Towards 95% Viral Suppression: Targeting HIV Drug Resistance, Adherence to Therapy, and Access to Care in Southern and East Africa

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Jennifer Anne Brown

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Prof. Dr Niklaus Labhardt & Prof. Dr Thomas Klimkait als Erstbetreuer

Prof. Dr Markus Affolter als Zweitbetreuer

Prof. Dr Gilles Wandeler als externen Experten

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Prof. Dr Marcel Mayor

Dekan

Contents

1. Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
AZT	Azidothymidine
CART-1	Comorbidities and Virologic Outcomes among Patients on Antiretroviral Therapy in Rural Lesotho (cohort)
CASCADE	Same-day ART Initiation Versus Standard of Care after Positive HIV-test Result in Rural Lesotho (randomised controlled trial)
CD4	Cluster of differentiation 4
DNA	Deoxyribonucleic acid
DO-REAL	Dolutegravir in Real Life in Lesotho (cohort)
GIVE MOVE	Genotype-Informed Versus Empiric Management Of VirEmia (randomised controlled trial)
HIV	Human immunodeficiency virus
HIV-1	HIV type 1
HIV-2	HIV type 2
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
ODYSSEY	A Randomised Trial of Dolutegravir (DTG)-Based Antiretroviral Therapy vs. Standard of Care (SOC) in Children With HIV Infection Starting First-line or Switching to Second-line ART (randomised controlled trial)
REVAMP	Resistance Testing to Improve Management of Virologic Failure in Sub-Saharan Africa (randomised controlled trial)
RNA	Ribonucleic acid
SESOTHO	Switch Either near Suppression Or THOusand (randomised controlled trial)
SIV	Simian immunodeficiency virus
SIVcpz	Simian immunodeficiency virus strains infecting chimpanzees
SIVsm	Simian immunodeficiency virus strains infecting sooty mangabeys
Swiss TPH	Swiss Tropical and Public Health Institute
U=U	Undetectable=untransmittable
UNAIDS	United Nations Programme on HIV/AIDS
VITAL	Viral Load Triggered ART Care in Lesotho (randomised controlled trial)

2. Summary

In 2015, the Joint United Nations Programme on HIV/AIDS (UNAIDS) set the ambitious '90-90-90' targets with the aim that by 2020, 90% of people living with HIV should know their status, 90% of those who know that they have HIV should receive antiretroviral therapy (ART), and 90% of those receiving ART should achieve viral suppression. Success along this care cascade prevents HIV-related infections and progression to AIDS, leads to a life expectancy similar to that of people who do not have HIV, and prevents the onward transmission of HIV. Globally, these targets were narrowly missed at 84-87-90. To get back on track towards ending the epidemic by 2030, the target was raised to '95-95-95' by 2025.

This thesis aims to address the 'third 95' – viral suppression – in southern and eastern Africa, with projects conducted in Lesotho and Tanzania. The projects presented here address outcomes along each step of the 'viral load cascade': among people receiving ART, the detection of an unsuppressed viral load triggers adherence counselling, repeat viral load testing, and possibly switching to a different ART regimen. Particular consideration is given to children and adolescents, who are at a heightened risk for poor treatment outcomes. In line with the major underlying causes of viraemia among people receiving ART, viral resistance, adherence to therapy, as well as access to healthcare – including appropriate diagnostic services and antiretrovirals – are crosscutting themes.

Some of the studies address ART provision: how does the timing of ART initiation affect long-term engagement in care and viral suppression; and how does the roll-out of new drug regimens impact viral suppression or adverse effects? Other studies question the algorithms that guide the management of viraemia: what threshold should be used to define viraemia; does the requirement for adherence counselling before switching ART facilitate the emergence (further) resistance; and, crucially, under what circumstances is resistance testing clinically beneficial, and how can it be implemented in resource-limited settings? Finally, we assess outcomes along the entire viral load cascade among children living with HIV.

All projects aim to inform clinical guidelines, country programmes, and healthcare implementers in order to contribute to improved care for people living with HIV in and beyond the project countries.

3. Background

3.1. A Brief History of HIV, Antiretroviral Therapy and Treatment Guidelines

On 5 June 1981, an article in the *Morbidity and Mortality Weekly Report* described five cases of pneumocystis pneumonia – a disease associated with severe immunodeficiency – occurring in young homosexual men[1]. This short article would become known as the first time the disease later named acquired immunodeficiency syndrome (AIDS) entered the scientific literature. Further reports of formerly rare conditions were quick to follow, yet scientific progress was hampered by a lack of funding and political unwillingness, which only changed gradually and through tremendous efforts of activists and patient organisations[2].

The causative agent of AIDS, the virus later renamed human immunodeficiency virus (HIV), was first isolated and reported by distinct research groups in 1983[3,4]. Belonging to the family of retroviruses, HIV infects immune cells carrying the cluster of differentiation 4 (CD4) receptor as well as a chemokine co-receptor; notably CD4+ T cells, macrophages, dendritic cells, and monocytes[5,6]. Despite initial expectations that a vaccine would be developed within two years (a goal that has not yet been realised almost forty years later)[7], it would take another four years for the United States Food and Drug Administration to approve the first drug for the treatment of advanced forms of HIV[8]. The nucleoside reverse transcriptase inhibitor (NRTI) zidovudine, also known as azidothymidine (AZT), improved short-term survival and slowed disease progression[9–11]. However, it initially had to be taken every four hours[9], caused severe side-effects (prescribed at far higher doses than today)[8], and did not improve long-term survival[12,13]. As further drug options gradually followed, the three viral enzymes proved to be major drug targets: the reverse transcriptase, which mediates reverse transcription of viral single-stranded ribonucleic acid (RNA) to double-stranded deoxyribonucleic acid (DNA) in the infected host cell, is targeted by NRTIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs; the first of which became available in 1996); the integrase, which mediates integration of the viral DNA into the host cell's genome, is targeted by integrase inhibitors (first available in 2007); and the protease, which mediates proteolysis of viral precursor proteins during maturation of the viral particle, is targeted by protease inhibitors (first available in 1995)[5,6,14]. Other drugs target viral attachment to, fusion with and entry into the host cell[14], but are not as broadly used[15].

Reports that triple-drug combinations of antiretroviral therapy (ART) significantly improved clinical outcomes were integrated into new clinical guidelines in 1996[14,16,17], leading to massive declines in morbidity and mortality – for those with access to these treatment regimens[18,19]. It was not until 2003 that the World Health Organization launched the '3 by 5 Initiative', aiming at 3 million people in low- and middle-income countries – half of the estimated number needing it[1] – receiving ART by 2005[20]. The target was missed with only 1.3 million people in low- and middle income countries receiving ART by the end of 2005 (up from 400 000 two years prior), and huge disparities in access

[1] It was estimated at the time that 6 million people in low- and middle income countries 'needed' access to ART[20]. The number of people living with HIV in low- and middle income countries was far higher (40.3 million people were living with HIV worldwide, of whom almost two thirds lived in sub-Saharan Africa)[21]; however, prior to 2015, treatment was only recommended for people with a certain level of immunodeficiency, according to a threshold which changed over time[22].

remained[20]. Nevertheless, this initiative was credited as '[establishing] ART as an essential public health intervention'[20].

Even after the introduction of triple-drug combination ART, treatment was initially only recommended for those with advanced disease, corresponding to a low blood count of CD4 cells. The CD4 count for starting ART was progressively raised due to increasing evidence that early treatment with modern triple-drug combinations improves clinical outcomes. Finally, in 2015, the World Health Organization announced that all people living with HIV should receive access to ART[22], an approach that became known as 'test-and-treat'. Total ART coverage is critical at the individual and population level: early and even same-day ART initiation leads to better clinical outcomes for people living with HIV[23–28]; in addition, the last decade has provided conclusive evidence that viral suppression eliminates the risk of onward transmission, giving rise to the slogan 'undetectable=untransmittable' (U=U)[29–32].

Today, most ART regimens worldwide consist of a three-drug regimen (often combined into a single pill to be taken once daily) consisting of two NRTIs (the 'backbone') and either an NNRTI, a protease inhibitor, or an integrase inhibitor[15]. The ART landscape is bound to keep on changing: there are several new drug options on the horizon[33]; simplifications to dual therapy[34,35], or even monotherapy in special cases[36], is becoming possible with more effective drugs; long-acting injectable drugs may help to address problems of adherence and subsequent development of viral drug resistance[37,38]; and there is a growing emphasis on increasing access to antiretroviral drugs as pre-exposure prophylaxis for the prevention of HIV infection[39].

3.2. Epidemiology of HIV

HIV originated from simian immunodeficiency viruses (SIV), affecting non-human primates, through several instances of zoonotic transmissions in west and central Africa[40]. While humans may acquire SIV, notably through activities relating to bush meat[40], it is usually rapidly suppressed by the human immune system[41]. However, several successive human-to-human transmissions may allow the virus to adjust to the human host[41]. Separate transmission events led to the different groups within the two types of HIV known today: HIV-1, which appears to have evolved from SIVcpz, affecting a subs-species of chimpanzee, and HIV-2, which is most closely related to and likely originated from SIVsm, affecting sooty mangabeys and is largely restricted to West Africa[40,42]. However, HIV-1 group M, which dominates the global HIV epidemic[42], is believed to have originated from a single transmission event in what is today the Democratic Republic of the Congo around the 1920s[40,43].

Since the start of the HIV epidemic, 79.3 million people worldwide have become infected with HIV and 36.3 million people have died from AIDS-related causes[44]. In 2020, 37.7 million people worldwide were living with HIV, of whom around 27% were not receiving ART and around a third had an unsuppressed viral load[39]. The same year saw 1.5 million new HIV infections, as well as 680 000 AIDS-related deaths[39] – missing the Joint United Nations Programme on HIV/AIDS (UNAIDS) target of reducing both annual mortality and new infections to below 500 000 by 2020.

Eastern and Southern Africa are jointly home to 55% of all people and two thirds of all children living with HIV[39]. This is also the only UNAIDS region where the majority of new infections do not occur within a number of high-risk minority populations, or 'key populations' – such as men who have sex with men, sex workers, people who inject drugs, transgender people, and the sex partners of people belonging to these risk categories – but rather, 72% of cases occur in the 'remaining population'[45],

with adolescent girls and women being at particular risk[39]. Even within this region, however, there are major discrepancies, with an adult HIV prevalence below 1% reported for four countries (Comoros, Madagascar, Eritrea, Ethiopia), and above 20% for three countries (Eswatini, Lesotho, Botswana).

Lesotho and Tanzania, where the projects presented here are conducted, have an adult HIV prevalence of 22.8% and 4.8%, respectively[45]. Despite their considerable differences, these two countries share a few key challenges when it comes to healthcare provision for the management of HIV. One such challenge is rurality combined with difficult terrain and/or limited road infrastructure[46,47]: from a healthcare perspective, service delivery to remote and often mountainous areas is challenging; from a patient perspective, accessing care can entail a high time and financial burden[48]. Another closely linked challenge is poverty: in Lesotho in 2017, almost half the population lived below the national poverty line, and over a quarter lived below the international poverty line (1.90 US dollars per day in 2011 purchasing power parity)[46]. In Tanzania in 2018, over a quarter of the population lived below the national poverty line and almost half below the international poverty line[47]. In both countries, poverty is higher in rural areas[46,47]. In 2017, HIV/AIDS remained the leading cause of death in Lesotho, and the fourth most common cause of death in Tanzania[49].

3.3. International HIV targets and the 'Third 95' of Viral Suppression

At the start of the 'test and treat' rollout in 2015, there was great optimism that this approach could end the HIV/AIDS epidemic – towards a vision of zero new HIV infections, zero AIDS-related deaths, and zero stigma – by 2030[50]. At the time, modelling suggested an end to the pandemic would be possible if the world achieved what became known as the 90-90-90 goal: by 2020, at least 90% of people living with HIV should know their status, at least 90% of those knowing they have HIV should receive ART, and at least 90% of those receiving ART should achieve viral suppression, leading to an overall viral suppression rate among all people living with HIV of 73%[51]. This was an ambitious goal at a time when global ART coverage was estimated at 37%, with limited available data on viral suppression[51].

The targets for 2020 were missed but drove substantial progress, with global numbers reaching 84-87-90 in 2020[39]. Eight countries – including hard-hit countries like Eswatini and Botswana – even exceeded the targets; 11 further countries – including Lesotho – did not achieve ≥90% for each step of the cascade but surpassed the goal for overall viral suppression[39].

The new UNAIDS target is to increase success along the HIV care cascade to '95-95-95' by 2025[50], which would lead to an overall rate of viral suppression of almost 86%. As mentioned above, viral suppression is key to stopping onward transmission[29–32], and can furthermore facilitate a life expectancy close to that of people without HIV, especially with early diagnosis and rapid initiation of ART[52].

The optimal virological threshold constituting viral suppression has been subject to debate. Increasing evidence suggests that even low levels of viral replication increase the risk of virological treatment failure[53–56] and AIDS-defining events or mortality[55,57]. Unlike many high-income countries[58,59], however, guidelines of the World Health Organization as well as Lesotho and Tanzania national guidelines employ a high virological threshold of 1000 copies/mL for defining treatment failure[15,60–62]. Failure to achieve viral suppression typically triggers intensified

adherence counselling followed by a repeat viral load test and, if the viral load remains unsuppressed, a switch to a second-line regimen[15,61,62].[2]

The work presented here focuses on strategies to improve outcomes along this 'viral load cascade' and improve viral suppression among people receiving ART. As particularly vulnerable age groups for treatment failure, special consideration is given to children and adolescents.

3.4. Key Topics for Viral Suppression

3.4.1. Viral Drug Resistance

Treatment failure is most often caused by viral drug resistance or suboptimal adherence to ART. The two mechanisms are closely linked, as partial adherence and consequent exposure to sub-therapeutic drug levels select for drug-resistant viral quasi-species. The lack of proof-reading ability of the viral reverse transcriptase enzyme and consequent high error rate of reverse transcription[63,64], combined with the high replication rate of HIV[65], give rise to the potential for rapid acquisition of resistance-associated mutations. In many cases, a single point mutation is sufficient to confer high-level resistance to an entire drug class.[3] For this reason, modern ART regimens generally use three drugs from two separate drug classes. The diversity borne from this high mutation rate is also a key challenge for vaccine development[5].

Resistance to medication can be acquired, i.e. develop within an individual initially infected with wild-type HIV, or transmitted, meaning that the individual was infected with HIV harbouring resistance-associated mutations[66]. Pre-treatment drug resistance refers to either transmitted resistance or to resistance acquired during a prior exposure to ART, e.g. in individuals returning to care after treatment interruption[66].

Before the recent roll-out of the integrase inhibitor dolutegravir, increasing rates of pre-treatment resistance to NNRTI-based ART were a major concern, with World Health Organization guidelines recommending adjustments of the standard first-line regimens if rates exceeded 10%[66]. Rates of pre-treatment resistance were particularly high among children below 18 months of age, with over 50% carrying NNRTI-resistant HIV[66]. Acquired NNRTI resistance was also a major threat[66]; evidence from Lesotho, including work contributing to this thesis (**chapters 5.1.5** and **5.1.6**), shows high rates of viral drug resistance to NNRTI-based first-line regimens among patients with incomplete viral suppression on ART[67–70].

Recently, the roll-out of dolutegravir-containing ART as a first-line regimen has been a game-changer. While resistance to dolutegravir does occur, it is rare compared to previous core agents in first-line regimens (notably the NNRTIs efavirenz and nevirapine), even in the context of compromised

[2] The World Health Organization 2021 guidelines introduce exceptions for switching away from NNRTI-based first-line ART, which can be considered after a single viral load exceeding 1000 copies/mL or sustained viraemia above 50 copies/mL. These guidelines furthermore employ a threshold of 50 copies/mL for starting adherence counselling and 1000 copies/mL for switching to second-line ART[15], whereas current guidelines of Lesotho and Tanzania employ a threshold of 1000 copies/mL for both actions[60,61]. Lesotho guidelines additionally state that resistance testing should precede switching to second-line ART if the first-line regimen contains a protease inhibitor or integrase inhibitor[60,61], though in reality, access to resistance testing remains extremely limited.

[3] See the Stanford HIV drug resistance database (https://hivdb.stanford.edu/hivdb/by-mutations/) for information on the impact of individual mutations on available drug options (accessed 13.11.2021).

susceptibility of the NRTI backbone[71,72]. Despite this evidence, as well as promising early outcomes of dolutegravir performance in a 'real-life' cohort within this thesis (**chapter 5.1.4**)[73], resistance testing is likely to remain relevant at least for population-level surveillance in the years to come[15,74], and its future role for individual patient management currently constitutes a research gap[15]. Access to resistance testing is extremely limited in many regions worldwide, notably in the regions with the highest burden of HIV. Therefore, in contrast with guidelines of several high-income countries[58,59], current World Health Organization guidelines only give consideration to but do not broadly recommend resistance testing for individual patient management[15].

Several projects within this thesis address questions relating to resistance. Most notably, the ongoing GIVE MOVE randomised controlled trial is looking into the clinical impact of resistance testing-informed onward treatment for children and adolescents on a failing ART regimen (**chapters 5.1.7**. and **5.2**)[75]. Further studies measure the prevalence of resistance-associated mutations in the context of low-level viraemia (**chapter 5.1.6**)[70], assess the risk of emergence of (additional) resistance-associated mutations during the adherence counselling period after an first high viral load (**chapter 5.1.5**)[69], and investigate the risk of resistance acquisition upon home-based same-day ART initiation among individuals who do not subsequently link to care (**chapter 5.1.8**)[76].

3.4.2. Adherence to Therapy

Adherence to ART is key to viral suppression, though many hurdles may cause patients to either not take medication, or to take it inconsistently. Key factors in adherence vary widely between populations and age groups; factors specific to children and adolescents are addressed in **chapter 3.4.3**.

In a systematic review and meta-analysis, the most common self-reported individual barriers to adherence included forgetting, being away from home, and changes to the daily routine, all three of which were reported more frequently by adolescents than by adults or children/caregivers[77]. Barriers related to health services, notably distance to clinics and stock-outs, were also commonly reported, as were treatment-related factors such as toxicity, side effects, pill burden, and palatability[77].

Further potential barriers include secrecy, stigma (including internalized stigma) and fear of discrimination[77–79]; depression or other psychiatric conditions[77,78,80]; use of alcohol and other substances[77,78,81]; feeling either sick or well (with feeling sick being a more frequent self-reported barrier)[77]; knowledge gaps[78]; financial barriers[78]; food insecurity[78,82]; and suboptimal quality of or dissatisfaction with healthcare services[77,78].

Within this thesis, adherence is addressed to varying degrees in several projects. Reporting of 24-month outcomes of the CASCADE randomised controlled trial on home-based same-day ART initiation investigated adherence-related factors upstream of the 'third' towards the 'second 95' – reasons for not linking to or not remaining in care (**chapter 5.1.8**)[76]. Another project, the DO-REAL cohort study, looked at overall viral suppression after the programmatic roll-out of dolutegravir-based ART in Lesotho, with the few instances of viraemia appearing to be caused by non-adherence rather than resistance (**chapter 5.1.4**)[73]. Within the same cohort, we also looked beyond the 'third 95' towards health-related quality of life on ART – which deserves attention in its own right, and can also be hypothesized to feed back into long-term adherence (**chapter 5.1.2**)[83].

3.4.3. Paediatric HIV

Early, sustained viral suppression is important for optimal neurocognitive development and growth in children[84–86]. However, children and especially adolescents are at particular risk for poor treatment outcomes[39,86–90]: in eastern and southern Africa, 72% of adults but only 43% of children living with HIV are virally suppressed[39]. Similarly, the proportion with viral suppression among those receiving ART is 92% for adults but only 75% for children in this region[39]. Similar differences between children and adults have been reported for Lesotho and Tanzania[45]. While this work focuses on viral suppression, it is important to acknowledge major gaps in HIV diagnosis, the 'first 95', among children, leaving over 40% of children living with HIV undiagnosed[39].

The underlying causes for poor paediatric treatment outcomes differ by age group. Young children falling into lighter weight bands typically have to take several pills – or combinations of pills with pellets, granules, or syrup – often twice daily[61,90]. This is problematic, as studies in adults have shown that a higher pill burden is associated with both lower adherence and lower viral suppression, and that twice-daily regimens are associated with moderately lower adherence compared to once-daily ART[91]. In addition to the higher pill burden and frequency of drug intake, correct administration of these formulations can be challenging[90].

Adolescents do not only report the same barriers reported by adults at a higher frequency[77], but also face unique challenges relating to disclosure, a lack of youth-friendly services, and transitioning to adult services[86]. Poor mental health, which has been linked to viral non-suppression[92,93], and stigma are key factors for this age group[79,94]. A cohort study including over a thousand adolescents in South Africa observed high rates of discrimination, which was associated with internalised stigma and disengagement from care[79]. A separate, large cohort study in South Africa found that only 37% of adolescents consistently reported past-week adherence over three interviews at roughly one-year intervals[95]. Considering the fact that those who left care were excluded from analysis[95], and the tendency of self-reporting to overestimate adherence[96], true adherence levels are likely even lower. Low adherence combined with reported high sexual risk-taking within this age group also increases the risk of onward transmission[97].

As incomplete adherence increases exposure to sub-therapeutic drug levels, children and adolescents are at a heightened risk for acquiring viral drug resistance. This is of particular concern considering the exceedingly high prevalence of transmitted resistance among infants and young children, further limiting treatment options[66].

Within this thesis, two studies focus specifically on children and adolescents. The ongoing GIVE MOVE randomised clinical trial (mentioned above) assesses the impact of resistance testing in these age groups, considering the increased vulnerability to treatment failure in this population (**chapters 5.1.7** and **5.2**)[75]. Separately, we evaluated outcomes along the viral load cascade – from detection of viraemia, to confirmatory viral load testing, to switching to second-line ART if indicated – identifying key gaps and suggesting potential ways to address them (**chapter 5.1.3**)[98].

4. Research Objectives and Project Overview

The main objective of my PhD was to generate evidence on programmatic as well as individualised approaches towards achieving the 'third 95' – viral suppression. All projects focus on Lesotho, the country with the second-highest prevalence of HIV worldwide; one project is conducted in parallel in Lesotho and Tanzania.

The studies presented here thus question specific aspects of ART provision:

1) The timing of ART initiation;
2) The impact of programmatic changes to first-line drug options on virological outcomes;
3) The requirement for adherence counselling if viraemia is detected during first-line ART;
4) The threshold to define virological treatment failure and switch to second-line ART;
5) The potential for resistance testing to inform the switch to second-line ART; and
6) The virological outcomes in children along this entire 'viral load cascade'.
7) Finally, we look beyond the 'third 95' to consider mental and somatic health outcomes associated with different ART regimens.

First, my colleagues and I considered how the timing of ART initiation impacts long-term virological outcomes. Previously, the CASCADE trial had shown that offering ART initiation on the day of home-based HIV diagnosis leads to better one-year care outcomes than requiring several sessions of counselling before starting ART, as was then required in the standard of care[23]. In a 24-month analysis of the CASCADE trial data, we no longer observed any difference between the study arms. Importantly, however, we were able to alleviate concerns that same-day ART initiation might later lead to increased defaulting from care, as had been suggested within one trial on clinic-based same-day ART[99]. We assessed reasons given for non-linkage to or non-retention in care: of note, only a minority of self-reported reasons were related to time or finances, whereas several participants listed non-structural reasons associated with scepticism surrounding the HIV diagnosis or ART, unwillingness or unreadiness to take ART, or perceived ill-treatment from healthcare professionals. We did observe individual cases where resistance-associated mutations were likely acquired in consequence of home-based same-day ART initiation without subsequent linkage to care; however, in the small sample size available to address this risk, such outcomes appeared to be rare. Overall, we concluded that the considerable benefits of same-day ART in terms of earlier linkage to care and viral suppression outweigh the risks (see publication in **chapter 5.1.8**)[76].[4]

Second, we assessed the impact of the recent roll-out of dolutegravir-based first-line ART on virological outcomes. HIV treatment in most low- and middle-income countries is far less individualized than in resource-rich settings; therefore, a programmatic transition from one drug to another can mean a regimen change for the majority of the patient population. With most people set to transition from efavirenz- to dolutegravir-based ART with no change to the NRTI backbone, there were open questions as to whether prior NRTI resistance might compromise dolutegravir-based first-line ART and, consequently, what the viral load threshold and maximal time since previous viral load testing should be in order to minimise the risk of functional dolutegravir monotherapy[100,101]. Moreover, until recently most data on dolutegravir stemmed from high-income countries and/or randomised trials,

[4] This research was cited in the World Health Organization 2021 consolidated guidelines on HIV management, as well as the International AIDS Society – USA Panel 2020 recommendations on antiretroviral drugs for HIV prevention and treatment[15,59].

whereas 'real-life' data from low- and middle-income countries was lacking[100,101]. In the DO-REAL cohort study, which includes over a thousand individuals who transitioned from NNRTI- (nevirapine or efavirenz) to dolutegravir-based ART, we observed favourable short-term virological outcomes: among individuals with complete viral load data, 99.5% and 98% had viral suppression to <1000 copies/mL and <100 copies/mL, respectively, four months after transitioning to dolutegravir-based ART (see publication in **chapter 5.1.4**)[73]. Even among those with viraemia ≥100 copies/mL at transition to DTG, 95% achieved resuppression to <100 copies/mL. All in all, the rate of viral suppression was high already at transition to dolutegravir and increased further after transition[73]. These results provide further support for the programmatic roll-out of dolutegravir, though more research is needed to investigate if these findings hold true in populations with lower baseline rates of viral suppression and without recent pre-transition viral load data[73]. Furthermore, it remains to be seen whether these positive outcomes can be maintained over time; we intend to report on longer-term outcomes of the DO-REAL once available.

Third, we assessed short-term dynamics of resistance-associated mutations, questioning whether the current approach of dealing with viraemia could lead to the emergence of (additional) drug resistance-associated mutations in people receiving NNRTI-based ART. In a post-hoc analysis of plasma samples collected in the previously completed CART-1 cohort study, we observed extensive high-level drug resistance already at the initial detection of viraemia, and a moderate accumulation of additional mutations by the time of confirmatory viral load testing after adherence counselling. This was associated with a non-significant decrease in genotypic susceptibility scores. These results led us to conclude that the risk of acquiring additional resistance-associated mutations during the adherence counselling period, which inherently entails delays in clinical decision-making, should be balanced against the benefit of avoiding unnecessary switches in people who achieve viral suppression after adherence counselling (see publication in **chapter 5.1.5**)[69]. Since the completion of this research, new World Health Organization guidelines have adjusted the viral load monitoring algorithm to allow for a switch away from NNRTI-based ART after a single unsuppressed viral load measurement, without strictly requiring prior adherence counselling[15].

Fourth, we questioned the viral load threshold for switching from NNRTI-based first-line ART to second-line ART. Cohort data[53–55,57], as well as the SESOTHO randomised controlled trial (conducted in the context of NNRTI-based first-line and protease inhibitor-based second-line ART)[56], showed evidence that the threshold for switching to second-line ART recommended by the World Health Organization at the time was too high, though data on the relative role of non-adherence and resistance was limited. In a genotyping study nested within SESOTHO, we found high levels of viral drug resistance among individuals with 'low-level' viraemia below the threshold for switching: among people on NNRTI-based ART, over 80% of participants with successfully sequenced samples and over half overall (sequencing success is often limited for low-level viraemia) harboured resistance-associated mutations necessitating a regimen switch (see publication in **chapter 5.1.6**)[70]. These findings provide further support for lowering the threshold to switch[70]. Indeed, the World Health Organization's 2021 guidelines lowered the threshold for considering switching specifically from NNRTI-based first-line ART from 1000 to 50 copies/mL[15,102], highlighting the timeliness of this research question.

Fifth, we are assessing if the decision whether and how to switch to second-line ART should be informed by genotypic resistance data, as opposed to viral load data only. We hope to answer this question through an ongoing open-label randomised clinical trial, the GIVE MOVE trial, currently

conducted in seven sites (with additional sites planned) in two countries, Lesotho and Tanzania. As children and adolescents are particularly prone to poor treatment outcomes, this trial focuses on these priority age groups. A cost-effectiveness analysis will be conducted if any benefit of resistance testing is observed. Our goal is that the results of this trial will inform policymakers on whether or not to prioritise the roll-out and scale-up of access to resistance testing for this population (see publication of the protocol in **chapter 5.1.7** and the current project status in **chapter 5.2**)[75].

Sixth, we assessed the entire viral load cascade – from detection of viraemia to outcomes after adherence counselling or switch – among children and younger adolescents with routinely detected viraemia while receiving treatment in Lesotho. In this study, conducted in the context of an open cohort study including all children receiving viral load testing in two districts of Lesotho, we identified substantial delays at each stage of the cascade (see accepted manuscript in **chapter 5.1.3**)[98], in line with previously reported outcomes among adults[103]. We propose interventions which may facilitate timely and appropriate clinical action in response to detecting viraemia.

Finally, we began to look beyond the 'third 95' of viral suppression, and towards health-related quality of life for people in care for HIV. With over 27 million (73%) people living with HIV now accessing ART, of whom over 90% are virally suppressed[39], looking at health and wellbeing outcomes beyond viral suppression may become increasingly important[104]. Both dolutegravir and efavirenz, which it is largely replacing, have been associated primarily with neuropsychiatric adverse effects[105–109]. In a separate analysis within the above-mentioned DO-REAL cohort, we show that transitioning from efavirenz- to dolutegravir-based ART did not greatly alter overall health screening outcomes, but did lead to a significant reduction of specific mental health symptoms that may greatly impact health-related quality of life, notably symptoms relating to sadness/depression, dreams/sleep, and nervousness/anxiety (see submitted manuscript in **chapter 5.1.2**)[83].

5. Results

5.1. Published or Submitted Work

5.1.1. List of Publications as (Co-) First Author

Submitted:

Brown JA, Nsakala BL, Mokhele K, Rakuoane I, Muhairwe J, Glass TR, Amstutz A, Tschumi N, Belus JM, Klimkait T, Labhardt ND. Dolutegravir in Real Life: self-reported mental and physical health outcomes after transitioning from efavirenz- to dolutegravir-based antiretroviral therapy in a prospective cohort study in Lesotho. *Under review.*

Published / accepted:

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** Equal contribution.*

5.1.2. Mental and Physical Health upon Transition to Dolutegravir, Under Review

Dolutegravir in Real Life: self-reported mental and physical health outcomes after transitioning from efavirenz- to dolutegravir-based antiretroviral therapy in a prospective cohort study in Lesotho

Running head: Mental and physical health upon transition to DTG

Jennifer A. Brown[1,2,3], Bienvenu L. Nsakala[4], Kuena Mokhele[4], Itumeleng Rakuoane[4], Josephine Muhairwe[4], Tracy R. Glass[1,3], Alain Amstutz[1,3,5], Nadine Tschumi[1,3], Jennifer M. Belus[1,3,6], Thomas Klimkait[2,3], Niklaus D. Labhardt[1,3,5,§]

1 Clinical Research Unit, Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland; 2 Molecular Virology Group, Department of Biomedicine, University of Basel, Basel, Switzerland; 3 University of Basel, Basel, Switzerland; 4 SolidarMed, Partnerships for Health, Lesotho; 5 Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland; 6 University of Maryland, College Park, MD, USA; § Corresponding author

Corresponding author:
Niklaus Daniel Labhardt
Socinstrasse 57, 4051 Basel, Switzerland
+41 79 870 18 59; n.labhardt@unibas.ch

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Under review

Abstract

Introduction:

Since 2018, the World Health Organization has recommended the preferential use of dolutegravir-(DTG)-containing antiretroviral therapy (ART) to treat HIV. Country programs across Africa have transitioned individuals from other first-line regimens, most often containing efavirenz (EFV), to DTG-based ART. As both EFV and DTG are associated with neuropsychiatric side effects, this prospective cohort study assessed mental health as well as common HIV/ART-related symptoms before and after transitioning from EFV to DTG based ART in Lesotho.

Methods:

The prospective DO-REAL cohort enrolled people starting DTG-based ART in hospital settings in Lesotho. In this analysis within DO-REAL, we include adult participants changing from tenofovir disoproxil fumarate (TDF) / lamivudine (3TC) / EFV to TDF/3TC/DTG within first-line therapy at Butha-Buthe Government Hospital in Lesotho. On the day of this substitution as well as 16 weeks thereafter, participants completed the Patient Health Questionnaire-9 (PHQ-9) for depression screening, the 12-item Short Form Health Survey (SF-12) to assess mental and physical health, and a modified version of the HIV Symptom Index (mHSI) to capture individual HIV/ART-related symptoms. Clinical data including weight was also assessed. Binary categorical variables were assessed with the McNemar test with Bonferroni corrections. ClinicalTrials.gov: NCT04238767.

Results and discussion:

Between February and December 2020, 1228 participants were enrolled. 1131 participants completed follow-up; of these, 60% were female, median age was 46 years (IQR 38-55), and the median time taking ART was 5.7 years (IQR 3.5-8.9). No change was observed for weight or overall PHQ-9 or SF-12 outcomes. However, three pre-specified symptoms in the mHSI decreased after transitioning to DTG: 'feeling sad/down/depressed' (bothered 6.0% vs 3.3% of participants at least 'a little' before vs after transition; adjusted p=0.048); 'feeling nervous/anxious' (7.4% vs 3.4%; adjusted p=0.0009); and 'nightmares, strange/vivid dreams' (6.3% and 3.5%; adjusted p=0.027). Individual PHQ-9 or SF-12 items also improved. The proportion considered symptom-free across all measures increased from 56/1102 (5%) to 126/1102 (11%; p<0.0001).

Conclusions:

Despite high self-reported mental and physical health while taking EFV, we observed no negative impact and a potential moderate improvement after transitioning to DTG. These findings provide further support for the preferential use of DTG in first-line regimens.

Under review

Introduction

The antiretroviral dolutegravir (DTG) is generally well-tolerated and has a high barrier to development of viral resistance[1]. Compared with efavirenz- (EFV)-based antiretroviral therapy (ART), DTG-containing regimens have been shown to have non-inferior[2–5] or superior[6,7] virologic outcomes and faster viral suppression[3,6,8]. Following an update of World Health Organization guidelines in 2018[9], countries across Africa have rolled out DTG-containing ART as the preferred regimen type for most people living with HIV. This has led to a partial phase-out of EFV, the previous core agent in most adult first-line regimens. In Lesotho in southern Africa, 2020 saw large-scale programmatic transitioning of people previously receiving EFV-containing ART to DTG-based ART within first-line treatment.

However, both drugs are associated with neuropsychiatric side effects. For EFV, these include problems related to sleep, anxiety, depression, irritability, confusion, fatigue, blurred vision, vertigo, nausea, headaches, and hallucinations[11–13]. A similar range of side-effects, notably insomnia, has been reported for DTG[14–17].

With several million people recently transitioned from EFV to DTG[18], ART programmes as well as clinicians should be sensitive to any implications this may have on side effect profiles. In this prospective, registered cohort study, we aimed to assess detailed psychological and somatic wellbeing before and after the programmatic shift from EFV- to DTG-based ART in Lesotho.

Methods

Study design and participants

Dolutegravir in Real Life in Lesotho (DO-REAL) is a prospective, registered cohort study that enrolled people living with HIV who were initiating DTG-based ART within routine hospital-based care in Lesotho[19,20].

In this study within DO-REAL, we assess the effect of transitioning from EFV- to DTG-based ART on self-reported psychological and somatic wellbeing. We include adults enrolled at Butha-Buthe Government Hospital who transitioned from tenofovir disoproxil fumarate (TDF) / lamivudine (3TC) / EFV to TDF/3TC/DTG within first-line ART from 10 February until 15 December 2020. Follow-up visits were conducted from 1 June 2020 to 19 May 2021, when data was closed for this analysis.

Setting

In 2019, Lesotho's national guidelines were amended to recommend DTG as the preferred core agent for most people living with HIV[10]. The major rollout of DTG took place in 2020 and was largely preceded by a transition in EFV dosing from 600 to 400 mg/day. Transitions from EFV to DTG occurred during routine clinic visits.

Procedures

Participants were enrolled on the day of transitioning to DTG and followed up after 16 (window: 8-32) weeks. At enrolment and follow-up, participants were interviewed using the Patient Health Questionnaire-9 (PHQ-9) for depression screening[22], a modified HIV symptom index (mHSI; original version[23]) questionnaire on pre-specified HIV-/ART-related symptoms, the Short Form 12 (SF-12) general health screening tool[24], and a short study-specific questionnaire. The PHQ-9[25–28], SF-12[2,29–32], and variations of the HIV symptom index[2,29,31,33–38] have been used in the context of HIV in various populations in sub-Saharan Africa. Questionnaires were translated to Sesotho. Height and weight were measured.

Data collection

Routine data was retrieved from medical records. Under supervision of the study physician, two study data collectors administered the questionnaires and entered data into the secured database of an ongoing open cohort study into which DO-REAL is nested[40].

Measures

The PHQ-9 contains nine questions with the answer options 'not at all' (scored 0), 'several days' (1), 'more than half the days' (2), and 'nearly every day' (3), leading to a score range of 0-27[22]. A tenth question on difficulties associated with reported problems does not contribute to the score, with answer options 'N/A', 'not difficult at all', or 'somewhat difficult', 'very difficult', or 'extremely difficult'.

The SF-12 contains 12 items, with varying answer options between the items. It generates separate scores for physical and mental health.

The original HIV symptom index contains 20 symptoms[23]; we included one additional symptom associated with EFV, namely 'Nightmares, strange or vivid dreams'[11–13]. The mHSI thus contains 21 items, with the answer options 'I do not have this problem', 'It does not bother me', 'It bothers me a little', 'It bothers me', and 'It bothers me a lot'.

Lastly, we assessed being symptom-free across all measures, i.e., answering all PHQ-9 items with 'not at all', all mHSI items with either 'I do not have this problem' or 'It does not bother me', and all SF-12 items with the best-possible answer option.

Statistical methods

To analyse PHQ-9 outcomes, we compared the categories 'none/minimal' (score 0-4), 'mild' (5-9), and 'moderate to severe' (10-27; wider range due to the low observed prevalence of depression). For the tenth question on associated impact/difficulties, we combined the categories 'somewhat difficult' and above.

For the mHSI, we report symptoms per participant and the number of participants reporting symptoms.

For the SF-12, we report raw sum scores for the physical and mental component as recommended by Hagell et al.[41], excluding item 1 (general health; affecting the physical component) due to a translation error. This leads to a possible range of 5-15 for the physical and 6-27 for the mental component, with higher scores indicating better health.

Under review

Furthermore, we report individual items as binary outcomes for the PHQ-9 (at least 'several days' vs 'not at all'), mHSI (bothers participant at least 'a little', vs 'I do not have this problem' or 'It does not bother me'), and SF-12 items (best-possible vs any other answer).

Results are summarised as frequencies and percentages for categorical variables, and as medians, interquartile ranges (IQRs) and ranges for continuous variables. Baseline characteristics are shown for participants with a follow-up visit within the window period (1131/1228 [92%]); results at baseline and follow-up are shown and statistically analysed where the respective measure was captured at both time points (PHQ-9: 1126/1131 [99.6%]; mHSI: 1112/1131 [98%]; SF-12: 1123/1131 [99%]; all available: 1102/1131 [97%]).

Baseline and follow-up outcomes were compared using McNemar tests or marginal homogeneity tests for variables with two or more than two variables, respectively. Individual items were analysed using conservative Bonferroni corrections to adjust for multiple testing within the respective questionnaire.

All analyses were done using Stata/MP v16.1.

Ethical considerations

Participants received study information in Sesotho and gave written informed consent. In the case of illiteracy, consent by thumb print and a witness signature were required. DO-REAL, as well as the cohort study in which it is nested, were approved by the National Health Research Ethics Committee in Lesotho (ID134-2016). DO-REAL was registered with ClinicalTrials.gov on 23 January 2020 (NCT04238767).

Results and discussion

Participant characteristics

Among 1,228 participants enrolled, 1,131 completed follow-up within the specified window by data closure. Of the remaining 97 participants, one died, eight transferred out, 14 withdrew consent, 17 were lost to follow-up, and 57 remained in care but no follow-up data was collected within the window period. The median time between baseline and follow-up was 16.0 weeks (IQR 14.6-17.0).

Baseline characteristics are shown in **Table 1**: the majority were female, median age was 46 years (38-55), and the median time taking ART was 5.7 years (3.5-8.9).

Table 1: Participant characteristics at baseline (transition to DTG) among participants with follow-up data. ART: antiretroviral therapy; IQR: interquartile range.

	Total (N=1131)	Female (N=678)	Male (N=453)
Demographics and clinical data			
Age in years, median (IQR) [range]	46 (38-55) [19-88]	45 (36-54) [23-88]	47 (40-57) [19-83]
Years taking ART, median (IQR) [range]	5.7 (3.5-8.9) [0.6-16.5]	5.9 (3.5-8.9) [0.8-16.5]	5.4 (3.3-9.0) [0.6-14.5]
Years since HIV diagnosis, median (IQR) [range] [a]	6.9 (3.6-10.5) [0.6-22.5]	7.3 (4.0-10.7) [0.8-16.3]	6.3 (3.4-10.1) [0.6-22.5]
Taking any co-medication (excluding traditional medicine), n (%) [b]	307 (27%)	183 (27%)	124 (27%)
Co-trimoxazole [c]	118 (10%)	44 (7%)	74 (16%)

Tuberculosis prophylaxis or treatment [d]	35 (3%)	19 (3%)	16 (4%)
Oral antidiabetic drugs [d]	21 (2%)	15 (2%)	6 (1%)
Antihypertensive drugs [d]	120 (11%)	91 (13%)	29 (6%)
Other [d]	50 (4%)	38 (6%)	12 (3%)
Taking traditional medicine, n (%)	26 (2%)	6 (1%)	20 (4%)
Pregnant, n (%) [e]	-	1 (0.2%)	-
Breastfeeding, n (%) [e]	-	1 (0.2%)	-

a Missing for 23 participants (nine female, 14 male)
b Missing for three participants (one female, two male)
c Missing for five participant (three female, two male)
d Missing for five participant (two female, three male)
e Among women aged <50 years (n=442); thereof missing for three

Body mass index

The median body mass index was 25.3 kg/m^2 (21.9-30.5) at baseline and 25.5 kg/m^2 (21.9-30.7) at follow-up. Overweight and obesity were particularly prevalent among women. No major changes between BMI categories were observed (**Table 2**).

Depression

At both baseline and follow-up, ≥93% of participants had a PHQ-9 score indicating no/minimal depressive symptoms (PHQ-9 score <5; **Table 2**). There was no change in overall PHQ-9 score category (p=0.34), though the reported impact of any problems associated with PHQ-9 symptoms had decreased at follow-up (p<0.0001).

Assessing binary outcomes (at least 'several days') of individual items, four items showed a significant change at follow-up, of which two remained significant after Bonferroni adjustment: 'trouble falling or staying asleep, or sleeping too much' (unadjusted p<0.0001, adjusted p=0.0004), and 'trouble concentrating on things, such as reading the newspaper or watching television' (unadjusted p<0.0001, adjusted p<0.0001), both of which decreased after transition to DTG (**Figure 1**). The most commonly reported item, 'feeling down, depressed, or hopeless', was reported by 236/1126 (21%) participants at baseline and 194/1126 (17%) at follow-up (unadjusted p=0.0121; adjusted p=0.109).

Table 2: Weight and mental health/wellbeing screening outcomes. The patient Health Questionnaire-9 (PHQ-9) is used for depression screening; the modified HIV symptom index (mHSI) identifies common HIV- or ART-related symptoms, and the 12-item Short Form Health Survey (SF-12) assesses self-reported physical and mental health. BMI: body mass index; IQR: interquartile range.

	Baseline			Follow-up		
	Total	**Female**	**Male**	**Total**	**Female**	**Male**
Weight and BMI						
Body mass index, median (IQR) [range] [a]	25.3 (21.9-30.5) [14.7-61.9]	28.3 (24.4-32.9) [16.2-61.9]	22.3 (20.4-25.1) [14.7-39.3]	25.5 (21.9-30.7) [14.4-61.5]	28.1 (24.3-32.9) [16.7-61.5]	22.6 (20.4-25.2) [14.4-37.3]
Body mass index category, n (%) [a]						
Very severely underweight (<15)	1 (0.1%)	0	1 (0.2%)	1 (0.1%)	0	1 (0.2%)
Severely underweight (≥15, <16)	4 (0.4%)	0	4 (1%)	3 (0.3%)	0	3 (1%)
Underweight (≥16, <18.5)	48 (4%)	14 (2%)	34 (8%)	42 (4%)	13 (2%)	29 (6%)
Optimal (≥18.5, <25)	482 (43%)	186 (27%)	296 (65%)	478 (42%)	183 (27%)	295 (65%)
Overweight (≥25, <30)	276 (24%)	195 (29%)	81 (18%)	298 (26%)	208 (31%)	90 (20%)
Moderately obese (≥30, <35)	201 (18%)	169 (25%)	32 (7%)	182 (16%)	155 (23%)	27 (6%)
Severely obese (≥35, <40)	83 (7%)	78 (12%)	5 (1%)	88 (8%)	80 (12%)	8 (2%)
Very severely obese (≥40)	36 (3%)	36 (5%)	0	438 (3%)	38 (6%)	0
Weight change in kg, median (IQR) [range] [a]	-	-	-	0 (-1 – +2) [-18 – +21]	0 (-1 – +2) [-18 – +18]	0 (-1 – +2) [-13 – +21]
PHQ-9	***N=1126*** [b]	***N=674*** [b]	***N=452***	***N=1126***	***N=674***	***N=452***

Under review

PHQ-9 score, median (IQR) [range]	0 (0-2) [0-23]	0 (0-2) [0-23]	0 (0-1) [0-12]	0 (0-1) [0-21]	0 (0-1) [0-14]	0 (0-1) [0-21]
PHQ-9 depression category, n (%)						
None/minimal (0-4)	1051 (93%)	628 (93%)	423 (94%)	1063 (94%)	635 (94%)	428 (95%)
Mild (5-9)	66 (6%)	41 (6%)	25 (6%)	52 (5%)	31 (5%)	21 (5%)
Moderate to severe (10-27)	9 (1%)	5 (1%)	4 (1%)	11 (1%)	8 (1%)	3 (1%)
Reported impact of PHQ-9 symptoms, n (%)						
N/A (all PHQ-9 questions answered 'not at all')	651 (58%)	381 (56%)	270 (60%)	718 (64%)	414 (61%)	304 (67%)
Not difficult at all	436 (39%)	266 (39%)	170 (38%)	395 (35%)	252 (37%)	143 (32%)
Somewhat, very or extremely difficult	41 (4%)	28 (4%)	13 (3%)	13 (1%)	8 (1%)	5 (1%)
mHSI	***N=1112***	***N=670***	***N=442***	***N=1112***	***N=670***	***N=442***
Number of pre-specified symptoms reported, median (IQR) [range]	2 (1-4) [0-17]	3 (1-4) [0-17]	2 (1-4) [0-16]	2 (1-4) [0-16]	2 (1-4) [0-16]	2 (1-3) [0-13]
Participants reporting at least one pre-specified symptom, n (%)	1008 (91%)	630 (94%)	378 (86%)	1002 (90%)	634 (95%)	368 (83%)
Number of pre-specified symptoms that bother the participant at least 'a little', median (IQR) [range]	0 (0-1) [0-15]	0 (0-1) [0-15]	0 (0-1) [0-14]	0 (0-1) [0-12]	0 (0-1) [0-12]	0 (0-1) [0-11]
Participants bothered at least 'a little' by at least one pre-specified symptom, n (%)	476 (43%)	298 (44%)	178 (40%)	503 (45%)	313 (47%)	190 (43%)
SF-12	***N=1123*** [b]	***N=670*** [b]	***N=453***	***N=1123***	***N=670***	***N=453***
Mental health raw sum score, median (IQR) [range] [c]	24 (21-25) [8-27]	23 (21-25) [8-27]	24 (22-25) [11-27]	24 (22-25) [9-27]	24 (22-25) [10-27]	24 (22-26) [9-27]
Physical health raw sum score excluding question 1, median (IQR) [range] [d]	15 (15 15) [5 15]	15 (15 15) [5 15]	15 (15 15) [9 15]	15 (15 15) [5 15]	15 (15 15) [5 15]	15 (15 15) [5 15]
PHQ-9, mHSI, SF-12	***N=1102***	***N=661***	***N=441***	***N=1102***	***N=661***	***N=441***
Considered Symptom-free across all measures	56 (5%)	35 (5%)	21 (5%)	126 (11%)	71 (11%)	55 (12%)

a Missing for one participant (female)
b PHQ-9 data, SF-12 mental health component data, and HIV items 8 and 9 excluded for three participants (all female) because they were known to have received a psychological/psychiatric intervention between baseline and follow-up as a consequence of their baseline visit
c Possible range: 6-27 (higher score indicates better mental health)
d Possible range: 5 15 (higher score indicates better physical health). Question 1 was omitted due to a translation error.

Symptoms and side effects

The median number of symptoms reported and proportion of participants reporting symptoms were similar before and after transition to DTG (**Table 2**).

However, in unadjusted testing of binary outcomes (symptom bothered participant at least 'a little'), a change was observed for five symptoms, of which three remained significant after adjusting: 'feeling sad, down or depressed' (unadjusted p=0.0023; adjusted p=0.048), 'feeling nervous or anxious' (unadjusted p<0.0001; adjusted p=0.0009), and 'nightmares, strange or vivid dreams' (unadjusted p=0.0013; adjusted p=0.027). All three had a lower prevalence at follow-up (**Figure 1**). At both time points, the most common symptom was 'muscle aches or joint pain' (11.0% vs 11.2%; adjusted p=1.00).

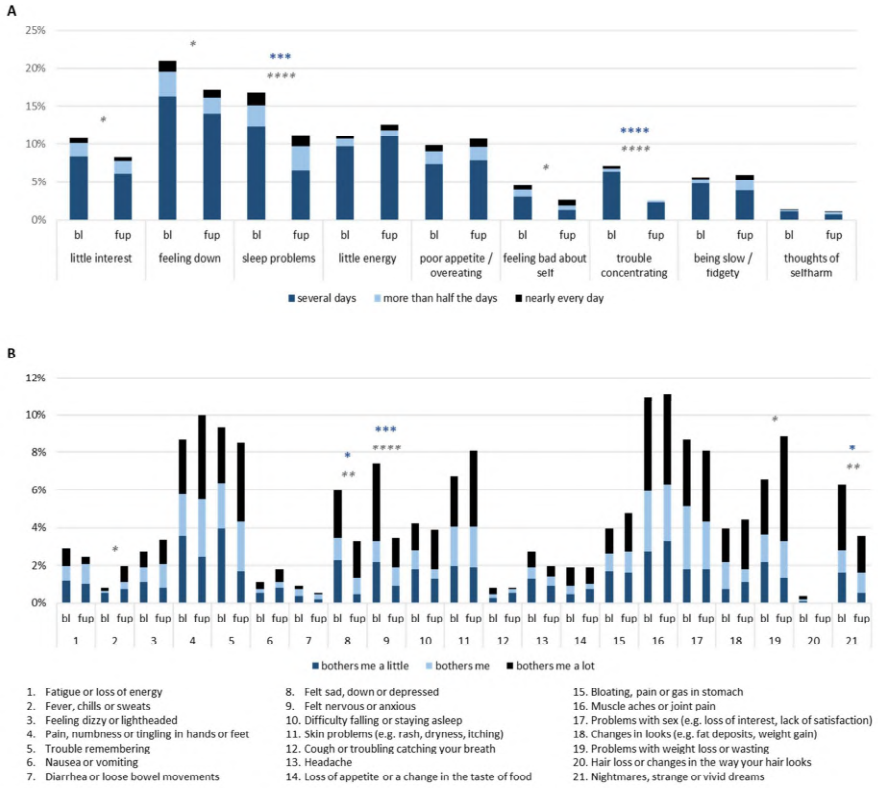

Figure 1: Individual PHQ-9 and mHSI items. A: Individual PHQ-9 items reported at baseline and follow-up as occurring at least 'several days' in the past two weeks (N=1126). Colour-coding shows the severity of any present items, whereas statistical analyses consider binary outcomes (at least 'several days', vs 'not at all'). **B:** Individual mHSI items bothering participants at least 'a little' at baseline and follow-up (N=1109 [items 8, 9] or N=1112 [all other items]; bl: baseline; fup: follow-up). Colour-coding shows the severity of any present items, whereas statistical analyses consider binary outcomes (at least 'a little' bothersome, vs 'I do not have this symptom' or 'It does not bother me'). **A and B:** McNemar tests to assess binary outcomes; adjusted for multiple testing within the respective questionnaire using Bonferroni corrections. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001. Italic grey asterisks indicate unadjusted p values; bold blue asterisks indicate adjusted p values.

General health

Neither the physical nor mental component raw sum scores of the SF-12 showed any meaningful change between baseline and follow-up (**Table 2**).

Assessing binary outcomes (proportion selecting the healthiest answer option), four items changed in unadjusted testing, of which three remained significant in adjusted testing: 'how much of the time have you had a lot of energy' (unadjusted p<0.0001; adjusted p<0.0001), 'how much of the time have

you felt downhearted and blue' (unadjusted p=0.0003; adjusted p=0.0036), and 'how much of the time have your physical health or emotional problems interfered with your social activities' (unadjusted p=0.0008; adjusted p=0.0096), all of which improved after transition to DTG.

Symptom-free across all measures

Among participants with a full dataset for all three screening tools, the proportion considered symptom-free ('not at all' indicated for all PHQ-9 items; 'I do not have this problem' or 'It does not bother me' for all mHSI items; best-possible answer for all SF-12 items) was 56/1102 (5%) at baseline and 126/1102 (11%) at follow-up (p<0.0001).

Strengths and limitations

This study has several limitations. First, the tools and translations were not validated in this population against clinical assessments, as the goal was to assess potential shifts rather than exact prevalence. Therefore, we do not recommend using these results for prevalence assessments. Second, external factors including the Covid-19 pandemic may have influenced health outcomes over time. Lastly, the inherent limitations of a before-and-after study design, notably the lack of a control group for temporal changes, apply.

The strengths of this study include its large sample size, the real-life setting, avoiding an impact of backbone drugs by considering only the most common backbone before and after transition, and the relatively short follow-up period which should minimize the impact of external factors. To our knowledge, this is the first study to provide detailed data on the shift of individual symptoms when changing from EFV- to DTG-based ART.

Previous data and future directions

The NAMSAL trial, with randomised ART-naïve patients to receive DTG or low-dose EFV, reported no difference between the prevalence of at least mild depression, the overall number of symptoms, and SF-12 outcomes between its study arms. This is in line with our findings; however, NAMSAL did not report on individual symptoms which may however be greatly relevant to affected individuals.

A separate cohort study reported a modest increase (with unchanged median) in PHQ-9 scores after transition to DTG-based ART[42], but excluded individuals with side effects on their pre-DTG regimen, potentially generating a bias against DTG.

Further studies are needed to validate the outcomes observed here, notably in cohorts with lower self-reported baseline health. Future studies should also assess paediatric populations transitioning from protease inhibitor- to DTG-based ART.

Conclusions

Under review

DO-REAL assessed mental and somatic wellbeing at, and sixteen weeks after, programmatic transition from TDF/3TC/EFV to TDF/3TC/DTG in Lesotho.

While this study did not identify major shifts in overall mental or self-reported physical health, its data show no negative impact of transitioning from EFV to DTG and suggest a potential moderate benefit of DTG with regard to specific neuropsychiatric symptoms. Notably, we observed improvements regarding feeling sad/down/depressed, nervousness/anxiety, concentration, dreams/sleep, energy (though not consistent across screening tools), associated difficulties or social impact, and being symptom-free across all measures. These results further support the current recommendation for the preferential use of DTG-containing first-line ART regimens.

Conflicts of interest:

TK reports advisory board membership fees from ViiV and Gilead for work outside of this study. NDL reports having received travel grants to attend IAS, AIDS, CROI conferences from Gilead Sciences Sarl. All other authors declare that they have no competing interests.

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Authors' contributions

NDL conceptualised the study. NDL and JAB designed the study with key input from BLN, AA, NT, TRG, JM, and TK. BLN managed the study onsite and oversaw all onsite procedures including consenting, data collection, and onsite data cleaning. KM and IR conducted consenting procedures (together with Reitumetse Peea and Kelebone Moqethei mentioned below), collected data from medical records, and conducted interviews. JAB conducted offsite data cleaning and analysed the data, with key input from NT, JMB, BLN, and NDL. NT and TRG provided statistical expertise, and NT cross-checked data analyses. JMB provided trainings on data collection and suicidality assessment for the site team, and provided further mental health expertise during analysis. AA provided clinical expertise at all project stages. JAB and NDL wrote the first version of the manuscript, and all authors contributed to the final version.

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Authors, highest degrees, and affiliations:

Josephine A. Muhairwe[1,2,*], MD, Jennifer A. Brown[3,4,5,*], MSc, MAS, Lipontso Motaboli[1], BSc, Bienvenu L. Nsakala[1], MD, Malebanye Lerotholi[6], BSc Hons, Alain Amstutz[3,5,7], MD, Thomas Klimkait[4,5], PhD, Tracy R. Glass[3,5], PhD, and Niklaus D. Labhardt[3,5,7,§], MD

1 SolidarMed, Partnerships for Health, Maseru, Lesotho

2 Institute of Global Health, University of Geneva, Geneva, Switzerland

3 Department of Medicine, Swiss Tropical & Public Health Institute, Basel, Switzerland

4 Molecular Virology, Department of Biomedicine, University of Basel, Basel, Switzerland

5 University of Basel, Basel, Switzerland

6 Ministry of Health of Lesotho, Maseru, Lesotho

7 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

* Contributed equally

§ Corresponding author:

Prof. Niklaus D. Labhardt, MD, MIH, DTM&H

Clinical Research Unit, Department of Medicine, Swiss Tropical and Public Health Institute

Socinstrasse 57, 4002 Basel, Switzerland

Accepted manuscript (Ped Infect Dis J)

Phone: +41 79 870 1859; Email: n.labhardt@unibas.ch

Email addresses of authors:

JAM: j.muhairwe@solidarmed.ch

JAB: jennifer.brown@swisstph.ch

LM: l.motaboli@solidarmed.ch

BLN: b.nsakala@solidarmed.ch

ML: 4malbe@gmail.com

AA: alain.amstutz@swisstph.ch

TK: thomas.klimkait@unibas.ch

TRG: tracy.glass@swisstph.ch

NDL: n.labhardt@swisstph.ch

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53 **Conflict of interest statement**

54 TK reports advisory board membership fees from ViiV and Gilead for work outside this
55 study. NDL reports having received travel grants to attend IAS, AIDS and CROI conferences
56 from Gilead Sciences Sarl. All other authors: none to declare.

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59

Abstract (max. 250 words; currently 248)

Background

Children living with HIV and taking antiretroviral therapy (ART) are a priority group for routine viral load (VL) monitoring. As per Lesotho guidelines, a VL ≥1000 copies/mL ("unsuppressed") should trigger adherence counselling and a follow-up VL; two consecutive unsuppressed VLs ("virologic failure") qualify for switching to second-line ART, with some exceptions. Here, we describe the pediatric VL cascade in Lesotho.

Methods

In a prospective open cohort study comprising routine VL results from 22 clinics in Lesotho, we assessed outcomes along the VL cascade for children who had at least one VL test from January 2016 through June 2020. Data was censored on February 10, 2021.

Results

In total, 1215 children received 5443 VL tests. The median age was 10 years (IQR 7-13) and 627/1215 (52%) were female. 362/1215 (30%) had at least one unsuppressed VL. A follow-up VL was available for 325/362 (90%), though only for 159/362 (44%) within six months of the first unsuppressed VL. Of those with a follow-up VL, 172/329 (53%) had virologic failure and 123/329 (37%) qualified for switching to second-line ART. Of these, 55/123 (45%) were ever switched, though only 9/123 (7%) were switched within 12 weeks of the follow-up VL. Delays were more pronounced in rural facilities. Overall, 100/362 (28%) children with an unsuppressed VL received a timely follow-up VL and, if required, a timely regimen switch.

Conclusions

Despite access to VL monitoring, clinical management was suboptimal. HIV programs should prioritize timely clinical action to maximize the benefits of VL monitoring.

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Since 2013, the World Health Organization (WHO) has recommended viral load (VL) testing as the preferred monitoring method for persons taking antiretroviral therapy (ART) and identified children living with HIV as a priority group for regular routine testing[1–4]. This recommendation is supported by low rates of viral suppression in children and adolescents compared with adults[5,6] as well as limited second- and third-line options available as pediatric formulations[7,8].

Lesotho adopted routine VL monitoring alongside the 'treat all' approach in 2016[9]. Children on ART should receive routine VL every six months and those with a VL ≥1000 copies/mL should follow a clearly defined care cascade including enhanced adherence counselling, follow-up VL testing and if required, switching to an effective second-line regimen, with prior resistance testing recommended for those taking protease inhibitor (PI-) or integrase strand transfer inhibitor (INSTI)-based ART according to national guidelines[9,10].

Studies of VL monitoring in children[6,11–14] and adults[15–20] using routine program data show, however, that the above-mentioned VL management algorithm is often not followed, resulting in high losses along the care cascade[15,16]. Here, we present programmatic data for children taking ART after the roll-out of decentralized routine VL monitoring in two districts in Lesotho, describing the care cascade for children with an elevated VL at 3 hospitals and 19 nurse-led clinics.

Materials and Methods

Study design and participants

The cohort study '*Implementation of routine viral load monitoring in rural Lesotho: a prospective cohort study on virologic outcomes among patients on ART in Lesotho*' includes people living with HIV who receive routine VL testing in Butha-Buthe and Mokhotlong districts, Lesotho. The cohort was launched in December 2015 and has been described previously[16]. Here, we present the VL cascade for children (<15 years at the time of the first VL) in this cohort with at least one recorded VL result from January 2016 through June 2020. Follow-up continued through February 10, 2021.

Setting

This study encompasses two urban hospitals, one rural hospital, and 19 rural nurse-led health centres in Butha-Buthe and Mokhotlong districts, which have a combined population of around 220,000[25]. The diagnostic laboratory of Butha-Buthe Government Hospital has conducted VL monitoring for Butha-Buthe district since December 2015, and for Mokhotlong district since January 2018.

VL cascade

Lesotho HIV guidelines, in alignment with the 2017 WHO guidelines, advise that all children taking ART should receive VL monitoring every six months. Children with a VL ≥1000 copies/mL should receive enhanced adherence counselling and a follow-up VL after 8-12 weeks. Upon resuppression to <1000 copies/mL or a ≥0.5 log drop, children should remain on an unchanged ART regimen, with continued adherence support for those with a ≥0.5 log drop without resuppression. Virologic failure (defined as two consecutive VLs ≥1000 copies/mL) should trigger a switch to an effective new regimen, though national guidelines recommend prior resistance testing in the case of PI- or INSTI-based regimens.[2,3,9,10].

46

47 *Outcomes and definitions*

48 Participants were categorized into mutually exclusive groups based on their VL results during
49 the study period: at least one VL ≥1000 copies/mL, all VLs <1000 copies/mL with at least one
50 VL ≥20 copies/mL, or all VLs <20 copies/mL. In line with current national recommendations,
51 we defined VLs >1000 copies/mL as unsuppressed and two consecutive VLs >1000 copies/mL
52 as virologic failure. A VL ≥20 copies/mL but <1000 copies/mL does not trigger clinical action
53 according to current guidelines in Lesotho, but does trigger a response according to the latest
54 2021 WHO and several regional and national guidelines, considering evidence that such low-
55 level viraemia is associated with worse virologic and clinical outcomes [21-24]. A VL <20
56 copies/mL is undetectable using the available VL platform at Butha-Buthe Government
57 Hospital.

58

59 We defined care to be "according to guidelines" if 1) a child received a follow-up VL within
60 <6 months of the first unsuppressed VL, and 2) the follow-up VL was <1000 copies/mL, and/or
61 there was a log drop ≥0.5 at the follow-up VL, and/or a child with a follow-up VL ≥1000
62 copies/mL was switched to a new regimen within 12 weeks after the follow-up VL test.
63 Moreover, children taking PI- or INSTI-based first-line ART were classified according to
64 guidelines as long as they received a follow-up VL within <6 months. Switch of regimen was
65 not evaluated as part of the definition for according to guidelines for these children, because a
66 resistance test prior to switch is recommended but often inaccessible.
67 We assessed the timeliness of sample transport (time from blood sampling to receipt at the VL
68 laboratory), processing (time from receipt to testing), and upload (time from testing to import
69 of the result into the database). The turn-around time was defined as transport plus processing.
70 Turnaround-times above 4 weeks were considered to be delayed.

71

Data collection

As per routine, EDTA blood samples were collected at the healthcare facilities, plasma separated at the three hospital laboratories, and VL testing was performed at Butha-Buthe Government Hospital using the COBAS® Ampliprep/COBAS® TaqMan HIV-1 Test, v2.0 or COBAS® 4800 system HIV-1 Test (Roche Diagnostics). VL results feed directly into the Laboratory Information System maintained by the Ministry of Health and were also uploaded to a secured online database alongside further patient information including demographic and treatment data.

Statistical Analyses

Results were summarized as frequencies and percentages for categorical variables and medians and interquartile ranges (IQRs) for continuous variables. Comparisons were made using chi-square tests for categorical variables and Wilcoxon rank sum test of medians for continuous variables. All analyses were done using Stata v14 (Stata Corp, College Station, TX, USA).

Ethical considerations

The cohort study has been approved by the National Health Research Ethics Committee of the Ministry of Health of Lesotho (ID 134-2016), and continues to receive annual renewals, with the latest approval dated 13 May 2021. The ethics committee gave a waiver of patient consent for the descriptive analysis of routinely collected data.

RESULTS

Participant characteristics

Within the study period, a total of 5443 VL tests were performed for 1215 children, with a median of four tests per person (IQR 2-6). At the time of the first recorded VL, 692/1215 (57%) were in care at rural health facilities, 627/1215 (52%) were female, median age was 10 years

(IQR 7-13), and median time taking ART was 3.6 years (IQR 1.6-6.5); furthermore, 896/1214 (74%) were taking NNRTI-based, 285/1214 (23%) PI-based, and 33/1214 (3%) INSTI-based ART. Compared with the two urban hospitals, children at rural health facilities received fewer VL tests during the study period (median of 3 versus 6, p<0.001).

VL outcomes and VL cascade

Overall, for 404/1215 (33%) children all VLs were <20 copies/mL, for 449/1215 (37%) all VLs were <1000 copies/mL but at least one VL was between 20-999 copies/mL, and 362/1215 (30%) had at least one VL ≥1000 copies/mL (Table 1). The proportion with at least one VL ≥1000 copies/mL was lower among children attending rural clinics (27% versus 34%, p=0.002). Among children with an unsuppressed VL, 325/362 (90%) ever had a follow-up VL result; 153/325 (47%) achieved viral resuppression to <1000 copies/mL and 172/325 (53%) were confirmed as having virologic failure. The rate of virologic failure was similar among children attending rural (54%) and urban clinics (52%, p=0.862).

Of those with virologic failure, 32/172 (19%) were taking PI- or INSTI-based ART, 17/172 (10%) were taking NNRTI-based ART but had a log drop >0.5 and were thus not switched; the remaining 123/172 (72%) qualified for switch to a new ART regimen as per guidelines. Of these, 55/123 (45%) were switched, but only 9/123 (7%) within 12 weeks of the confirmed virologic failure. Both the proportion switched (30% vs 59%) and the proportion switched within 12 weeks of the follow-up VL (3% vs 11%) were lower among rural than among urban facilities (p=0.005) (Table 1). The median time from the first elevated VL to the switch was 58 weeks (IQR 42-74).

Among those switched, 52/55 (95%) had a VL result after switch, 20/52 (38%) within 6 months after switching. 47/52 (90%) achieved resuppression to <1000 copies/mL and 25/52 (48%) to ≤20 copies/mL after switch; these rates were similar in rural and urban clinics.

124

125 *Proportion managed according to guidelines*

126 Among children with a first unsuppressed VL, 159/362 (44%) had a follow-up VL within 6
127 months. This fraction was smaller for children attending rural (31%) than urban (57%, p<0.001)
128 facilities. Of those who received a timely follow-up VL, 66/159 (42%) achieved resuppression
129 to <1000 copies/mL (managed according to guidelines), 34/159 (21%) had ongoing viremia
130 which was considered to have been managed according to guidelines (0.5 log drop or on
131 PI/INSTI regimen), and 59/159 (37%) had ongoing viremia which was not managed according
132 to guidelines (Figure 1). Thus overall, 100/362 (28%) children with a first unsuppressed VL
133 were managed according to guidelines (Figure 1). This proportion was 17% in rural and 38%
134 in urban facilities (p<0.001).

135

136 *Turn-around time of VL results.*

137 The median turn-around time was 9 days (IQR 5-18) and turn-around was delayed (≥4 weeks)
138 for 637/5443 (12%) samples (Table 2). The frequency of delays varied strongly by year,
139 peaking in 2019 at 389/1383 (28%) samples followed by a sharp reduction in 2020, when
140 18/1530 (1%) samples were delayed. In samples from rural facilities, median turn-around time
141 was higher (13 vs 7 days; p<0.001) and delays were more frequent (18% vs 7%, p<0.001) than
142 in those from urban facilities. This difference appears to be driven by the median transport time,
143 which was higher in rural (6 days) than urban (0 days; p<0.001) facilities. Once in the VL
144 laboratory, median processing time was similar for samples from rural (6 days) or urban (5
145 days; p=0.0001) facilities. There was no evidence to suggest that the initial delay in transport
146 caused delays in taking the follow-up sample, as the median time to a follow-up VL was 6
147 months both in cases with or without a delay of the initial sample.

148

149 **DISCUSSION**

In this prospective cohort study on the VL cascade in children in Lesotho, we found that among children with a first unsuppressed VL, only 28% were managed correctly according to WHO and Lesotho National Guidelines. While management of viremia – notably timely follow-up after an unsuppressed VL and treatment switch after virologic failure while taking NNRTI-based ART – was suboptimal regardless of location, the situation was slightly better at the two urban hospitals, where children living with HIV have access to medical doctors working in specialized, non-governmental organization-led pediatric clinics.

This study confirms that despite the scale-up of routine VL monitoring, VL data is not yet optimally utilized to improve patient outcomes, and the majority of unsuppressed VLs currently do not trigger timely and appropriate action. These findings are unlikely to be heavily impacted by the Covid-19 pandemic considering the timing of data closure: the first major Covid-19 wave hit Lesotho from December 2020 to February 2021, whereas for this analysis we only considered children with a high VL until the end of June 2020, and only 17 children in this study had their first high VL in 2020. Furthermore, delays in turn-around time varied greatly by year and were particularly rare in 2020/2021.

The great variability of turn-around times over years suggests a lack of resilience at the laboratory level and high susceptibility to unforeseen events such as instrument breakdown, highlighting the need for laboratory system strengthening [26–28]. However, we have also shown that subsequent delays along the VL care cascade were largely independent from delays in VL testing. VL monitoring therefore must be combined with strategies to ensure that unsuppressed VLs trigger appropriate clinical action[29–32]. One promising approach to address both laboratory delays and appropriate use of VL results is point-of-care VL testing within tiered laboratory networks[27], as included in the latest WHO guidelines[4]. Point of care VL testing not only reduces sample transport and processing time but allows for clinical action based on the VL result to start on the same day. In the STREAM randomized controlled trial, point-of-care VL testing increased 12-month retention in care and 12-month viral

suppression, and led to faster detection of virologic failure and, if indicated, faster switch to second-line ART[33].

Appropriate use of VL data may also be supported by eHealth-based approaches [34] and further differentiation of service delivery. An ongoing study is testing the possibility of using VL data to guide differentiated care in Lesotho, with less intensive follow-up of those with stable viral suppression and reallocation of resources towards those with treatment failure[35]. This is a key opportunity to adapt HIV management to individual patients' needs while simultaneously increasing cost-effectiveness of HIV programs[36].

This study has several limitations. First, it includes children who received at least one VL measurement, while children without any VL tests would be missed. Second, as continuation of PI- or INSTI-based ART may be the best or often the only available regimen even in the case of virologic failure, we assume that even those with virologic failure while taking PI- or INSTI-based ART were still managed to the best extent possible under the current conditions. Increased access to resistance testing and to appropriate second-line regimens will however be crucial to ensure optimal care for children with treatment failure taking PI- or INSTI-based first-line ART. Third, there may be clinically valid reasons (such as clear evidence of non-adherence) for delaying follow-up VL testing or switch, which we cannot assess here. Fourth, there are aspects of optimal clinical management, such as the timing and quality of providing adherence counselling, which we cannot assess within this analysis. Finally, underlying causes of suboptimal clinical management have not been explored.

Our findings are in line with previous studies assessing various parts of the VL cascade in children have shown poor outcomes, including lower rates of viral suppression while in active care compared with adults[6], lower rates of resuppression after an adherence intervention upon detection of viremia than in adults[11], and limited switching to second-line ART upon treatment failure[12–14].

In conclusion, this data from a real-life setting in Lesotho shows suboptimal management of viremia for children taking ART. A lack of timely follow-up after a first unsuppressed VL as well as reluctance to switch to second-line ART reduce the potential benefits of scaling up VL monitoring and endanger health outcomes. The cascade was poorer among children attending rural facilities. Future studies should assess models of care that facilitate follow-up, including through innovative uses of information technology, to ensure that VL results trigger timely and appropriate clinical action. Studies assessing models of care targeting more rural populations could provide evidence to reduce the rural-urban gap.

Conflict of interest statement

TK reports advisory board membership fees from ViiV and Gilead for work outside this study. NDL reports having received travel grants to attend IAS, AIDS and CROI conferences from Gilead Sciences Sarl. All other authors: none to declare.

Authorship

The cohort on which this study is based was designed and established by TRG, NDL, JAM, and TK. This study was conceptualized by NDL and designed by JAM, JAB, TRG and NDL. LM, BLN, AA, and ML contributed to data collection. TRG analyzed the data with key input from JAM, JAB, and NDL. JAM, JAB, and NDL, wrote the first draft manuscript. All authors reviewed and approved the final manuscript.

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348

349 **Figure 1. Outcomes among children with at least one unsuppressed viral load.**

350 **Table 1: Viral load outcomes.**

351 **Table 2: Sample processing times by health center location**

352

Figure 1. Outcomes among children with at least one unsuppressed viral load.

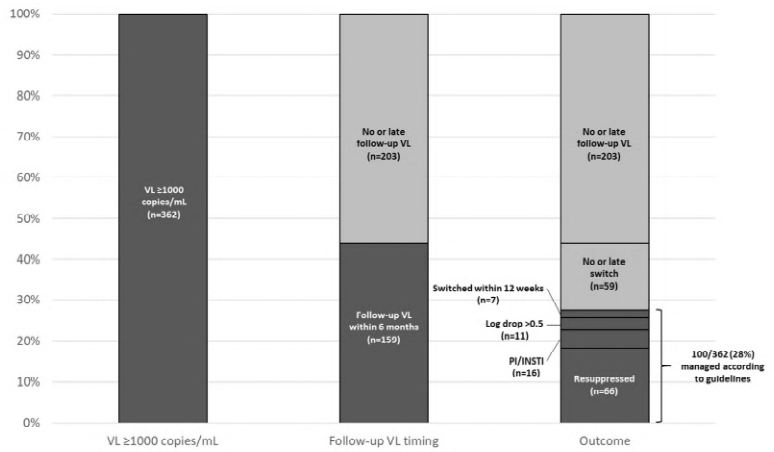

Table 1: Viral load outcomes.

	All	Rural	Urban	Rural vs urban
Children, n	1215	692	523	
Viral load tests per person, median (IQR) [range]	4 (2-6) [1-12]	3 (2-5) [1-11]	6 (4-8) [1-12]	p<0.001
Viral load in copies/mL, n (%)				p=0.002
Always ≤20	404 (33%)	256 (37%)	148 (28%)	
Always <1000, at least one >20	449 (37%)	252 (36%)	197 (38%)	
At least one ≥1000	362 (30%)	184 (27%)	178 (34%)	
Of those with a viral load ≥1000 copies/mL and at least 6 months follow-up:	*N=362*	*N=184*	*N=178*	
Had follow up viral load, n (%)	329 (91%)	160 (87%)	169 (95%)	p=0.008
Did not have follow up viral load, n (%)	33 (9%)	24 (13%)	9 (5%)	
Of those with a viral load ≥1000 copies/mL and a follow up viral load:	*N=329*	*N=160*	*N=169*	
Time to follow up viral load in months, median (IQR) [range]	6 (5-9) [0.7-49]	8 (5-14) [0.8-49]	5 (4-7) [0.7-28]	p<0.001[1]
<3, n (%)	36 (11%)	14 (9%)	22 (13%)	
3-<6, n (%)	123 (37%)	43 (27%)	80 (47%)	
6-<9, n (%)	87 (26%)	39 (24%)	48 (28%)	
9-<12, n (%)	28 (9%)	19 (12%)	9 (5%)	
>12, n (%)	55 (17%)	45 (28%)	10 (6%)	
Follow up viral load in copies/mL, n (%) [2]				p=0.862
≤20	85 (26%)	39 (25%)	46 (27%)	
21-999	68 (21%)	34 (22%)	34 (20%)	
≥1000	172 (53%)	84 (54%)	88 (52%)	
Of those with a viral load ≥1000 copies/mL but WITHOUT a follow up viral load:	*N=33*	*N=24*	*N=9*	
Time from unsuppressed viral load to data censoring in months, median (IQR) [range]	24 (18-36) [9-55]	20 (16-33) [9-51]	35 (24-38) [16-55]	p=0.09
6-<9, n (%)	1 (3%)	1 (4%)	0	
9-<12, n (%)	2 (6%)	2 (8%)	0	
≥12, n (%)	30 (91%)	21 (88%)	9 (100%)	
Of those with a viral load ≥1000 copies/mL and follow up viral load ≥1000 copies/mL:	*N=172*	*N=84*	*N=88*	
Did not require a switch to second line ART, n (%)	49 (28%)	24 (29%)	25 (28%)	p=0.98
Required a switch to second-line ART. n (%) [3]	123 (72%)	60 (71%)	63 (72%)	
Taking NNRTI-based ART, n (%)	140 (82%)	69 (82%)	71 (81%)	p=0.38
Taking PI-based ART, n (%)	30 (17%)	15 (18%)	15 (17%)	

Taking INSTI-based ART, n (%)	2 (1%)	0	2 (2%)	
Of those with a viral load ≥1000 copies/mL and follow up viral load ≥1000 copies/mL who required a switch:	*N=123*	*N=60*	*N=63*	
Switched within <12 weeks of follow-up VL, n (%)	9 (7%)	2 (3%)	7 (11%)	p=0.005
Switched ≥12 weeks after follow-up VL, n (%)	46 (37%)	16 (27%)	30 (48%)	
Not switched, n (%)	68 (55%)	42 (70%)	26 (41%)	
Of those taking PI- or INSTI-based ART with a viral load ≥1000 copies/mL and follow up viral load ≥1000 copies/mL:	*N=32*	*N=15*	*N=17*	
No third VL and second VL was ≥6 months before data censoring, n (%)	6 (19%)	0	6 (35%)	p=0.02
Switched to second-line ART, n (%)	0	0	0	
No switch, no third VL but second VL was <6 months before data closure, n (%)	2 (6%)	1 (7%)	1 (6%)	
No switch, received a third VL, n (%)	24 (75%)	14 (93%)	10 (59%)	
Of those taking NNRTI-based ART with a viral load ≥1000 copies/mL and follow up viral load ≥1000 copies/mL:	*N=140*	*N=69*	*N=71*	
No switch or sufficient log drop, and follow-up VL was ≥12 weeks before data censoring, n (%)	68 (49%)	42 (61%)	26 (37%)	P=0.005
Switched to second-line ART, n (%)	55 (39%)	18 (26%)	37 (52%)	
No switch, had >0.5 log drop (on 1st line) , n (%)	17 (12%)	9 (13%)	8 (11%)	
No switch or sufficient log drop, but follow-up VL was <12 weeks before data censoring, n (%)	0	0	0	
Of those switched:	*N=55*	*N=18*	*N=37*	
Weeks to switch (from 1st viral load ≥1000 copies/mL), median (IQR) [range]	58 (42-74) [18-160]	57 (42-75) [28-160]	59 (46-73) [18-138]	p=0.86
Weeks to switch (from 2nd viral load ≥1000 copies/mL), median (IQR) [range]	30 (19-45) [4-138]	31 (19-48) [10-138]	30 (21-44) [4-91]	p=0.68
Of those with a viral load upon switching	*N=53*	*N=17*	*N=36*	
1st viral load on 2nd line in copies/ml, n (%) [4]				p=0.94
≤20	25 (48%)	8 (47%)	17 (49%)	
21-999	22 (42%)	7 (41%)	15 (43%)	
≥1000	5 (10%)	2 (12%)	3 (9%)	
1st viral load on 2nd line obtained within 6 months of switch, n (%)	20 (38%)	5 (29%)	15 (42%)	P=0.39

[1] "Binary analysis: within six months vs not within six months"

[2]Four (three rural, one urban) had a date of follow-up viral load but the result was missing

[3] Defined as: taking NNRTI-based ART and not achieving a log drop >0.5

[4] One (urban) had a date of follow-up viral load but the result was missing

2

Table 2: Sample processing times by health center location

	All[1]	Rural	Urban
Children, n	1215	692	523
Total viral load results, n	5443	2415	2998
2016	459	171	288
2017	767	345	422
2018	1184	498	686
2019	1383	616	766
2020	1530	756	756
2021 (until 10 February)	120	29	80
Turnaround time (sampling until testing)			
Total samples taking >28 days, n (%)	637 (12%)	423 (18%)	214 (7%)
2016	120 (26%)	115 (67%)	5 (2%)
2017	85 (11%)	81 (23%)	4 (1%)
2018	25 (2%)	19 (4%)	6 (1%)
2019	389 (28%)	195 (32%)	194 (25%)
2020	18 (1%)	13 (2%)	5 (1%)
2021 (until 10 February)	0	0	0
Time in days, median (IQR)	9 (5-18) [0-485]	13 (7-22) [0-485]	7 (2-13) [0-449]
Transport time (sampling until registration)			
Samples taking >2 weeks, n (%)	429 (8%)	343 (14%)	85 (3%)
Time in days, median (IQR)	1 (0-7)	6 (2-11) [0-479]	0 (0-2) [0-443]
Processing time (registration until testing)			
Samples taking >2 weeks, n (%)	982 (18%)	464 (19%)	518 (17%)
Time in days, median (IQR)	5 (1-11)	6 (1-13)	5 (1-10)

[1] Rural vs urban data missing for 30 samples

5.1.4. Cohort on programmatic transition to DTG-based ART, HIV Med, 2021

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SHORT COMMUNICATION

Viral suppression after transition from nonnucleoside reverse transcriptase inhibitor- to dolutegravir-based antiretroviral therapy: A prospective cohort study in Lesotho (DO-REAL study)

Jennifer A. Brown[1,2,3] | **Bienvenu L. Nsakala**[4] | **Kuena Mokhele**[4] | **Itumeleng Rakuoane**[4] | **Josephine Muhairwe**[4] | **Lorena Urda**[2,3] | **Alain Amstutz**[1,3,5] | **Nadine Tschumi**[1,3] | **Thomas Klimkait**[2,3] | **Niklaus D. Labhardt**[1,3,5]

[1]Clinical Research Unit, Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland

[2]Molecular Virology Group, Department of Biomedicine, University of Basel, Basel, Switzerland

[3]University of Basel, Basel, Switzerland

[4]SolidarMed, Partnerships for Health, Maseru, Lesotho

[5]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

Correspondence
Niklaus D. Labhardt, Socinstrasse 57, 4051 Basel, Switzerland.
Email: n.labhardt@unibas.ch

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Abstract

Objectives: Since 2018, the World Health Organization has recommended dolutegravir (DTG)-containing antiretroviral therapy (ART) for most people living with HIV. Country programmes across Africa have subsequently transitioned from other, mostly nonnucleoside reverse transcriptase inhibitor (NNRTI)-based ART to DTG-based ART. This study aims to assess the virological impact of programmatic transitioning to DTG-based ART in Lesotho.

Methods: The prospective Dolutegravir in Real-Life in Lesotho (DO-REAL) cohort enrols people living with HIV initiating or transitioning to DTG-based ART in Lesotho. Here, we present data from participants who transitioned from NNRTI- to DTG-based ART between February and December 2020. Blood samples collected at transition and at 16 weeks' follow-up (window 8–32 weeks) were used for viral load (VL) and resistance testing.

Results: Among 1347 participants, follow-up data was available for 1225. The majority (60%) were female, median age at transition was 47 years [interquartile range (IQR): 38–56], and median (IQR) time since ART initiation was 5.9 (3.5–9.0) years. Among those with complete VL data, the rate of viral suppression to < 100 copies/mL was 1093/1116 (98%) before, 1073/1116 (96%) at, and 1098/1116 (98%) after transition. Even among those with a VL ≥ 100 copies/mL at transition, 42/44 (95%) achieved suppression to < 100 copies/mL at follow-up. Seven participants had a VL ≥ 1000 copies/mL at follow-up and did not harbour any integrase mutations associated with resistance to DTG.

Conclusions: The high levels of viral suppression observed are encouraging regarding virological outcomes upon programmatic transitioning from NNRTI- to DTG-based ART.

KEYWORDS
HIV, integrase inhibitors, observational study, Southern Africa, viraemia

INTRODUCTION

Antiretroviral therapy (ART) containing dolutegravir (DTG) is recommended as the preferred regimen type of most people living with HIV [1,2]. DTG-based ART leads to superior [3–5] or non-inferior [6–9] virological outcomes and faster viral suppression [3,4,7] compared with commonly available NNRTI-based regimens, has a high barrier to development of drug resistance [3,10], and a favourable tolerability profile [3]. For these reasons, countries including Lesotho are recommending DTG-containing regimens not only for people newly diagnosed with HIV, but also for people transitioning from other, mostly nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens to DTG-containing ART [11].

While DTG-containing ART has good virological outcomes when used in triple and dual drug regimens [3–10,12,13], DTG monotherapy is associated with the development of drug resistance and treatment failure [10], and should therefore be restricted to special circumstances [14]. This has caused concerns that DTG functional monotherapy among ART-experienced people – which could occur through just substituting the NNRTI for DTG in the case of viral resistance to the other two drugs in a three-drug regimen – might likewise adversely impact treatment outcomes [10,15]. Moreover, instances of mutations associated with resistance to DTG have been described in randomized trials even in the context of triple therapy in people with and without prior exposure to first-generation integrase strand transfer inhibitors (INSTIs) [10,16].

Consequently, there is uncertainty regarding the optimal modality for the roll-out of DTG, with open questions including the requirement for previous viral suppression, the viral load (VL) threshold allowing for transition, and the timing of VL measurement before transition. This study aims to assess virological outcomes upon programmatic transitioning from NNRTI- to DTG-based ART in a routine care setting in Lesotho, southern Africa.

MATERIALS AND METHODS

The prospective, registered Dolutegravir in Real Life in Lesotho (DO-REAL) cohort study enrolled people living with HIV initiating or transitioning to a DTG-containing ART regimen at two hospitals in Butha-Buthe district, Lesotho; for logistical reasons, one hospital was dropped after enrolling only seven participants.

According to the Lesotho guidelines, patients with a routine VL < 1000 copies/mL were to be transitioned to a first-line DTG-containing regimen typically maintaining the same nucleoside reverse transcriptase inhibitor (NRTI) backbone, whereas patients with two consecutive VLs ≥ 1000 copies/mL with enhanced adherence counselling before the follow-up measurement were to be transitioned to a second-line DTG-containing regimen with an altered NRTI backbone. With regard to the safety signal indicating an increased risk of neural tube defects in the context of DTG taken around the time of contraception [17], which has since been corrected downwards [18], Lesotho guidelines recommend an approach based on informed choice for female adolescents and women of childbearing potential [11].

In this analysis, we only include participants who transitioned from NNRTI- to DTG-containing first- or second-line ART in the ART clinic at Butha-Buthe Government Hospital in Lesotho between 10 February 2020 and 31 December 2020. Data were closed for analysis on 5 May 2021. Participants were enrolled into the study on the day of transition and had a follow-up study visit 16 weeks (window: 8–32 weeks) thereafter. Study participants received routine services, with the exception of participant interviews and the collection of a non-routine blood sample at both time points. Samples were transferred to the hospital laboratory for VL testing with a detection limit of 20 copies/mL (cobas® 4800 system; F. Hoffman-La Roche AG, Basel, Switzerland) and stored at −80°C. Resistance testing was performed at the Molecular Virology laboratory, Department of Biomedicine, University of Basel in Basel, Switzerland (RNA extraction, cDNA conversion and amplification) and at SEQ-IT GmbH & Co. KG in Kaiserslautern, Germany (deep sequencing with Illumina MiSeq platform; Illumina Inc., San Diego, CA, USA). Variants occurring with a nucleotide frequency ≥ 10% were considered during analysis. Sequences were uploaded to the Stanford HIV Drug Resistance Database (v.9.0) to assess the presence of resistance-associated mutations. Patient data were retrieved from medical records and participant interviews, and collected in the established secured database of an ongoing open cohort study in Lesotho [19], which also contains participants' prior routine VL results.

The pill count was calculated as a measure of adherence, and was calculated as follows: $\text{Pill count} = \frac{\text{number of pills dispensed} - \text{number of pills returned}}{\text{number of pills to be taken per day} \times \text{number of days since dispensing}} \times 100\%$. A pill count of 95–100% is considered as good adherence.

Results are summarized as frequencies and percentages for categorical variables, and medians and interquartile ranges (IQRs) for continuous variables. All analyses were done using Stata/MP v.16.1.

All participants received study information in Sesotho and gave written informed consent. In the case of illiteracy, consent was given by thumbprint and a witness signature was required. DO-REAL, as well as the open cohort study in which it is nested [19], were approved by the National Health Research Ethics Committee of Lesotho (ID134-2016). The prospective DO-REAL cohort was registered with ClinicalTrials.gov on 23 January 2020 (NCT04238767).

RESULTS

Among 1347 individuals transitioning from NNRTI- to DTG-containing ART who were enrolled to the cohort and meet the criteria for this analysis, 16-week follow-up data were available for 1225 (91%) by data closure. Of the remaining 122, 99 remained in care (68 at the same hospital but with no follow-up data collected within the window, 20 at the same hospital but withdrew from the study, 11 transferred out); 22 were recorded as lost to follow-up; and one died (this participant had a VL < 20 copies/mL both before and at transition). According to routine data, the 1225 enrolled and with follow-up data represent > 70% of eligible patients at the clinic.

Table 1 shows characteristics of participants who attended a 16-week follow-up visit (we did not observe any differences in baseline characteristics between participants with vs. those without a follow-up visit within the window period; data not shown). The majority were female, median (IQR) age at transition to DTG was 47 (38–56) years, and median time since ART initiation was 5.9 (3.5–9.0) years. The median time between receiving a previous VL and transitioning to DTG was 16 (12–24) weeks. The vast majority (98%) transitioned from efavirenz (EFV)-containing ART, with only 2% transitioning from nevirapine (NVP)-containing ART. Nine participants (1%) transitioned to second-line ART as a consequence of treatment failure, whereas all others were considered to have transitioned within first-line ART. Both before and after transition, the backbone consisted of lamivudine (3TC)/tenofovir disoproxil fumarate (TDF) for > 93% of participants. Adherence was higher in women than men, especially at transition (Table 1).

A routine VL result from before transition, a VL at transition, and a VL at follow-up were available for 1217/1225 (99%), 1174/1225 (96%) and 1166/1225 (95%) participants, respectively. Data on VL were available at all three time points for 1116/1225 (91%) participants. Figure 1 shows

TABLE 1 Participant characteristics and adherence among participants who attended a follow-up visit

N = 1225	At transition to DTG	At follow-up
Baseline characteristics		
Female	731 (60%)	
Age (years)	47 (38–56)	
Time taking ART (years)	5.9 (3.5–9.0)	
Time since HIV diagnosis (years)	7.1 (3.9–10.6)	
Time since last viral load[a] (weeks)	16 (12–24)	
ART immediately before transition		
ABC/3TC/EFV	28 (2%)	
AZT/3TC/EFV	37 (3%)	
TDF/3TC/EFV	1135 (93%)	
ABC/3TC/NVP	1 (0.1%)	
AZT/3TC/NVP	13 (1%)	
TDF/3TC/NVP	11 (1%)	
ART immediately after transition		
ABC/3TC/DTG	41 (3%)	
AZT/3TC/DTG	10 (1%)	
TDF/3TC/DTG	1174 (96%)	
Transition type		
Programmatic transition within first-line ART	1216 (99%)	
Switch to second-line ART due to treatment failure	9 (1%)	
Backbone changed at transition	71 (6%)	
Adherence		
Good adherence defined as pill count 95–100%[b]	967 (82%)	882 (90%)
Among women (*N* = 731)	607 (85%)	527 (91%)
Among men (*N* = 494)	360 (76%)	355 (89%)
ART missed on two or more consecutive days in past month[c]	98 (8%)	80 (7%)
Among women (*N* = 731)	44 (6%)	41 (6%)
Among men (*N* = 494)	54 (11%)	39 (8%)
Viral load category		
Overall[d]	*N* = 1174	*N* = 1166
< 20 copies/mL	1029 (88%)	1053 (90%)
20–99 copies/mL	98 (8%)	94 (8%)
100–999 copies/mL	25 (2%)	12 (1%)
≥ 1000 copies/mL	22 (2%)	7 (1%)
Overall, among women[e]	*N* = 696	*N* = 698

(Continues)

TABLE 1 (Continued)

N = 1225	At transition to DTG	At follow-up
< 20 copies/mL	620 (89%)	640 (92%)
20–99 copies/mL	52 (7%)	48 (7%)
100–999 copies/mL	14 (2%)	5 (1%)
≥ 1000 copies/mL	10 (1%)	5 (1%)
Overall, among men[f]	*N* = 478	*N* = 468
< 20 copies/mL	409 (86%)	413 (88%)
20–99 copies/mL	46 (10%)	46 (10%)
100–999 copies/mL	11 (2%)	7 (2%)
≥ 1000 copies/mL	12 (3%)	2 (0.4%)
If VL at transition < 20 copies/mL[g]		*N* = 987
< 20 copies/mL		912 (92%)
20–99 copies/mL		62 (6%)
100–999 copies/mL		8 (1%)
≥ 1000 copies/mL		5 (1%)
If VL at transition 20–99 copies/mL[g]		*N* = 93
< 20 copies/mL		70 (75%)
20–99 copies/mL		20 (22%)
100–999 copies/mL		3 (3%)
≥ 1000 copies/mL		0
If VL at transition 100–999 copies/mL[g]		*N* = 24
< 20 copies/mL		20 (83%)
20–99 copies/mL		3 (13%)
100–999 copies/mL		1 (4%)
≥ 1000 copies/mL		0
If VL at transition ≥ 1000 copies/mL[g]		*N* = 20
< 20 copies/mL		14 (70%)
20–99 copies/mL		5 (25%)
100–999 copies/mL		0
≥ 1000 copies/mL		1 (5%)

Note: For continuous variables, brackets indicate the interquartile range.

Abbreviations: 3TC, lamivudine; ABC, abavacir; AZT, azidothymidine; DTG, dolutegravir; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate.

[a]Missing for 10 participants.

[b]Missing for 41 participants (21 female, 20 male) at transition and 248 (155 female, 93 male) at follow-up.

[c]Self-reported; missing for three participants (all female) at transition.

[d]Missing for 51 participants at transition to DTG and 59 participants at follow-up.

[e]Missing for 35 participants at transition to DTG and 33 participants at follow-up.

[f]Missing for 16 participants at transition to DTG and 26 at follow-up.

[g]VL missing at transition to DTG and/or at follow-up for 101 participants.

the VL dynamics between these three time points (categories: < 20, 20–99, 100–999 and ≥ 1000 copies/mL). Table 1 shows the VL category at follow-up stratified by VL category at transition to DTG . At all assessed VL categories at transition, ≥ 94% of participants had a VL < 100 copies/mL at follow-up and ≥ 68% of participants had a VL < 20 copies/mL at follow-up.

Among all participants with VL data at follow-up, 19/1166 (2%) had a follow-up VL ≥ 100 copies/mL including 7/1166 (1%) with a follow-up VL ≥ 1000 copies/mL. Among those with a follow-up VL ≥ 100 copies/mL, 17/19 had a VL < 100 copies/mL at or before transition and remained on an unchanged NRTI backbone, and 2/19 had a VL ≥ 100 copies/mL at transition (544 and 22 000 copies/mL) and had an NRTI backbone change at transition to DTG. Sequencing of the sample taken at transition (544 copies/mL: not sequenced; 22 000 copies/mL: reverse transcriptase sequenced) and/or follow-up sample (271 and 17 900 copies/mL; reverse transcriptase and integrase regions sequenced for both) identified a K103N mutation conferring resistance to EFV and NVP for one participant, and no mutations associated with NRTI or INSTI resistance were detected. Of the seven with a follow-up VL ≥ 1000 copies/mL, none harboured any mutations associated with DTG resistance in the integrase region; sequencing of the reverse transcriptase region was successful for four samples and no resistance-associated mutations were detected.

DISCUSSION

In order to assess virological outcomes within programmatic transitioning to DTG-based ART, this prospective cohort study assessed virological outcomes among people routinely changing from NNRTI- to DTG-based ART in a government hospital setting in Lesotho.

The vast majority of participants, including those with viraemia at transition, achieved viral suppression by follow-up. Furthermore, viraemia detected at follow-up was unlikely to be caused by drug resistance: among 19 participants with viraemia ≥ 100 copies/mL at follow-up, only two had viraemia ≥ 100 copies/mL at transition and neither harboured NRTI resistance (assessed at transition and/or follow-up); furthermore, among seven with viraemia ≥ 1000 copies/mL at follow-up, none harboured any resistance-associated mutations in the integrase region, suggesting suboptimal adherence as the cause of viraemia. These results support the virological effectiveness of pragmatic transitioning taking into account the patient's most recent available data.

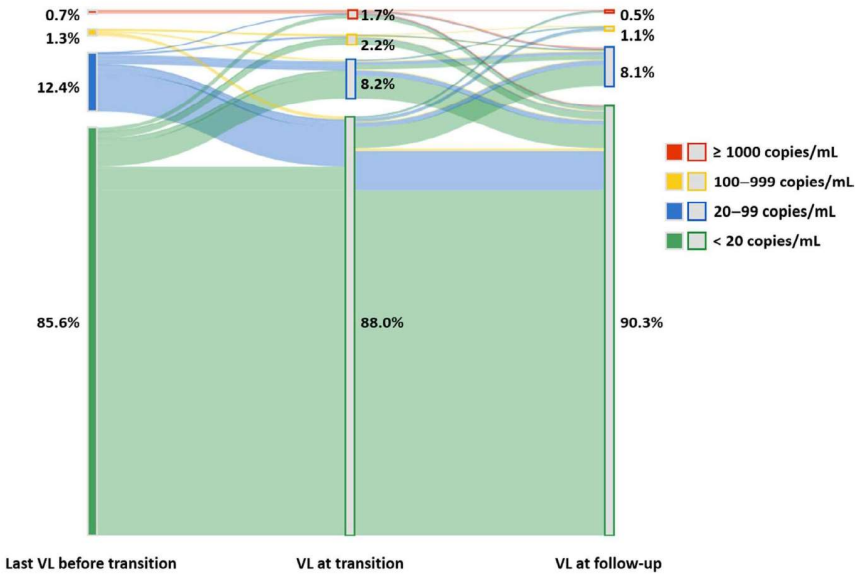

FIGURE 1 Viral load (VL) dynamics. The VL of the last measurement pre-transition from an nonnucleoside reverse transcriptase inhibitor (NNRTI)- to a dolutegravir (DTG)-based antiretroviral therapy (ART), the VL at transition, and the VL at follow-up are shown among participants with VL data at all three time points ($N = 1116$). The median time between the last VL before transition and transition to DTG was 16 weeks [interquartile range (IQR): 12–24; range: 1–191]; the median time between transition and follow-up was 16 (IQR: 16–18; range: 8–32). The colours of the nodes indicate the VL category at that time point; colour coding of the flows corresponds to the respective participant's VL pre-transition

This study has several limitations. First, 122 participants had no follow-up data and a further 59 had no follow-up VL. While the majority of these remained in care, their 16-week virological status is unknown. Second, it is not yet certain whether the viral suppression observed during this short-term follow-up will remain stable over time. Third, the median time between the most recent VL before transition and the transition to DTG was only 16 weeks in this cohort. A longer time-frame between the previous VL test and transition would probably increase the proportion of participants with viraemia and, subsequently, NRTI resistance at transition, with unclear implications on viral suppression after transition to DTG. Fourth, the high rate of viral suppression at transition in this study limits its capacity to assess the impact of NRTI backbone resistance on viral suppression after transition to DTG-based ART. Given the high rate of viral suppression at transition in this cohort – with > 96% having a VL < 100 copies/mL – caution is advised in extrapolating these results to populations with higher rates of viraemia or with a greater time differential between the last available VL and the time of transition to DTG. Further studies as well as longer-term follow-up are needed to assess the short- and long-term efficacy of DTG in the context of NRTI resistance and functional monotherapy. The major strength of this study is that it included > 70% of patients transitioned in this clinic and was conducted in a routine healthcare setting with minimal intervention of the study on clinical procedures and clinical decision-making. Its representativeness is supported by the fact that the rates of viral suppression observed among people in care are in line with the national average of 97% [20].

Overall, these results are encouraging regarding the short-term virological effectiveness of programmatic transitioning from NNRTI- to DTG-based ART.

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CONFLICTS OF INTEREST

TK reports advisory board membership fees from ViiV and Gilead for work outside of this study. NDL reports having received travel grants to attend IAS, AIDS and CROI conferences from Gilead Sciences Sarl. All other authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

NDL conceptualized this study. JAB and NDL designed the study with key input from BLN, AA, NT, JM and TK. BLN managed the study on site and oversaw all on-site procedures including consenting, data collection and on-site data cleaning. KM and IR conducted consenting procedures (together with Reitumetse Peea and Kelebone Moqethei, mentioned above) and collected data from medical records; KM, BLN and Reitumetse Peea collected blood samples. JAB conducted off-site data cleaning and analysed the data. LU performed resistance testing. JAB and NDL wrote the first version of the manuscript. All authors provided feedback on the manuscript and approved the final version.

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5.1.5. Rising HIV Drug Resistance During EAC, Open Forum Infect Dis, 2021

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MAJOR ARTICLE

Emergence of Human Immunodeficiency Virus-1 Drug Resistance During the 3-Month World Health Organization-Recommended Enhanced Adherence Counseling Period in the CART-1 Cohort Study

Jennifer A. Brown,[1,2,3] Herbert A. Mbunkah,[4,5] Thabo I. Lejone,[6] Isaac Ringera,[6] Molisana Cheleboi,[7] Thomas Klimkait,[2,3] Karin J. Metzner,[4,5] Huldrych F. Günthard,[4,5] Niklaus D. Labhardt,[1,2,8] Roger D. Kouyos,[4,5,a] and Nadine Tschumi[1,2,4,5,a]

[1]Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]University of Basel, Basel, Switzerland, [3]Department of Biomedicine, University of Basel, Basel, Switzerland, [4]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland, [5] Institute of Medical Virology, University of Zurich, Zurich, Switzerland, [6]SolidarMed, Swiss Organization for Health in Africa, Maseru, Lesotho, [7]Seboche Mission Hospital, Seboche, Lesotho, [8]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

Background. In resource-limited settings, the World Health Organization recommends enhanced adherence counseling (EAC) for individuals with an unsuppressed human immunodeficiency virus (HIV)-1 viral load (VL) and to remeasure VL after 3 months to avoid unnecessary regimen switches. In cases in which this follow-up VL remains unsuppressed, a regimen switch is indicated. We aimed to assess levels of HIV-1 drug resistance before and after the EAC period among people with ongoing viremia (≥80 c/mL) after EAC.

Methods. We included adult participants of the CART-1 cohort study conducted in Lesotho who had a VL ≥80 c/mL after EAC. Paired plasma samples (before and after EAC) were analyzed by next-generation sequencing. We assessed the prevalence of resistance-associated mutations and viral susceptibility scores to each participant's antiretroviral therapy (ART) regimen (range, 0–3; 3 indicates complete susceptibility).

Results. Among 93 participants taking nonnucleoside reverse-transcriptase inhibitor-based ART with an initial VL ≥1000 copies/mL who received a follow-up VL test after EAC, 76 still had a VL ≥80 copies/mL after EAC, and paired samples were available for 57 of 76. The number of individuals without full susceptibility to any drug in their regimen increased from 31 of 57 (54.4%) before to 36 of 57 (63.2%) after EAC. Median susceptibility scores dropped from 0.5 (interquartile range [IQR] = 0.25–) to 0.25 (IQR = 0.25–1) during the EAC period (P = .16).

Conclusions. Despite high levels of resistance before EAC, we observed a slight decline in susceptibility scores after EAC. The risk of further accumulation of resistance during EAC has to be balanced against the benefit of avoiding unnecessary switches in those with spontaneous resuppression after EAC.

Keywords. drug resistance; genotypic resistance testing; HIV; sub-Saharan Africa.

Sub-Saharan Africa (SSA) is home to 25.6 million people with human immunodeficiency virus (HIV)-1 (PWH), corresponding to 68% of the global burden of the epidemic [1]. The number of PWH who receive antiretroviral therapy (ART) rose rapidly across SSA to an estimated 16.4 million by the end of 2018 [1–3]. However, the emergence of HIV drug resistance (HIVDR) is a key threat to the achievements made by improving access to ART in SSA and endangers the success of the universal test-and-treat strategy [4, 5]. Projections show that ~890 000 acquired immune deficiency syndrome (AIDS) deaths and costs of US ~$6.5 billion attributable to HIVDR are expected in SSA between 2016 and 2030 [6]. Currently, for PWH with a viral load (VL) ≥1000 copies/mL (c/mL) while on first-line ART in resource-limited settings, the World Health Organization (WHO) recommends adherence counseling with a follow-up VL test within 3 to 6 months [7]. According to guidelines in Lesotho, nurse-administered enhanced adherence counseling (EAC) sessions should begin as soon as possible after treatment failure and focus on reviewing a patient's adherence, identifying potential barriers to good adherence, and developing and implementing an adherence plan. Only a sustained VL ≥1000 c/mL after EAC triggers a switch to second-line ART, generally consisting of ritonavir-boosted lopinavir

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[a]R. D. K. and N. T. contributed equally to this work.
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Correspondence: Nadine Tschumi, Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Rämistrasse 100, CH-8091 Zürich, Switzerland; Clinical Research Unit, Department of Medicine, Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4051 Basel, Switzerland (nadine.tschumi@swisstph.ch).

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plus 2 nucleoside reverse-transcriptase inhibitors (NRTIs) at the time of this study.

The rationale for this approach is to avoid unnecessary regimen switches for PWH where ongoing poor adherence rather than drug resistance is the cause of unsuppressed VL, notably in settings where drug resistance testing or drug level testing are unavailable. Several cohort studies from resource-limited settings endorse this approach, reporting up to 50% resuppression after EAC [8]. This may both save additional costs of second-line treatment and avoid further complications on second-line regimens, such as a higher pill burden or side effects. Likewise, in patients who do require switching to second-line ART due to ongoing viremia despite EAC, increased treatment literacy after EAC might help prevent the emergence of drug resistance on the new ART regimen.

However, the risk of the current EAC approach is that PWH with drug resistance, who qualify for switch to second-line ART and who are already taking their medication correctly, are delayed from receiving appropriate care. In these patients, ongoing viral replication may drive the accumulation of further HIVDR, thereby limiting future treatment options. Furthermore, many ART programs subsequently fail to switch PWH with virologic failure promptly [9], potentially leading to morbidity, mortality, and onward transmission of HIV [10–14].

The CART-1 study (NCT02126696) assessed virologic outcomes among patients attending routine ART care at 10 clinics in Lesotho [15, 16]. This substudy includes participants who had an unsuppressed VL, received EAC, and then had a second VL. Comparing resistance-associated mutations (RAMs) detectable through next-generation sequencing (NGS) before and after EAC, we assess to what extent the "waiting period" during EAC contributed to an accumulation of further RAMs, with the hypothesis that further RAMs might emerge during this period.

METHODS

Patient Consent Statement

The "Comorbidities and Virologic Outcome among Patients on Antiretroviral Therapy in Rural Lesotho" (CART-1, NCT02126696) [15] study was approved by the National Health Research Ethics Committee of the Ministry of Health of Lesotho (ID 01-2014) and the "Ethikkommission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland (ID 2014-029). Participants provided written informed consent.

Participants and Sample Collection

Samples were obtained from the CART-1 study conducted in 2014. CART-1 involved a cross-sectional assessment of routine VL among patients on first-line ART attending care at 10 facilities in 2 districts of Lesotho, followed by a cohort study involving patients with a VL ≥80 c/mL. Clinical and virological outcomes of this cohort have been published [15]. Among 110 adult participants with an initial VL ≥1000 c/mL, 3 (2.7%) died, 5 (4.6%) switched to second-line ART after their first VL, and 9 (8.2%) were lost to follow-up. Of the remaining 93, 17 (18.3%), 12 (12.9%), and 64 (68.8%) had a follow-up VL of <80, 80–999, and ≥1000 c/mL, respectively. We were able to obtain and analyze plasma samples, which had been stored at −80°C, for 10 of 12 and 47 of 64 participants with a follow-up VL of 80–999 c/mL (FUVL80-999 group) and ≥1000 c/mL (FUVL ≥1000 group), respectively.

Sample Preparation and Sequencing

We used Ilumina MiSeq NGS (Illumina, San Diego, CA) to analyze the viral protease and reverse-transcriptase regions in paired plasma samples. Ribonucleic acid was extracted from up to 1 mL blood plasma using the Maxwell RSC Viral Total Nucleid Acid Purification Kit (Promega Corporation, Madison, WI). All subsequent laboratory procedures were performed according to a validated protocol, which has been described elsewhere [17], with the slight modification that only 1 complementary deoxyribonucleic acid fragment was synthesised (primer 3' UNI-KS-A/G-4) as a template for amplification of both the protease and the reverse-transcriptase regions.

Analysis of Drug-Susceptibility Scores Before and After Enhanced Adherence Counseling

Consensus sequences for each sample were obtained by Minvar 2.2 using the nucleotide present at maximum frequency at each position [18]. Using these consensus sequences, we obtained drug resistance scores to each drug in a patient's 3-drug regimen from the Stanford HIV Drug Resistance Database [19, 20]. Based on the resistance scores, we calculated drug-specific susceptibility scores. High-level resistance, intermediate resistance, low-level resistance, potential low-level resistance, and susceptible were assigned values of 0.00, 0.25, 0.50, 0.75, and 1.00, respectively, as has been described elsewhere [21]. Values for each drug of a participant's 3-drug regimen (possible range: 0.00–1.00) were added to obtain their overall regimen susceptibility score (possible range: 0.00–3.00), with lower scores indicating lower susceptibility (higher resistance). We used a sign test to compare regimen susceptibility scores before and after EAC in the full dataset as well as stratified by VL after EAC, ie, in the FUVL80-999 and the FUVL ≥1000 group.

Analysis of Frequency of Viral Drug Resistance Before and After Enhanced Adherence Counseling

We determined the presence of RAMs and their patient-level variant frequencies using Minvar 2.2 [18]. We considered changes in frequencies of major RAMs, according to IAS-USA 2019 edition [22], at all genomic positions in which major RAMs were observed with a frequency of >5%, the lowest threshold recommended by Huber et al [18] for analyses using MinVar. If different major RAMs, according to Wensing et al [22], were present at a given position, we considered the sum of their

frequencies. This analysis was carried out for the full dataset as well as separately for the FUVL ≥1000 and the FUVL80-999 group. We used paired *t* tests to compare these variant frequencies before and after EAC.

RESULTS

Study Population

Table 1 displays characteristics of the FUVL ≥1000 and FUVL80-999 populations. All participants were on an ART regimen consisting of 1 non-NRTI (NNRTI), either efavirenz or nevirapine, and a NRTI backbone, consisting of lamivudine with either zidovudine or tenofovir disoproxil fumarate. During EAC, the median VL in the FUVL ≥1000 and FUVL80-999 groups changed from a median of 13 971 c/mL and 3515 c/mL to 9738 c/mL and 476 c/mL, respectively.

Prevalence of Resistance-Associated Mutations

Among the participants studied here, ie, individuals whose VL did not suppress to <80 c/mL after EAC, 53 of 57 (93.0%) harbored at least 1 major NNRTI RAM and 50 of 57 (87.7%) harbored at least 1 major NRTI RAM already before EAC (Figure 1). Stratified by subsequent VL, 43 of 47 (91.5%) in the FUVL ≥1000 group and 10 of 10 (100.0%) in the FUVL80-999 had 1 major RAM before EAC, and 33 of 47 (70.2%) and 6 of 10 (60.0%), respectively, had 4 or more (Supplementary Figure S1). Although the number of participants without a single major RAM remained unchanged after EAC (Figure S1), 16 of 57 (28.1%) participants accumulated major RAMs in genomic positions that had not harbored RAMs before EAC. The total number of major RAMs detected throughout the study population increased from 278 (231 in the FUVL ≥1000 group and 47 in the FUVL80-999 group) before EAC to 287 (241 and 46) after EAC. The total number of RAMs (major and accessory) increased from 405 (343 in the FUVL ≥1000 group and 62 in the FUVL80-999 group) to 425 (363 and 62) (Supplementary Table S1).

Change of Susceptibility Score to Current Antiretroviral Therapy Regimen During Enhanced Adherence Counseling

Thirty-one of 57 (54.4%) were not fully susceptible to any drug in their regimen already before EAC, which increased to 36 of 57 individuals (63.2%) after EAC. After EAC, susceptibility scores (1) remained unchanged for 49 participants, (2) increased for 2 participants, and (3) decreased for 6 participants. Median susceptibility scores decreased from a 0.5 (interquartile range [IQR] = 0.25–1) before to 0.25 (IQR = 0.25–1, $P = .16$) after EAC (Figure 2). In the FUVL ≥1000 group, median regimen-level susceptibility scores remained unchanged at 0.25 (IQR = 0.25–1, $P = .06$) before and after EAC. Forty of

Table 1. Characteristics of FUVL80-999 and FUVL≥1000 populations

	FUVL80-999	FUVL≥1000
N	10	47
Female, n (%)	5 (50)	34 (72)
Age in years, median [IQR]	43.4 [38.8–54.4]	41.1 [30.0–49.4]
Pill count in percent, median [IQR][a,b]	100 [100–100]	98 [96–100]
WHO stage, n (%)[c]		
1	4 (40)	14 (30)
2	4 (40)	18 (38)
3	2 (20)	12 (26)
4	0 (0)	2 (4)
VL before EAC in copies/mL, median [IQR]	3515 [1852–15424]	13971 [5973–25869]
VL after EAC in copies/mL, median [IQR]	476 [318–600]	9738 [4644–21033]
ART regimen, n (%)		
AZT/3TC/EFV	4 (40)	11 (23)
AZT/3TC/NVP	3 (30)	13 (28)
TDF/3TC/EFV	3 (30)	19 (40)
TDF/3TC/NVP	0 (0)	4 (9)
Time on ART in years, median [IQR][d]	4.7 [4.0–5.3]	4.5 [2.5–6.5]
Days between VL before and after EAC, median [IQR][e]	100.0 [92.0–103.5]	105.0 [98.0–115.8]
CD4 cell count before EAC (median [IQR])	408 [250–569]	350 [208–466]

[a]Number of pills presumably taken since the last visit (ie, number of pills provided at last visit minus number of pills remaining in pill bottle) divided by the number of pills that should have been taken since the last visit, multiplied by 100%.

[b]Missing: 2 participants in FUVL≥1000 population.

[c]Missing: 1 participants in FUVL≥1000 population.

[d]Missing: 1 participants in FUVL80-999 population.

[e]Missing: 3 participants in FUVL80-999 population and 5 participants in FUVL≥1000 population.

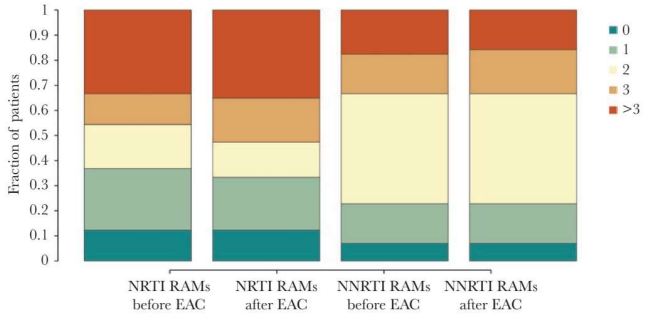

Figure 1. Fraction of participants with respective number of genomic positions with at least 1 major resistance-associated mutation (RAM) (with a variant frequency >5%) at respective time point[1]. [1] Observed major RAMs: M41L, A62V, K65R, D67N, K70E, K70R, V75I, F77L, Y115F, M184V, M184I, L210W, T215Y, T215F, K219Q, K219E, L100I, K101P, K101E, K103N, K103S, V106M, V108I, E138A, E138G, E138Q, Y181C, Y188C, Y188L, Y188H, V179L, G190A, G190S, H221Y, P225H, M230L, M230I, D30N. EAC, enhanced adherence counseling; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor.

the 47 individuals (85.1%) in this group experienced no change in susceptibility scores over the course of the EAC period, whereas 6 of 47 (12.8%) had a decreased score after EAC and 1 of 47 (2.1%) had an increased score after EAC (Supplementary Figure S2). The characteristics and RAMs of the 7 individuals experiencing a change in susceptibility are listed in Table 2. Only 6 of 47 (12.8%) individuals in the FUVL ≥1000 group were fully susceptible to their current regimen, and 28 of 47 (59.6%) harbored RAMs conferring resistance to all the drugs in their regimen already before EAC. In the FUVL80-999 group, the median susceptibility score did not change between the time point of the first (median = 1, IQR = 0.44–1) and the follow-up (median = 1, IQR = 0.63–1, $P = .32$) VL test. In this group, the susceptibility score remained unchanged in 9 of 10 (90.0%) individuals and increased in 1 of 10 (10.0%) individual (Supplementary Figure S3). The characteristics and RAMs of the individual with changed susceptibility scores are listed in Table 2. None of the participants in FUVL80-999 group had HIV-1 that was fully susceptible to their regimen, and 3 of 10 (30.0%) harbored HIV-1 that was not fully susceptible to any of the drugs in their regimen.

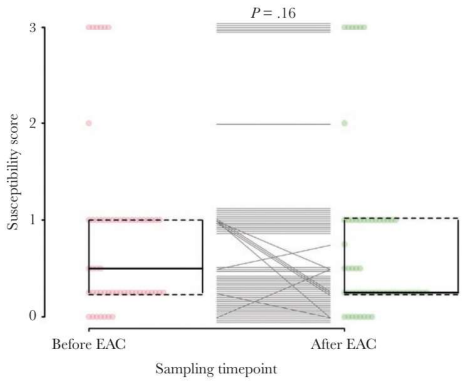

Figure 2. Susceptibility scores before and after enhanced adherence counseling ([EAC] N = 57). Gray lines connect samples of the same individual. Box plots indicate the median (bold horizontal line) and the interquartile range (dashed horizontal line). The *P* value was derived using sign test.

Change of Variant Frequency During the Enhanced Adherence Counseling Period

We found major RAMs with a patient-level variant frequency of >5% at 26 genomic positions (Supplementary Figure S4). Of these, mutations in 12 (M41L, A62V, K65R, D67N, K70E/R, V75I, F77L, Y115F, M184I/V, L210W, T215F/Y, K219E/Q) positions are associated with resistance to NRTIs, 13 (L100I, K101E/P, K103N/S, V106A/M, V108I, E138A/G/Q, V179L, Y181C/I, Y188C/H/L, G190A/S, H221Y, P225H, M230L/I) are associated with NNRTI resistance, and 1 (D30N) is associated with protease inhibitor resistance. Although mean variant frequencies did not change significantly over the course of the EAC period, we observed a trend ($P < .1$) towards increased variant frequency after EAC for A62V, V106A/M, and K219E/Q and towards decreased frequency for G190A/S. The stratified analyses of the FUVL ≥1000 and the FUVL80-999 group are shown in Supplementary Figure S5 and Supplementary Figure S6, respectively.

DISCUSSION

This study examines the evolution of HIVDR during the WHO-recommended EAC period in a resource-limited setting, thereby assessing the risk of further resistance

Table 2. Characteristics and RAMs of Participants With a Change in Susceptibility Scores Before Versus After EAC[a]

Population	ID	VL Before EAC [c/mL]	VL After EAC [c/mL]	ART Regimen	Susceptibility Score Before EAC	Susceptibility Score After EAC	Number of Fully Active Drugs Before EAC	Number of Fully Active Drugs After EAC	Major RAMs Before EAC (Frequency)	Major RAMs After EAC (Frequency)
FUVL80–999	1	1933	944	TDF/3TC/EFV	0	0.5	0	0	K65R (0.65), D67N (0.32), K70R (0.39), L100I (0.17), K103N (0.99), V108I (0.57), M184V (1), K219E (0.38), K219Q (0.62)	D67N (1), K70R (1), K103N (1), V108I (1), M184V (1), K219E (0.5), K219Q (0.5). No longer observed: K65R (0), L100I (0).
FUVL ≥1000	2	20 369	2747	AZT/3TC/EFV	1	0.5	1	0	K70R (0.18), K103N (0.7), K103S (0.3), V106M (0.32), V108I (0.68), M184V (1), H221Y (0.75)	D67N (0.09), K70R (0.75), K103N (0.24), K103S (0.76), V106M (0.87), V108I (0.11), M184V (1), K219Q (0.1), H221Y (0.11)
	3	28 595	12 156	AZT/3TC/NVP	1	0.25	1	0	K101E (1), M184V (1), G190A (1), T215Y (0.32)	K101E (1), M184V (1), G190A (1), T215Y (0.83)
	4	26 586	15 446	TDF/3TC/EFV	1	0.25	1	0	A62V (0.08), K65R (0.19), D67N (0.07), K103N (1), V106M (1), M184V (0.81), M184I (0.19)	A62V (0.72), K65R (0.74), K70E (0.22), K103N (0.94), V106M (0.94), Y115F (0.22), M184V (0.92). No longer observed: D67N (0), M184I (0).
	5	2603	2463	AZT/3TC/EFV	0.25	0	0	0	M41L (0.75), K103N (1), E138A (1), M184V (1), Y188L (1), L210W (0.43), T215Y (1)	M41L (0.96), K103N (1), E138A (1), M184V (1), Y188L (1), L210W (0.68), T215Y (1)
	6	3643	10 517	TDF/3TC/EFV	0.5	0.75	0	0	D67N (0.96), K70R (0.91), K103N (0.11), V108I (0.77), Y181C (0.93), M184V (0.86), G190A (0.9), K219Q (0.89)	D67N (1), K70R (0.51), K103N (0.12), V106M (0.48), V108I (0.39), Y181C (1), M184V (1), G190A (0.5), K219Q (1)
	7	20 7772	13 592	TDF/3TC/EFV	1	0.25	1	0	A62V (0.31), K65R (0.33), D67N (0.17), K103N (0.62), K103S (0.38), V106M (1), M184V (0.97)	A62V (1), K65R (1), K103N (0.39), K103S (0.61), V106M (1), M184V (1). No longer observed: D67N (0).
	8	7360	1271	AZT/3TC/NVP	1	0	1	0	D67N (0.98), K70R (0.36), K101E (0.98), V106M (1), E138A (1), M184V (1), K219Q (0.36)	D67N (0.88), K70R (0.83), K101E (0.98), V106M (1), E138A (1), M184V (1), K219Q (0.83)

Abbreviations: ART, antiretroviral therapy; AZT, zidovudine; EAC, enhanced adherence counselling; EFV, efavirenz; NVP, nevirapine; RAM, resistance-associated mutation; TDF, tenofovir disoproxil fumarate; VL, viral load; 3TC, lamivudine.

[a]Red and green indicates an increase and decrease, respectively, in measured variant frequency.

accumulation with this clinical standard of care. Previously, before EAC, we observed extremely high levels of resistance, and the majority (54.4%) of participants already harbored HIV-1 with at least partial resistance to all the drugs in their ART regimen. The median regimen-level susceptibility scores dropped nonsignificantly from 0.5 to 0.25 during the EAC period, indicating an increase in resistance. Of note, because RAMs that drop below the limit of detection may remain archived in the latent reservoir [23, 24], the increase in susceptibility scores observed in a small subset of participants might not correspond to an actual disappearance of drug resistance. The results reported here align with an observation by Kantor et al [25] of additional emergence of drug resistance in patients on second-line (lopinavir/ritonavir-based) ART after a median of 55 days.

This study is not without limitations. The number of included participants was limited by the availability of well documented patient samples (from the CART-1 study), and the sample size in the FUVL80-999 group was low. Furthermore, the CART-1 participants may not be representative of patients living in resource-limited settings today for several reasons. First, this study relies on samples from 2014 and does not include any patients on protease inhibitors or integrase strand-transfer inhibitors. Nevertheless, this study includes drugs that have been taken by the vast majority of people on ART in resource-limited settings, and that may remain relevant for those for whom integrase inhibitors are contraindicated. Second, participants received their first-ever VL test in the context of this study despite having been on ART for a median of 4.6 years, meaning that they most likely had already spent extensive time on a failing regimen. Indeed, the rate of resuppression observed in CART-1 is higher than in another study from Uganda with participants receiving their first-ever VL test after extensive time on ART [26], but it is low compared with data from a recent systematic review, which showed that approximately half of the PWH with an initial unsuppressed VL resuppressed after adherence counseling [8]. Today, routine (generally yearly) VL monitoring might lead to accelerated clinical action, although resistance levels are lower than those observed in this study. On the one hand, identifying viremia earlier could lead to higher rates of resuppression than observed in CART-1. On the other hand, it is possible that the further emergence of resistance and loss of clinically relevant drug options would be even greater in patients with new treatment failure due to HIVDR.

Taking into consideration that only 17 of 93 (18.3%) participants in the CART-1 study resuppressed to <80 c/mL, and considering the high levels of drug resistance among those who did not resuppress, our results indicate that the vast majority of participants with initial viremia in CART-1 would have benefitted from a rapid switch to second-line ART, provided sufficient counseling was given around the time of the switch to promote high onward adherence. Of note, all participants with partial resuppression to 80–999 c/mL had extensive drug resistance, making it unlikely that they will achieve long-term suppression on first-line ART and calling into question the WHO cutoff of 1000 c/mL for viral suppression. It is also worth noting that in CART-1, among 110 patients with an initial ≥1000 c/mL, 17 never received a second VL: however, 5 were switched after their first VL, 3 died (AIDS-related), and 9 were lost to follow-up. These 2 latter groups may have fared better upon an immediate intervention. In settings with high loss to follow-up and where patients may struggle to pay transport fees to the healthcare facility, additional clinical visits and delays in switching to second-line ART may lead to increased attrition from care. In Lesotho, in routine clinical care, only 40% of patients with an unsuppressed VL receive a follow-up VL within 6 months, and only 25% of those receiving a follow-up VL either resuppress or are switched to second-line ART within 3 months [9]. Apart from leading to higher morbidity and mortality [10–14], such delays increase the time window in which HIV-1 drug resistance can emerge and onward transmission of resistant HIV-1 may take place.

CONCLUSIONS

In summary, we observed extremely high levels of HIVDR already before EAC, as well as a slight further increase in resistance mutations and a nonsignificant decrease in regimen-level susceptibility at follow-up. These results indicate that most participants would have benefitted from earlier detection of treatment failure followed by a rapid switch to second-line ART, and they suggest that the current treatment algorithm bears a certain risk of further accumulation of drug resistance, potentially jeopardizing future treatment options. However, these findings should be balanced against the benefit EAC has in achieving resuppression without regimen switch and thus avoiding unnecessary treatment changes.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. N. T. and R. D. K. conceptualized and designed this study. Samples were made available by N. D. L. and had been collected and initially processed by N. D. L., T. I. L., I. R., and M. C. H. A. M. developed the resistance testing methodology. J. A. B. performed all laboratory procedures in the present study. N. T. conducted all bioinformatics and statistical procedures. J. A. B. and N. T. analyzed the data, with key input from T. K., K. J. M., R. D. K., H. F. G., and N. D. L. T. K. and K. J. M. provided virological and diagnostic expertise. R. D. K. advised on statistical methodology. H. F. G. and N. D. L. provided clinical expertise. Conclusions were jointly developed among all coauthors. J. A. B. and N. T. wrote the first draft of the manuscript, and all coauthors approved the final version.

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5.1.6. Drug Resistance During Low-Level HIV Viraemia, J Antimicrob Chemother, 2021

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Extensive drug resistance during low-level HIV viraemia while taking NNRTI-based ART supports lowering the viral load threshold for regimen switch in resource-limited settings: a pre-planned analysis from the SESOTHO trial

Jennifer Anne Brown [1,2,3], Alain Amstutz [1,3,4], Bienvenu Lengo Nsakala[5], Ulrike Seeburg[2,3], Fiona Vanobberghen [1,3], Josephine Muhairwe [5], Thomas Klimkait [2,3] and Niklaus Daniel Labhardt [1,3,4]*

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland;* [2]*Molecular Virology, Department of Biomedicine, University of Basel, Basel, Switzerland;* [3]*University of Basel, Basel, Switzerland;* [4]*Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland;* [5]*SolidarMed, Partnerships for Health, Maseru, Lesotho*

*Corresponding author. E-mail: n.labhardt@swisstph.ch

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Objectives: WHO guidelines on ART define the HIV-1 viral load (VL) threshold for treatment failure at 1000 copies/mL. The *Switch Either near Suppression Or THOusand* (SESOTHO) trial, conducted in Lesotho from 2017 to 2020, found that patients with persistent viraemia below this threshold (100–999 copies/mL) benefit from switching to second-line ART. This pre-planned nested study assesses the prevalence of resistance-associated mutations (RAMs) in SESOTHO trial participants.

Methods: The SESOTHO trial [registered at ClinicalTrials.gov (NCT03088241)] enrolled 80 persons taking NNRTI-based first-line ART with low-level HIV-1 viraemia (100–999 copies/mL) and randomized them (1:1) to switch to a PI-based second-line regimen (switch) or continue on first-line therapy (control). We sequenced relevant regions of the viral *pol* gene using plasma samples obtained at enrolment and 36 weeks. RAMs were classified with the Stanford HIV Drug Resistance Database.

Results: Sequencing data were obtained for 37/80 (46%) participants at baseline and 26/48 (54%) participants without viral suppression to <50 copies/mL at 36 weeks (21 control participants and 5 switch participants). At baseline, 31/37 (84%) participants harboured high-level resistance to at least two drugs of their current regimen. At 36 weeks, 17/21 (81%) control participants harboured resistance to at least two drugs of their current regimen, while no PI-associated resistance was detected in the 5 switch participants with ongoing viraemia.

Conclusions: Among persons with low-level viraemia while taking NNRTI-based first-line ART enrolled in the SESOTHO trial, the majority harboured HIV-1 with RAMs that necessitate ART modification. These findings support lowering the VL threshold triggering a switch to second-line ART in future WHO guidelines.

Introduction

Persistent HIV viraemia below 1000 copies/mL is associated with adverse health outcomes compared with complete viral suppression, including a higher risk of virological treatment failure[1–3] and AIDS-defining events or mortality.[3] However, low-level viraemia (defined here as 100–999 copies/mL) does not trigger clinical action according to current WHO guidelines.[4,5]

WHO guidelines, that inform clinical management in most resource-limited settings, recommend that two consecutive viral load (VL) results ≥1000 copies/mL, with adherence support between the measurements and reported good adherence, should trigger a switch from first-line ART.[4] The recently published *Switch Either near Suppression Or THOusand* (SESOTHO) randomized controlled trial challenged this threshold by showing that lowering the threshold for switching to 100 copies/mL led to a higher proportion of participants with low-level viraemia achieving viral suppression (<50 copies/mL) at 36 weeks.[6] From a clinical perspective, these results support a lower threshold for defining virological failure and switching to second-line ART in future WHO guidelines.

However, questions remain regarding the underlying causes of low-level viraemia; notably, whether viraemia is driven by non-adherence or viral drug resistance. By sequencing baseline and 36 week samples from the SESOTHO trial, we aimed to understand the role of resistance-associated mutations (RAMs) in low-level viraemia.

Methods

We retrieved plasma samples from the SESOTHO trial, registered at ClinicalTrials.gov (NCT03088241).[6,7] This parallel-group, open-label randomized controlled trial enrolled 80 HIV-positive people receiving first-line ART containing an NNRTI (efavirenz or nevirapine) who had two consecutive VLs ≥100 copies/mL, with the second VL between 100 and 999 copies/mL. Participants were randomized (1:1) to switch to a second-line ART regimen based on WHO[4,5] and national[8,9] guidelines under consideration of national availability of antiretroviral drugs, i.e. switched to ritonavir-boosted lopinavir-based second-line ART (switch) or remained on first-line ART as per guidelines (control). Participants were followed-up for 36 weeks. The primary endpoint, a documented VL <50 copies/mL at 36 weeks, was achieved by 22/40 (55%) participants in the switch arm, but only 10/40 (25%) in the control arm. Consenting procedures have been described[6] and included consent for resistance testing. Participants were enrolled at eight healthcare facilities in Lesotho from August 2017 to August 2019 and the trial was completed in May 2020.

Plasma samples taken at baseline and 36 weeks were stored at −80°C at Butha-Buthe Government Hospital, Lesotho, and after trial completion shipped to the University of Basel, Switzerland, for analysis. For resistance testing, up to 2 mL of plasma was centrifuged at 20 800 **g** for ≥30 min. A portion of the supernatant was removed, leaving the lower 300 µL with concentrated virus for RNA extraction using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Promega, Madison, WI, USA). Two methods were used for reverse transcription and amplification: either an RT–PCR and subsequent nested PCR covering the relevant protease and reverse transcriptase region according to an in-house protocol, or a cDNA conversion followed by semi-nested PCRs of two fragments jointly covering codons 1–99 of the protease and 1–321 of the reverse transcriptase, adapted from Mbunkah *et al.*[10] Either of the protocols was used in a first attempt; if no PCR product was obtained, reverse transcription and amplification were repeated at least once using the latter method. Amplification success was verified by gel electrophoresis and PCR products were sent to SEQ-IT GmbH, Kaiserslautern, Germany, for sequencing using the MiSeq platform (Illumina Inc., San Diego, CA, USA). Variants occurring with a nucleotide frequency ≥10% were considered during analysis. Sequences were uploaded to the Stanford HIV Drug Resistance Database (v8.9-1) to assess the presence of RAMs and levels of resistance to antiretroviral drugs in the participants' regimens. All further analyses were performed using Stata v16.0. Resistance data are reported separately for enrolment and 36 weeks, followed by an assessment of the combined data among those with resistance information at either or both timepoints.

Results

Resistance data at enrolment

Samples from the enrolment visit were available for 74/80 (93%; 37/40 per arm) participants and resistance testing was successful for 37/74 (50%) samples. The median last VL result before enrolment was 425 copies/mL (IQR = 200–723; range = 109–950) in samples that could be sequenced and 300 copies/mL (IQR = 143–424; range = 109–914) in samples that could not be sequenced (Wilcoxon rank-sum test: $P = 0.08$).

Among participants with resistance data at enrolment, 6/37 (16%) had no detectable RAMs and the remaining 31/37 (84%) had high-level resistance to at least two drugs in their current regimen, including the NNRTI [Table 1 and Table S1 (available as Supplementary data at *JAC* Online)].

Resistance data at 36 weeks

Among participants with a documented VL ≥50 copies/mL at 36 weeks, 35/43 (81%) samples were available and resistance testing was successful for 26/35 (74%); 21/26 (81%) in the control arm and 5/9 (56%) in the switch arm (Table 1). The median VL was 612 copies/mL (IQR = 162–1170; range = 58–9560) for the 26 samples that could be sequenced and 165 copies/mL (IQR = 94–293; range = 92–2820) for the nine samples where sequencing failed (Wilcoxon rank-sum test: $P < 0.0001$).

Among those with resistance data in the control arm, all but two (19/21; 90%) participants had mutations associated with NNRTI resistance and thus qualified for switch to second-line ART. Most of these participants (15/19; 79%) still had viraemia <1000 copies/mL. Eighteen of the 21 (86%) participants reported never having missed a dose within the last 4 weeks.

In the switch arm, none had PI-associated resistance mutations and 4/5 (80%) had three fully active drugs in their regimen. See Table 1 and Table S1.

Combined resistance data at enrolment and 36 weeks

Considering both timepoints, 49/80 (61%) participants had at least one resistance result; 42/49 (86%) had detectable resistance mutations associated with NNRTI resistance and 41/49 (84%) with NRTI resistance (Table S1).

Figure 1 shows the 36 week virological and resistance outcomes of participants with resistance data at baseline. Among those in the control arm with a resistance result at baseline, only 2/16 (13%) achieved viral suppression to <50 copies/mL at 36 weeks while remaining on first-line ART; one was susceptible to all drugs at baseline and the other achieved viral suppression (to <20 copies/mL) despite having intermediate resistance to one NRTI and high-level resistance to the other two drugs (K65R, L100I, Y115F and Y188L). After completion of the trial, this latter participant's next routine VL was again in the range of low-level viraemia.

For 14 participants (10 control participants and 4 switch participants) with ongoing viraemia at 36 weeks, both baseline and 36 week resistance data were available. In the control arm, no major changes were observed, except for one participant with three fully active drugs at enrolment and none at 36 weeks. Among four participants in the switch arm, one had no RAMs at either timepoint. The other three harboured NRTI and NNRTI resistance at enrolment and, while NNRTI resistance was still detected in all three at 36 weeks, NRTI resistance was only detected for one. None of these four participants had any PI-associated resistance mutations.

Discussion

The threshold for virological failure of 1000 copies/mL in current WHO guidelines has been subject to debate.[11,12] The recently published SESOTHO trial challenges this threshold by showing that patients with sustained viraemia between 100 and 999 copies/mL while taking NNRTI-based first-line ART benefit from switching to second-line ART.[6] As low-level viraemia may be caused by viral resistance, we investigated the role of drug resistance in this trial.

We found high levels of drug resistance, with NNRTI resistance detected in the majority of participants at one or both timepoints.

Table 1. Participant characteristics and results of genotypic resistance testing at baseline and 36 weeks

	Control arm (*N* = 40)	Switch arm (*N* = 40)	Total (*N* = 80)
HIV sequences available at baseline, 36 weeks or both	*N* = 27	*N* = 22	*N* = 49
female	18 (67%)	16 (73%)	34 (69%)
age at enrolment (years), median (IQR) [range]	41 (34–51) [5–75]	40 (29–50) [13–73]	41 (32–50) [5–75]
child (<16 years)	3 (11%)	1 (5%)	4 (8%)
time since ART initiation at enrolment (years), median (IQR) [range]	7.1 (4.0–9.3) [1.4–12.1]	7.4 (4.8–8.9) [3.3–13.2]	7.3 (4.4–8.9) [1.4–13.2]
Enrolment			
available HIV sequences at enrolment	*N* = 16	*N* = 21	*N* = 37
core agent			
EFV	14/16 (88%)	18/21 (86%)	32/37 (86%)
NVP	2/16 (13%)	3/21 (14%)	5/37 (14%)
NRTI backbone			
TDF/3TC	13/16 (81%)	15/21 (71%)	28/37 (76%)
ZDV/3TC	2/16 (13%)	3/21 (14%)	5/37 (14%)
ABC/3TC	1/16 (6%)	3/21 (14%)	4/37 (11%)
last VL before enrolment (copies/mL), median (IQR) [range]	399 (215–761) [167–897]	486 (122–723) [109–950]	425 (200–723) [109–950]
three ARVs predicted fully active	4/16 (25%)	2/21 (10%)	6/37 (16%)
one ARV predicted fully active	1/16 (6%)	3/21 (14%)	4/37 (11%)
one ARV predicted partially active	9/16 (56%)	10/21 (48%)	19/37 (51%)
no active ARVs	2/16 (13%)	6/21 (29%)	8/37 (22%)
36 weeks			
available HIV sequences at 36 weeks	*N* = 21	*N* = 5	*N* = 26
core agent			
EFV	14/21 (67%)	0	14/26 (54%)
NVP	7/21 (33%)	0	7/26 (27%)
LPV/r	0	5/5 (100%)	5/26 (19%)
NRTI backbone			
TDF/3TC	15/21 (71%)	1/5 (20%)	16/26 (62%)
ZDV/3TC	5/21 (24%)	4/5 (80%)	9/26 (35%)
ABC/3TC	1/21 (5%)	0	1/26 (4%)
self-reported number of ART doses missed in last 4 weeks[a]			
none	18/21 (86%)	2/4 (50%)	20/25 (80%)
at least one	3/21 (14%)	2/4 (50%)	5/25 (20%)
VL (copies/mL), median (IQR) [range]	568 (269–986) [58–9560]	1170 (162–3330) [138–7040]	612 (162–1170) [58–9560]
three ARVs predicted fully active	2/21 (10%)	4/5 (80%)	6/26 (23%)
two ARVs predicted fully and one predicted partially active[b]	0	1/5 (20%)	1/26 (4%)
two ARVs predicted fully active[c]	2/21 (10%)	0	2/26 (8%)
one ARV predicted fully active	2/21 (10%)	0	2/26 (8%)
one ARV predicted partially active	11/21 (52%)	0	11/26 (42%)
no active ARVs	4/21 (19%)	0	4/26 (15%)

EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; ZDV, zidovudine; ABC, abacavir; LPV/r, lopinavir/ritonavir; ARV, antiretroviral.
[a]Missing for one participant in the switch arm.
[b]Switch arm participant with incomplete sequencing of the reverse transcriptase region (only codons 1–154 sequenced).
[c]Including one control arm participant with incomplete sequencing of the reverse transcriptase region (only codons 1–155 sequenced).

In line with previous data from this setting,[13] most participants required a switch to second-line ART. At enrolment, the high prevalence of the M184I mutation and/or M184V mutation indicates likely recent drug exposure in these participants, since M184I/V decreases viral fitness and can become undetectable in the absence of antiretroviral pressure.[14] Conversely, the lack of RAMs

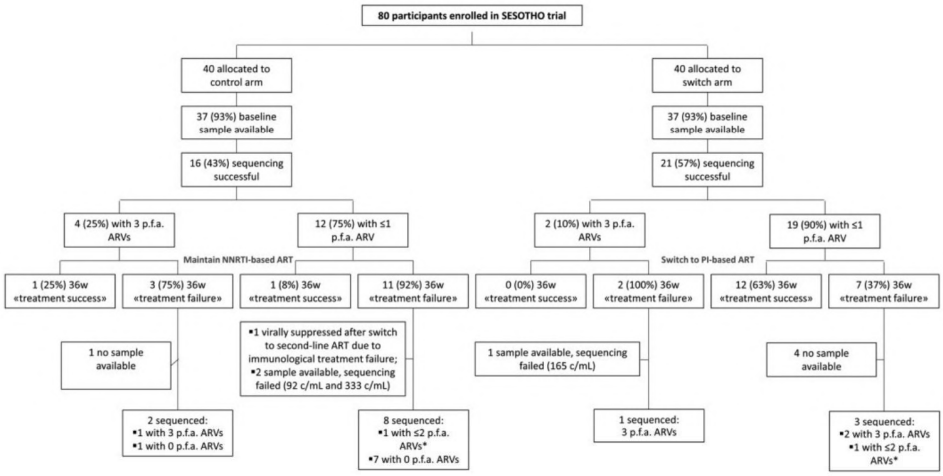

Figure 1. Baseline resistance, 36 week viral load and 36 week resistance among the 37 participants with resistance data available at baseline. 36w, 36 week; ARV, antiretroviral; c/mL, copies/mL; p.f.a., predicted fully active. *Incomplete sequencing of the reverse transcriptase region (only codons 1–154 or 1–155 sequenced).

associated with PI resistance among those with ongoing viraemia after switching to PI-based ART suggests that non-adherence was the major driver of viraemia at 36 weeks in the switch arm, though the sample size was small. This is in line with our expectations; in the EARNEST trial, even after 144 weeks, only 2% of participants taking a regimen consisting of ritonavir-boosted lopinavir plus NRTIs developed PI resistance.[15]

Overall, these findings endorse lowering the VL threshold below 1000 copies/mL. US, European and South African guidelines set the threshold for treatment failure at 50 or 200 copies/mL.[16–18] Our data indicate that, even below 200 copies/mL, low-level viraemia may be associated with drug resistance. Among the nine participants with a VL between 100 and 199 copies/mL before enrolment and with successful sequencing, seven harboured HIV with NRTI and NNRTI resistance at enrolment; among the seven follow-up samples with a VL between 50 and 199 copies/mL and successful sequencing, six harboured treatment-relevant RAMs (Table S1). Though too small to be conclusive, these numbers suggest that sustained low-level viraemia, irrespective of its level, may indicate resistant HIV strains.

This study has several limitations. First, resistance data were only available for 50% of baseline and 74% of 36 week samples. A limited success rate of resistance testing was expected considering the low viral concentration. Nevertheless, we cannot exclude a bias towards overestimating the prevalence of RAMs at baseline if only the sequenced samples are considered, as higher baseline VLs likely corresponded both with higher sequencing success and higher prevalence of RAMs. Second, all participants were on NNRTI-based first-line ART at enrolment—a type of regimen that is gradually being phased out with the roll-out of the integrase inhibitor dolutegravir. When used in combination therapy, resistance to dolutegravir is extremely rare.[19] Nevertheless, according to a recent estimate, NNRTIs were still used in 47% of adult first-line ART regimens in 2020 and will remain in use for 1.7 million adults, representing 7% of adult first-line regimens, in 2024.[20]

In summary, in this pre-planned sub-study of the SESOTHO trial that enrolled patients taking NNRTI-based ART with low-level viraemia, the majority of participants showed high levels of drug resistance to their first-line regimen. Combined with the improved viral suppression upon switching to second-line ART reported in the SESOTHO trial,[6] these data further support lowering the VL threshold for treatment switch in upcoming WHO guidelines.

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Transparency declarations

T.K. reports advisory board membership fees from ViiV and Gilead for work outside of this study. N.D.L. reports having received travel grants to attend IAS, AIDS and CROI conferences from Gilead Sciences Sarl. All other authors: none to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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STUDY PROTOCOL Open Access

Genotype-Informed Versus Empiric Management Of VirEmia (GIVE MOVE): study protocol of an open-label randomised clinical trial in children and adolescents living with HIV in Lesotho and Tanzania

Jennifer Anne Brown[1,2,3], Isaac Ringera[4], Ezekiel Luoga[5], Molisana Cheleboi[6], Namvua Kimera[5], Josephine Muhairwe[4], Buntshi Paulin Kayembe[7], Mosa Molapo Hlasoa[7], Lorraine Kabundi[7], Ching Wey David Yav[7], Buoang Mothobi[4], Lineo Thahane[7,8], Alain Amstutz[1,3,9], Nadine Bachmann[1,3], Getrud Joseph Mollel[5], Moniek Bresser[1,3], Tracy Renée Glass[1,3], Daniel Henry Paris[1,3], Thomas Klimkait[2,3], Maja Weisser[1,3,5,9] and Niklaus Daniel Labhardt[1,3,9*]

Abstract

Background: Globally, the majority of people living with HIV have no or only limited access to HIV drug resistance testing to guide the selection of antiretroviral drugs. This is of particular concern for children and adolescents, who experience high rates of treatment failure. The GIVE MOVE trial assesses the clinical impact and cost-effectiveness of routinely providing genotypic resistance testing (GRT) to children and adolescents living with HIV who have an unsuppressed viral load (VL) while taking antiretroviral therapy (ART).

Methods: GIVE MOVE is an open-label randomised clinical trial enrolling children and adolescents (≥6 months to <19 years) living with HIV with a VL ≥400 copies/mL (c/mL) while taking first-line ART. Recruitment takes place at sites in Lesotho and Tanzania. Participants are randomised in a 1:1 allocation to a control arm receiving the standard of care (3 sessions of enhanced adherence counselling, a follow-up VL test, continuation of the same regimen upon viral resuppression or empiric selection of a new regimen upon sustained elevated viremia) and an intervention arm (GRT to inform onward treatment). The composite primary endpoint is the occurrence of any one or more of the following events during the 36 weeks of follow-up period: i) death due to any cause; ii) HIV- or ART-related hospital admission of ≥24 h duration; iii) new clinical World Health Organisation stage 4 event (excluding
(Continued on next page)

* Correspondence: n.labhardt@swisstph.ch
[1]Swiss Tropical and Public Health Institute, Basel, Switzerland
[3]University of Basel. Basel, Switzerland
Full list of author information is available at the end of the article

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lymph node tuberculosis, stunting, oral or genital herpes simplex infection and oesophageal candidiasis); and iv) no documented VL <50 c/mL at 36 weeks follow-up. Secondary and exploratory endpoints assess additional health-related outcomes, and a nested study will assess the cost-effectiveness of the intervention. Enrolment of a total of 276 participants is planned, with an interim analysis scheduled after the first 138 participants have completed follow-up.

Discussion: This randomised clinical trial will assess if the availability of resistance testing improves clinical outcomes in children and adolescents with elevated viremia while taking ART.

Trial registration: This trial is registered with ClinicalTrials.gov (NCT04233242; registered 18.01.2020). More information: www.givemove.org.

Keywords: HIV, Genotypic resistance testing, Drug resistance, Randomised clinical trial, Antiretroviral therapy, Treatment failure, Children, Adolescents, Sub-Saharan Africa

Background

Almost three million children and adolescents worldwide are living with HIV [1]. Every day, almost 1000 children and adolescents are newly infected and over 300 die from HIV/AIDS-related causes [1]. Eastern and Southern Africa are particularly affected, accounting for 65% of the epidemic in children and adolescents [1]. While substantial progress has been made towards providing antiretroviral therapy (ART) to all people living with HIV, which can suppress viral replication and prevent onward transmission of HIV [2–4], children and adolescents suffer high rates of treatment failure: among those younger than 15 years who receive ART, reported rates of treatment failure in Eastern and Southern Africa range from 10% (Eswatini) to over 50% (Eritrea, Mozambique, South Sudan) [5].

Treatment failure can be caused by non-adherence to therapy, viral drug resistance, or a combination of both, requiring differentiated clinical management. Without resistance testing, healthcare providers cannot definitively determine whether treatment failure is caused by drug resistance, necessitating an urgent switch of drug regimen, or non-adherence, in which case underlying causes must be addressed and unnecessary switching must be avoided to preserve the limited future treatment options.

Access to genotypic resistance testing (GRT) to detect viral drug resistance is lacking in most low-income settings [6]. As national HIV programs in sub-Sahara Africa struggle with limited resources, the question if resistance testing is of real clinical benefit or rather a "nice to have" is important as it impacts resource allocation within programs. The World Health Organisation (WHO) recommends resistance testing only upon confirmed treatment failure on second-line ART and/or protease-inhibitor-based ART, and even then only after a lengthy process of enhanced adherence counselling followed by a confirmatory viral load (VL) test [7].

A recent systematic review on the impact of genotypic and/or phenotypic resistance testing in ART-experienced individuals only found randomised clinical trials published before 2007, all conducted in Europe, the USA, or South America, only two of which included children and/or adolescents [8]. This review reported a potential slight reduction of virologic failure where resistance testing was available, but little or no difference in mortality, CD4 cell count, progression to AIDS, or adverse events. Among three modelling studies on the cost-effectiveness of GRT in southern Africa, published between 2011 and 2014, conclusions differed greatly [9–11].

Three ongoing randomised clinical trials (in addition to the trial presented here) will assess the usefulness of resistance testing in sub-Saharan Africa: the REVAMP study, conducted in South Africa and Uganda, is assessing the feasibility, effectiveness, and cost-effectiveness of GRT upon detection of viremia in adults taking non-nucleoside reverse transcriptase inhibitor- (NNRTI-)based first-line ART [12]. A trial in Tanzania, including all age groups, implements GRT upon confirmation of treatment failure after enhanced adherence counselling [13]. Finally, the Opt4Kids trial assesses the impact of a combination of point-of-care VL testing and targeted resistance testing among children on first-line ART in Kenya [14].

We report here the protocol of the trial: *Genotype-Informed* Versus *Empiric Management Of VirEmia (GIVE MOVE) in HIV-Infected Children and Adolescents on Antiretroviral Therapy: An Open-Label Randomised Clinical Trial*. GIVE MOVE is among the first randomised clinical trials assessing the clinical impact of providing GRT to children and adolescents with viremia while on first-line ART, with key differences in study design compared to the above-mentioned ongoing trials.

Methods

Aim

The GIVE MOVE trial assesses whether timely provision of GRT upon detection of viremia improves health outcomes for children and adolescents on first-line ART

when compared to the current standard of care. In the case of an observed clinical benefit, the cost-effectiveness of this intervention will be assessed. Combined, these results will provide evidence on whether the availability of GRT should be prioritised for children and adolescents living with HIV in resource-limited settings.

Design and study setting

GIVE MOVE is a multi-centre, parallel-group (1,1 allocation), open-label, superiority randomised clinical trial conducted in Lesotho and Tanzania. These two countries are home to 21,000 and 150,000 children and adolescents living with HIV [1], respectively, and have a reported adult HIV prevalence of 22.8% in Lesotho and 4.8% in Tanzania [5].

Enrolment will take place at four sites. In Lesotho, these are the Satellite Centres of Excellence of the Baylor College of Medicine Children's Foundation Lesotho ('Baylor Clinics') located in Hlotse, Butha-Buthe, and Mokhotlong. In Tanzania, the study is conducted at the One-Stop Clinic of the Chronic Diseases Clinic Ifakara at Saint Francis Referral Hospital in Ifakara, Kilombero District. In both countries, additional sites have been identified for potential inclusion at a later stage.

GRT takes place at the laboratory of Seboche Mission Hospital in Butha-Buthe district, Lesotho and at the laboratory of the Ifakara Health Institute in Ifakara, Tanzania. All other laboratory diagnostics are conducted at laboratories associated with the respective sites.

Participants

Potential participants are identified through pre-screening of routine medical records.

Inclusion criteria are: being in care in a study site; age ≥6 months and <19 years; latest HIV VL result ≥400 copies/mL (c/mL); being on a first-line ART regimen (defined as never having had a regimen change due to virologic failure); having been on an unchanged ART regimen for ≥6 months; phlebotomy for the latest VL test done <3 months before screening; and written informed consent.

Exclusion criteria are: an indication for treatment switch according to WHO guidelines at screening; initiation of the first session of enhanced adherence counselling >2 weeks prior to screening; an intention to transfer out of the study site (and not into a different study site) within 3 months after randomisation; already being enrolled in another study if judged as non-compatible by the (Local) Principal Investigator; being pregnant or breastfeeding at screening (no exclusion based on pregnancy or breastfeeding after enrolment); acute illness requiring hospitalisation at screening (no exclusion based on hospitalisation after enrolment); and having received an HIV resistance test in the last 12 months.

Consent procedures, screening, and randomisation

Consent is provided by the participant if aged ≥16 years (Lesotho) or ≥18 years (Tanzania), and by the caregiver for younger participants. Minors aged ≥6 years additionally receive age-appropriate study information and provide informed assent. Written informed consent and, where applicable, written informed assent is a prerequisite for participation in this study. Details on consenting procedures are listed in the declarations below. Formal screening, including a non-routine pregnancy test for female adolescents aged ≥12 years, takes place only after consent (and assent, if applicable) has been obtained.

Eligible and consenting individuals are enrolled and randomised in a 1:1 ratio to the intervention and control arms. Randomisation is stratified by country (Lesotho or Tanzania), age at enrolment ([≥6 months to <12 years] or [≥12 years to <19 years]), and type of ART regimen at enrolment (NNRTI-, protease inhibitor- (PI-), or integrase strand transfer inhibitor- (INSTI-)based regimen), using permuted blocks with varying block size. Randomisation is automated using the electronic data capture software MACRO version 4.8.1 (Elsevier) once eligibility and consent have been confirmed and entered into the database, thereby maintaining concealment of allocation.

Control and intervention arm

The control arm is largely based on the WHO and national guidelines [7, 15, 16]: Participants in the control arm receive (at least) three sessions of adherence counselling followed by a second VL test. Onward treatment is determined by the outcome of this second VL test: sustained viremia ≥400 c/mL triggers a switch to a second-line ART regimen selected based on empiric criteria according to national guidelines [15, 16], whereas viral resuppression to <400 c/mL results in continuation of the current regimen.

This cut-off of 400 c/mL for viral suppression was selected based on the growing body of evidence that the cut-off of 1000 c/mL currently recommended by the WHO [7] and the national guidelines of the project countries [15, 16] may be too high [17–21]. While guidelines suggest the confirmatory VL test should be delayed and adherence counselling should continue in the case of 'ongoing poor adherence', the GIVE MOVE protocol allows for this only upon evidence of non-adherence defined as i) a pill count <90%, or ii) a self-reported period of no drug intake of ≥2 days during the past 4 weeks.

Participants in the intervention arm receive an intervention package consisting of: i) GRT by Sanger sequencing completed by an in-country laboratory (target

turn-around time: 2 weeks); ii) review of the GRT result by at least three members of a GRT Expert Committee, providing a recommendation for onward treatment (target turn-around time: 1 week); iii) GRT-informed choice of onward therapy; and iv) GRT-informed further adherence counselling.

The main study visits for each arm are shown in Fig. 1. Additionally, a '6 months post decision visit' takes place 24 weeks (range: 20—28 weeks) after the decision on onward therapy, i.e. after the visit in which the follow-up VL result (control arm) or the GRT result (intervention arm) become available. Depending on the timing, this '6 months post decision visit' is either combined with another study visit or conducted separately. Any additional visits and laboratory tests taking place according to the standard of care or clinical necessity (including but not limited to: more frequent clinical visits upon pregnancy; check-up visits after modifications to the ART regimen; clinical indication) are recorded. Missing participants will be traced, contacted and encouraged to return back to care. The study procedures at each study visit are shown in the SPIRIT diagram in Fig. 2.

Endpoints

The composite primary endpoint is the occurrence of any one or more of the events i) death due to any cause during the follow-up period (36 weeks), ii) HIV- or ART-related hospital admission of ≥24 h duration (possibly, probably or definitely related to HIV or ART, judged by the endpoint committee blinded to the study arm) during follow-up, iii) new clinical WHO stage IV event (excluding lymph node tuberculosis, stunting, oral or genital herpes simplex infection and oesophageal candidiasis; judged by the endpoint committee blinded to the study arm) during follow-up, and iv) no documentation of a suppressed VL (<50 c/mL) at 36 weeks follow-up (window: 32–44 weeks).

The secondary and exploratory endpoints are listed in Table 1.

The first four secondary endpoints are the individual components of the composite primary endpoint.

Sample size

We hypothesise that 35% of participants in the control arm will reach the primary endpoint. With $\alpha = 0.05$ and 80% power, a sample size of 276 participants is needed to detect a clinically relevant reduction in the primary endpoint of 15% in the intervention arm.

Planned analyses

Analysis and reporting will follow CONSORT guidelines [22] and intention-to-treat (ITT) principles including all participants as randomised. In addition, a per-protocol analysis of the primary endpoint will include all randomised participants who completed the study without a protocol violation. A flowchart will describe the inclusion and follow-up of participants by study arm. Baseline characteristics will be described by study arm with summary statistics such as median and interquartile range or number and percentage; no formal testing between arms will be performed [23]. The primary endpoint as well as categorical secondary and exploratory endpoints will be assessed using a logistic regression model, reporting odds ratios and risk differences with standard errors estimated using the delta method [24]. The exploratory endpoint of time until documented viral suppression will be assessed with Cox proportional hazard models, reporting hazard ratios. All estimates will be reported with 95% confidence intervals. All models will be adjusted for the stratification factors of country, age, and ART regimen at enrolment. Subgroup analyses are planned by country (Lesotho or Tanzania), sex (female or male), age ([≥6 months to <12 years] or [≥12 years to <19 years]), and ART regimen at enrolment (NNRTI-, PI-, or INSTI-based regimen).

Interim analysis

An interim analysis for efficacy and inefficacy is planned once 138 participants (50% of the target number of participants) have completed the 9-month study visit and/or reached the primary endpoint. The trial may be concluded early for success if a significant difference between the study arms is achieved for the composite primary endpoint. We will use the conservative Haybittle-Peto stopping level of $p = 0.001$ [25]. The trial may be stopped for inefficacy if the odds ratio is greater than 1 and the two-sided 95% confidence interval does not contain the alternative hypothesis (i.e., odds ratio of 0.57, [26]). More details are provided in the statistical analysis plan.

The interim analysis will be conducted by an independent statistician. The results will be reviewed by a Data Safety Monitoring Board, who will issue a recommendation to continue or stop the trial to the Steering Committee. For this recommendation, additional information (i.e. new emerging evidence) may also be taken into account. The Steering Committee will vote on and thereby determine the continuation or termination of the trial. In the event of a tie, the Sponsor/Chief Investigator will cast the deciding vote. If the decision is taken to stop the trial, recruitment will be suspended but participants who are already enrolled will continue to be followed for the primary and secondary outcomes.

Data collection and management

Data is captured online in electronic case report forms in the password-protected MACRO database, which generates an audit trail. On the electronic report forms,

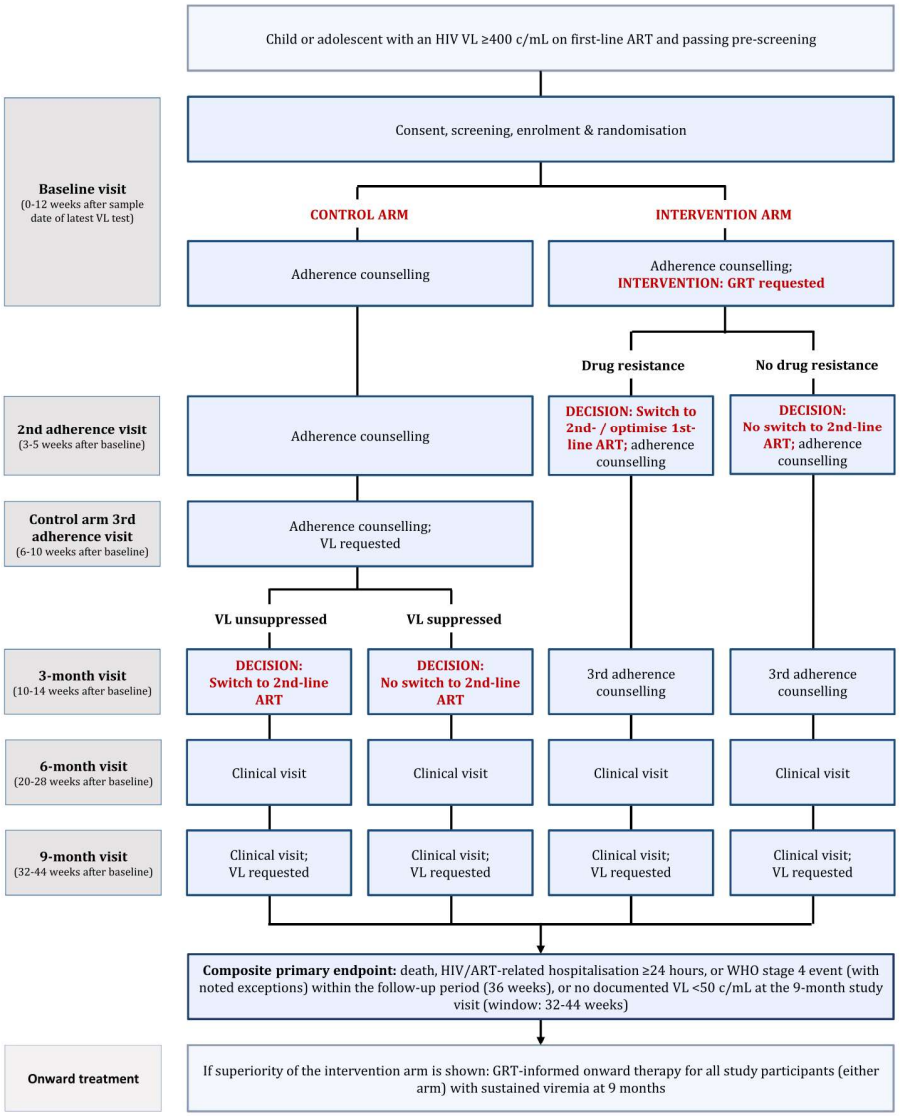

Fig. 1 Flow chart of GIVE MOVE treatment algorithm and study visits. Detailed procedures at each study visit are listed in Fig. 2. GRT: genotypic resistance testing; VL: viral load

Study visit	Enrolment	Post-allocation					Close-out
	Baseline	2nd adherence visit	Control arm 3rd adherence visit	3-month	6-month	6 months post-decision[1]	9-month
Timepoint	0	4 weeks (range: 3-5 weeks)	8 weeks (range: 6-10 weeks)	12 weeks (range: 10-14 weeks)	24 weeks (range: 20-28 weeks)	24 weeks after decision[1] (range: 20-28 weeks)	36 weeks (range: 32-44 weeks)
Enrolment:							
Informed consent / assent	X						
Screening and enrolment	X						
Randomisation	X						
Interventions:							
Intervention arm:							
Phlebotomy for GRT	Intervention arm						
GRT-informed onward therapy		Intervention arm					
Control arm:							
Phlebotomy for follow-up VL[2]			Control arm				
VL-informed, empirically selected onward therapy[2]				Control arm			
Both arms:							
Decision on onward therapy		Intervention arm		Control arm[2]			
Adherence counselling	X	X[3]	Control arm	Intervention arm[3]			
Additional visits and tests as required in standard care							
Assessments:							
Sociodemographic data	X						
Medical history[4]	X						
Growth and nutrition indicators[5]	X						X
Clinical information[6]	X	X	Control arm	X	X	X	X
ART and co-medication	X	X	Control arm	X	X	X	X
Adherence	X	X	Control arm	X	X	X	X
Pregnancy[7]	X						X
CD4 cell count	X						X
Full blood count	X						
Hepatitis B surface antigen	X						
Serum creatinine	X						
Viral load							X
Sample storage for post-hoc analyses	X			X	X	X	X

Fig. 2 SPIRIT diagram of study procedures. Footnotes: [1] Conducted 24 weeks (range: 20–28 weeks) after the visit in which the follow-up viral load result (control arm) or the GRT result (intervention arm) is first available; may coincide with another study visit. [2] May be delayed upon evidence of poor adherence, defined as a pill count of <90% and/or a self-reported period of no drug intake of ≥2 days in the last 4 weeks. [3] In intervention arm: informed by GRT result. [4] Clinical information at ART initiation; previous ART regimens; exposure to prevention to mother-to-child transmission strategies. [5] Height, weight, middle upper arm circumference (if aged <5 years), nutritional status. [6] WHO stage; co-morbidities; symptoms and side-effects; new hospitalisation. [7] Female participants aged ≥12 years. GRT: genotypic resistance testing; VL: viral load

participants are identified by a unique identifier and no participant names are stored in the database. A paper-based participant identification list, the Informed Consent/Assent Forms, and paper-based source documents are kept under lock and key at each study site. All study data and documentation will be archived for at least 10 years after completion of the study.

Plasma collection and storage

Participants will undergo phlebotomy at enrolment and 2 (control arm only), 3, 6 and 9 months after enrolment (see Spirit diagram in Fig. 2). Study-related phlebotomy will be limited to age-appropriate volumes per blood draw, defined as ≤5 mL for participants <5 years; ≤10 mL for participants ≥5 and <10 years; ≤15 mL for

Table 1 Secondary and exploratory endpoints

Endpoint	Definition	Timeframe
Secondary endpoints		
Death due to any cause	Proportion of participants confirmed dead during the follow-period among all participants enrolled	Within 36 weeks after enrolment
HIV- or ART-related hospitalisation of ≥24 h duration	Proportion of participants with HIV- or ART-related hospital admission(s) of ≥24 h duration (possibly, probably or definitely related to HIV or ART, judged by the endpoint committee blinded to the study arm) during the follow-up period among all participants enrolled	Within 36 weeks after enrolment
New clinical WHO stage 4 event(s)	Proportion of participants with new clinical WHO stage 4 event(s) (excluding lymph node tuberculosis, stunting, oral or genital herpes simplex infection and oesophageal candidiasis, judged by the endpoint committee blinded to the study arm) among all participants enrolled	Within 36 weeks after enrolment
Without documentation of a suppressed VL	Proportion of participants without documentation of a VL <50 c/mL at 9 months among all participants enrolled	32—44 weeks after enrolment
Loss to follow-up	Proportion of participants with no documented clinic visit at 9 months among all participants enrolled	32—44 weeks after enrolment
Observed virologic failure	Proportion of participants with a VL ≥50 c/mL among all participants with a VL result at 9 months	32—44 weeks after enrolment
Composite endpoint at 6 months after the decision on onward treatment	Proportion of participants among all participants enrolled experiencing any one or more of the events i) death due to any cause within 24 weeks of the decision on onward treatment, ii) HIV- or ART-related hospital admission of ≥24 h duration (possibly, probably or definitely related to HIV or ART, judged by the endpoint committee blinded to the study arm) within 24 weeks of the decision on onward treatment, iii) new clinical WHO stage IV event (excluding lymph node tuberculosis, stunting, oral or genital herpes simplex infection and oesophageal candidiasis; judged by the endpoint committee blinded to the study arm) within 24 weeks of the decision on onward treatment, and iv) no documentation of a suppressed VL (<50 c/mL) at 6 months (20–28 weeks) after the choice of onward treatment. The time point of the decision on onward treatment is defined as the first visit in which the follow-up VL result (control arm) or the GRT result (intervention arm) is available.	i-iii): within 24 weeks after the decision on onward therapy; iv): 20—28 weeks after the decision on onward therapy
Exploratory endpoints		
Time to documented viral suppression	Time to achieving a VL <50 c/mL; considering VL testing done with samples from the 3-, 6- and 9-month study visits in both arms	Assessed at 3- (10—14 weeks after enrolment), 6- (20—28 weeks after enrolment), and 9-month study visit (32—44 weeks after enrolment)
Drug regimen switches in the absence of major resistance-associated mutations and/or non-switches in the presence of major resistance-associated mutations	Proportion of participants with ART regimen switches in the absence of major resistance-associated mutations and/or non-switches in the presence of major resistance-associated mutations among all participants enrolled (as identified by Sanger sequencing, according to the Stanford HIV drug resistance database).	Assessed at enrolment and at 9-month study visit (32—44 weeks after enrolment)
Proportion with new resistance-associated mutations emerged within the study period	Proportion of participants with new resistance-associated mutations emerged within the study period among all participants enrolled	Change from enrolment to 9-month study visit (32—44 weeks after enrolment)

participants ≥10 and <15 years; and ≤25 mL for participants ≥15 years [27]. The sites receive training and written guidance on the safe blood volumes in paediatric patients and the prioritisation of laboratory procedures in the event that insufficient blood is available to perform all tests as per protocol.

Biological material is identified by the participant's study ID and processed or stored at −80 °C at the laboratory site in charge. Consent is collected for further use of samples in future studies, subject to approval from the relevant ethics committee(s).

Monitoring

In Lesotho, GIVE MOVE is monitored by the Monitoring Group of the Clinical Operations Unit at the Swiss Tropical and Public Health Institute, as well as monitors from SolidarMed Lesotho who are supervised by this group. In Tanzania, the trial is monitored by the Ifakara Health Institute. The first two participants per site and approximately 10% of the total number of participants will undergo 100% source data verification. The remaining participants will undergo source data verification of all key data as defined in the monitoring plan. For each study site a site initiation visit, regular routine monitoring visits and a close-out visit are planned.

In addition, the Baylor Clinics are audited on a half-yearly basis by the Baylor College of Medicine Children's Foundation Lesotho.

Ethical considerations

This study has been approved by the relevant ethics committees and, if applicable, other authorities in the project countries. In addition, a Swiss ethics committee provided a statement confirming the trial meet ethical requirements. Details are listed in the declarations below.

All participants/caregivers are informed that participation is voluntary and that they may withdraw from the study at any time. Participants do not receive any form of remuneration, though transport costs to the study site are compensated for participants and up to one caregiver.

Pregnancy (assessed by a pregnancy test in female adolescents aged ≥12 years during screening) or breastfeeding at enrolment are exclusion criteria; however, pregnancy after enrolment does not lead to exclusion. Participants who become pregnant during the study period will receive additional services including more frequent visits and additional laboratory testing in accordance with the national guidelines [15, 16]. If births occur during the study period, the HIV status and health of the new-born will be recorded.

The following serious adverse events will be captured and reported to the ethics committees: any untoward medical occurrence that i) results in death or is life-threatening; ii) requires in-patient hospitalisation or prolongation of existing hospitalisation; iii) results in persistent or significant disability or incapacity; or iv) causes a congenital anomaly or birth defect.

In the case that the intervention proves beneficial, all participants will receive GRT if they still have an elevated VL at study closure.

Nested study on cost-effectiveness

A nested study will assess the cost and cost-effectiveness of GRT. For this purpose, the number of clinical visits and care received at each visit (e.g. counselling, clinical exam with a doctor/nurse), the number and duration of hospitalisations, concomitant medication, and all requested laboratory tests are recorded for each participant.

Trial registration

This trial has been registered with ClinicalTrials.gov (NCT04233242; registered 18.01.2020; https://clinicaltrials.gov/ct2/show/NCT04233242). Further information is available on the trial website: www.givemove.org.

Discussion

Evidence to guide the management of treatment failure in children and adolescents in low-income settings is desperately lacking, endangering the UNAIDS vision of an AIDS-free generation by 2030 [28].

Early, successful ART is key to child development as it reduces mortality and morbidity, improves neurocognitive and growth outcomes [29, 30], and preserves future therapeutic options. However, intention-to-treat analyses report that 20—30% of children and adolescents have an unsuppressed VL 1 year after starting first-line ART [31, 32]. Similarly, a recent systematic review showed that after undergoing enhanced adherence counselling upon detection of viremia, subsequent resuppression was achieved by a little over half (50.4%) of adults, but only 31.2 and 40.4% of children and adolescents, respectively [33]. Even among children whose ART regimen is switched to second-line, resuppression rates remain low [34].

The GIVE MOVE trial assesses the feasibility, clinical impact, and cost-effectiveness of GRT to guide the clinical management of viremia despite first-line ART in children and adolescents. We hypothesise that GRT will substantially improve treatment outcomes by allowing for differentiated care customised according to the individual child/adolescent's health situation and needs, i.e. targeted adherence support for those without drug resistance and a rapid switch to an optimised ART regimen (with potential additional adherence support) in those with drug-resistant HIV, as well as by reducing the time to appropriate clinical action.

This trial has several limitations. Given the nature of the intervention, blinding of participants or healthcare professionals is not possible. Furthermore, the trial cannot make full use of the potential of GRT to reduce time to clinical decision-making: due to the ethical necessity of consenting participants before conducting any non-routine procedures, enrolment and phlebotomy for GRT take place ideally at the first clinic visit after a routine VL test (generally after 1 month). In clinical practice, however, it would be possible to use blood remaining after VL testing to immediately conduct GRT if viremia is detected, and provide both the VL and the GRT result at the subsequent clinic visit. Thus, the GIVE MOVE trial will likely underestimate the potential benefit of reducing time to clinical decision-making.

However, this trial also has several strengths. The multi-site approach, as well as the fact that this pragmatic trial relies heavily on existing infrastructure at the study sites and the logistical capacities of the in-country partners, will increase external validity. The inclusion of participants on newer INSTI-based ART regimens (notably dolutegravir-based regimens) ensures that results will remain relevant for years to come as dolutegravir-based ART becomes increasingly available [35], and the focus on children and adolescents ensures that the needs of particularly vulnerable age groups are addressed.

In conclusion, the GIVE MOVE trial will assess if the availability of GRT for children and adolescents with unsuppressed VLs while taking ART improves clinical outcomes and if it is cost-effective. While funding for national HIV programs in Africa is stagnating or even decreasing, it is more important than ever that resource allocation gives highest priority to evidence-based interventions. Data from GIVE MOVE will provide evidence to program managers and policymakers for the decision on whether access to GRT is an intervention to which further resources should be allocated.

Trial status

The trial was launched at the first site (Baylor Clinic Hlotse) on 20.02.2020, and the first participant was enrolled on 03.03.2020. As per 06.10.2020, all sites are following study protocol v1.3 (dated 27.02.2020), 33 participants have been enrolled, and all four sites have enrolled at least one participant. Enrolment is expected to continue until mid- to late 2021, with a subsequent follow-up period of up to 11 months.

Abbreviations

ART: Antiretroviral therapy; c/mL: Copies per millilitre; GIVE MOVE: Genotype-Informed Versus Empiric Management Of VirEmia; GRT: Genotypic resistance testing; HIV: Human immunodeficiency virus; INSTI: Integrase strand transfer inhibitor; ITT: Intention-to-treat; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor; VL: Viral load; WHO: World Health Organisation

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Authors' contributions

NDL is the sponsor/chief investigator, first conceptualised this trial, and acquired funding. JAB is the principal investigator, wrote the first version of the study protocol and this manuscript, and acquired funding. NDL and JAB designed this trial, with significant input from TRG, TK, MW, AA, NB, JM, EL, and GJM. TRG is the trial statistician and responsible for the statistical analysis plan. MB is the principal data manager. IR is the study manager overseeing recruitment and data quality. BM provides expertise and operational support on quality assurance and documentation. JM, BPK, MMH, LT, MW, and DHP provide expertise and coordinating support. MC and NK provide diagnostic expertise and support, and are responsible for GRT in Lesotho and Tanzania, respectively. TK provides further diagnostic expertise. EL, LK, and CWDY are local principal investigators. NDL, JAB, JM, BPK, MMH, IR, MW, EL, TK and TRG form the GIVE MOVE Steering Committee. The authors read and approved the final manuscript.

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Availability of data and materials

Results of this research will be disseminated at the district and/or national level to stakeholders within the project countries, as well as at the international level through peer-reviewed publications and academic conferences.

Upon publication of the trial results, a subset of the key pseudo-anonymised individual participant data collected during the study, along with a data dictionary, will be made available through the data repository Zenodo. Case report forms and other key study documents will also be made available upon publication of results. The full dataset will be made available upon request to the Department of Medicine at the Swiss Tropical and Public Health Institute and after signing a data confidentiality agreement.

Ethics approval and consent to participate

This trial has been approved by the National Health Research Ethics Committee in Lesotho (ID229–2019), the Institutional Review Board of the Ifakara Health Institute (12–2020), the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol IX/3442), and the Tanzania Medicines and

Medical Devices Authority (TMDA0020/CTR/0003/03). In addition, the Ethikkommission Nordwest- und Zentralschweiz in Switzerland provided a statement confirming the trial meets all requirements for a Swiss research project (Req-2019-01275).
Written informed consent and, where applicable, written informed assent is a prerequisite for participation in this study. The consenting process is conducted by an authorised member of the study team based at the respective site. Before deciding whether or not to consent, participants/caregivers are given detailed information about the study, and are informed that they will continue to receive the standard of care if they choose not to participate and that they may withdraw their consent at any time. Participants aged ≥16 years (Lesotho) or ≥18 years (Tanzania) fill in an Informed Consent Form. For children and younger adolescents below these age thresholds, a caregiver fills in a separate Informed Consent Form for caregivers. Within the approved study protocol, a caregiver is defined as a parent, a legal guardian, or an adult aged ≥18 years and living in the same household as the participant. In addition, participants aged ≥12 and <16 years (Lesotho) or ≥12 and < 18 years (Tanzania) fill in an Informed Assent Form for Adolescents, and those aged ≥6 and <12 years fill in a simplified Informed Assent Form for Children. All forms are available in English as well as Sesotho (Lesotho) or Swahili (Tanzania). In the case of illiteracy, consent/assent is given by thumb print, and a witness signature is required.

Consent for publication
Not applicable.

Competing interests
TK reports advisory board membership fees from ViiV and Gilead for work outside of this study. All other authors declare that they have no competing interests.

Author details
[1]Swiss Tropical and Public Health Institute, Basel, Switzerland. [2]Molecular Virology, Department of Biomedicine, University of Basel, Basel, Switzerland. [3]University of Basel, Basel, Switzerland. [4]SolidarMed, Partnerships for Health, Maseru, Lesotho. [5]Ifakara Health Institute, Ifakara, Tanzania. [6]Seboche Mission Hospital, Seboche, Lesotho. [7]Baylor College of Medicine Children's Foundation Lesotho, Maseru, Lesotho. [8]Baylor College of Medicine, Houston, TX, USA. [9]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland.

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Publisher's Note

5.1.8. 24-Month Outcomes After Same-Day ART, Clin Infect Dis, 2020

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MAJOR ARTICLE

Engagement in Care, Viral Suppression, Drug Resistance, and Reasons for Nonengagement After Home-Based Same-Day Antiretroviral Therapy Initiation in Lesotho: A Two-Year Follow-up of the CASCADE Trial

Alain Amstutz,[1,2,3,a] Jennifer Anne Brown,[1,2,4,a] Isaac Ringera,[5] Josephine Muhairwe,[5] Thabo Ishmael Lejone,[5] Thomas Klimkait,[2,4] Tracy Renée Glass,[1,2] and Niklaus Daniel Labhardt[1,2,3]

[1]Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]University of Basel, Basel, Switzerland, [3]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland, [4]Molecular Virology, Department of Biomedicine, University of Basel, Basel, Switzerland, and [5]Partnerships for Health, Lesotho

Background. The CASCADE trial showed that compared with usual care (UC), offering same-day (SD) antiretroviral therapy (ART) during home-based human immunodeficiency virus testing improved engagement in care and viral suppression 12 months after diagnosis. However, questions remain regarding long-term outcomes and the risk of propagating drug resistance.

Methods. After completion of the primary endpoint at 12 months, participants not in care in both arms were traced and encouraged to access care. At 24 months, the following outcomes were assessed in both arms: engagement in care, viral suppression, and reasons for nonengagement. Furthermore, we explored the acquisition of drug resistance mutations (DRMs) among SD arm nonlinkers.

Results. At 24 months, 64% (88/137) in the SD arm vs 59% (81/137) in the UC arm were in care (absolute difference [AD], 5%; 95% confidence interval [CI], −6 to16; $P = .38$) and 57% (78/137) vs 54% (74/137) had documented viral suppression (AD, 3%; 95% CI, −9 to 15; $P = .28$). Among 36 participants alive and not in care at 24 months with ascertained status, the majority rejected contact with the health system or were unwilling to take ART. Among 8 interviewed SD arm nonlinkers, 6 had not initiated ART upon enrollment, and no acquired DRMs were detected. Two had taken the initial 30-day ART supply and acquired DRMs.

Conclusions. SD ART resulted in higher rates of engagement in care and viral suppression at 12 months but not at 24 months. Leveling off between both arms was driven by linkage beyond 12 months in the UC arm. We did not observe compensatory long-term disengagement in the SD arm. These long-term results endorse SD ART initiation policies.

Keywords. same-day ART; rapid ART initiation; Lesotho; HIV infection; retention in care.

Human immunodeficiency virus (HIV) care and treatment programs globally adopted the recommendation to initiate lifelong antiretroviral therapy (ART) for all people living with HIV regardless of CD4 cell count [1]. A major challenge in implementing a universal test-and-treat strategy in sub-Saharan Africa is low linkage to care among individuals diagnosed as living with HIV [2–6]. This is particularly pronounced in the context of community-based testing, where less than half of those newly diagnosed link to care [7, 8]. Accelerated ART initiation, including starting ART on the day of confirmed HIV diagnosis, is a promising strategy to close the gap between testing and start of treatment. Despite 1 trial showing a trend toward higher loss to follow-up after rapid ART initiation [9], several recent randomized clinical trials from resource-limited settings [10–12] and 2 systematic reviews [13, 14] have concluded that this strategy can improve patient and program outcomes by increasing linkage to care, engagement in care, and sustained viral suppression. As a result, the World Health Organization (WHO) currently recommends offering immediate ART initiation [15].

The CASCADE trial, conducted in Lesotho, southern Africa, was the first of its kind to demonstrate the feasibility and effectiveness of home-based same-day (SD) ART initiation during a door-to-door HIV testing campaign. Offering SD ART start to individuals found to be living with HIV resulted in a significantly higher proportion linked to care within 3 months, as well

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[a]A. A. and J. A. B. contributed equally to this article and share first authorship.

Correspondence: N. D. Labhardt, Clinical Research Unit, Department of Medicine, Swiss Tropical and Public Health Institute, Socinstrasse 57, 4051 Basel, Switzerland (n.labhardt@swisstph.ch).

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as engaged in care and virally suppressed 12 months after the home-based HIV diagnosis [16].

However, knowledge gaps still remain relating to SD ART initiation. These include the long-term outcome, emergence of drug resistance among those who subsequently do not link to care, and data on reasons for nonlinkage to care despite the offer of SD ART. In this follow-up study among participants of the CASCADE trial, we aim to shed light on these knowledge gaps.

METHODS

Study Design and Participants

The CASCADE trial was a parallel-group, open-label, randomized clinical trial that assigned individuals who tested positive for HIV during a home-based HIV testing campaign to either the SD or usual care (UC) treatment arm (1:1 allocation). Study participants in the SD arm were offered home-based ART initiation on the day of HIV diagnosis after point-of-care baseline tests, a counseling session, and a readiness assessment. In the SD arm, 98% were considered ready, accepted SD ART start, and received a 30-day ART supply according to the national ART guidelines. In the UC arm, participants underwent a minimum of 2 pre-ART counseling sessions at the health facility with the subsequent offer to start ART, which was the standard of care in Lesotho at the time of enrollment. Consenting adults living with HIV who were ART-naive (aged ≥18 years) were eligible. The trial was conducted in the catchment area of 6 health facilities in the Butha-Buthe district in rural northern Lesotho. The detailed study protocol and the 12-month outcomes have been published [16, 17]. After assessment of the primary endpoint at 12 months, those not in care were contacted and encouraged to (re-) engage in care at the clinic. First contact attempts were made through phone (if available) and, if unsuccessful, were followed by physical tracing through village health workers, health facility staff, and/or the study nurse.

The original study protocol was approved by a Swiss Ethics Committee and the National Health and Research Ethics Committee of Lesotho. The study protocol was subsequently amended to conduct a 24-month follow-up and approved by the National Health and Research Committee of Lesotho. Participants provided written informed consent for a blood draw. Illiterate participants provided a thumb print, and a witness (independent to the trial and aged >21 years), chosen by the participant, cosigned the form. Informed consent was provided in the local language, Sesotho, and the participant received a copy of the consent form.

Procedures

At 24 months, the status of all participants was systematically assessed by the local study nurse responsible for the main trial. The following information sources were searched: the patient files at each health facility in the study district, the laboratory information system of the Ministry of Health, and the district-wide viral load (VL) database of the research consortium. Participants who were not in care (more than 2 months late for ART refill) were traced by the study nurse by phone (if available) or through home visits in collaboration with the clinic staff and the village health workers. Self-reported transfers to another clinic were followed up with the corresponding health facility to confirm the participants' status. Participants from both arms who had disengaged from care by 24 months and who could be contacted were interviewed about reasons for leaving care.

SD arm participants who had never linked to care and were successfully traced were interviewed using a structured questionnaire to assess their reasons for not linking to care, their adherence to the initial 30-day ART supply, and their history of ART exposure before or since enrollment. Furthermore, they underwent venous blood draw to perform drug resistance testing. The EDTA blood samples were transported within 1 day to the hospital laboratory of the study district (Butha-Buthe Government Hospital) where plasma was separated and frozen at −80°C. Thereafter, plasma aliquots were shipped to a reference laboratory in Switzerland. Drug resistance was assessed using next-generation sequencing (NGS). HIV RNA was extracted using the Maxwell Viral Total Nucleic Acid Purification Kit (PROMEGA Corporation, Fitchburg, WI). Workup and NGS were conducted according to the protocol established by Mbunkah et al [18]. NGS data were processed using MinVar version 2.2.2 [19]. Drug resistance mutations (DRMs) were identified according to the Stanford HIV drug resistance database (www.hivdb.stanford.edu). In order to minimize the risk of false-negative results when assessing the potential harm of transient exposure to ART without subsequent linkage to care, an NGS cutoff of 1% was used.

Outcomes

In this study, we aimed to assess at 24 months (i) among all study participants: engagement in care, reasons for nonengagement, and viral suppression and (ii) among SD arm nonlinkers or late linkers: the acquisition of DRMs. Engagement in care was defined as at least 1 clinic visit in the 24-month follow-up window (range, 22–28 months) and included participants who transferred to any other health facility with a known outcome (documented proof of visit or laboratory report). Viral suppression was defined as <100 copies/mL, which is in line with the definition used in the CASCADE trial [16, 17].

Statistical Analyses

Participants were analyzed according to their randomization arm. The proportions with viral suppression and engagement in care were compared using the Pearson χ^2 or Fisher exact test

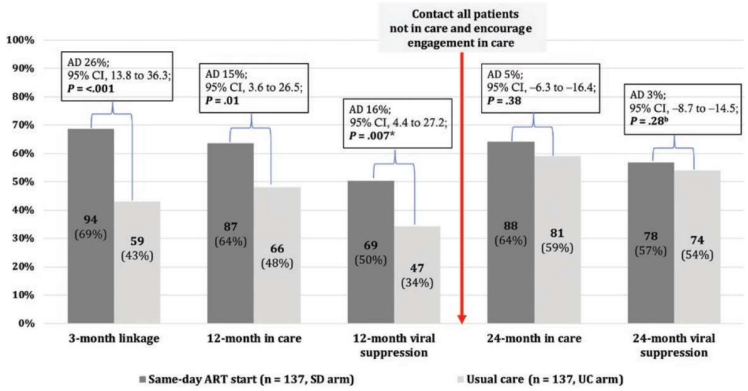

Figure 1. Care cascade in the CASCADE trial until 24-month follow-up. *In each arm, 10.2% (14/137) of participants had no documented viral load result despite having attended the health facility within the predefined outcome window. [b]Five participants in the UC arm and 4 participants in the SD arm had no documented viral load result despite having attended the health facility within the predefined outcome window. Abbreviations: AD, absolute difference; ART, antiretroviral therapy; CI, confidence interval; SD, same-day; UC, usual care.

and presented as absolute differences (ADs) with 95% confidence intervals (CIs) estimated using the Newcombe-Wilson score method [20]. Baseline characteristics potentially associated with engagement in care at 24 months were analyzed using a multivariate logistic regression model, followed by backward stepwise selection based on the Wald test (*P* value cutoff point of .15) and are presented as odds ratios (ORs) with their respective 95% CIs. As a sensitivity analysis, forward stepwise selection was performed with the same selection criteria. For all tests, complete case analysis and 2-sided *P* values with the significance level set at 0.05 were used. All analyses were performed in Stata (version 14, StataCorp, Austin, TX). The CASCADE trial has been registered on clinicaltrials.gov (NCT02692027).

RESULTS

From 22 February 2016 to 17 July 2016, 274 participants (137 per arm) were recruited. Baseline characteristics have been published [16]. The 24-month follow-up window started on 23 December 2017 and closed on 11 November 2018. Figure 1 displays the care cascade from enrollment until the 24-month follow-up. Of the 274 study participants, 64% (88/137) in the SD arm vs 59% (81/137) in the UC arm were in care 24 months after enrollment (AD, 5%; 95% CI, −6 to 16; *P* = .38), and 57% (78/137) vs 54% (74/137) had documented viral suppression (AD, 3%; 95% CI, −9 to 15; *P* = .28).

Table 1 shows the detailed status of all study participants at 24 months. Figure 2 shows the dynamics of engagement in

Table 1. Overall 24-Month Status in Both Arms

24-Month Status	Same-Day Arm (N = 137), n (%)	Usual Care Arm (N = 137), n (%)	Total (N = 274), n (%)
In care at 24 months, n (%)	88 (64)	81 (59)	169 (62)
In care with suppressed VL[a]	78 (57)	74 (54)	152 (56)
In care with unsuppressed VL[b]	6 (4)	2 (2)	8 (3)
In care but without VL in 24-month window[c]	4 (3)	5 (4)	9 (3)
Not in care at 24 months, n (%)	49 (36)	56 (41)	105 (38)
Dead	3 (2)	1 (1)	4 (2)
Lost to follow-up	14 (10)	18 (13)	32 (12)
Unconfirmed transfer out	17 (12)	16 (12)	33 (12)
Traced, alive, no transfer out reported[d]	15 (11)	21 (15)	36 (13)

Abbreviation: VL, viral load.

[a]Including 11 confirmed transfers out.

[b]Including 1 confirmed transfer out.

[c]Including 2 confirmed transfers out.

[d]Contact with participant in person or by telephone, or status confirmed by village health worker or relative. For details, see Supplement Table 2.

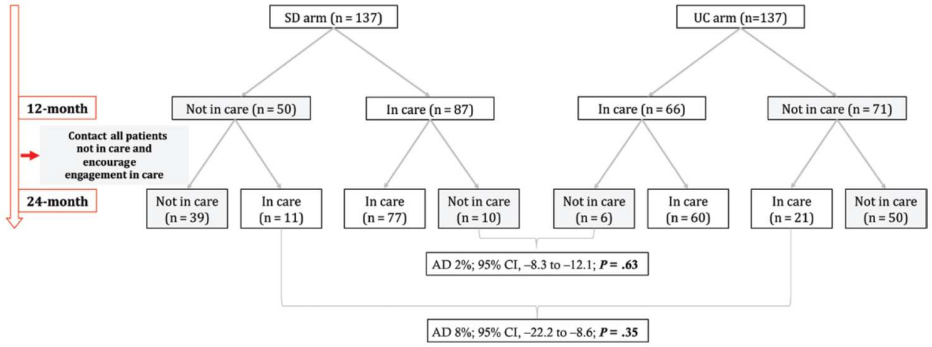

Figure 2. Dynamics of engagement in and disengagement from care in the CASCADE trial. Abbreviations: AD, absolute difference; CI, confidence interval; SD, same-day; UC, usual care.

and disengagement from care during the 24-month follow-up period. Between month 12 and month 24, disengagement from care occurred at a similar rate in the SD (11%; 10 of 87) and the UC (9%; 6 of 66) arms (AD, 2%; 95% CI, −8 to 12, $P = .63$). New engagement in care during this period was higher in the UC arm (30%, 21/71) than the SD arm (22%, 11/50) (AD, 8%; 95% CI, −22.2 to 8.6; $P = .35$), though this difference was not statistically significant. In the logistic regression model, older age and known to be living with HIV before the home-based HIV testing campaign at enrollment were associated with higher engagement in care at 24 months (Table 2). Forward stepwise selection as a sensitivity analysis yielded the same results.

Reasons for nonengagement in care for those alive and not in care at 24 months who could be traced are listed in Supplementary Table 1. The main reasons cited included rejection of contact with the healthcare system, rejection of ART due to skepticism related to the HIV diagnosis or ART, and unwillingness/unreadiness to take ART; only a minority of reasons were structural.

In the SD arm, 43 participants had not linked to care within 3 months and were thus potentially exposed to transient ART. Their 24-month outcomes are shown in Table 3. Ten (23%) linked to care after 3 months and remained in care at 24 months. Among these 10, 9 had VL measurement within the 24-month window and 7 of these had a suppressed VL. The other 2 had a VL of 144 and 3180 copies/mL.

Among those in the SD arm who never linked to care, 8 could be reached and agreed to an interview and phlebotomy for drug resistance testing. Their self-reported adherence to the initial 30-day supply of ART, reasons for not linking, as well as baseline and 24-month NGS drug resistance results are listed in Table 4. Among those 8, 2 reported initial adherence to the 30-day ART supply. In comparison to baseline, new DRMs were detectable in both (participant CA194: V106M; participant CA336: K103N, P225H) at 24 months. The remaining 6 stated that they never started ART; in those participants, no new DRMs were detected at 24 months.

DISCUSSION

The CASCADE trial has shown that offering SD ART initiation after home-based HIV testing significantly increases the proportion of patients engaged in care with viral suppression 12 months after diagnosis [16]. In this follow-up study, we assessed the 24-month status of care among all participants of the CASCADE trial, the emergence of drug resistance among those receiving home-based SD ART who subsequently did not link to care, and their reasons for not linking to care. To our knowledge, we are the first to report on 24-month outcomes after SD ART initiation in resource-limited settings.

Two years after testing positive for HIV, a significant difference in engagement in care and viral suppression was no longer observed between the SD and UC arms. Equalization between both arms appears to be mainly driven by higher rates of later linkage in the UC arm, as we did not observe higher rates of disengagement from care in the SD arm. Previous studies in pregnant women report higher mid- and long-term disengagement from care upon SD ART initiation [21]. Our findings do not endorse the fear of a compensatory higher attrition from care after SD ART initiation. The reason for the increased late linkage in the UC arm is likely multifactorial. Participants in either arm who were not in care at 12 months were traced and encouraged to (re-) engage in care, which might have had a greater effect in the UC arm. On the other hand, the passing of time without any

Table 2. Association Between Baseline Characteristics and Engagement in Care at 24 Months

		Multivariate Logistic Regression (LR χ^2 = 32.92, P = .01)			Backward Selection (P Value Cutoff = .15)		
Baseline Characteristic	n (%)	aOR (95% CI)	β Coefficient	P Value	aOR (95% CI)	β Coefficient	P Value
Same-day arm vs usual care arm	137 (50)	1.28 (.74–2.23)	0.25	.379	…	…	
Age (per year), median (interquartile range)	39 (28–52)	1.04 (1.02–1.07)	0.04	.001	1.02 (1.00–1.04)	0.02	.017
Female vs male	180 (66)	1.48 (.77–2.87)	0.39	.241	…	…	
Marital status[a]							
Single	35 (13)	1	–	–	…	…	…
Married/lives with partner	177 (65)	0.73 (.31–1.75)	−0.31	.484	…	…	
Widowed	60 (22)	0.21 (.06–.67)	−1.57	.008	…	…	
Completed years of school							
Primary not completed	132 (48)	1	–	–	…	…	
Primary completed	120 (44)	1.45 (.76–2.77)	0.37	.262	…	…	
Secondary completed	18 (7)	0.61 (.19–1.98)	−0.49	.412	…	…	
Tertiary completed	4 (1)	0.69 (.08–5.93)	−0.37	.734	…	…	
Employment							
In Lesotho with regular income	54 (20)	1	–	–	…	…	
Outside Lesotho	9 (3)	0.29 (.05–1.52)	−1.25	.142	…	…	
No regular income	211 (77)	0.61 (.29–1.27)	−0.50	.183	…	…	
Known living with HIV vs newly diagnosed living with HIV	71 (26)	2.55 (1.26–5.18)	0.94	.009	2.43 (1.31–4.51)	0.89	.05
Plan to disclose to someone[b]							
Yes	235 (86)	1	–	–	…	…	
No, not for the moment	19 (7)	0.73 (.25–2.09)	−0.32	.557	…	…	
I don't know yet	13 (5)	1.76 (.46–6.74)	0.56	.412	…	…	
World Health Organization stage[c]							
I (asymptomatic)	211 (77)	1	–	–	…	…	
II (oligosymptomatic)	48 (18)	1.36 (.64–2.90)	0.31	.419	…	…	
III (advanced)	11 (4)	1.96 (.48–8.07)	0.67	.351	…	…	
CD4 cell count levels, cells/μL[c]							
<200	44 (16)	1	–	–	…	…	
200–349	76 (28)	2.22 (.94–5.26)	0.80	.070	…	…	
≥350	150 (55)	1.55 (.69–3.48)	0.44	.284	…	…	

Complete-case regression analysis (N = 257).

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; LR, likelihood ratio; (–)N/A, freestanding dashes.

[a]Data from 2 participants missing.

[b]Data from 7 participants missing.

[c]Data from 4 participants missing.

additional intervention likely had an equalizing effect given the natural cycling of patients in and out of care. Overall rates of 24-month engagement in care and viral suppression observed in our study are in line with previous reports in which we analyzed data from before the test-and-treat era, thus likely missing the pre-ART disengagement from care [22–24].

To our knowledge, there are no reports assessing the potential for rapid community-based ART initiation to cause harm in recipients of SD ART who are transiently exposed to ART but do not link to care. It is encouraging to note that 7 of 9 SD arm late linkers to care for whom VL results were available achieved viral suppression (Table 3). Furthermore, self-reported accounts from the 8 interviewed nonlinkers in the SD arm indicate that 6 did not take any of the initial ART supply and thus had no risk of developing drug resistance, which was confirmed by NGS. The remaining 2 declared full initial adherence; both had acquired therapy-related DRMs still detectable at 24 months that had not existed at enrollment. However, this risk also exists upon attrition from care after UC ART initiation. More importantly, the risk of acquiring DRMs has to be viewed in light of the overall benefit that home-based SD ART decreases the time to linkage to care and successful therapy (Figure 1). This is an essential factor not only for individual health but also to prevent further HIV transmission.

The systematic assessment of the status of all study participants, including verifying self-reported transfer out of care as well as collecting valuable but hard-to-obtain data about

Table 3. 24-Month Outcomes of Same-Day Arm Late Linkers and Nonlinkers

Outcome	Total (N = 43), n (%)
Late linkers: linked to care >3 months after enrollment	Subtotal: 13 (30)
In care at 24 months[a]	10 (23)
In care with suppressed VL	7
In care with unsuppressed VL	2
In care but without VL in 24-month window	1
Dead	1 (2)
Lost to follow-up	2 (5)
Unconfirmed transfer out	0 (0)
Nonlinkers: never linked to care	Subtotal: 30 (70)
Dead	2 (5)
Lost to follow-up	6 (14)
Unconfirmed transfer out	9 (21)
Traced, alive, no transfer out reported[b]	13 (30)
Only village health worker reached or participant reached but did not agree to interview and phlebotomy	5
Reached and agreed to interview and phlebotomy (see Table 4)	8

Abbreviations: VL, viral load.

[a]Including confirmed transfer out.

[b]Contact with participant in person or by telephone, or status confirmed by village health worker.

participants who never linked after SD ART start, are among the strengths of this study. Our study has, however, several limitations. The study was conducted in the catchment areas of 6 facilities in 1 rural district in Lesotho, and generalizability may be limited. Furthermore, the assessment of the risk of developing therapy resistance as a consequence of unstructured treatment stop was based on very few individuals. Reasons given for nonlinkage to or disengagement from care should be considered exploratory. Finally, it is not clear if the similar outcomes in both study arms are due to the active tracing of participants not engaged in care at 12 months (after completion of the primary endpoint) or simply due to the additional time available to link to care.

Although both arms of the CASCADE trial had higher rates of linkage to care than reported in previous studies [7, 8], almost one-third of SD arm participants did not link to care. More research is needed to explore strategies to increase engagement in care directly after home-based SD ART. The 2 SD arm participants who did not link to care after taking their first 30-day supply of ART noted transport costs and perceived poor treatment by healthcare professionals, respectively, as their reasons. One promising approach for such participants may be decentralized, community-based ART refills following the SD ART initiation, whereby participants are directly linked to a nearby village health worker for subsequent ART refills [25–29].

In conclusion, our findings endorse the current WHO recommendation to offer rapid or even SD ART initiation to individuals diagnosed as living with HIV. Our findings do not indicate that higher initial linkage to care through SD ART would result in substantially higher attrition from care beyond 12 months. Furthermore, the risk of developing drug resistance through SD ART in those who do not link to care exists but appears to be low. However, offering SD ART during home-based HIV testing campaigns alone does not lead to sufficient linkage to care nor sufficient engagement in care. Additional strategies

Table 4. Individual 24-Month Outcomes of a Subsample of Same-Day Arm Participants Who Never Linked to Care

Patient	Self-reported Adherence to Initial 30-Day ART Supply	Reasons for Nonlinkage to Care After Same-Day ART Offer	Resistance-Associated Mutations (Prevalence in %) at Baseline[a]	Resistance-Associated Mutations (Prevalence in %) at 24 Months[a]
1	All pills taken; full adherence (once daily)	Cost of transport to clinic	K103N (23)	K103N (84) V106M (18)
2	All pills taken; likely mistakenly taken twice daily	Perceived poor treatment from healthcare professionals	None	K103N (100) P225H (14)
3	No pills taken	Reliance on traditional medicine	n.d.	None
4	No pills taken	Fear of being on medication; not feeling ready for lifelong treatment	n.d.	n.d.
5	No pills taken[b]	Fear of being on medication; not feeling well-informed about ART regimen	K103N (100)	K103N (100)
6	No pills taken	Fear of being on medication; not feeling certain that he/she would be able to take medication correctly	None	None
7	No pills taken	Reliance on traditional medicine; travel to South Africa for work	None	None
8	No pills taken	Not believing in human immunodeficiency virus diagnosis	n.d.	n.d.

Abbreviations: ART, antiretroviral therapy; n.d., no data.

[a]Excluding polymorphic resistance-associated mutations that are prevalent at similar frequencies (within 5 percentage points) at baseline and 24 months or, if baseline data are not available, could not be selected by the given drug regimen (ie, polymorphic protease or integrase mutations).

[b]Participant indicated prior exposure to antiretroviral drugs in the context of prevention of mother-to-child transmission strategies.

are needed to address the remaining individual challenges in linking to and remaining in care.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. N. D. L. was the principal investigator of the CASCADE trial. A. A., J. A. B., and N. D. L. were responsible for the design of the follow-up study. J. M., I. R., and T. I. L. coordinated the local project implementation. A. A. and I. R. were responsible for data and biological sample collection. A. A., J. A. B., and N. D. L. analyzed the data. T. R. G. contributed to the statistical analyses. J. A. B. and T. K. were responsible for the virologic analyses conducted in Switzerland. J. A. B. performed next-generation sequencing and analyzed the laboratory data. A. A. and J. A. B. wrote the first draft of the manuscript with input from N. D. L. All authors commented on the manuscript and read and approved the final manuscript for publication.

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Disclaimer. The funders had no role in study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit the manuscript for publication. Data from this study, including deidentified participant data, study protocol, and informed consent documents, will be made available to researchers. To access data, researchers should contact the corresponding author. Researchers will need to present a concept sheet for their proposed analysis. This will have to be reviewed and approved by all coauthors. The coauthors will consider overlap of the proposed project with active or planned analyses and the appropriateness of study data for the proposed analysis.

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5.2. Ongoing work: the GIVE MOVE trial

Children and adolescents are at particular risk for treatment failure (see **chapter 3.4.3**). This may be caused by suboptimal medication intake or viral resistance to their current drug regimen, with different clinical interventions needed depending on the underlying cause. However, access to HIV-1 resistance testing to inform clinical interventions is extremely limited in many of the regions with the highest burden of disease. The *Genotype-Informed Versus Empiric Management Of VirEmia* (GIVE MOVE) trial assesses the need for introducing routine resistance testing for children and adolescents living with HIV.

GIVE MOVE is an ongoing, open-label randomised clinical trial conducted in two countries, Lesotho and Tanzania. It currently has seven active sites (six in Lesotho, one in Tanzania), with additional sites in Tanzania planned. The study aims to enrol 276 children and adolescents with HIV viraemia while in care and receiving ART. Participants are randomised 1:1 to a control arm, receiving viral load-informed onward care, and an intervention arm, receiving onward treatment informed by resistance testing and the recommendations of an Expert Committee. The composite primary endpoint is the occurrence of i) death, ii) a new WHO stage 4 event (with some exclusions), or iii) an HIV- or ART-related hospitalisation lasting ≥24 hours through the 36-week follow-up period; or iv) not having a documented viral load <50 copies/mL at 36 weeks follow-up.

The study protocol has been published (see **chapter 5.1.7**[75]) and the trial is registered with ClinicalTrials.gov (NCT04233242). Updates are available on the trial website, www.givemove.org.

The first site was activated on 20 February 2020 and the first participant was enrolled on 3 March 2020. As per 3 November 2021, 175 participants – 63% of the target sample size – have been enrolled. An amendment has been submitted to the relevant authorities slightly modifying enrolment criteria in light of newer drug regimens and logistical considerations, and adding three additional sites in Tanzania. A pre-planned interim analysis will take place once 50% of participants have completed the 36-week study visit and/or reached the primary endpoint; as the 50% mark for recruitment was reached on 5 July 2021, this interim analysis is currently scheduled for mid-2022.

At present, the internal study team consists of over forty team members (a Steering Committee including myself as Principal Investigator; site teams of the currently active sites; as well as clinical, statistics, data management, and diagnostic co-investigators). In addition, the trial has defined an independent statistician to conduct the interim analysis, a Data Safety Monitoring Board, and Expert Committee on genotypic resistance testing; and an Endpoint Committee. The trial is regularly monitored by monitors at the Swiss Tropical and Public Health Institute and SolidarMed (Lesotho), or the Ifakara Health Institute (Tanzania).

Figure 1 shows a timeline of the major milestones achieved so far. Error! Reference source not found. shows the cumulative recruitment since the start of the trial.

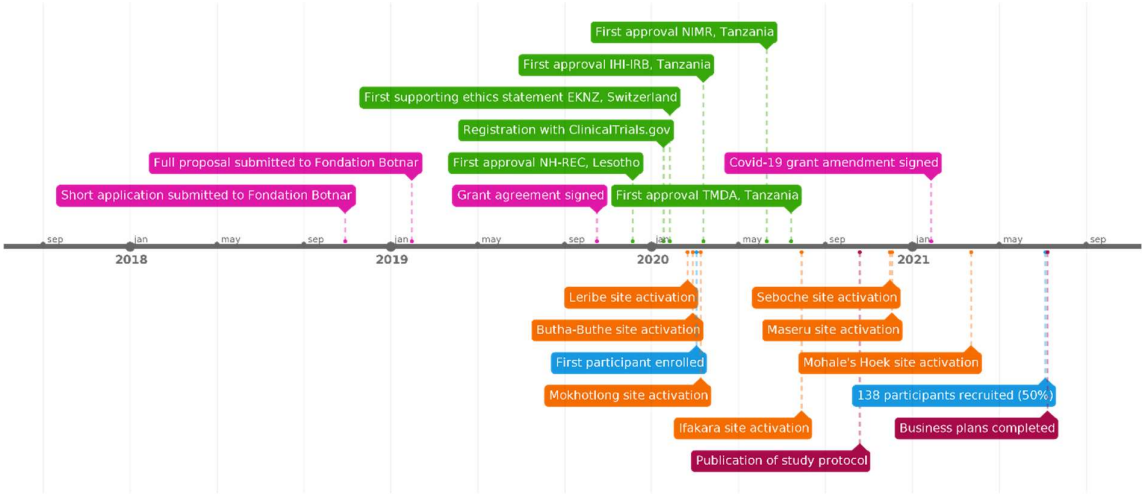

Figure 1: Timeline of major milestones until 3 November 2021. Milestones related to funding, ethics approvals, site activations, recruitment, and output are shown in pink, green, orange, blue, and red, respectively. NH-REC: National Health Research Ethics Committee (Lesotho); EKNZ: Ethikkommission Nordwest- und Zentralschweiz (Switzerland); IHI-IRB: Ifakara Health Institute Institutional Review Board (Tanzania); NIMR: National Institute for Medical Research (Tanzania); TMDA: Tanzania Medicines and Medical Devices Authority (Tanzania).

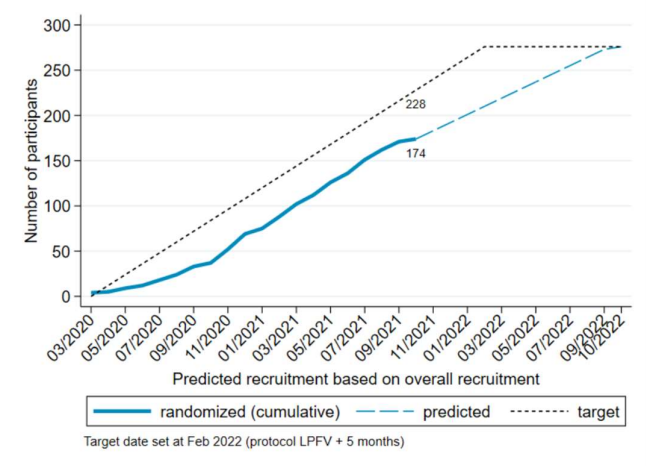

Figure 2: Cumulative recruitment until 3 November 2021. The Covid-19 pandemic caused several delays, especially (though not only) at the beginning of the trial. More recently, monthly recruitment rates have often aligned with the target. The planned addition of new sites in Tanzania is expected to considerably boost recruitment.

Among participants enrolled up to 3 November 2021, the majority are female (58%, vs male), live in Lesotho (82%, vs Tanzania), were between 12 and 19 years old at enrolment (53%, vs below 12 years old), and were taking integrase inhibitor-based ART at enrolment (54%, vs 39% taking protease inhibitor-based ART and 7% taking NNRTI-based ART).

One sub-component of this trial is to assess the feasibility of resistance testing in relatively rural settings. This diagnostic test is cost- and time-intensive, and requires extensive infrastructure as well as technical expertise. Unfortunately, resistance testing has been hampered by numerous challenges, most notably relating to supply chain. Nevertheless, a business plan with a detailed, easily-adjustable

costing analysis was developed to guide the respective resistance testing laboratories involved in GIVE MOVE in each country with regards to future pricing.[5] A cost-effectiveness analysis is planned if the intervention package proves to be clinically beneficial.

[5] This process was led by Olivia Kitau at the Ifakara Health Institute in Tanzania, with support from Namvua Kimera at the Ifakara Health Institute laboratory, Molisana Cheleboi at the Seboche Mission Hospital laboratory in Lesotho, Guy de Coulon formerly at SolidarMed Tanzania, and myself.

6. Discussion

6.1. Results of this thesis in context

Recent years have shown tremendous progress in HIV care, with several countries in sub-Saharan Africa reaching the 90-90-90 targets, or narrowly missing it but reaching the target for overall viral suppression[30]. The studies presented here highlight important steps in these improvements; however, they also demonstrate that key gaps in access to adequate healthcare services remain, even for people who are already in care.

This thesis contains projects covering all stages of health facility-based HIV treatment: my colleagues and I have considered questions relating to when to start ART, how to introduce new first-line regimens, how to handle viraemia during first-line ART, when and how to switch to second-line ART, the success of this entire viral load care cascade in children, and mental and somatic health considerations that look beyond the 'third 95' of viral suppression.

Integrating these results as well as the current literature, the following chapters consider the current standard of care, potential gaps, and possible ways forward across four parameters of relevance to sustained viral suppression: ease of access to healthcare services; adherence to ART; the role of diagnostics; and novel antiretrovirals.

6.2. The Way Forward for Access – Reaching Care Remains a Burden

Even when HIV care is provided free of charge, accessing services can place a high financial and time burden on patients[48]. The Covid-19 pandemic has further exacerbated access barriers[110,111]. Interventions to improve access can adopt different approaches, notably including i) decentralising services, or ii) reducing the frequency of health facility visits. As such interventions can directly impact both engagement in care and viral suppression, both must be taken into account in their evaluation.

The CASCADE trial, conducted in the context of a door-to-door HIV testing campaign, integrated aspects of both approaches: pre-ART services were decentralised to allow for home-based same-day ART initiation, though subsequent care was clinic-based[23]. Together with less-frequent clinic visits due to multi-month ART dispensing, this approach led to increased linkage to care as well as higher 12-month engagement in care and viral suppression compared to the standard of care[23].

When my colleagues and I analysed the 24-month CASCADE data, outcomes between the study arms had equalised, driven by a greater net increase in engagement in care in the control arm in the second year of the trial[76]. We could not be certain to what extent prior tracing of participants who were not in care at 12 months had had an equalising effect. Nevertheless, the earlier engagement in care observed in the intervention arm – allowing less time for disease progression or onward transmission – without greater subsequent attrition from care led us to endorse the trial intervention[76]. The only remaining (albeit minor[6]) concern was related to the few instances of acquired drug resistance observed among individuals who had taken the initial (efavirenz-based) ART supply without

[6] Though the sample size to address this risk was small, our data suggested that only a minority of participants in the intervention arm who did not link to care ever took any medication and developed drug resistance[76]; furthermore, defaulting from care also occurs after initial linkage, and the trial had shown clear overall benefits of the intervention in terms of twelve-month engagement in care and viral suppression[23].

subsequently linking to care[76]. This risk would be far lower today considering the recent roll-out of dolutegravir, which has a higher barrier to development of resistance[71], providing further support for home-based same-day ART.

Despite the trial's success, three-month linkage to care (69%) as well as 12- (64%) and 24-month engagement in care (64%) remained suboptimal even with same-day ART[23,76], necessitating additional interventions. Expanding on the approach of decentralised services, subsequent work in Lesotho attempted to fill this gap through the offer of village health worker-mediated ART refills[112]. However, uptake was lower than expected and no difference in viral suppression was observed between this intervention and clinic-based ART refill, potentially showing the limitations of task shifting[112]. A separate trial in Lesotho found that community-based ART dispensing was non-inferior to facility-based dispensing[113], and was associated with cost savings for providers and patients[48].

Within our research group, one ongoing project is taking the second above-mentioned approach, decreasing the frequency of clinic visits. The VITAL trial is testing a viral load-driven differentiated service delivery model, allowing for less-frequent ART supply among virally suppressed patients and a reallocation of resources towards individuals needing intensified support[114]. There is reason to believe that this approach may be successful: a recently published trial enrolling over nine thousand participants established the non-inferiority of six-monthly facility-based ART supply compared with three-monthly refills or the standard of care with regard to 12-month retention in care[115]. This was accompanied by lower provider and patient costs[115]. However, data on viral suppression as well as long-term outcomes were lacking in this trial – a research gap which VITAL is poised to fill[115].

6.3. The Way Forward for Adherence – No One-Size-Fits-All

Within this thesis, extensive high-level resistance observed already at the first detection of viraemia during NNRTI-based ART, combined with the risk of acquiring additional resistance-associated mutations during ongoing exposure to a failing regimen, called into question whether the requirement for adherence counselling before switching to second-line ART was justified[69]. Indeed, recent World Health Organization guidelines loosened this requirement specifically for people taking NNRTI-based first-line ART[15]. However, these considerations change with the roll-out of dolutegravir: the increasing availability of antiretrovirals with a high barrier to resistance is bound to increase the proportionate role of suboptimal adherence as a cause of treatment failure, at least in the short term. Within this thesis, the high observed tolerability of dolutegravir-based ART in the DO-REAL cohort is a promising sign for long-term adherence[83]; nevertheless, the few cases of ongoing viraemia after transition from NNRTI- to dolutegravir-based ART appeared to be driven by suboptimal adherence rather than drug resistance (though longer-term follow-up will be important)[73]. Preliminary data from the GIVE MOVE trial also suggest an important role for adherence in viraemia during treatment with modern ART regimens.

The diversity of factors influencing adherence to ART (described in chapters **3.4.2** and **3.4.3**) poses a challenge to delivering suitable interventions, meaning that there cannot be a 'one-size-fits-all' approach[77], at least with the currently available drug options. It is therefore unsurprising that adherence interventions typically have small effects, and that packages comprising multiple interventions generally yield superior outcomes than single interventions[116]. Interventions that have shown moderate positive results in a systematic review and network meta-analysis included text

messaging, counselling, supporters, and combined behavioural and cognitive interventions, though effects tend to wane once interventions end[116].

Unfortunately, none of the studies presented in this thesis systematically assessed reasons for or predictors of non-adherence. While a great number of studies investigating adherence barriers already exist[77,78], the diversity of such barriers might necessitate a certain geographical and contextual granularity to inform subpopulation-specific intervention packages. This diversity of barriers might also make it more efficient to address adherence in combination with other health issues that impact adherence but deserve to be addressed in their own right: for example, among people with depression or with hazardous alcohol use, tailored approaches using behavioural interventions or cognitive behavioural therapy can reduce depression or alcohol use as well as improve adherence[117–120]. Within our research group, several nested studies within the VITAL trial are assessing the prevalence of and designing interventions for depression and alcohol use, both of which have been linked to reduced adherence[81,92,93]. As VITAL only enrols adults, separate studies will be needed to better investigate and address adherence challenges faced by children, adolescents, and their caregivers.

While there is a clear need for broader adherence packages, new diagnostic technologies may revolutionise adherence monitoring to inform the use of downstream diagnostics, and novel drug options show promise to substantially ease the burden of adherence itself. Such approaches are discussed in the following two chapters.

6.4. The Way Forward for Diagnostics – Novel Approaches to Inform Treatment

Within this thesis, an analysis of routine viral load data in an ongoing open cohort study identified significant gaps along the paediatric viral load cascade, with notable delays in clinical action after detection of an unsuppressed viral load[98]. Similar outcomes were previously described among adults[103]. Unfortunately, understanding the underlying causes of delays was largely beyond the scope of our study. However, significant year-on-year differences in turnaround times at the laboratory level suggest a need for laboratory system strengthening and building in more redundancies[98].

However, even under optimal conditions, patients typically only get their results one to three (with multi-month ART dispensing) months after phlebotomy when they next return to the clinic. In theory, delays between blood draw and clinical action could be completely eliminated by point-of-care viral load testing, as included in the latest World Health Organization guidelines[15]. In a randomised controlled trial, this approach led to improved viral suppression and retention in care, as well as faster switching to second-line ART where indicated[121]. As described in 2018 Lancet series on pathology and laboratory medicine, point-of-care viral load testing can play a key role within tiered laboratory systems – but cannot compensate for weak healthcare systems[122].

When viral load testing detects viraemia, the decision whether or not to adjust an ART regimen has long been challenging in settings with no or limited access to resistance testing. In the time of NNRTI-based first-line ART, viraemia was commonly associated with viral drug resistance necessitating a switch to second-line ART, as observed in two studies contributing to this thesis[69,70]. On the other hand, switching non-adherent individuals to second-line ART would expose them to further drugs in a context of limited treatment options. Until recently, unnecessary switching would moreover have made adherence even more challenging, as it meant moving from a once-daily (NNRTI-based) to a twice-daily (ritonavir-boosted lopinavir-containing) regimen.

Despite this apparent need for resistance testing, the recently-published REVAMP randomised controlled trial saw no benefit in providing genotypic resistance testing over the standard of care, i.e. repeat viral load testing, to inform onward treatment in terms of nine-month viral suppression[123]. This is an important message with regards to cost-effective allocation of funding. However, REVAMP enrolled adults with an unsuppressed viral load while taking NNRTI-based ART, which limits generalisability to many paediatric and to newer adult first-line regimens[123]. The ongoing GIVE MOVE trial, presented within this thesis, aims to fill this gap for children and adolescents[75]. The timeliness of this trial is demonstrated by research gaps identified in the most recent consolidated guidelines of the World Health Organization[15]:

> '*Considering the very low levels of drug resistance, the role of drug resistance testing is unclear in a treatment failure algorithm for people living with HIV receiving [dolutegravir]-based treatment to minimize unnecessary switches from this regimen. Additional data for children and adolescents would support optimized treatment monitoring in these populations for which drug resistance is a critical issue.*'

Indeed, the need for resistance testing in low- and middle-income countries is bound to change with increasing availability of dolutegravir, in multiple ways. The roll-out of dolutegravir further decreases the probability that pre-treatment resistance testing will be prioritised globally: despite being recommended in some high-income countries[58], there is evidence from high-income settings arguing against the need for or cost-effectiveness of pre-treatment testing for integrase resistance[124,125]. Furthermore, recent evidence suggests good performance of dolutegravir even in the context of extensive NRTI resistance[72], further decreasing the need for pre-treatment resistance testing. The drug's high barrier for development of resistance will also mean that a smaller proportion of people with treatment failure on dolutegravir-based ART will require a treatment switch than was the case with efavirenz[71]. Therefore, strict implementation of current World Health Organization guidelines, which require a switch of regimen upon two successive viral load results ≥1000 copies/mL[15], would lead to many unnecessary switches from generally well-tolerated, once-daily dolutegravir-based ART to more complex, twice-daily ritonavir-boosted lopinavir-based ART[15], at least in countries where alternative second-line options are not available.[7] While resistance to dolutegravir does emerge even in the context of standard triple-drug ART[71,72,128], its low prevalence might favour a more conservative approach to switching away from dolutegravir-based ART in the absence of resistance testing. On the other hand, excessive reluctance to switch might gradually allow for the prevalence of dolutegravir resistance to increase to a greater extent than was observed in resource-rich settings, where genotypic testing is readily available to rapidly respond to any emergent resistance and thereby limit its spread.

Before the roll-out of dolutegravir, modelling studies reached highly divergent conclusions regarding the cost-effectiveness of resistance testing upon treatment failure in low- and middle-income settings[129–131], and evidence from the dolutegravir era is missing. One approach that might greatly improve the cost-effectiveness of resistance and even viral load testing is prior point-of-care testing

[7] Ritonavir-boosted atazanavir and ritonavir-boosted darunavir are other recommended second-line core agents for people switching from dolutegravir-based first-line ART[15]. Neither are currently readily available in Lesotho or Tanzania, where the projects presented within this thesis were conducted. However, the adult market share of ritonavir-boosted atazanavir in low- and middle-income countries is rapidly increasing compared to ritonavir-boosted lopinavir[126], and ritonavir-boosted darunavir is expected to become available in the near future at comparable prices to ritonavir-boosted lopinavir[127].

for the presence of antiretrovirals in patient samples as a proxy for adherence[132–139]. In addition to saving costs, this technology has the potential to improve clinical outcomes through faster recognition of non-adherence and initiation of adherence interventions, or faster provision of resistance testing or switch to a new regimen in the case of viraemia despite measurable drug levels[137]. Innovative trials combining point-of-care testing for antiretrovirals, point-of-care viral load testing, and resistance testing with the aim to improve viral suppression are currently underway[133].

6.5. The Way Forward for Antiretrovirals – the Impact of New Treatment Options

As shown throughout this work, dolutegravir has markedly changed the treatment landscape. Compared with efavirenz, previously the most common core agent in first-line regimens, dolutegravir has been shown to lead to non-inferior[140–143] or superior[144–146] as well as faster[141,144,145] viral suppression among adults. While long-term routine data from resource-limited settings is still needed, data presented within this thesis indicate good short-term viral suppression and tolerability of dolutegravir after its programmatic introduction in Lesotho[73,83]. As this analysis included individuals who are not at particular risk for treatment failure – ART-experienced adults in care in an urban, NGO-supported hospital setting with high access to viral load testing – further research should investigate to what extent these findings hold true in populations with lower baseline rates of viral suppression and without recent pre-transition viral load data.

For paediatric patients, recent data from the ODYSSEY randomised controlled trial shows superior viral suppression with dolutegravir compared with the standard of care, for both first- and second-line ART[128,147]. The roll-out of paediatric dolutegravir for children weighing below 20 kilograms is now imminent in many countries in sub-Saharan Africa, which is hugely promising for paediatric virological outcomes while simultaneously reducing costs to the health system[90]. Moving forward, it will be important to better assess tolerability, adverse effect profiles, as well as patient and caregiver preferences with regard to paediatric regimens. This will help to determine to what extent dolutegravir can fully replace other paediatric first-line regimens, as has largely been the case among adults, or whether alternative options should remain available. Unfortunately, even with dolutegravir, cohort data from mostly high-income countries has shown suboptimal viral suppression among children, adolescents and young adults, though integrase resistance was only observed in few cases[148–152]. This suggests that the roll-out of paediatric dolutegravir might still not suffice to reach the ‘third 95’ target among younger age groups.

With the major cause for viraemia shifting increasingly from viral resistance towards adherence, novel long-acting or extended release antiretrovirals may play a key role in curbing treatment failure. Recent trials have established the non-inferiority of monthly injections of cabotegravir and rilpivirine as compared with standard daily oral ART[153,154], as well as the non-inferiority of dosing every two months instead of monthly[155]. Furthermore, the overwhelming majority of study participants indicated a preference for long-acting injectable regimens and for longer dosing intervals[153–155].

Long-acting cabotegravir and rilpivirine has since been included in some clinical guidelines[58,59], though not yet those of the World Health Organization[15]. Moving forward, it will be essential to ensure these new formulations meet the needs of and become available to the populations most impacted by HIV[37,38]. This will depend critically on costs and implementation constraints[38,156,157], with modelling data for Kenya suggesting that potential cost-effectiveness for

adolescents and young adults if these regimens can be provided at less than twice the cost of oral ART[156].

Though not without risks and challenges[37], these novel regimen types could greatly increase the convenience of taking ART. By eliminating the most important patient-reported barriers to adherence – including forgetting, being away from home, changes to routine, stigma, depression, pill burden, or substance use – long-acting injectable ART might be a game-changer for viral suppression[77].

7. Conclusions

While major progress has been made, reaching 95% viral suppression across all geographic regions and population groups, and especially among children and adolescents, will be challenging. The work presented here addresses several key aspects relating to viral suppression.

Starting slightly upstream of the 'third 95', the assessment of long-term outcomes after home-based same-day ART, which has since been cited by several guidelines[15,59], led to a strengthened recommendation for this approach but also showed that same-day ART is not sufficient to ensure linkage to and retention in care[76].

Once in care, patients until recently received NNRTI-based ART regimens that were effective, though vulnerable to the development of drug resistance. Accordingly, high levels of resistance to NNRTIs were observed in this work, including in the context of low-level viraemia[69,70]. This, combined with delays observed along the viral load cascade among children taking predominantly NNRTI-based ART[98], highlighted the need for less conservative switching away from NNRTI-based regimens. Indeed, the latest World Health Organization guidelines have substantially lowered the barriers for switching away from NNRTI-based ART[15].

The recent roll-out of dolutegravir – a drug that is more robust regarding the risk of viral resistance – substantially impacts considerations on clinical management on viraemia. Two studies addressing the transition to dolutegravir-based ART among adults show positive outcomes with regard to short-term viral suppression and – looking beyond the 'third 95' – adverse effects[73,83]. However, longer-term follow-up of virological outcomes, including potential emergent resistance, is needed. The availability of new drug options with a high barrier to resistance will also impact the need for and cost-effectiveness of resistance testing. Evidence to quantify the possible impact of resistance testing is lacking for the main current first-line ART regimens as well as for the age groups with the greatest need, namely children and adolescents. Here, the ongoing GIVE MOVE trial will assess the feasibility, clinical impact and, if beneficial, cost-effectiveness of routine resistance testing[75].

As countries approach 95-95-95, distinct new research agendas are becoming clear. On the one hand, another major push is needed along the HIV care cascade as well as in preventive services, as the world is not on track to reach zero new infections by 2030[39]. Novel approaches – including strategies to decentralise and differentiate services delivery[114,162], intelligent combinations of novel and existing diagnostic approaches[133], and long-acting or extended release antiretrovirals for treatment and prevention[37,38,153–155] – show promise towards making an end to the epidemic possible.

On the other hand, with 25 million people now on ART with viral suppression, it is also time to look beyond the 'third 95' of viral suppression and towards quality of life. For adults, dealing with comorbidities and co-medication as well as promoting healthy aging will increasingly shape the research agenda[104]. For younger children, the roll-out of paediatric dolutegravir is now imminent, though information on adverse effects, convenience, and preference is scarce. Further data to inform guidelines on the provision of paediatric ART is thus urgently needed.

Achieving zero new infections and zero new deaths by 2030 will require targeted interventions reaching those most at risk. However, involvement of all of society will be needed to achieve the third goal for 2030 – creating a world with zero stigma.

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[10] The 'half' visit was cut short due to Covid-19.

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[11] As different from yours as they may be.

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10. Curriculum Vitae

Personal Information

Name	Jennifer Anne Brown
Date of birth	10.10.1991
Nationality	Swiss / South African
Address	Hochstrasse 85, 4053 Basel, Switzerland
Email	jennifer.brown@swisstph.ch
LinkedIn	jennifer-brown-1
Orcid	0000-0003-1874-6153

Education

Since 03/2018 **PhD Student in Medical Biological Research**

University of Basel, Basel, Switzerland /
Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland

Research interests: HIV care cascade in southern Africa; paediatric HIV; HIV drug resistance and resistance testing; programmatic transition to dolutegravir-based antiretroviral therapy; access to healthcare; laboratory system strengthening

Research settings: Lesotho (five districts); Tanzania (Ifakara)

Academic affiliations: HIV and Chronic Disease Care, Clinical Research Unit, Department of Medicine, Swiss TPH; Molecular Virology, Department of Biomedicine, University of Basel

Supervision: Prof. Niklaus Labhardt (Swiss TPH) & Prof. Thomas Klimkait (University of Basel)

09/2016—06/2019 **MAS in Development and Cooperation**

ETH Zurich, Zurich, Switzerland

09/2014—10/2016 **MSc in Biology, specialisation Cell Biology**

ETH Zurich, Zurich, Switzerland

09/2011—09/2014 **BSc in Biology**

ETH Zurich, Zurich, Switzerland

Experience

Since 03/2018 **PhD Student in Medical Biological Research**

University of Basel, Basel, Switzerland / Swiss TPH, Basel, Switzerland

See Education.

02/2017—11/2017 **Project Assistant Molecular HIV Diagnostics, Towards 90-90-90 Research Consortium**

Swiss TPH, Basel, Switzerland (supervision) /
University of Basel, Basel, Switzerland (one month) /
SolidarMed, Butha-Buthe, Lesotho (eight months)

Internship within the MAS in Development and Cooperation.

05/2016—06/2016 **Assistant Researcher, Laboratory of Food Microbiology**

ETH Zurich, Zurich, Switzerland

Additional Qualifications

08/2021	**Good Clinical Practice for Investigators and Study Teams Certificate** Swiss TPH, Basel, Switzerland
02/2019	**Good Clinical Practice Certificate, Sponsor-Investigator Level** University Hospital Basel, Basel, Switzerland
01/2019	**Good Clinical Practice Certificate, Investigator Level** University Hospital Basel, Basel, Switzerland

Languages

English	Mother tongue
German	Mother tongue
French	Limited working proficiency

Funding

Contribution to funding proposals	**Lead in writing proposal to Fondation Botnar** (REG-19-008) – CHF 700 000 Project funding for the GIVE MOVE randomised clinical trial. Lead in drafting the two-stage funding proposal. Grant awarded to Prof. Niklaus Labhardt (2019).
Conference registration scholarships	**IAS 2021** (11th International AIDS Society Conference on HIV Science) **CROI 2021** (28th Conference on Retroviruses and Opportunistic Infections) **EACS 2019** (17th European AIDS Conference)

Conference Presentations (as presenting author)

07/2021 **International AIDS Society (IAS) 2021, virtual;** e-poster presentation:

Brown JA, Nsakala BL, Mokhele K, Rakuoane I, Peea R, Glass TR, Muhairwe J, Amstutz A, Bachmann N, Klimkait T, Labhardt ND. High rates of viral suppression sixteen weeks after transition from EFV- to DTG-based ART regardless of viral load at transition: the DO-REAL cohort study in Lesotho. Abstract No. A-IAS2021-00434.

03/2021 **Conference on Retroviruses and Opportunistic Infections (CROI) 2021, virtual;** Science Spotlight presentations:

Brown JA, Nsakala BL, Seeburg U, Vanobberghen F, Muhairwe J, Klimkait T, Labhardt ND. Drug resistance during low-level HIV viremia supports lowering threshold for switch. Abstract No. 1205.

Brown JA, Nsakala BL, Mokhele K, Rakuoane I, Peea R, Tarumbiswa T, Muhairwe J, Glass TR, Amstutz A, Bachmann N, Belus JM, Klimkait T, Labhardt ND. Dolutegravir in real life: quality of life outcomes in a cohort study in Lesotho. Abstract No. 1013.

11/2019 **European AIDS Clinical Society (EACS) 2019, Basel, Switzerland;** moderated e-poster presentation:

Bachmann N, **Brown JA**, Mbunkah HA, Klimkait T, Metzner KJ, Günthard HF, Kouyos RD, Labhardt ND. The evolution of HIV-1 drug resistance during the 3-month WHO-recommended enhanced adherence counselling period. Abstract No. PE17/3.

07/2019 **International AIDS Society (IAS) 2019, Mexico City, Mexico;** poster presentation:

Brown JA, Amstutz A, Ringera I, Muhairwe J, Lejone TI, Bachmann N, Klimkait T, Glass TR, Labhardt ND. Non-linkage to care after same-day ART initiation - reasons and acquisition of drug resistance: 24-month follow-up of non-linkers to care from the CASCADE trial in Lesotho. Abstract No. MOPED635.

Publications

Submitted:

1. **Brown JA**, Nsakala BL, Mokhele K, Rakuoane I, Muhairwe J, Glass TR, Amstutz A, Tschumi N, Belus JM, Klimkait T, Labhardt ND. Dolutegravir in Real Life: self-reported mental and physical health outcomes after transitioning from efavirenz- to dolutegravir-based antiretroviral therapy in a prospective cohort study in Lesotho. *Under review.*

Published / accepted:

2. Muhairwe JA*, **Brown JA***, Motaboli L, Nsakala BL, Lerotholi M, Amstutz A, Klimkait T, Glass TR, Labhardt ND. The suboptimal paediatric HIV viral load cascade: multi-district cohort study among children taking antiretroviral therapy in Lesotho, Southern Africa. Pediatr Infect Dis J; accepted.
3. **Brown JA**, Nsakala BL, Mokhele K, Rakuoane I, Muhairwe J, Urda L, Amstutz A, Tschumi N, Klimkait T, Labhardt ND. Viral suppression after transition from nonnucleoside reverse transcriptase inhibitor- to dolutegravir-based antiretroviral therapy: a prospective cohort study in Lesotho (DO-REAL study). HIV Med. 2021; published online ahead of print. doi: 10.1111/hiv.13189.
4. **Brown JA**, Mbunkah HA, Lejone TI, Ringera I, Cheleboi M, Klimkait T, Metzner KJ, Günthard HF, Labhardt ND, Kouyos RD*, Tschumi N*. Emergence of HIV-1 drug resistance during the 3-month WHO-recommended enhanced adherence counselling period in the CART-1 cohort study. Open Forum Infect Dis. 2021; 8(5):ofab046. doi: 10.1093/ofid/ofab046.
5. **Brown JA**, Amstutz A, Nsakala BL, Seeburg U, Vanobberghen F, Muhairwe J, Klimkait T, Labhardt ND. Extensive drug resistance during low-level HIV viraemia while taking NNRTI-based ART supports lowering the viral threshold for regimen switch in resource-limited settings: a pre-planned analysis from the SESOTHO trial. J Antimicrob Chemother. 2021;76(5):1294-1298. doi: 10.1093/jac/dkab025.
6. **Brown JA**, Ringera I, Luoga E, Cheleboi M, Kimera N, Muhairwe J, Kayembe BP, Molapo Hlasoa M, Kabundi L, Yav CWD, Mothobi B, Thahane L, Amstutz A, Bachmann N, Mollel GJ, Bresser M, Glass TR, Paris DH, Klimkait T, Weisser M, Labhardt ND. Genotype-Informed Versus Empiric Management Of VirEmia (GIVE MOVE): study protocol of an open-label randomized clinical trial in children and adolescents living with HIV in Lesotho and Tanzania. BMC Infect Dis. 2020;20(1):773. doi: 10.1186/s12879-020-05491-9.
7. Lejone TI, Kopo M, Bachmann N, **Brown JA**, Glass TR, Muhairwe J, Matsela T, Scherrer R, Chere L, Namane T, Labhardt ND, Amstutz A. PEBRA trial - effect of a peer-educator coordinated preference-based ART service delivery model on viral suppression among adolescents and young adults living with HIV: protocol of a cluster-randomized clinical trial in rural Lesotho. BMC Public Health. 2020;20(1):425. doi: 10.1186/s12889-020-08535-6.
8. Amsutz A*, **Brown JA***, Ringera I, Muhairwe J, Lejone TI, Klimkait T, Glass TR, Labhardt ND. Engagement in care, viral suppression, drug resistance and reasons for non-linkage after home-based same-day ART initiation in Lesotho: a two-year follow-up of the CASCADE trial. Clin Infect Dis. 2020;71(10):2608-2614. doi: 10.1093/cid/ciz1126.
9. Courlet P, Decosterd LA, **Brown JA**, Alves Saldanha S, Marzolini C, Cavassini M, Stoeckle M, Csaijka C, Labhardt ND, Calmy A, Swiss HIV Cohort study. Emtricitabine and lamivudine concentrations in saliva: a simple suitable test for treatment adherence. J Antimicrob Chemother. 2019;74(8):2468-70. doi: 10.1093/jac/dkz181.
10. Jawaid A, **Brown JA**, Schulz PE. Diabetes mellitus in amyotrophic lateral sclerosis: Dr Jekyll or Mr Hyde? Eur J Neurol. 2015;22(11):1419-20. doi: 10.1111/ene.

** equal contribution.*

Basel, 15 November 2021