CD4^+$ count <200 cells/mm^3), as these are the PWH at risk of acquiring PJP. These participants would receive a PFT at baseline, followed by regular PFTs. At baseline and during each follow-up PFT, data would be collected on them, including on PJP status and the confounders used in our cross-sectional study, but also on confounders we could not account for (i.e., history of prior bacterial pneumonias, inhaled or intravenous drug use, HCV infection, and underlying pulmonary vascular or interstitial lung disease).[123,127,130] Respiratory symptoms should also be assessed at baseline and during follow-up, to assess their progression over time and the specific effect of pulmonary impairment risk factors on them. These longitudinal data, which overcome the potential survival bias and unaddressed confounders in our cross-sectional study, can then be analyzed using mixed models with repeated measures. In this way, the effect of PJP (if any) on the TLCO-z-score over time can be multivariably analyzed, shedding more light on this interesting and timely topic.

While PJP is relatively rare in PWH today, the same cannot be said for infection with HBV. HBV co-infection is relatively common in PWH due to shared routes of transmission, with global estimates indicating that approximately 5-20% of PWH are affected.[131] The introduction of tenofovir, an NRTI, has significantly facilitated the treatment of HBV co-infection in PWH, leading to better clinical outcomes.[132] However, there is concern that the convenience of tenofovir-containing regimens may have led to decreased attention to the treatment and monitoring of HBV in PWH. This concern becomes even more relevant in light of newer ART regimens that do not include tenofovir, such as dual or long-acting injectable therapy.[21,22,133] In **Chapter 9**, we presented the results of a proof-of-concept quality assessment and improvement study focusing on HBV care in PWH. In this study, we first assessed the quality of guideline-adherent HBV care in PWH and those with HIV/HBV co-infection. We identified 28.8% of our study population with HIV as non-immune for HBV infection, making them candidates for HBV vaccination. In individuals with HIV/HBV co-infection, hepatitis delta virus (HDV) antibody screening, hepatitis B surface antigen (HBsAg) monitoring and hepatocellular carcinoma (HCC) surveillance were found to be substandard. We performed

the missing laboratory tests to optimize monitoring and screening for co-infections and found that, in people with HIV/HBV co-infection, 11.8% had HDV antibodies and 10% had HBsAg seroclearance.

The results of our study underscore the importance of continued attention to HBV care in PWH and highlight critical areas for improvement. It is intriguing to note that despite the existence of HBV vaccination for more than 30 years in the Netherlands[134], nearly 30% of our study population remains unvaccinated. This can only partly be attributed to the restriction of free HBV vaccines for risk groups such as men who have sex with men (MSM) in the Netherlands[134], as our study found that 19.1% of MSM were still unvaccinated. Additionally, 3% of this group was not on tenofovir, placing them at highest risk of HBV infection.[135] Thus, a more proactive approach to HBV vaccination appears to be a promising option for enhancing HBV care in this group. In those already co-infected with HIV/HBV, a significant disparity in guideline adherence was observed across various aspects of HBV care. Particularly concerning was the substandard implementation of HDV antibody screening, HBsAg monitoring, and HCC surveillance, given the implications of these measures in terms of increased risk of end-stage liver disease and high mortality in case of (missed) HDV co-infection and HCC.[136,137] Our study marks the first step in raising awareness as we have informed caregivers and conducted subsequent laboratory testing to address missing data. In my opinion, the next step in improving HBV care involves the implementation of targeted healthcare interventions. For instance, in the case of HDV, reflex testing could be used, where a positive HBsAg result immediately triggers HDV antibody screening in PWH. In Spain, implementation of this has resulted in five times more HDV diagnoses, albeit in people with HBV mono-infection.[138] In light of this study and our own findings, we have chosen to implement this in the UMC Utrecht and are currently evaluating its impact. Moreover, interventions for HCC surveillance could include automated checklists for every newly diagnosed person with HIV/HBV co-infection to assess HCC indication, as well as automated reminders or educational programs for both caregivers and those with HIV/HBV co-infection.[139]

As the number of new HBV infections in PWH continues to decline as a result of increased HBV vaccination and a preventive effect of tenofovir[102], attention to HBV (co-)infection among caregivers will diminish. The dangers of inattention are illustrated by a recent case-series study of HBV infection or reactivation in PWH after switching to dual therapy, in which 2 cases of fulminant hepatitis were reported after switching to non-tenofovir ART, even requiring liver transplantation in 1 case.[140] It is therefore imperative to continue prioritizing care and attention for this vulnerable group. In the upcoming years, improving national awareness for people with HIV/HBV co-infection is of great importance. The availability of the ATHENA cohort[31], a comprehensive database with information on all PWH under care in the Netherlands (including on HBV co-infection) offers an opportunity for some of the

targeted interventions mentioned above. Since our study in the UMC Utrecht highlights the suboptimal state of three specific HBV areas, the focus should be on alerting caregivers nationwide regarding these areas. In this regard, the recent efforts to address HDV in the Netherlands are of interest[141], especially considering the emergence of new therapeutic options[142].

I would like to conclude this discussion with some final reflections on the future of HIV and HIV care as a whole. In the three years since I started researching HIV, substantial changes have already taken place. On the treatment front, in August 2020, INSTI-based regimens such as Triumeq and Biktarvy were well-established as preferred ART regimens, while dual and long-acting injectable therapy had just been approved or were about to be approved for use in PWH. Since then, even newer ART agents that target entirely different mechanisms have been under development or even already approved for use, such as non-nucleoside reverse transcriptase translocation inhibitors (Islatravir)[143] and capsid inhibitors (Lenacapavir)[144]. The search for new treatments thus continues and is far from complete. In the coming years, I anticipate that INSTI-based regimens will continue to be the preferred treatment for most PWH, with an increasing portion of the growing HIV population adopting them due to the considerable benefits mentioned earlier. However, as also previously mentioned, INSTIs (and TAF) have been increasingly linked to excessive weight gain[44], showing it's crucial to continue development in other ART classes as well. DOR serves as an important example in this regard. Despite appearing less effective than BIC or DTG, there will always be PWH who require alternatives from non-INSTI classes due to factors such as excessive weight gain, intolerance or virologic resistance. It's vital not to concentrate all our efforts in one area for the sake of these individuals. Moreover, DOR represents a substantial step forward compared to earlier NNRTIs like EFV and rilpivirine, illustrating that advances in efficacy and tolerability are clearly still possible in currently less prescribed anchor classes such as NNRTIs and PIs.

Another intriguing development is long-acting injectable therapy, which is currently approved for use in PWH on either a one- or two-month basis.[15,16,133,145] Because this requires more frequent hospital visits compared with oral treatment, along with injection site reactions such as pain, relatively few PWH currently choose this option. In addition, the fact that most PWH have been on an effective oral ART regimen without adverse effects for many years makes them reluctant to switch. However, efforts are underway to extend the interval of long-acting injectable therapy to, for example, six months.[146] This extension does pose potential challenges regarding the risk of VF (with subsequent emergent NNRTI and INSTI resistance). Given that this risk may be higher with longer dosing intervals[146], I believe caution should be exercised when assessing extending the interval. Nevertheless, if it proves feasible in the years ahead, I expect a significant number of PWH will choose this ultra-long-acting option, as it would reduce their hospital visits to the current frequency on

oral ART, while eliminating the need to take daily pills.

Although this is beyond the scope of my dissertation, it is interesting to discuss progress made and future challenges in other areas of HIV care. In the area of prevention, for example, the introduction and scale-up of pre-exposure prophylaxis (PrEP) has led to a substantial decrease in new HIV infections. In the Netherlands, this is reflected in an absolute decrease in the number of new HIV infections among MSM (from 256 in 2020 to 218 in 2021), with a smaller proportion involving recent (rather than non-recent) HIV infections (namely 27% in 2021 versus 37% in 2020).[102,147] A recent HIV infection is defined as a negative test in the past 12 months, and since the MSM who tested regularly were able to use PrEP, the decrease in recent HIV infection indicates its good efficacy among MSM. Even more recently, only 9 new HIV infections were reported in 2022 in Amsterdam, with PrEP presumably also playing a major role.[148] Despite these successes, prevention challenges remain: men and sex workers who have sex with men are high-risk groups in the Netherlands for whom PrEP is currently available, but long waiting lists exist due to funding problems. Progress such as in PrEP has unfortunately not been made in all areas, as demonstrated by recent setbacks in the development of an HIV vaccine.[150] An HIV vaccine does not seem likely in the coming years, underscoring the importance of continued access to HIV prevention and treatment worldwide. In that regard, it is sad to see that regional disparities continue to increase.[151] There are wide disparities globally in the response to the HIV epidemic: while the number of new HIV infections decreased by about 40% in sub-Sahara Africa in the last 10 years, in contrast, in eastern Europe, central Asia, Middle East and north Africa the number increased by 27-33%.[152] Particularly marginalized populations that face high levels of stigma, such as people who inject drugs, transgender people or MSM are at the center of the increasing incidence in these regions. In the coming years, these regions and particularly key populations should therefore be one of the main focus areas for large-scale implementation of current HIV prevention and treatment strategies. Finally, while prevention is key in ending the HIV epidemic, the penultimate goal for the 39 million now living with HIV is undoubtedly a cure. Encouragingly, recent reports of the sixth person potentially being cured of HIV by allogeneic hematopoietic stem cell transplantation (the first person by way of a CCR5 wild-type donor) hold promise from a research perspective.[153] I look forward to seeing what progress will be made in these areas of HIV care in the coming years.

CONCLUSION

This dissertation encompasses research aimed at optimizing the treatment and monitoring of HIV and HIV-associated co-morbidities. A wide range of topics has been addressed, including the significant difference between virologic suppression observed in clinical trials and real-world settings, potential effects of contemporary ART beyond virologic suppression,

such as HIV-associated immune activation, viral blips, residual viremia and neurocognitive impairment, as well as treatment and monitoring of PJP and HBV co-infection. Throughout these studies, it is evident that while substantial progress has been made in improving the management of these conditions, there is still much work to be done. More than 40 years ago, the world was confronted with the worst health crisis we had ever seen. Today, PWH on ART can expect to live nearly as long as those without HIV, with a significantly improved quality of life. This paradigm shift has allowed us to move beyond virologic suppression and focus on broader aspects of HIV care. However, the “last mile” is often the most challenging, and we must continue to push forward to achieve not just 95-95-95, but the ultimate goal as HIV caregiver: 100-100-100-100. Although the road to ending the HIV epidemic is still long and challenging, I believe that with continued effort and dedication, we can realize a future where HIV is no longer a global health crisis, but a manageable and controllable condition for those living with it.

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SUMMARY

Background

Over four decades ago, the world witnessed the beginning of a global health crisis unprecedented in history: the Human Immunodeficiency Virus (HIV) epidemic. The initial 15 years were marked by significant setbacks and difficult progress in the treatment of HIV. However, a turning point came with the advent of combination antiretroviral therapy (cART). Currently, people with HIV (PWH) on cART experience minimal side effects, and cART is remarkably effective in suppressing HIV - the longstanding ultimate objective. These advances in antiretroviral therapy (ART) now allow us to expand our perspective. In this dissertation, I present a series of studies dedicated to optimizing treatment and monitoring of both HIV and HIV-associated co-morbidities beyond virologic suppression.

Optimizing treatment and monitoring of HIV

In the first part of this dissertation, several studies are presented on optimizing treatment and monitoring of HIV. Although virologic suppression is widely achieved in the Western world today, important challenges remain. These include the substantial differences between virologic suppression achieved in clinical trials versus the real world, as well as the potential effects of contemporary ART beyond virologic suppression, such as HIV-associated immune activation, viral blips, residual viremia (RV), and neurocognitive impairment.

In [**Chapter 2**], we investigated the real-world virologic effectiveness and tolerability of switching to doravirine (DOR)-based ART in a nationwide matched cohort study of Dutch PWH. DOR is a non-nucleoside reverse transcriptase inhibitor (NNRTI) approved for use as anchor agent in PWH since 2018. We observed non-inferior virologic effectiveness between those who switched to DOR-based ART and well-matched controls on non-DOR regimens after 104 weeks. Although more PWH who switched to DOR-based ART modified their regimen within the first year, the tolerability of the regimens was ultimately similar between the two groups at the end of the study period. In comparison to the registration trial conducted in a similar ART-experienced population switching to DOR-based ART, the percentage of individuals discontinuing DOR-based ART due to toxicity was five times higher in our study (12.4% after 104 weeks versus 2.7% after 144 weeks). Nevertheless, considering the non-inferior effectiveness and similar tolerability observed between study groups, we concluded that our findings indicate that DOR-based triple therapy is a non-inferior, effective and well-tolerated ART option for ART-experienced PWH considering a regimen switch.

In the following chapter, we presented the results of a cohort study in which we investigated viral blips and the potential role of RV in its etiology, as well as their associated factors [**Chapter 3**]. Viral blips are temporary elevations of HIV plasma viral load above the

detection limit of standard assays and RV is the detectable viremia below the commonly used assay threshold of 50 cop/mL (i.e., virologic suppression). We observed that RV was indeed associated with subsequent viral blips in virologically suppressed PWH on triple therapy regimens. Importantly, higher preceding RV was associated with higher odds of subsequent blips during follow-up. When investigating factors associated with RV as an outcome, we found that protease inhibitor (PI)-based regimens, compared to NNRTI- and integrase strand transfer inhibitor (INSTI)-based regimens, were associated with higher odds of RV. In addition, certain factors previously linked to the viral reservoir, such as Fiebig stage at ART initiation, zenith plasma viral load, lowest recorded $CD4^+$ count, and time since ART initiation, were also associated with RV. Interestingly, while PI-based regimens were associated with the highest odds of subsequent viral blips (consistent with the observed association between PI-based regimens with the highest odds of RV), INSTI-based regimens were associated with the lowest odds of blips, in contrast to NNRTI-based regimens, which were associated with the lowest odds of RV. The findings of this study therefore suggest that viral blips have a multifactorial origin and that the effect of cART on blips is not solely determined by changes in RV.

In **[Chapter 4]**, in a mini review, we elaborated on the effect of triple, dual and mono ART on HIV-associated immune activation in PWH. HIV-associated immune activation is a hyperactive inflammatory state that ultimately leads to T-cell depletion and is associated with numerous comorbidities, including cardiovascular disease. We first addressed the different markers of HIV-associated immune activation, focusing on soluble and T-cell activation and apoptosis markers. Afterwards, we delved into studies assessing these markers in PWH on ART. Studies regarding monotherapy show that a switch to monotherapy is associated with increased T-cellular markers and that the pattern in terms of soluble markers is conflicting. Importantly, given the virologic inferiority of monotherapy compared to triple therapy, this will not be an issue of importance in the coming years. However, dual ART regimens have been on the market since 2018: studies in PWH switching to dual therapy show no consistent increase in soluble markers compared to triple therapy, but there are no studies that specifically examined the T-cell markers. Given this paucity of evidence, we concluded that there is insufficient evidence that dual therapy are non-inferior to triple therapy in terms of suppressing HIV-associated immune activation.

In the final chapters of this part, we shifted our focus towards neurocognitive impairment: a common condition in PWH, collectively known as HIV-associated neurocognitive disorders (HAND), which substantially impacts the quality of life. HAND is comprised of three subtypes: HIV-associated dementia, mild neurocognitive disorder, and asymptomatic neurocognitive impairment. It is hypothesized that cART itself contributes to the development of HAND by exerting a neurotoxic effect. Of particular interest in this context is efavirenz (EFV), an NNRTI notorious for neurocognitive side effects such as dizziness or insomnia. In **[Chapter 5]**, using

data from the ESCAPE trial in which the impact of discontinuing EFV on neurocognition in PWH was assessed, we investigated the effect of EFV on reward processing using blood oxygen level dependent (BOLD) functional MRI (fMRI). Reward processing consists of several neurocognitive processes such as processing the outcome of a reward and anticipating future rewards, and is crucial for decision-making and goal-directed behavior. BOLD fMRI can detect localized changes in cerebral blood flow and oxygenation, thus providing insight into regional neuronal activity related to reward processing. Our results indicate that discontinuation of EFV did not result in any significant alterations in reward processing in neurocognitive asymptomatic male PWH. The findings of this study are especially reassuring in light of the already increased prevalence of depression and apathy in PWH, conditions associated with impaired reward processing.

Lastly, we examined response inhibition and additionally explored potential neural mechanisms of cognitive improvement in the same population **[Chapter 6]**. Response inhibition reflects the ability to suppress irrelevant or interfering information or impulses. By combining neuropsychological assessment (NPA) and BOLD fMRI findings, we assessed potential neural mechanisms underlying the observed cognitive improvement in attention and processing speed during NPA. Although no significant differences were observed in response inhibition after discontinuing EFV, we did find noteworthy interactions between changes in attention and processing NPA Z-scores and neuronal activity in subcortical functioning in multiple regions. Our findings suggest that EFV's effect on attention and processing speed is, at least partially, mediated by reactive inhibition and thus affects these key subcortical areas involved in executive functioning, working memory and attention. Additionally, discontinuing EFV did not appear to have a substantial effect on response inhibition, which is reassuring given that impaired response inhibition has been associated with gambling and substance abuse: disorders already common in PWH.

Optimizing treatment and monitoring of HIV-associated co-comorbidities

In the second part of this dissertation, I present the results of two studies focused on HIV-associated co-morbidities. In addition to HIV itself, co-morbidities associated with HIV are a large driver of health-related quality of life for PWH. This is especially important in light of the increasing prevalence of HIV-associated co-morbidities, in part due to an aging population. To achieve good health-related quality of life for PWH, it is therefore crucial to optimize not only the treatment and monitoring of HIV, but also that of HIV-associated comorbidities.

In **[Chapter 7]**, we investigated whether prior *Pneumocystis jirovecii* pneumonia (PJP) was associated with long-term pulmonary impairment in PWH. Employing a cross-sectional design, we enrolled two study groups: one consisting of PWH who had experienced PJP over a year ago, and the other comprising well-matched controls without a history of PJP. We evaluated pulmonary dysfunction using a single pulmonary function test and observed

that past PJP was not associated with diffusion impairment, nor with obstructive or restrictive impairment. However, we did find that current smoking (compared to never smoking) was independently associated with all three types of impairment. Notably, over 25% of PWH in our study had diffusion impairment. Our findings thus suggest that PJP-related pulmonary damage recovers in the long-term or that its contribution, in the presence of persistent pulmonary impairment from smoking or HIV infection, is marginal. These findings offer reassurance to those with past PJP. However, given the substantial proportion of PWH with diffusion impairment and the observed correlation with smoking, once again, it emphasizes the critical importance of smoking cessation.

Next, in **[Chapter 8]**, we responded to a correspondence we received after publication of our study on PJP. In this correspondence, several unaddressed confounders were mentioned and the possibility of survival bias due to our study's cross-sectional design was pointed out. In our response, we acknowledged these limitations (which were mentioned already in the original study) and elaborated on the effect of PJP in the modern cART era. We emphasized that in reviewing studies conducted in the cART era that examined risk factors (including PJP) for diffusion impairment, none provided evidence that past PJP alone was independently associated with diffusion impairment. These findings aligned with results from our study: a history of PJP seems to consistently lose significance as an independent contributing factor in the presence of other risk factors, including those mentioned in the correspondence. We therefore conclude that there is currently no convincing evidence indicating a significant impact of past PJP alone on diffusion impairment in the context of optimally treated HIV.

In the following **[Chapter 9]**, we presented the results of a proof-of-concept quality improvement study in which we investigated and improved guideline-adherent hepatitis B virus (HBV) care in PWH. We first assessed the quality of guideline-adherent HBV care regarding screening, therapy and monitoring in PWH and with HIV/HBV co-infection. We identified 28.8% of our study population with HIV as non-immune for HBV infection, making them candidates for HBV vaccination. In individuals with HIV/HBV co-infection, hepatitis delta virus (HDV) antibody screening, hepatitis B surface antigen (HBsAg) monitoring and hepatocellular carcinoma (HCC) surveillance were found to be substandard. Missing laboratory tests were then performed to optimize monitoring and screening for co-infections, showing that in people with HIV/HBV co-infection, 11.8% had HDV antibodies and 10% had HBsAg seroclearance. This study demonstrated the feasibility and added value of evaluating HBV care components and performing missing laboratory tests, identifying a large number of HBV vaccination candidates and HDV antibody screening, HBsAg monitoring and HCC surveillance as key areas for improvement.

Finally, in **[Chapter 10]**, we discussed the main findings of this dissertation and presented perspectives for the future. We addressed knowledge gaps and made recommendations for new research projects.

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NEDERLANDSE SAMENVATTING VOOR NIET-INGEWIJDEN

DANKWOORD / ACKNOWLEDGEMENTS

LISTS OF PUBLICATIONS

CURRICULUM VITAE

NEDERLANDSE SAMENVATTING VOOR NIET-INGEWIJDEN

HIV: van verleden tot heden

Het Humaan Immuundeficiëntie Virus (HIV) werd 40 jaar geleden ontdekt toen bleek dat vijf jonge mannen zonder medische voorgeschiedenis een ernstige longontsteking hadden die niet voorkomt bij mensen met een gezond immuunsysteem. HIV bleek witte bloedcellen te infecteren wat ervoor zorgt dat het immuunsysteem minder goed kan werken. Het werd duidelijk dat het virus kon worden overgedragen via verschillende routes, waaronder bloedtransfusies, seksueel contact en de geboorte. Mensen met een vergevorderde HIV-infectie kregen allerlei ernstige infectieziekten en vormen van kanker omdat het immuunsysteem slecht werkte: dit stadium van vergevorderde HIV-infectie kwam bekend te staan als AIDS ('Acquired Immunodeficiency Syndrome'). Helaas bleek het ontwikkelen van een goede behandeling tegen HIV in het begin erg moeilijk, waardoor veel (jonge) mensen met HIV uiteindelijk AIDS kregen en stierven aan de gevolgen hiervan. In 1996 zorgde de introductie van een combinatietherapie bestaande uit drie HIV-remmers, ook wel antiretrovirale middelen genoemd ('cART'), voor een ware revolutie in de behandeling van HIV. Het leidde tot een substantiële toename van de levensverwachting van mensen met HIV. Ondanks het succes bleef de behandeling van mensen met HIV in de vroege dagen van cART uitdagend omdat zij dagelijks veel pillen moesten slikken met vaak ernstige of soms zelfs dodelijke bijwerkingen.

Vandaag de dag heeft het landschap van de HIV-behandeling een opmerkelijke transformatie ondergaan. De meeste soorten combinaties van HIV-remmers ('regimes') bestaan nu uit één pil per dag met minimale bijwerkingen en zijn zeer effectief in het onderdrukken van HIV. Daarbij is de levensverwachting van mensen die behandeld worden voor HIV tegenwoordig bijna vergelijkbaar met die van mensen zonder HIV en is hun kwaliteit van leven sterk verbeterd. Hoewel er meer mensen dan ooit met HIV leven - de huidige schattingen zijn 39,0 miljoen wereldwijd in 2022 - is het sentiment hoopvoller in vergelijking met de donkere beginperiode. Historisch gezien was het onderdrukken van HIV ('virologische onderdrukking') het alomvattend doel bij HIV zorg, omdat dat levens redde. Dankzij de verbetering van HIV medicijnen hebben we nu echter de mogelijkheid om ons perspectief te verbreden. Dit proefschrift focust op het verbeteren (optimaliseren) van de behandeling en monitoring van zowel HIV als bijkomende ziektes ('co-morbiditeiten') die vaak bij HIV voorkomen.

Optimaliseren van behandeling en monitoring van HIV

In het eerste deel van dit proefschrift worden verschillende studies over het optimaliseren van de behandeling en monitoring van HIV gepresenteerd. Hoewel virologische onderdrukking tegenwoordig veel wordt bereikt in de westerse wereld, blijven er belangrijke uitdagingen bestaan.

In [**Hoofdstuk 2**] hebben we gekeken naar hoe goed doravirine (een bepaalde HIV-remmer) werkt en hoe verdraagbaar dit medicijn is bij mensen met HIV in Nederland. Sinds de goedkeuring in 2018 is doravirine een belangrijke optie geworden in de behandeling van HIV. Uit onze bevindingen bleek dat doravirine na 104 weken behandeling net zo goed werkte als andere HIV-remmers die gebruikt worden. Daarnaast was het geruststellend om te zien dat, hoewel doravirine gebruikers vaker van HIV-behandeling wisselden vanwege bijwerkingen in het eerste jaar dan mensen met HIV die geen doravirine gebruikten, er na 2 jaar in beide groepen hetzelfde aantal van HIV-behandeling was gewisseld vanwege bijwerkingen: doravirine bleek dus net zo goed te worden verdragen als andere HIV-remmers na twee jaar.

In [**Hoofdstuk 3**] richt ons onderzoek zich op voorbijgaande verhogingen van het aantal virusdeeltjes bij mensen met HIV die al HIV-remmers gebruikten. Bij mensen met HIV die onder behandeling zijn, wordt het HIV-virus in het bloed meestal niet gedetecteerd ('virus ondetecteerbaar'). De meeste HIV-tests spreken van 'virus ondetecteerbaar' als er minder dan 50 virusdeeltjes per milliliter bloed zijn. Soms worden virusdeeltjes echter boven deze limiet gemeten. We onderzochten deze episodes van tijdelijke toename van het aantal virusdeeltjes, ook wel 'viral blips' genoemd, op hun mogelijke oorzaken. We ontdekten dat het optreden van viral blips gerelateerd was aan de eerdere hoogte van het aantal virusdeeltjes in het bloed *onder* de eerder genoemde grens van 50 virusdeeltjes per milliliter. Deze 'restwaarde' van virusdeeltjes (ook wel 'residuele viremie' genoemd) wordt dus niet gemeten door normale HIV-testen, maar kan wel worden gemeten door extra gevoelige HIV-testen. Het bleek dat hoe hoger de residuele viremie in het bloed bij een eerdere bloedcontrole, hoe groter de kans op een virale blip bij de volgende bloedcontrole. Daarnaast vonden we dat verschillende individuele factoren, zoals het type HIV-behandeling en de mate van aantasting van het immuunsysteem door HIV, van invloed waren op de kans op virale blips.

In [**Hoofdstuk 4**] richten wij ons op onnodige activering van het immuunsysteem door HIV, wat 'HIV-geassocieerde immuun activatie' wordt genoemd. Deze immuun activatie is belangrijk omdat het in verband is gebracht met verschillende bijkomende ziekten, zoals hart- en vaatziekten. Het is dus zaak om dit zo veel mogelijk te beperken bij mensen met HIV. In dit hoofdstuk bespreken we de verschillende studies die HIV-geassocieerde immuun activatie hebben onderzocht, specifiek in relatie tot HIV-behandeling. De behandeling van HIV bestaat meestal uit 3 HIV-remmers (triple therapie), maar tegenwoordig zijn ook regimes van 2 HIV-remmers goedgekeurd (duale therapie). Daarnaast wordt behandeling met slechts 1 HIV-remmer bestudeerd in onderzoekssettings (monotherapie). Uit het literatuuroverzicht blijkt dat er meer immuun activatie optreedt bij monotherapie vergeleken met triple therapie. Tot slot blijkt dat het onduidelijk is of er meer of minder immuun activatie optreedt bij duale therapie vergeleken met triple therapie.

De focus verschuift in **[Hoofdstuk 5]** naar neurocognitieve problemen (zoals moeite met concentreren) bij mensen met HIV, wat HIV-geassocieerde neurocognitieve stoornissen (HAND) wordt genoemd. HAND heeft een grote invloed op de kwaliteit van leven. Door gegevens van een eerder onderzoek naar de effecten van het stoppen met efavirenz, een bepaalde HIV-remmer, te analyseren, konden wij aantonen dat het stoppen van deze behandeling geen negatief effect had op een specifieke hersenfunctie, namelijk de verwerking van beloning. We onderzochten de verwerking van beloning met behulp van 'BOLD fMRI': een type MRI-scan waarmee neurocognitie kan worden onderzocht. Deze uitkomst was geruststellend omdat een verminderde beloningsverwerking verband houdt met depressie en apathie (gebrek aan emotie), welke vaker dan gemiddeld voorkomen bij mensen met HIV.

In **[Hoofdstuk 6]** onderzochten we een ander deel van neurocognitie bij mensen met HIV, namelijk het vermogen om impulsen te controleren, ook wel responsinhibitie genoemd. Wij combineerden resultaten van de BOLD fMRI met neuropsychologische testen (uitgevoerd door neuropsychologen) om dit te onderzoeken. Daarin ontdekten wij dat veranderingen in de BOLD fMRI resultaten samenhingen met veranderingen in de resultaten van bepaalde neuropsychologische tests. Daarnaast ontdekten wij dat stoppen met efavirenz geen negatief had op responsinhibitie. Dit laatste was geruststellend omdat er een verband bestaat tussen verminderde responsinhibitie en gedrag zoals gokken en middelenmisbruik, dat vaker voorkomt bij mensen met HIV.

Optimaliseren van behandeling en monitoring van HIV-geassocieerde comorbiditeiten
Het tweede deel van mijn proefschrift bevat twee studies die zich richten op ziekten die vaak gepaard gaan met HIV ('comorbiditeiten'). Naast HIV zelf, hebben deze comorbiditeiten een grote invloed op de kwaliteit van leven. Voor optimale kwaliteit van leven van mensen met HIV is het daarom essentieel om niet alleen de behandeling en monitoring van HIV te verbeteren, maar ook die van de gerelateerde ziekten.

In **[Hoofdstuk 7]** richten wij ons op mogelijke lange termijn schade aan de longen als gevolg van een eerdere longontsteking veroorzaakt door een schimmel genaamd *Pneumocystis jirovecii* bij mensen met HIV. We verzamelden gegevens van twee groepen: één bestaande uit mensen met HIV die meer dan een jaar eerder een zogenaamde *Pneumocystis jirovecii* pneumonie (PJP) hadden gehad, en een groep controlepersonen zonder voorgeschiedenis van PJP. Alle mensen ondergingen een longfunctieonderzoek, dat wij vervolgens vergeleken tussen beide groepen. We vonden geen verband tussen eerdere PJP en specifieke longfunctiestoornissen. Interessant genoeg was roken wel geassocieerd met verschillende vormen van longfunctiestoornissen. Het was opvallend dat meer dan een kwart van de mensen met HIV in ons onderzoek enige mate van longbeperking had. Dit benadrukt het belang van stoppen met roken.

[**Hoofdstuk 8**] is een reactie op een brief die wij ontvingen na de publicatie van het onderzoek naar PJP. De auteurs van de brief beargumenteerden dat ons onderzoek enkele belangrijke factoren over het hoofd zag en dat de opzet van de studie mogelijk tot enigszins vertekende resultaten had geleid. In ons antwoord erkennen wij dat de studie inderdaad mogelijke beperkingen had, zoals wij ook in het oorspronkelijke artikel hadden besproken. Vervolgens bespreken we hoe PJP van invloed is op mensen die moderne HIV-behandelingen krijgen. Als we kijken naar recente studies, dan toont geen enkele studie aan dat een eerdere PJP-infectie een probleem is voor de longen. Onze studie bevestigde dit ook: vroegere PJP lijkt niet samen te hangen met ernstige longproblemen, wanneer andere risicofactoren voor longfunctiestoornissen worden meegerekend. Daarom concluderen we dat er geen bewijs is dat PJP-infectie in het verleden op zichzelf longproblemen veroorzaakt bij mensen die een moderne, goede HIV-behandeling krijgen.

Vervolgens presenteren we in [**Hoofdstuk 9**] de resultaten van een onderzoek waarin wij ons richtten op het verbeteren van de kwaliteit van zorg wat betreft hepatitis B-virus (HBV) bij mensen met HIV. Wij beoordeelden hoe goed de richtlijnen werden nageleefd voor HBV zorg bij mensen met HIV en (co-)infectie met zowel HIV als HBV. Opmerkelijk was dat bijna een derde van de mensen met HIV geen immuniteit tegen HBV had en dus in aanmerking kwam voor vaccinatie tegen HBV. Bij mensen met een co-infectie met HIV/HBV schoot daarnaast de screening op hepatitis delta virus en de controle op specifieke biologische markers en leverkanker tekort. Door ontbrekende laboratoriumtesten na te bepalen, konden wij meer licht werpen op de aanwezigheid van bepaalde markers. We concluderen dat er behoefte was aan verbetering op specifieke gebieden wat betreft de HBV-zorg in mensen met HIV.

Tot slot bespreek ik in [**Hoofdstuk 10**], de discussie, de belangrijkste bevindingen van dit proefschrift en perspectieven voor de toekomst. Ik ben ingegaan op kennis die nog mist en heb aanbevelingen gedaan voor nieuwe onderzoeksprojecten om de gezondheid en het welzijn van mensen met HIV verder te verbeteren.

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CURRICULUM VITAE

Patrick George August Oomen was born August 18, 1993, in Delft, the Netherlands. After graduating from the Christelijk Lyceum Delft in 2011, he studied Medicine at Utrecht University. His first steps in research began with a scientific internship at the Department of Acute Internal Medicine, University Medical Center Utrecht (UMCU), in 2018. Under the supervision of Jan Willem Uffen, Jan Jelrik Oosterheert and Karin Kaasjager, he investigated the prognostic value of red blood cell distribution width in patients with suspected infection. This experience sparked his strong interest in infectious disease research.

Patrick obtained his medical degree in early 2019, after a senior internship in Internal Medicine at the Ziekenhuis Gelderse Vallei in Ede. He then started working as a physician in the Department of Internal Medicine at the Diakonessenhuis in Utrecht. During this period, he set up a database on antibiotic use and, supervised by Jacob Dutilh, Susan Logtenberg and Sanjay Sankatsing, published a study on the appropriate prescription of antibiotics at the emergency department.

In August 2020, under the supervision of Berend van Welzen and Andy Hoepelman, he started working on his PhD dissertation (‘HIV: optimizing treatment and monitoring beyond virologic suppression’) at the Department of Infectious Diseases at the UMCU. He combined his PhD research with the Postgraduate Master Epidemiology, from which he graduated cum laude in early 2023. In December 2023, Patrick started his training as a resident Internal Medicine at the Ziekenhuis Gelderse Vallei in Ede.