Long-term effects of HIV treatment in sub-Saharan Africa: from access to quality
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As HIV treatment programs in sub-Saharan Africa mature, there are rising concerns about the long-term sustainability and quality of these programs. Increasing levels of HIV drug resistance have been measured in sub-Saharan Africa, and could jeopardize long-term treatment success.

This thesis presents the results of prospective cohort studies in adults and children, assessing the quality of HIV treatment programmes and the emergence of HIV drug resistance in sub-Saharan Africa.
Long-term effects of HIV treatment in sub-Saharan Africa
from access to quality

Sonia Boender
Long-term effects of HIV treatment in sub-Saharan Africa: from access to quality.
Academic thesis, University of Amsterdam, the Netherlands.

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Long-term effects of HIV treatment in sub-Saharan Africa

from access to quality

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ABBREVIATIONS

3TC lamivudine
AZT azidothymidine (also referred to as ZDV)
ADR acquired drug resistance
AIDS acquired immunodeficiency syndrome
AIGHD Amsterdam Institute for Global Health and Development
ANC antenatal care
ART combination antiretroviral therapy
ARV antiretroviral (drug)
CRF circulating recombinant forms
DNA deoxyribonucleic acid
DRM drug resistance mutation
EDCTP European and Developing Countries Clinical Trials Partnership
EFV efavirenz
HAART highly active antiretroviral therapy
HIV human immunodeficiency virus
HIVDR HIV drug resistance
HR hazard ratio
IAS-USA International Antiviral Society-USA
ITT intention-to-treat
JCRC Joint Clinical Research Centre
LAASER Linking Asian and African Societies for an Enhanced Response to HIV/AIDS
LMIC low- and middle-income country
LUTH Lagos University Teaching Hospital
LTFU loss to follow-up
MARCH Monitoring Antiretroviral Resistance in Children
MTCT mother-to-child transmission
NNRTI non-nucleoside reverse transcriptase inhibitor
NRTI nucleoside reverse transcriptase inhibitor
NVP nevirapine
OR odds ratio
OT on-treatment
PASER PanAfrican/PharmAccess Studies to Evaluate Resistance
PASER-M PASER-Monitoring
PCR polymerase chain reaction
PDR pretreatment drug resistance
PI protease inhibitor
PMTCT prevention of mother-to-child transmission
RNA ribonucleic acid
TAM thymidine analogue mutation
TASER TREAT Asia Asian Studies to Evaluate Resistance
VL HIV viral load
WHO World Health Organization
ZDV zidovudine (also referred to as AZT)
General introduction
INTRODUCTION

The human immunodeficiency virus (HIV) epidemic remains a major global health burden. In 2014, more than 36.9 [34.3-41.4] million people were living with HIV worldwide [1]. Access to antiretroviral treatment has changed the HIV epidemic drastically. Because of this treatment, HIV-positive people can now live long and healthy lives. An estimated 7.8 million HIV-related deaths were averted between 2000 and 2014 [2]. As of June 2015, an estimated 15.8 million people had accessed to treatment, which is an exponential increase from 200,000 people in 2002 [3]. More than 11 million people access HIV treatment in sub-Saharan Africa, the region that carries the largest HIV-burden. Increasing levels of HIV drug resistance in sub-Saharan Africa could jeopardize the long-term treatment success [4–6]. As HIV treatment programs in sub-Saharan Africa mature, there are rising concerns about the long-term sustainability and quality of these programs.

HIV: the human immunodeficiency virus

HIV is a retrovirus, belonging to the genus *Lentiviridae* which is characterised by a long incubation period (*lentus* = slow). There are two types of HIV: HIV-1, which is most common globally, and HIV-2 which is less pathogenic and mostly found in West-Africa [7]. HIV-1 can be divided in four groups: M, N, O, and P. HIV-1 group M is responsible for the global epidemic, and is the virus generally referred to as simply ‘HIV’. HIV-1 group M can be divided into nine different subtypes: A, B, C, D, E, F, G, H, J and K. These subtypes can also form hybrid viruses, known as circulating recombinant forms (CRFs). For example CRF02_AG is a subtype A/G recombinant, which is circulating widely in West and Central Africa [8,9]. Subtype B is the predominant subtype in Western Europe, Australia and North America. As a result, most HIV research has focused on subtype B, while it is responsible for only 12% of the infections worldwide. In contrast, less is known about other subtypes like A and C, which are frequently found at a global scale and the dominant subtypes found in sub-Saharan Africa [1,10].

HIV is an enveloped virus, enclosing viral enzymes, a capsid and core with two single RNA strands. The viral enzymes are involved in HIV replication: reverse transcriptase, converting RNA to DNA; integrase, integrating the viral DNA into the host DNA; and protease, cleaving the viral proteins (Figure 1). Every HIV virion produces ~100,000,000,000 new virions per day [11]. The replication of HIV through reverse transcriptase is error-prone. With each life cycle, at least one random mutation in the HIV genome occurs. This creates a large pool of different versions of HIV, so
called ‘quasispecies’. The process of continuous viral alteration complicates drug and vaccine development.

HIV can be transmitted from one person to the other through body fluids [12]. The most common ways of transmission are sexual intercourse, needle sharing, using unsterilized medical equipment, occupational exposure (e.g. needlestick injury), or through blood transfusion. Additionally, vertical transmission of HIV from mother to child can occur during pregnancy, delivery and through breastmilk [13].

After infection, HIV replicates within and destroys cells of the human immune system, which are mostly CD4+ T-cells, macrophages and dendritic cells. Early HIV-infection is often asymptomatic, or characterised by flu-like symptoms for about a week, often going unnoticed. This is followed by an asymptomatic period of on average 8-10 years [14]. Without treatment, the immune system will deteriorate, leading to the most advanced stage of infection: acquired immunodeficiency syndrome (AIDS). When a person has AIDS, the level of CD4+ T cells has become dangerously low and the immune system is no longer able to fight off (opportunistic) infections, eventually leading to death.

**HIV treatment: combination antiretroviral therapy**

HIV was discovered as the cause of AIDS in 1983 [15,16]. In 1987, the first antiretroviral (ARV) drug became available on the market: azidothymidine (AZT), which is a nucleoside reverse transcriptase inhibitor (NRTI). Despite the impressive survival benefit of HIV-positive people taking AZT, the effect was of limited duration as HIV developed resistance against the drug [17]. The new ARVs prescribed as mono- or duo-therapy also lost their effect due to the development of drug resistance.

In 1996, the introduction of highly active antiretroviral therapy (HAART; currently referred to as ART) drastically improved the life-expectancy of HIV-positive people [16]. New drug classes, non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI), were developed, each targeting different viral enzymes involved in HIV replication (Figure 1). ART, consisting of a triple-therapy combination of three different ARVs from two different drug classes, was able to suppress HIV replication for a long time with minimum development of drug resistance [16]. After the introduction of NNRTI- and PI-based ART, new ARV drug classes have been developed: integrase inhibitors, fusion/entry inhibitors and maturation inhibitors.
HIV treatment in sub-Saharan Africa: the public health approach

While the year 1996 was a turning point for HIV-positive people living in resource-rich settings, ARVs were not available nor affordable in Africa at that time. By the end of 2001, 40 million people were estimated to be living with HIV worldwide; 25.8 million were living in sub-Saharan Africa. Three million people died of AIDS in that same year [18]. The impact of the epidemic reverted society’s development, as the estimated infection rates continued to rise, and life-expectancy continued to fall. Therefore, the 2001 United Nations General Assembly Special Session on HIV/AIDS called for urgent global action in the ‘Declaration of Commitment on HIV/AIDS’ [19]. At that time, HIV treatment guidelines were directed at resource-rich settings and based on individual patient management. Medical doctors could prescribe every ARV available, and high-end laboratory technology was available to closely monitor treatment response. This personalised approach was not feasible in resource-limited settings such as sub-Saharan Africa, where doctors and laboratory capacity were (and still are) limited. This prompted the development of the ‘public health approach’ to providing HIV treatment by the World Health Organization (WHO) in 2002. [20–29].

Figure 1. HIV replication and drug targets.
Adaptation of illustration by Thomas Splettstoesser (www.scistyle.com).
Key elements of the public health approach include task-shifting to lower-level health care workers, administration of standard ART regimens and minimal laboratory monitoring [20]. It has allowed the rapid scale-up of access to ART to millions of people, with efficient use of resources. However, the lack of laboratory monitoring within this approach has raised concern that treatment failure could go unnoticed.

**Treatment monitoring**

HIV/AIDS disease progression and ART success can be measured through clinical, immunological and viral load monitoring. Clinical monitoring is the assessment of clinical symptoms, and/or opportunistic infections. Immunological monitoring assesses the CD4⁺ T cell count (in short, CD4 count), which is a measure of immune function. Viral load monitoring comprises the quantification of the HIV RNA load in copies per millilitre plasma. This is a direct measurement of the number of viral copies in the blood. Typically, virological failure is the first sign of treatment failure (Figure 2). When the viral load in the body increases, the immune system suffers the consequences and the CD4 count will decrease, eventually leading to immunological failure. Subsequently, a person will soon suffer new or recurrent clinical event(s), which is defined as clinical failure. The 2002-2015 WHO definitions of treatment failure are summarized in the Table 1 [21–29].

Historically, the public health approach relies on clinical monitoring which allows drug resistance mutations to accumulate before clinical failure is diagnosed (Figure 2).
While viral load monitoring is increasingly being recommended as the preferred ART monitoring tool [21–29], it is often unavailable in resource limited settings, increasing the chance that drug resistance may develop [30].

**Specific issues in children**

Vertical transmission of HIV is preventable, and effective interventions have virtually eliminated new HIV infections in children in resource-rich settings [31]. The prevention of mother-to-child transmission (PMTCT) consists of a collection of measures to prevent perinatal HIV transmission [32]: routine HIV testing for all pregnant women; reduction of the maternal viral load through short-course ARVs or ART during pregnancy and delivery; elective caesarean delivery; and the use of formula feeding or

<table>
<thead>
<tr>
<th>Table 1. Summary of the World Health Organization antiretroviral treatment guidelines for a public health approach.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>When to start</strong></td>
</tr>
<tr>
<td>Adults CD4 counts ≤200 cells/mm³ CD4 counts ≤350 cells/mm³</td>
</tr>
<tr>
<td>Children All: age-specific CD4 criteria &lt;2 years old: all;</td>
</tr>
<tr>
<td>2.5 years old: ≤750 cells/mm³ or 25%</td>
</tr>
<tr>
<td>&lt;5 years old: all</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td><strong>What to start</strong></td>
</tr>
<tr>
<td>Adults NNRTI + 2NRTI</td>
</tr>
<tr>
<td>Children NNRTI + 2NRTI ≤3 years old: PI + 2NRTI;</td>
</tr>
<tr>
<td>≥3 years old: NNRT + 2NRTI</td>
</tr>
<tr>
<td><strong>Switch to</strong></td>
</tr>
<tr>
<td>Adults PI + 2NRTI</td>
</tr>
<tr>
<td>Children Stay on PI + 2NRTI, adherence counselling ≤3 years old:</td>
</tr>
<tr>
<td>INI + 2NRTI;</td>
</tr>
<tr>
<td>≥3 years old: PI + 2NRTI</td>
</tr>
<tr>
<td><strong>Treatment monitoring</strong></td>
</tr>
<tr>
<td>Adults Clinical and immunological monitoring, targeted viral load monitoring if available</td>
</tr>
<tr>
<td>Children</td>
</tr>
<tr>
<td><strong>Diagnosis of treatment failure</strong></td>
</tr>
<tr>
<td>Clinical New or recurrent WHO stage 4 condition</td>
</tr>
<tr>
<td>Immunological CD4 below baseline; or 50% fall form peak value;</td>
</tr>
<tr>
<td>or persistent CD4 &lt;100 cells/mm³</td>
</tr>
<tr>
<td>Virological No recommendation VL ≥10,000 VL ≥5,000 VL ≥1,000</td>
</tr>
<tr>
<td>recommendation      cps/ml    cps/ml     cps/ml</td>
</tr>
</tbody>
</table>

INI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, viral load; WHO, World Health Organization.
following strict breastfeeding guidelines. For HIV-exposed infants, a short course of ARVs is administered, followed by virological testing for early infant diagnosis. Through PMTCT, the risk of perinatal transmission of HIV can be reduced from 15-45% without intervention, to <2% with optimal PMTCT in resource-rich settings [32–34].

Despite the success of PMTCT, an estimated 2.6 [2.4-2.8] million children were living with HIV globally in 2014, the majority (90%) of whom live in sub-Saharan Africa [1]. Although HIV-infection in children follows a similar pattern as in adults, children face specific challenges. The persistence of maternal antibodies in children under 18 months means a virological diagnostic test (DNA PCR) is required which is not readily available in resource-limited settings. This makes HIV diagnosis in this age group difficult. Without ART, over one-third of children will die before reaching two years of age [35]. Therefore, early HIV diagnosis and immediate ART initiation has been recommended by the WHO since 2010; irrespective of clinical or immunological status (see Table 1) [26–28]. However, in 2015 only an estimated 32% [30-34%] of HIV-infected children have access to treatment, compared to 41% [38-46%] of the adult population [1].

When a child has been diagnosed and is enrolled in HIV care, only limited paediatric ARV drug options and formulations are available [36]. Some children have been exposed to ARVs through PMTCT before initiation of ART, which increases their chances of having already acquired drug resistant HIV strains and in turn, limits their treatment options. The fact that children rapidly change their body weight makes correct dosing challenging, increasing the risk of treatment failure and of developing drug resistance. HIV-infected children also have higher viral loads and faster disease progression compared to adults [37]. Furthermore, children rely on their caregivers for adherence and retention in care [38,39].

**Figure 3. HIV drug resistance.**

ART, antiretroviral therapy
Drug resistance

HIV drug resistance refers to the ability of HIV to replicate in the presence of drugs that usually suppress its replication [4]. HIV drug resistance can impede the successful suppression of HIV by ART [40]. Several mutations in the viral reverse transcriptase, protease, integrase and envelope have been found to be associated with a poor response to ART [41,42]. Drug resistant HIV can be acquired or transmitted (Figure 3). Acquired drug resistance occurs in HIV-positive people who are taking ARV drugs, for example ART or ARVs for PMTCT. Often in the context of insufficient drug levels, HIV continues residual viral replication and builds up drug-resistant variants through a process of selection and mutation. Transmitted drug resistance occurs when previously uninfected people become newly infected with these drug-resistant viruses. Pretreatment drug resistance refers to drug resistance in those who are about to initiate ART. Several factors lead to the development of HIV drug resistance: viral factors, drug specific factors, patient factors and programmatic factors [4,43]:

1. Viral factors
   Due to the high replication rate and error-prone transcription by HIV, drug resistant variants are created on a daily basis. Pretreatment drug resistance is associated with increased risk of virological failure, and further development of drug resistance [44,45]. The risk of virological failure and drug resistance development may also vary across HIV subtypes [46,47].

2. Drug specific factors
   Some ARVs have a higher genetic barrier than others. In drugs with a low genetic barrier (such as NNRTIs), one mutation can be enough to develop drug resistance, compared to drugs with a high genetic barrier (such as PIs), requiring multiple mutations. Also, side effects (nausea, diarrhoea) caused by ARVs can be a reason for poor adherence or malabsorption of the drugs. Furthermore, drug-drug interaction can cause suboptimal ARV drug levels, for example rifampicin for tuberculosis treatment interacts with ARVs [48].

3. Patient factors
   Suboptimal adherence is one of the main reasons for suboptimal drug levels, which in turn leads to virological failure and the emergence of drug resistance [49]. HIV-associated stigma and fear might negatively affect adherence and retention in care. Adherence and timely clinic attendance are especially challenging for children, who depend on their caregiver [50].
4. Programmatic factors
Suboptimal program functioning can increase the risk of drug resistance [51,52]. Drug stock-outs and limited human resources can affect adherence and retention in care. Lack of adequate treatment monitoring in the WHO public health approach can lead to delayed switch practices. Drug resistance mutations will accumulate when treatment failure is diagnosed late and ART regimen switches are delayed; particularly in the absence of viral load monitoring.

RATIONALE FOR THIS THESIS

Due to the increased availability of ART for HIV in sub-Saharan Africa, there are rising concerns about the long-term sustainability of these ART programs. Increased levels of pretreatment HIV drug resistance have already been measured in sub-Saharan Africa and jeopardize treatment success [4,5,30,53–57]. This thesis presents data on the long(er)-term effects of HIV treatment in sub-Saharan Africa. We aimed to assess the issues pertinent to the next challenge of large-scale ART programs: access to quality HIV care for adults and children.

The research objectives of this thesis are:
- To assess factors associated with the timing of HIV treatment initiation in children.
- To evaluate the prevalence and predictors of pretreatment HIV drug resistance in children.
- To summarize long-term HIV suppression rates in adults and children on first-line ART in low- and middle-income countries.
- To assess the long(er)-term effects of pretreatment HIV drug resistance on morbidity, mortality and ART regimen switches in adults.
- To assess the remaining HIV drug susceptibility after failing standard first- and second-line ART in adults and children.

Research setting
PASER, Pan African Studies to Evaluate Resistance (formerly known as PharmAccess African Studies to Evaluate Resistance) was established in 2006 as a multi-country capacity building and research program, for the assessment and prevention of HIV drug resistance in sub-Saharan Africa. PASER was established in coordination with WHO’s HIV drug resistance prevention strategy and contributes to the WHO Global HIV Drug Resistance Network (HIVResNet), a global network advising WHO on the control and surveillance of HIV drug resistance [59]. While WHO focussed on the public sector, PASER chose to focus on the private sector (profit and not-for-profit)
to complement WHO efforts. The primary PASER objectives were to build capacity in the monitoring and surveillance of HIV drug resistance in Africa, and to disseminate information, perform advocacy and realize policy support (coordinated with WHO HIVResNet). PASER was coordinated by PharmAccess Foundation and the Amsterdam Institute for Global Health and Development.

The PASER network and its sister network TASER in Asia (TREAT Asia Asian Studies to Evaluate Resistance) were part of the bi-regional network called LAASER (Linking Asian and African Societies for an Enhanced Response to HIV/AIDS), with the primary aim to build capacity on the surveillance and monitoring of HIV drug resistance [58].

Figure 4. The PASER network.
Over the past several years, the PASER program on African adults has been supplemented with a similar studies on paediatric ART and HIV drug resistance: Monitoring Antiretroviral Resistance in Children (MARCH).

**The PASER cohort**

The PASER-Monitoring observational cohort enrolled HIV-positive adults in thirteen public and private clinical sites in six countries: Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe (Figure 4). At each site, 240 HIV-positive adults were enrolled when initiating standard first-line ART, or when switched to second-line ART because of presumed first-line treatment failure. A total of ~3000 participants were followed prospectively and received routine HIV care according to national guidelines, which were in line with the WHO HIV treatment guidelines; see Table [21–29].

**The MARCH cohort**

The aim of MARCH was to strengthen the capacity of HIV drug resistance monitoring in children, and to optimize care and treatment guidelines for paediatric ART programmes in sub-Saharan Africa. The MARCH cohort was a prospective, multi-centre, observational cohort study of 460 HIV-infected children under 12 years of age at clinical sites already participating in the established PASER network (Figure 4). PASER and MARCH were conducted using harmonized study protocols. In 2010, the MARCH cohort enrolled 360 children when initiating first-line ART, or switching to second-line ART at three clinical sites of the Joint Clinical Research Centre in Uganda. At each site in Kampala, Fort Portal and Mbale, 120 children ≤12 years of age were enrolled. In 2012, the MARCH cohort enrolled an additional 100 children initiating first-line ART at the Lagos University Teaching Hospital in Nigeria. All children received routine care according to national guidelines which were in line with WHO guidelines; see Table [21–29].

**OUTLINE OF THIS THESIS**

Part I of this thesis describes pretreatment challenges of HIV-infected children in sub-Saharan Africa. First, we assess the reasons for late entrance into HIV care, with advanced disease, among children in Uganda (Chapter 2). Next, we assess the prevalence and patterns of pretreatment HIV drug resistance in children from both Uganda (Chapter 3) and Nigeria (Chapter 4). Furthermore, we summarize the published literature on prevalence of pretreatment HIV drug resistance in children in sub-Saharan Africa (Chapter 4).
Part II describes the long-term outcomes of first-line ART. We systematically review the long-term virological suppression rates in HIV-infected children (Chapter 5) and adults (Chapter 6) receiving first-line ART in low- and middle-income countries. Additionally, we assess the effect of pretreatment HIV drug resistance on mortality, progression to AIDS and switches to second-line ART in adults on first-line ART (Chapter 7).

Part III examines the potential future ARV drug options for people who are failing standard first- and second-line ART. First, we describe the remaining drug susceptibility in both adults and children experiencing continued virological failure on first-line ART (Chapter 8). Next, we describe the risk of virological failure on second-line ART and quantify the need for and requirements of third-line ART (Chapter 9).

In the epilogue, we pay tribute to the work of the late Professor Joep Lange (Chapter 10). His efforts were fundamental in enabling access to ART in Africa and Asia, and he was one of the co-initiators of the PASER cohort.
REFERENCES


Part I

Starting HIV treatment: pretreatment challenges
Barriers to initiation of pediatric HIV treatment in Uganda: a mixed methods study.


AIDS Research and Treatment
Volume 2012, Article ID 817506, 10 pages.
ABSTRACT

Although the advantages of early infant HIV diagnosis and treatment initiation are well established, children often present late to HIV programs in resource-limited settings. We aimed to assess factors related to the timing of treatment initiation among HIV-infected children attending three clinical sites in Uganda. Clinical and demographic determinants associated with early disease (WHO clinical stage 1-2) or late-disease (stage 3-4) stage at presentation were assessed using multilevel logistic regression. Additionally, semi-structured interviews with caregivers and health workers were conducted to qualitatively explore determinants of late-disease stage at presentation. Of 306 children initiating first-line regimens, 72% presented late. Risk factors for late presentation were age below 2 years old (OR 2.83, p=0.014), living without parents (OR 3.93, p=0.002), unemployment of the caregiver (OR 4.26, p=0.001), lack of perinatal HIV prophylaxis (OR 5.66, p=0.028), and high transportation costs to the clinic (OR 2.51, p=0.072). Forty-nine interviews were conducted, confirming the identified risk factors and additionally pointing to inconsistent referral from perinatal care, caregivers’ unawareness of HIV symptoms, fear and stigma as important barriers. The problem of late disease at presentation requires a multi-factorial approach, addressing both health system and individual-level factors.
INTRODUCTION

Despite the effectiveness of antiretroviral prophylaxis for the prevention of mother-to-child transmission (PMTCT) of HIV, approximately 370,000 children were newly infected with HIV in 2009. An estimated 2.5 million children are currently infected with HIV worldwide, of whom 2.3 million reside in sub-Saharan Africa [1]. HIV-infected infants have much higher rates of disease progression and mortality than adults or older children, even with a relatively high percentage of CD4 T-lymphocytes [2,3]. Without treatment, over 50% of HIV-infected children are estimated to die before the age of two [4].

Despite the increased mortality in young infants, children in resource-limited settings generally initiate ART at an older age and with advanced disease [4,5]. In 2008, the CHER trial in South Africa demonstrated a 76% mortality reduction among infants in whom antiretroviral treatment (ART) was initiated before 12 weeks of age, regardless of HIV-symptoms or immunodeficiency, compared with those deferring therapy [6]. Based on these important findings, the World Health Organization (WHO) guidelines currently recommend that all HIV-infected children under the age of two should initiate treatment [7]. The WHO estimates that only 32% of HIV-infected children in East Africa requiring ART are currently treated [8].

Although government policies and efforts by international donors seek to make antiretrovirals (ARVs) freely available to children through national ART programs, other factors are holding back further scale-up of pediatric ART in Africa. A wide array of such factors or barriers has been put forward in the literature including health system and personal level barriers [9,10]. The development of new strategies to overcome these barriers is essential to reduce child morbidity and mortality, thereby contributing to the achievement of the Millennium Development Goal 4 [11]. However, limited structured research has been performed and there is little setting-specific insight into health care barriers.

The Joint Clinical Research Centre (JCRC) is a main provider of HIV care and treatment in Uganda. It was founded in 1990 as a strategic partnership with the Ministry of Health and Makerere University Medical School. The national JCRC network – consisting of more than 50 clinical sites – has over 20 years of experience with ART, from conducting clinical trials to nationwide roll-out programs since 2003. This study describes the characteristics of children initiating HIV care at three JCRC clinical sites based in Kampala, Fort Portal and Mbale. Drawing on both quantitative and qualitative data sources, we aimed to identify the most important factors influencing the timing of pediatric ART initiation.
Chapter 2

METHODS

Population, setting and study design

We used an observational study design with a mixed methodology. This allowed us to triangulate findings from both participants and methods, and generate a deeper understanding of the barriers to initiation of pediatric HIV care. The present study was performed as part of the Monitoring Antiretroviral Resistance in Children (MARCH) observational cohort, monitoring HIV-infected children (below 12 years old) initiating ART at three JCRC sites. The clinical sites in Kampala, Fort Portal and Mbale are Regional Centers of Excellence and provide ART for both adults and pediatric patients. The Kampala site, based in the national capital, houses JCRC headquarters and mainly serves an urban population. The sites in Fort Portal and Mbale are located in district capitals, serving the Rwenzori region in Western Uganda and the entire Eastern region of Uganda, respectively [12]. People attending these two clinical sites come from both urban and rural areas (Table 1).

| Table 1. Characteristics of the Joint Clinical Research Centre (JCRC) sites. |
|-----------------|-----------------|-----------------|
| Location        | Kampala         | Fort Portal     | Mbale           |
| Population a    | 1,659,600       | 47,100          | 91,800          |
| HIV prevalence b| 5.9%            | 5.9%            | 5.9%            |
| Catchment area   | Urban           | Urban and rural | Urban and rural |
| Number of adults in care at JCRC (% of total) c  |
| Kampala         | 15306 (85.7)    | 5880 (87.3)     | 3027 (85.7)     |
| Fort Portal     | 858 (12.7)      | 2575 (43.8)     | 2505 (82.8)     |
| Mbale           | 506 (14.3)      | 340 (39.6)      | 349 (69.0)      |
| Number of children in care at JCRC (% total) c   |
| Kampala         | 2553 (14.3)     | 858 (12.7)      | 506 (14.3)      |
| Fort Portal     | 349 (39.6)      | 349 (69.0)      | 340 (39.6)      |
| Mbale           | 349 (69.0)      | 349 (69.0)      | 340 (39.6)      |
| Number of adults receiving ART at JCRC (% of adults in care) c |
| Kampala         | 6096 (39.8)     | 2575 (43.8)     | 2505 (82.8)     |
| Fort Portal     | 2575 (43.8)     | 2575 (43.8)     | 2505 (82.8)     |
| Mbale           | 2505 (82.8)     | 2505 (82.8)     | 2505 (82.8)     |
| Number of children receiving ART at JCRC (% of children in care) c |
| Kampala         | 888 (34.8)      | 340 (39.6)      | 340 (39.6)      |
| Fort Portal     | 340 (39.6)      | 349 (69.0)      | 349 (69.0)      |
| Mbale           | 349 (69.0)      | 349 (69.0)      | 349 (69.0)      |


b Source: UNAIDS Epidemiological Factsheet Uganda 2009.

c Source: Monitoring and Evaluation records at JCRC, 2011.

The sample-size was calculated based on the MARCH study objective to monitor HIV drug resistance. This cross-sectional, observational sub-study aims to identify the most important factors influencing the timing of pediatric ART initiation at the three sites. Potential participants were informed of the study and screened for eligibility by the study staff at each clinic. All children that initiated ART were included; previous use of ARVs for the purpose of therapy (i.e. ART or mono/duo therapy) was an exclusion criterion. Previous use of ARVs for PMTCT was allowed. The ethical committees of
Barriers to initiation of pediatric HIV treatment in Uganda

JCRC and the Academic Medical Center of the University of Amsterdam approved the study protocol. The parent(s)/guardian(s) of all eligible children provided written informed consent. Children above the age of eight who were aware of their HIV status provided written informed assent. Routine socio-demographic, clinical and laboratory data were collected using electronic case report forms, which were aggregated in a web-based data system. Whenever possible, the health status and medication use of the mother was also captured.

**Quantitative methods**

Group comparisons for categorical data were performed using the chi-square test, and for continuous data using the student’s t-test. Nutritional status was assessed by means of the WHO Child Growth Standards: WHO Anthro version 3.2.2 (age 0-5) and WHO Reference 2007 for height and weight (age 5-19) [13,14]. Severe immunodeficiency was classified according to the WHO guidelines: CD4 cell percentage <25% or CD4 cell count <1500 cps/mm^3 below 12 months old; CD4 cell percentage <20% or CD4 cell count <750 cps/mm^3 between 12-35 months old; CD4 cell percentage <15% or CD4 cell count <350 cps/mm^3 above 35 months old [15]. WHO clinical staging was used to define early-disease stage or late-disease stage at presentation: children in stage 1 (asymptomatic) or 2 (mild symptoms) were considered to have early-disease stage, and children in stage 3 (advanced symptoms) or 4 (severe symptoms) were considered to have late-disease stage [16].

Multivariate logistic regression analysis with random intercepts was used to examine risk factors for late-disease stage at presentation, while accounting for clustering of observations within sites. Results are expressed as odds ratios (ORs) with 95% confidence intervals (CIs) and p-values, with two-sided p-values <0.05 considered statistically significant. A sensitivity analysis was performed, excluding children with unknown source of HIV infection. All analyses were performed with Stata version 10 (StataCorp LP, TX, USA).

**Qualitative methods**

Qualitative semi-structured interviews with both JCRC health workers and caregivers of children attending JCRC clinical sites were conducted to explore participants views of key findings from the quantitative results, such as the late (disease stage) presentation of children for ART. Interviews consisted of open-ended questions to explore perceived barriers to ART initiation. Topics covered in the interview were the referral system, quality of care, HIV testing and treatment protocols, characteristics of the caregiver, transport to the clinic, pharmacy and laboratory facilities. Pilot interviews were held with three local physicians; questions were adapted if necessary to ensure that
they were appropriate for all participants. A separate questionnaire aimed at health workers, testing ART guideline knowledge, was developed in collaboration with a pediatric infectious disease specialist.

Interviews were conducted by the first author (TSB) in July 2011 at the three clinical sites. All caregivers of children below 12 years of age were identified by the doctor or counselor during a regular follow-up visit. Data were collected until the saturation point [17] was reached; we are therefore confident that the findings presented are internally valid. All health workers - pediatricians, clinicians, nurses, counselors and adherence officers - involved in pediatric HIV care at JCRC where interviewed at all 3 clinics, to maximize health worker representation and internal validity.

All interviews took place in private settings where other people could not hear the respondents' answers. For interviews with caregivers, a local trained counselor assisted with translation and/or interpretation of questions. Interviews were recorded and transcribed in English. Using the framework approach for qualitative analysis [17], key issues and themes emerging from the data were identified, and responses were compared and contrasted among the different groups of study participants. Findings from the qualitative study were interpreted using the Andersen’s Behavioral Model of Health Services Use [18–20].

RESULTS

Quantitative results

Participant characteristics

Between January 2010 and May 2011, 310 children initiating first-line ART were enrolled in the MARCH study (92 from Kampala, 113 from Fort Portal and 105 from Mbale). After excluding a protocol violation (n=1) and children with missing data on eligibility criteria (n=3), 306 participants were included in the analysis. The median age was 4.8 years and 50% (n=152) were boys (Table 2). The reported source of HIV infection was mother-to-child transmission (MTCT) in 284 (93%) participants. In 22 (7%) children, the source of infection was unknown. HIV-status was known for 208 (68%) of their mothers, of whom 195 (94%) were HIV-infected, 1 (0.5%) was uninfected and 12 (6%) were unaware their HIV status. Among the HIV-infected mothers, 45% were on ART, 49% were not on ART, and for the remainder ART usage was unknown.
Table 2. Clinical and demographic characteristics of the ‘Monitoring of Antiretroviral Resistance in Children’ cohort participants.

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 306)</th>
<th>Kampala (n = 91)</th>
<th>Fort Portal (n = 112)</th>
<th>Mbale (n = 103)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>152 (49.8)</td>
<td>43 (47.3)</td>
<td>55 (49.1)</td>
<td>54 (52.9)</td>
<td>0.719</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td>4.8 (2.2-8.6)</td>
<td>4.0 (1.5-8.5)</td>
<td>4.2 (1.9-8.5)</td>
<td>5.8 (3.0-8.7)</td>
<td>0.127</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 years old</td>
<td>76 (24.8)</td>
<td>32 (35.2)</td>
<td>29 (25.9)</td>
<td>15 (14.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>2-5 years old</td>
<td>84 (27.5)</td>
<td>18 (19.8)</td>
<td>37 (33.0)</td>
<td>29 (28.2)</td>
<td></td>
</tr>
<tr>
<td>5-12 years old</td>
<td>146 (47.7)</td>
<td>41 (45.1)</td>
<td>46 (41.1)</td>
<td>59 (57.3)</td>
<td></td>
</tr>
<tr>
<td>Age at (first) confirmed HIV+ test</td>
<td>3.7 (1.6-6.8)</td>
<td>3.0 (1.3-7.2)</td>
<td>3.3 (1.1-6.8)</td>
<td>4.5 (2.7-6.8)</td>
<td>0.264</td>
</tr>
<tr>
<td>WHO clinical stage</td>
<td>Stage 3 and 4</td>
<td>221 (72.2)</td>
<td>36 (39.6)</td>
<td>92 (82.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HIV-TB co-infection</td>
<td>Pulmonary tuberculosis</td>
<td>31 (10.1)</td>
<td>18 (19.8)</td>
<td>6 (5.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Severe immunodeficiency a</td>
<td>CD4 count-for-age</td>
<td>67 (31.0)</td>
<td>36 (40.0)</td>
<td>9 (21.4)</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>CD4%-for-age</td>
<td>102 (51.3)</td>
<td>57 (63.3)</td>
<td>18 (42.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Viral load, median log10 cps/mL (IQR) c</td>
<td>5.0 (4.4-5.5)</td>
<td>5.2 (4.7-5.6)</td>
<td>5.1 (4.2-5.5)</td>
<td>4.7 (4.1-5.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Main reason for ART initiation d</td>
<td>HIV diagnosis &lt;24 months</td>
<td>30 (9.8)</td>
<td>20 (22.0)</td>
<td>9 (8.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td></td>
<td>Immunological status</td>
<td>102 (33.3)</td>
<td>55 (61.4)</td>
<td>25 (22.3)</td>
<td>22 (21.4)</td>
</tr>
<tr>
<td></td>
<td>WHO clinical stage</td>
<td>174 (56.9)</td>
<td>16 (17.6)</td>
<td>78 (69.6)</td>
<td>80 (77.7)</td>
</tr>
<tr>
<td>Time between HIV test and ART initiation, median days (IQR)</td>
<td>&lt;2 years old</td>
<td>45 (19-85)</td>
<td>40 (18-66)</td>
<td>48 (23-103)</td>
<td>43 (26-86)</td>
</tr>
<tr>
<td></td>
<td>2-5 years old</td>
<td>97 (26-400)</td>
<td>117 (34-309)</td>
<td>197 (29-546)</td>
<td>64 (15-180)</td>
</tr>
<tr>
<td></td>
<td>5-12 years old</td>
<td>258 (29-802)</td>
<td>242 (28-634)</td>
<td>296 (35-757)</td>
<td>216 (21-848)</td>
</tr>
<tr>
<td>PMTCT exposed</td>
<td>Yes</td>
<td>14 (4.6)</td>
<td>11 (12.1)</td>
<td>3 (2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drugs for PMTCT</td>
<td>Single dose NVP</td>
<td>9 (2.9)</td>
<td>6 (6.6)</td>
<td>3 (2.7)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NVP</td>
<td>4 (1.3)</td>
<td>4 (4.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AZT</td>
<td>2 (0.7)</td>
<td>2 (2.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1 (0.3)</td>
<td>1 (1.1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Yes</td>
<td>24 (7.9)</td>
<td>10 (11.1)</td>
<td>12 (10.7)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>“Is there enough food in the household?”</td>
<td>Yes</td>
<td>300 (98.0)</td>
<td>87 (95.6)</td>
<td>110 (98.2)</td>
<td>103 (100.0)</td>
</tr>
</tbody>
</table>

Data are presented as n (%) unless otherwise indicated.

a Severe immunodeficiency, defined as CD4 percentage <25% or CD4 count <1500 cps/mm³ below 12 months old, CD4 percentage <20% or CD4 count < 750 cps/mm³ between 12-35 months old and CD4 percentage <15% or CD4 count <350 cps/mm³ above 35 months old.

b CD4 count based on n=218, CD4 percentage based on n=201.

c Viral load based on n=184.

d Main reason for initiation as indicated by clinician.
At first presentation, 72% of participants were in WHO clinical stage 3 or 4 (40% in Kampala, 82% in Fort Portal and 90% in Mbale). Severe immunodeficiency according to a decreased CD4 cell count-for-age was present in 31% of children and in 51% when based on CD4 cell percentage-for-age. Severe immunodeficiency was more prevalent in Kampala compared to Fort Portal and Mbale (Table 2). There was a poor correlation between clinical staging and immunological status: of children in clinical stage 3 or 4, 47% also had severe immunodeficiency according to CD4 cell percentage-for-age.

In children below 5 years of age, the prevalence of weight-for-age z-score < -2 standard deviation (SD) was 43%; height-for-age z-score <-2 SD was found in 62% (Table 3). Of children in clinical stage 3 or 4, 70% had HIV-related malnutrition. The nutritional status of children did not differ significantly between the clinical sites.

Table 3. Nutritional status of the ‘Monitoring of Antiretroviral Resistance in Children’ cohort participants at presentation.

<table>
<thead>
<tr>
<th></th>
<th>&lt; 5 years old</th>
<th>5-12 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight (WAZ &lt; -2 SD)</td>
<td>42.7 (34.6-50.7)</td>
<td>24.5 (15.2-33.7)</td>
</tr>
<tr>
<td>Severe underweight (WAZ &lt; -3 SD)</td>
<td>26.1 (18.9-33.3)</td>
<td>12.8 (5.5-20.0)</td>
</tr>
<tr>
<td>Stunting (HAZ &lt; -2 SD)</td>
<td>62.0 (53.9-70.1)</td>
<td>39.0 (30.4-47.5)</td>
</tr>
<tr>
<td>Severe stunting (HAZ &lt; -3 SD)</td>
<td>40.0 (31.8-48.2)</td>
<td>18.4 (11.5-25.5)</td>
</tr>
<tr>
<td>Wasting (WHZ &lt; -2 SD)</td>
<td>21.7 (14.9-28.4)</td>
<td>NA</td>
</tr>
<tr>
<td>Severe wasting (WHZ &lt; -3 SD)</td>
<td>7.0 (2.7-11.3)</td>
<td>NA</td>
</tr>
<tr>
<td>BMI-for-age z-score (&lt; -2 SD)</td>
<td>16.3 (10.2-22.3)</td>
<td>8.6 (3.6-13.7)</td>
</tr>
<tr>
<td>Mid-upper arm circumference n (%) *</td>
<td>≤ 13.5 cm</td>
<td>55 (37.9)</td>
</tr>
</tbody>
</table>

Data are presented as percentage with 95% confidence interval (CI) unless otherwise indicated. No significant differences were found between clinical sites. Reference data used are WHO Anthro version 3.2.2, January 2011 for age 0-5 and Reference 2007 for age 5-19 [13, 14, 49]. 33 z-scores were excluded from analysis because of biological implausibility (WAZ n = 9, HAZ n = 19, WHZ n = 3, BMI-for-age n = 2). *Mid-upper arm circumference only applicable for children 1-5 years old (n = 148). WAZ, weight-for-age z-score, HAZ, height-for-age z-score, WHZ, weight-for-height z-score, BMI, body mass index (in kg/m²).
Table 4. Risk factors for late disease stage at presentation (WHO stage 3 or 4).

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th></th>
<th>Multivariate analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.99</td>
<td>0.56-1.74</td>
<td>0.963</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-12 years old</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-5 years old</td>
<td>1.20</td>
<td>0.60-2.41</td>
<td>0.612</td>
<td>1.90</td>
</tr>
<tr>
<td>0-2 years old</td>
<td>1.45</td>
<td>0.72-2.92</td>
<td>0.303</td>
<td>2.83</td>
</tr>
<tr>
<td>Current living situation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>With both parents</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>With one parent</td>
<td>1.39</td>
<td>0.71-2.73</td>
<td>0.340</td>
<td>2.05</td>
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<td>With siblings/relatives/other</td>
<td>2.46</td>
<td>1.14-5.31</td>
<td>0.021</td>
<td>3.93</td>
</tr>
<tr>
<td>caregiver/in institution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>0.80</td>
<td>0.22-2.94</td>
<td>0.738</td>
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<tr>
<td>Grandparent</td>
<td>3.07</td>
<td>1.06-8.92</td>
<td>0.039</td>
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<tr>
<td>Other relative/friend</td>
<td>0.92</td>
<td>0.45-1.91</td>
<td>0.826</td>
<td></td>
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<td>Institution</td>
<td>3.09</td>
<td>0.54-17.64</td>
<td>0.203</td>
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</tr>
<tr>
<td>Mother’s health status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sick</td>
<td>1.66</td>
<td>0.49-5.60</td>
<td>0.416</td>
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</tr>
<tr>
<td>Deceased</td>
<td>1.59</td>
<td>0.71-3.58</td>
<td>0.260</td>
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</tr>
<tr>
<td>Unknown</td>
<td>1.10</td>
<td>0.28-4.24</td>
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<tr>
<td>Father’s health status</td>
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</tr>
<tr>
<td>Healthy</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sick</td>
<td>1.27</td>
<td>0.43-3.74</td>
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<tr>
<td>Deceased</td>
<td>1.78</td>
<td>0.82-3.85</td>
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<td>Unknown</td>
<td>2.17</td>
<td>0.81-5.81</td>
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<tr>
<td>Highest level of education of the</td>
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<td>caregiver</td>
<td>Illiterate</td>
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<td>Literate/primary school</td>
<td>0.77</td>
<td>0.32-1.83</td>
<td>0.550</td>
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<td>0.55</td>
<td>0.21-1.44</td>
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<td>Post-secondary school</td>
<td>0.49</td>
<td>0.17-1.45</td>
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<tr>
<td>Occupation of the caregiver</td>
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<td></td>
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<tr>
<td>Wage-employed</td>
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<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Self-employed</td>
<td>1.82</td>
<td>0.78-4.26</td>
<td>0.166</td>
<td>2.50</td>
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<tr>
<td>None/at home/student</td>
<td>3.24</td>
<td>1.45-7.34</td>
<td>0.005</td>
<td>4.26</td>
</tr>
<tr>
<td>PMTCT experienced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>5.07</td>
<td>1.24-20.79</td>
<td>0.024</td>
<td>5.66</td>
</tr>
<tr>
<td>Time between HIV+ diagnosis and ART</td>
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<td></td>
</tr>
<tr>
<td>initiation</td>
<td>0-31 days</td>
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<td></td>
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<tr>
<td></td>
<td>&gt; 30 days</td>
<td>0.93</td>
<td>0.50-1.73</td>
<td>0.818</td>
</tr>
<tr>
<td>Transportation time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 hour</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour or more</td>
<td>0.85</td>
<td>0.45-1.58</td>
<td>0.606</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 2

Table 4. Risk factors for late disease stage at presentation (WHO stage 3 or 4). (continued)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI P-value</td>
<td>OR 95% CI P-value</td>
</tr>
<tr>
<td>Transportation costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile (UGX 500-1500)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd quartile (UGX 1500-3000)</td>
<td>1.07 0.51-2.23 0.857</td>
<td>0.96 0.43-2.14 0.915</td>
</tr>
<tr>
<td>3rd quartile (UGX 3000-4000)</td>
<td>0.89 0.34-2.33 0.818</td>
<td>0.85 0.30-2.38 0.756</td>
</tr>
<tr>
<td>4th quartile (UGX 4000-15000)</td>
<td>2.09 0.85-5.15 0.108</td>
<td>2.51 0.92-6.85 0.072</td>
</tr>
<tr>
<td>Waiting time at clinic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 hours</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2 hours or more</td>
<td>0.83 0.35-1.98 0.676</td>
<td></td>
</tr>
</tbody>
</table>

Multilevel univariate and multivariate logistic regression analysis with random intercepts to examine factors associated with late-disease at presentation (WHO stage 3 or 4), accounting for clustering of observations within sites.

a UGX 500 = $0.18; UGX, Uganda Shilling.

Risk Factors for Late Disease Stage at Presentation: Quantitative Results

Compared to children with early-disease stage at presentation, children with late-disease stage at presentation were more likely to be younger, to have an unemployed caregiver and to have higher transportation costs. They were less likely to be living with both parents or to have a history of uptake of PMTCT services (Table 4). Late-disease stage at presentation was not associated with sex, the caregiver’s health status or education, transportation time, time between HIV-positive diagnosis and ART initiation or waiting time at the clinic. The sensitivity analysis, excluding 22 children in whom MTCT was not confirmed, yielded similar associations (data not shown).

Qualitative Results

Participant Characteristics

Interviews were conducted with 19 health workers and 30 caregivers. Twenty-one caregivers were HIV-infected, six were uninfected and three reported they were unaware of their status. Among the HIV-infected caregivers, sixteen caregivers were in care at JCRC and the remaining caregivers were in care at another clinic. Five health workers were specialized pediatricians; others were clinicians (n=2), nurses (n=6), counselors (n=4) or adherence officers (n=2) with pediatric training. The caregivers included those of children participating in the MARCH study and those of other clinic attendants aged 0-12 years old. All but four adults accompanying a child to the clinic were the primary caregivers. The caregivers were mostly self-employed; six were unemployed.
Barriers to initiation of pediatric HIV treatment in Uganda

Health System Factors: Resources and Organization

Kampala is the only site with a separate pediatric outpatient clinic. All sites have access to local laboratory facilities (including HIV-DNA PCR testing for infants <18 months of age), first- and second-line ARVs, and ready-to-use therapeutic food products (i.e. Plumpy’nut). According to the physician respondents, the clinic’s capital and labor resources are sufficient to take care of all children attending the clinic. Occasional stock-outs of specific drugs or fixed-dose combinations were reported, in which case drugs are borrowed from other clinics or doctors prescribe different formulations to reconstruct the same regimen. When pharmacy stocks are low, ARV prescriptions are given for one month at a time rather than the regular three months.

All doctors were aware that ART should be initiated in infants below the age of two years, irrespective of CD4 count or clinical condition. The general consensus among health workers was that enough qualified personnel are available at the clinic, although the workload is high. There was often insufficient time for thorough counseling, which can result in longer waiting times for patients and caregivers attending the clinic. Health workers evaluated the pediatric HIV care delivered at JCRC as better in comparison to other clinics. However, health workers also noted that it is necessary to spread information about the clinic in the community.

“When we sit here and wait for people to come, we can wait for a long time. We have to go out there and tell about the available services so they can choose to come”

Female counselor, Mbale.

Health workers reported that many children are referred to JCRC after visiting private clinics, local hospitals and sometimes traditional healers or herbalists for recurrent infections. There are no antenatal care (ANC) services at JCRC, and therefore only HIV-infected pregnant women who are already attending the JCRC adult clinic are immediately linked with pediatric care. Referral from external ANC clinics is limited. In Fort Portal, the regional general hospital offering ANC is adjacent to the JCRC clinic, which facilitates referral of HIV-infected pregnant women.

Family-centered care is not routinely offered at JCRC, but health workers encourage parents to bring their other children and family members. Disclosure issues play a role as health workers construct a family tree of the HIV status of the family members and ask to bring in any children with unknown status. When children and caregivers come for HIV testing, they receive a ticket with a number to match their test results. By using this method, people are assured of anonymous testing, thereby reducing fear of
disclosure. Health workers’ recommendations for improving access to care are listed in the Box 1.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Improve antenatal care attendance and linkage to ART clinics.</td>
</tr>
<tr>
<td>ii</td>
<td>Involve men during antenatal care.</td>
</tr>
<tr>
<td>iii</td>
<td>Increase awareness of care and treatment services.</td>
</tr>
<tr>
<td>iv</td>
<td>Improve community outreach for HIV.</td>
</tr>
</tbody>
</table>

**Box 1.** Health workers’ recommendations to improve timely access to ART for children.

**Population Factors: Living Situation and Transport**

Both health workers and caregivers reported that children without parents are living under poorer conditions and present later in care. When the mother is receiving HIV care, the child is more likely to present early. The effect of living in an institution (e.g. orphanage) can go both ways. Some institutions bring their children early because they recognize the importance of HIV testing and can provide transport; others have fewer resources and do not prioritize HIV testing. Health workers pointed out that caregivers’ financial constraints and lack of employment are important barriers to access health care. More highly educated caregivers seem to visit earlier, but taking time off work can be an obstacle. Some caregivers fear to disclose to their employers, and thus have a hard time justifying their absence to attend clinic visits. The unemployed have more time, but lack the money to visit the clinical sites.

“*When the caregiver is employed, they have the advantage of money for transport, but they can be too busy at work to come. For the unemployed it is difficult to pay for transport.*”

Female counselor, Fort Portal.

Transportation costs are prohibitively high when considered in comparison to the need for food. Furthermore, having to travel long distances to reach the clinic makes it difficult for people to leave and return home within one day, especially when no or limited public transport is available. This problem was more frequently reported in Fort Portal and Mbale compared to Kampala.

“*To some people transportation costs matter. For the ones living in the villages; they do not have the income, but they do have time.*”

HIV-positive mother of a five-year-old boy, Fort Portal.

“*My daughter lived in the villages and it was too far for her. That is why I am taking care of her daughter now. The mother is dead now. Just being a housewife, raising money for transport is hard.*”

HIV-negative grandmother of a ten-year-old girl, Mbale.
Population Factors: Knowledge, Stigma and Fear

According to health workers, many women are delivered by traditional birth attendants, and are not tested for HIV during pregnancy. This increases the likelihood that a child’s infection remains unnoticed; the child may only be tested after becoming clinically ill or after the loss of one or both parents. Caregivers have often visited other clinics for the child’s frequent infections before enrolling at the JCRC clinic. Health workers described that health-seeking behavior among caregivers can be delayed due to lack of knowledge or denial of HIV symptoms. Caregivers also reported that HIV is something people do not think about, or do not want to think about. They are often not ready to disclose their or their child’s HIV status to others. Health workers recommended involving men more actively in ANC, in order to improve the uptake of PMTCT measures and enrolment of HIV-exposed children in pediatric HIV care.

“Men need more involvement, include men to PMTCT, now only very few come. They are the biggest decision makers in the home. This would strengthen adherence too.”

Female pediatric counselor, Fort Portal.

Health workers explained that sensitization campaigns by means of radio and advertisements were helpful to spread information about HIV prevention and care, and decrease stigma. At the same time, they acknowledge stigma experienced by caregivers remains an important barrier to care.

“HIV is not a taboo anymore. There used to be a lot of stigma, in the ‘90 and early ‘00. There’s been a lot of sensitization for HIV. You see more and more people test and seek care.”

Male doctor, Mbale.

“HIV is not a taboo anymore, but there’s stigma. People don’t want to associate with HIV. They fear to come to the clinic because someone might see them, which affects adherence. Stigma also delays the start of ART.”

Female counselor, Fort Portal.

Fear was a major factor reported by caregivers as a barrier to visiting ART clinics. People fear to be seen at the clinic and fear that other people get to learn about their HIV status. Fear of disclosure appeared to be more common in the smaller towns compared to the city, as evidenced by the responses from both health workers and caregivers.
“I never got married and feared to tell my mother. She is harsh and will tell everybody to stigmatize me. Nobody knows about my and my daughter’s HIV status. They discriminate you, even at work.”

HIV-positive mother of a three-year-old girl, Mbale.

DISCUSSION

This mixed-methods study examined factors influencing the timing of ART initiation among children attending HIV clinics in Uganda. Even though ART is now free and widely available in Uganda, 72% of the children in this study presented with advanced HIV disease at their initial visit. The main risk factors for this late-disease stage at presentation identified in our study - from both quantitative and qualitative data - included lack of HIV-specific perinatal care, living without parents, financial constraints of the caregiver, caregivers’ unawareness of HIV symptoms, stigma and fear. Our study adds insight into the challenges of identifying HIV-infected infants and children sooner and recruiting them into care. In the setting of the JCRC network of HIV treatment sites in Uganda, linkage to the ANC systems and psychosocial support are recognized as priorities to improve pediatric access. Even though JCRC sites are at the high-end with respect to resources, infrastructure, staff and available diagnostics, late-disease stage at presentation was a frequent and important problem among children initiating ART. The barriers identified in our study are therefore likely of national relevance and applicable to other HIV clinics in Uganda.

Health system factors

The linkage between ANC and pediatric ART clinics was found to be inconsistent. This lack of coordination across services is similar to previous studies investigating barriers to timely pediatric ART initiation in resource-limited settings [21,22]. Failure to diagnose HIV in pregnancy, to provide PMTCT services and to follow-up the HIV-exposed infant represent missed chances for prevention of HIV transmission. As previous research has also shown, integrating antenatal services, PMTCT, early infant diagnosis, and pediatric HIV care greatly improves outcomes for HIV-infected infants in resource-limited settings [23–27].

As ANC is not performed at JCRC, this challenge could be addressed by closer collaboration between JCRC’s Centers of Excellence and outside ANC providers. HIV-infected women should be routinely referred to have their infants tested post-delivery and actively followed-up to ensure they receive the results. Health workers at JCRC have suggested collaborating with traditional birth attendants to reach women who do not visit regular ANC service centers. Additionally, improving male involvement in
ANC was proposed, as men could be decision-makers in seeking care for the child. Studies have shown that male attendance in ANC is a cost-effective strategy to increase PMTCT uptake, and is associated with reduced MTCT and infant mortality [28–30].

We examined the time between HIV test and ART initiation and found that it was increased in older children. It is possible that the first HIV-positive test was performed outside of JCRC, with a subsequent referral delay for ART initiation. Secondly, children might have been tested at JCRC in early-disease stage and started ART later when immunological or WHO stage criteria were met. This is in line with the high numbers of children in care at JCRC in whom ART is not yet initiated (Table 1). Regression analysis showed that lag time between the first HIV diagnosis and ART initiation was not a significant risk factor for late-disease stage at presentation.

Although the clinics in Fort Portal and Mbale have similar resources as the Kampala clinic, the latter was found to have a higher percentage of early-disease stage presenters. The clinic’s urban setting likely contributes to improved accessibility and stigma was reported less frequently in the qualitative study compared to the other sites. Health workers at all JCRC clinics were well-trained and consistently adhered to current pediatric HIV guidelines. The clinics could give more attention to community outreach and active case finding in order to increase parents’ awareness and to identify HIV-infected children before the onset of symptoms. Community outreach could be targeted specifically at the most vulnerable children, such as those in orphanages. Alternatively, outreach could be performed by screening infants at immunization clinics [31].

Rates of underweight and stunted children were alarmingly high, which concurs with previous reports among HIV-infected children in Uganda [32,33]. Malnutrition was a common clinical stage 3 or 4 defining symptom, and therefore timely referral to HIV care is perhaps the most critical nutritional intervention. JCRC routinely provides therapeutic foods. Additionally, nutritional education for caregivers and sufficient supply of micronutrients are important to decrease rates of underweight and stunting [34].

**Population factors**

When examining individual-level barriers to care, the child’s living situation was found to be an important determinant. This corresponds with earlier studies in which orphans were more likely to initiate ART at an older age with lower baseline CD4 levels and more advanced WHO staging [35,36]. In addition, unemployment of the caregiver and high travel costs were risk factors for late-disease stage at presentation.
in quantitative analysis. The qualitative interviews in our study confirmed these socio-economic factors as important barriers, especially in the smaller towns. Prior studies from Uganda and other resource-limited settings have reported similar findings, suggesting that interventions such as community outreach, transportation refunds, home-based ART distribution or outreach clinics in orphanages might be useful in overcoming these issues [37–41].

Other personal factors observed in the interviews were caregivers’ unawareness of HIV symptoms, stigma and fear, confirming that these personal beliefs discourage people from seeking ART [10,21]. In addition to lack of knowledge of HIV symptoms, unawareness of free HIV services also impedes timely presentation [42]. Media campaigns designed to inform people about ANC, HIV testing and free ART for children, could be improved at relatively low cost [43,44].

One of this study’s strengths was the mixed method approach by which quantitative data could be contextualized and confirmed by qualitative study. Methodological triangulation increased the credibility of the findings and enhanced comprehensiveness of the study, creating a deeper understanding of the barriers to initiation of pediatric HIV care [45–48]. A limitation of the study is the cross-sectional design, making it difficult to establish causal relations in the quantitative analysis. In the qualitative study, there is a risk of socially desirable answers during the interviews.

Finally, different types of selection bias may have affected our study. First, late-disease stage at presentation could have been overestimated as many children were referred to JCRC after visiting other clinics; the disease stage at presentation in the referring clinics was not part of our study. Second, our study did not take into account the HIV-infected children that died before reaching the clinic. Seeing as the median age was over 4 years old in our cohort, this population consisted of mostly medium and slow progressors. Younger children appeared to be a risk factor for late-disease stage at presentation but this finding is subject to survival bias and should be interpreted accordingly. Additionally, other risk factors identified might not apply to children with fast disease progression. The specific barriers experienced by these children and their caregivers should be evaluated in longitudinal studies of HIV-exposed children.

In conclusion, although first-line ART has become widely available for HIV-infected children in Uganda, this alone does not ensure timely access. The problem of late-disease stage at presentation requires a multi-factorial approach, prioritizing community and orphanage outreach programs and linkage of ANC systems to ART providers. Knowledge of these factors and their potential solutions is important in
order to help health workers and ART program planners to create interventions to reach HIV-infected infants as early as possible and avoid preventable child mortality.

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FUNDING SOURCE

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REFERENCES


HIV drug resistance among children initiating first-line antiretroviral treatment in Uganda.


AIDS Research and Human Retroviruses.
February 2016, ahead of print.
ABSTRACT

Background
There are limited data on primary human immunodeficiency virus drug resistance (HIVDR) in pediatric populations. This study aimed to assess the prevalence of primary HIVDR and associated risk factors among children initiating first-line antiretroviral therapy (ART) in Uganda.

Methods
At three Ugandan clinics, children (age <12 years) requiring ART were recruited between January 2010 and August 2011. Before starting ART, blood was collected for viral load and pol gene sequencing. Drug resistance mutations were determined using the 2010 International AIDS Society–USA mutation list. Risk factors for HIVDR were assessed with multivariate regression analysis.

Results
Three hundred nineteen HIV-infected children with a median age of 4.9 years were enrolled. Sequencing was successful in 279 children (87.5%). HIVDR was present in 10% of all children and 15.2% of children <3 years. Nucleoside reverse transcriptase inhibitors (NRTIs), non-NRTI (NNRTI), and dual-class resistance was present in 5.7%, 7.5%, and 3.2%, respectively. HIVDR occurred in 35.7% of prevention of mother-to-child transmission (PMTCT)–exposed children, 15.6% in children with unknown PMTCT history, and 7.7% among antiretroviral-naive children. History of PMTCT exposure [adjusted odds ratio (AOR): 2.6, 95% CI: 1.3–5.1] or unknown PMTCT status (AOR: 3.8, 95% CI: 1.1–13.5), low CD4 (AOR: 2.2, 95% CI: 1.3–3.6), current breastfeeding (AOR: 7.4, 95% CI: 2.6–21), and current maternal ART use (AOR: 6.4, 95% CI: 3.4–11.9) emerged as risk factors for primary HIVDR in multivariate analysis.

Conclusion
Pretreatment HIVDR is high, especially in children with PMTCT exposure. Protease inhibitor (PI)–based regimens are advocated by the World Health Organization, but availability in children is limited. Children with (unknown) PMTCT exposure, low CD4 count, current breastfeeding, or maternal ART need to be prioritized to receive PI-based regimens.
INTRODUCTION

The rapid scale-up of antiretroviral therapy (ART) regimens for human immunodeficiency virus (HIV), including prevention of mother-to-child transmission (PMTCT), in resource-limited countries is unprecedented [1–3]. With increased access to PMTCT, the total number of children being born with HIV has significantly decreased. However, those children who do become infected after PMTCT failure are at particular risk of HIV drug resistance (HIVDR), as a result of non–nucleoside reverse transcriptase inhibitors (NNRTIs) used in maternal or pediatric drug regimens [4,5]. Therefore, the World Health Organization (WHO) currently recommends initiating ART with a protease inhibitor (PI)–based regimen in all children younger than 3 years [6].

In Uganda, it is currently estimated that 200,000 children are HIV infected. Of those, 70,000 (35%) are on ART, with the remaining number expected to start ART in the near future. Ugandan guidelines [7,8] recommend providing ART to all HIV-infected children younger than 15 years regardless of CD4 cell count. NNRTI-based regimens are used, while for children younger than 2 years with reported PMTCT exposure, ritonavir-boosted lopinavir (LPV/r)–based regimens are preferred (in contrast to the WHO 2013 consolidated ART guidelines [6]) due to costs and limited availability of PIs. At the time of enrolling participants in this study, the national recommended choice of ART combinations [9] was two nucleoside reverse transcriptase inhibitors (NRTIs) plus one NNRTI, either efavirenz or nevirapine, depending on CD4 count and CD4% threshold for different age categories. Efavirenz was only prescribed to children older than 3 years. For infants exposed to PMTCT and since August 2010 for all PMTCT-exposed children up to 24 months of age [10], a PI-based regimen was prescribed.

The prevalence and patterns of HIVDR with or without PMTCT exposure history in routine programs have not been well described [11]. This is mainly due to the lack of laboratory facilities, affordability, and trained personnel for HIVDR testing [12,13]. Available data in the sub-Saharan settings indicate that use of single-dose nevirapine (sdNVP), male gender, lower baseline immunological profiles, poor adherence, and breastfeeding are important predictors of HIVDR among children who have started on treatment [12–14]. The data on factors associated with the presence of HIVDR besides previous exposure to antiretroviral (ARV) among children before ART initiation are sparse [15,16], yet baseline HIVDR is a critical indicator of the future success of ART as programs aim for universal access. The aim of this study was to evaluate the prevalence of and risk factors for primary HIVDR among newly diagnosed HIV-infected Ugandan children younger than 12 years. This was considered of particular importance in light
of the relatively longer history of ART in Uganda compared to other African countries and the observed high baseline HIVDR prevalence of 13.8% in its adult population [17].

**MATERIALS AND METHODS**

**Study design and population**

Data reported here are based on the baseline assessments of children enrolled into the MARCH (Monitoring Antiretroviral Resistance in Children) study in Uganda. The study was conducted as a multicenter prospective observational cohort of HIV-1–infected children who initiated on ART from early 2010 to August 2011 at the Joint Clinical Research Centre (JCRC) study sites based in Kampala (central region), Fort Portal (western region), and Mbale (eastern region). The JCRC and its Regional Centers of Excellence are the main providers of HIV care and treatment in Uganda, with more than 20 years of experience in providing ART, training in HIV care, conducting clinical trials, and undertaking nationwide rollout of programs in ART care. Site and cohort characteristics have been described elsewhere [18].

Potential participants were informed of the study and screened for eligibility by the study staff at each clinic. Children, aged up to 12 years who initiated ART, were included. Study participants were patients who attended the clinic as identified HIV-infected patients from the Early Infant Diagnosis (EID) national program that only started in late 2009. The older children were mainly self-referrals brought in by parents or guardians, referrals from other healthcare facilities, HIV-infected children from the Centers’ diagnostic services, or children proactively sought by the Centers’ community outreach programs. This program was intended to identify HIV-infected children who were not accessing existing ART programs and link them to treatment.

For those starting first-line regimens, a history of ART (i.e., three-drug regimen or mono/duo therapy) was an exclusion criterion. However, children with previous use of ARVs for PMTCT were included. Children who were failing on first-line therapy and initiating second-line therapy were included in the study, although they are not part of this current cross-sectional analysis. The ethical committees of the JCRC, the Uganda National Council for Science and Technology, and the Academic Medical Center of the University of Amsterdam approved the study protocol. The parent(s)/guardian(s) of all eligible children provided written informed consent. Children older than 8 years who were aware of their HIV status provided written informed assent as per Ugandan research guidelines. Routine sociodemographic, clinical, and laboratory data were
collected using electronic case report forms, which were aggregated in a Web-based data system. Whenever available, the health status and medication use of the mother were also captured.

**Laboratory procedures**

Routine laboratory results, including CD4 cell count and hemoglobin, were obtained from local laboratory records and/or clinical notes. Before initiation of ART, an additional phlebotomy was performed, and EDTA-anticoagulated plasma specimens were stored at −80°C and batch shipped to the JCRC reference laboratory in Kampala for determination of reference HIV RNA, as well as genotypic resistance testing on all specimens with HIV RNA above 1,000 copies/ml. For HIV RNA determination, the COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche, Branchburg, NJ) was used. For HIV-1 genotyping, an in-house sequencing method encompassing the whole of protease and codons 1–300 of reverse transcriptase with a Beckman Coulter CEQ 8000 Analyzer (Beckman Coulter, Inc., Fullerton, CA) was used [19]. Sequences were assembled and manually edited using BioEdit version 7.0. All final sequences were submitted to the ViroScore database (Advanced Biological Laboratories SA, France) for data storage. Drug resistance mutations (DRMs) were scored according to the 2010 International AIDS Society–USA list [20]. Subtypes were determined using the SCUEAL HIV-1 subtyping tool [21] and additional analysis with the REGA algorithm version 2.0 [22] once required.

**Statistical analysis**

Group comparisons for categorical data were performed using the chi-square or Fisher’s exact tests and for continuous data using the Wilcoxon rank-sum test. Nutritional status was assessed by means of the WHO Child Growth Standards: WHO Anthro version 3.2.2 (age 0–5 years) and WHO Reference 2007 for height and weight (age 5–19 years) [23–25]. Immunodeficiency for age was classified according to the 2010 WHO guidelines; children were considered immunodeficient if they had a CD4 cell percentage <25% for those younger than 5 years and a CD4 cell count <350 cells/mm³ for the ones older than 5 years [10]. A second immunodeficiency threshold using total lymphocyte count (TLC) was based on the 2006 WHO guidelines using the following cutoffs: TLC <4,000 cells/mm³ for those younger than 11 months, TLC <3,000 cells/mm³ for those between 12 and 35 months, TLC <2,500 cells/mm³ for those between 36 and 59 months, and TLC <2,000 cells/mm³ for those older than 5 years [26].

Univariate and multivariate logistic regression was performed to identify factors associated with the presence of at least one drug resistance mutation at initiation of first-line ART. Explanatory variables considered in the analysis were age, sex, previous
ARV exposure (ARV naive, PMTCT exposed, or unknown), immunodeficiency for age, breastfeeding (past or current), maternal ART usage, maternal previous ARV use, WHO clinical stage, weight-for-age, height-for-age z-scores, and HIV RNA load. Explanatory variables that were associated with the outcome variables (p < .10) in univariate analysis were forwarded to the multivariate model using a step forward procedure. Biologically plausible interactions were examined. Results were expressed as ORs with 95% confidence intervals (CIs) and p values, with p < .05 regarded statistically significant. Analyses were performed using the statistical software package STATA version 10 (STATA Corp LP, College Station, TX). All statistical inferential frameworks were based on the two-sided p value, and statistical significance was based on the 5% error rate.

RESULTS

Between January 2010 and August 2011, 372 children at three JCRC sites were enrolled into the study, of which 319 initiated first-line ART regimens. Recruited patients had a median age of 4.9 years [interquartile range: 2.3–9.0], and half (49.8%) were female. Most children had history of prior breastfeeding (69.9%) or current breastfeeding at the time of screening (8.2%). Current ART use was reported by 81 (28.3%) mothers, and 17 (6.1%) mothers had received drugs for PMTCT for previous pregnancies and/or pregnancy of the child who participated in the MARCH study. Most children (83.5%) had no history of PMTCT exposure, and in 11.5% of children, PMTCT exposure was unknown. Of the children with unknown PMTCT exposure, 38% had lost both their parents. Among children with reported PMTCT history (n = 14, 5%), eight had received sdNVP and six had received extended-course prophylaxis. Thirty-seven (13.3%) children were orphans, and 156 (55.9%) had mothers as their primary caregivers. Most (71.3%) patients had advanced disease (WHO stages 3 and 4) at screening, and CD4% or cell count for age was below treatment threshold (24–59 months CD4 < 750/CD4% < 25% and >5 years CD4 ≤ 350) among 60.6%, according to the WHO 2010 guidelines [10].

Eighteen children had a viral load <1,000 copies/ml at baseline. HIV sequencing was successfully performed in 279 children (87.5%, Fig. 1). At baseline, the prevalence of any DRM was 28/279 (10.0%) among this population and 14/92 (15.2%) among children <3 years of age. Baseline characteristics are summarized in Table 1, stratified by the presence of any DRMs. Most (53.8%) patients had HIV-1 subtype A, 29.4% had HIV-1 subtype D, and 3.6% had HIV-1 subtype C or G, yet 13.3% had circulating recombinant forms or unique recombinant forms.
Table S1 (Supplementary Data are available online at www.liebertpub.com/aid) and Figure 2 present the mutational patterns among the enrolled patients initiating first-line ART stratified by previous ARV exposure. Overall, any mutation was found in 10.0% of children: In children with PMTCT exposure, with unknown PMTCT history, or who reported ARV naive, HIVDR was present in 35.7%, 15.6%, and 7.7%, respectively. In children younger than 3 years, the overall rate of any HIVDR mutation was 15.2% (14/92): In young children with PMTCT exposure, with unknown PMTCT history, or who reported ARV naive, HIVDR was present in 38.5% (5/13), 20% (2/10), and 10.1% (7/69), respectively. NRTI mutations were detected among 16/279 (5.7%) patients—10 of whom were ARV-naive patients, three were PMTCT-exposed patients, and three patients had unknown ART exposure status. Thymidine analogue mutations were detected among 8/279 (2.9%) patients—six of whom were ARV-naive patients, yet two had an unknown ART exposure. Most prevalent NRTI mutations found were M184V/I (n = 11) and K219Q (n = 4). NNRTI mutations were detected among 21/279 (7.5%) patients—13 of whom were ARV-naive patients, four were PMTCT-exposed patients, and four patients had unknown ART exposure status. Most prevalent NNRTI
Table 1. Baseline Characteristics of Children Initiating First-Line ART, Stratified by the Presence of any Drug Resistance Mutations

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 279)</th>
<th>No DRM (n = 251)</th>
<th>At least one DRM (n = 28)</th>
<th>p</th>
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<td>Age—median [IQR]</td>
<td>4.9 [2.3–9.0]</td>
<td>5.0 [2.4–9.1]</td>
<td>3.0 [1.0–7.0]</td>
<td>0.037a</td>
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<td>&lt;2 years</td>
<td>65 (23.3)</td>
<td>55 (21.9)</td>
<td>10 (35.7)</td>
<td>0.101b</td>
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<td>≥2 years</td>
<td>214 (76.7)</td>
<td>196 (78.1)</td>
<td>18 (64.3)</td>
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<tr>
<td>Sex, female</td>
<td>139 (49.8)</td>
<td>125 (49.8)</td>
<td>14 (50.0)</td>
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<td>WHO clinical stage 3 or 4</td>
<td>199 (71.3)</td>
<td>182 (72.5)</td>
<td>17 (60.7)</td>
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<td>Weight-for-age z-score&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Underweight (&lt;−2 SD)</td>
<td>82 (35.5)</td>
<td>72 (35.0)</td>
<td>10 (40.0)</td>
<td>0.618b</td>
</tr>
<tr>
<td>Severe (&lt;−3 SD)</td>
<td>47 (20.4)</td>
<td>40 (19.4)</td>
<td>7 (28.0)</td>
<td>0.314b</td>
</tr>
<tr>
<td>Height-for-age z-score&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stunting (&lt;−2 SD)</td>
<td>139 (52.5)</td>
<td>120 (50.4)</td>
<td>19 (70.4)</td>
<td>0.049b</td>
</tr>
<tr>
<td>Severe (&lt;−3 SD)</td>
<td>81 (30.6)</td>
<td>68 (28.6)</td>
<td>13 (48.2)</td>
<td>0.036b</td>
</tr>
<tr>
<td>CD4 cell count (in ≥5 year)</td>
<td>362.5 [229–695]</td>
<td>404 [249–695]</td>
<td>169 [56–506]</td>
<td>0.076a</td>
</tr>
<tr>
<td>CD4% or cell for age, below treatment threshold</td>
<td>106 (39.4)</td>
<td>99 (40.7)</td>
<td>7 (26.9)</td>
<td>0.171b</td>
</tr>
<tr>
<td>Viral load (log&lt;sub&gt;10&lt;/sub&gt;)</td>
<td>5.2 [4.6–5.6]</td>
<td>5.1 (4.6–5.6)</td>
<td>5.4 (4.6–5.8)</td>
<td>0.459a</td>
</tr>
<tr>
<td>Previous ARV exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None/ARV naive</td>
<td>233 (83.5)</td>
<td>215 (85.7)</td>
<td>18 (64.3)</td>
<td>0.002b</td>
</tr>
<tr>
<td>PMTCT&lt;sup&gt;g&lt;/sup&gt;</td>
<td>14 (5.0)</td>
<td>9 (3.6)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>32 (11.5)</td>
<td>27 (10.8)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
<tr>
<td>Primary caregiver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>156 (55.9)</td>
<td>136 (54.2)</td>
<td>20 (71.4)</td>
<td>0.483b</td>
</tr>
<tr>
<td>Father</td>
<td>19 (6.8)</td>
<td>18 (7.2)</td>
<td>1 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Both parents died</td>
<td>37 (13.3)</td>
<td>35 (13.9)</td>
<td>2 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>67 (24.0)</td>
<td>62 (24.7)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, past</td>
<td>195 (69.9)</td>
<td>181 (72.1)</td>
<td>14 (50.0)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Yes, current</td>
<td>23 (8.2)</td>
<td>15 (6.0)</td>
<td>8 (28.6)</td>
<td></td>
</tr>
<tr>
<td>None or unknown</td>
<td>61 (21.9)</td>
<td>55 (21.9)</td>
<td>6 (21.9)</td>
<td></td>
</tr>
<tr>
<td>Mother current ART</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-line ART</td>
<td>74 (26.5)</td>
<td>61 (24.3)</td>
<td>13 (46.4)</td>
<td>0.021b</td>
</tr>
<tr>
<td>Second-line ART</td>
<td>5 (1.8)</td>
<td>3 (1.2)</td>
<td>2 (7.1)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>160 (57.3)</td>
<td>150 (59.8)</td>
<td>10 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>40 (14.3)</td>
<td>37 (14.7)</td>
<td>3 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Mother previous ART</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, PMTCT</td>
<td>17 (6.1)</td>
<td>15 (6.0)</td>
<td>2 (7.1)</td>
<td>0.041b</td>
</tr>
<tr>
<td>Yes, ART</td>
<td>3 (1.1)</td>
<td>1 (0.4)</td>
<td>2 (7.1)</td>
<td></td>
</tr>
</tbody>
</table>
HIV drug resistance among children in Uganda

Table 1. Baseline Characteristics of Children Initiating First-Line ART, Stratified by the Presence of any Drug Resistance Mutations (continued)

<table>
<thead>
<tr>
<th>HIV-1 subtype</th>
<th>Total (n = 279)</th>
<th>No DRM (n = 251)</th>
<th>At least one DRM (n = 28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>139 (50.0)</td>
<td>124 (49.4)</td>
<td>15 (53.6)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>120 (42.9)</td>
<td>111 (44.2)</td>
<td>9 (32.1)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>150 (53.8)</td>
<td>136 (54.2)</td>
<td>14 (50.0)</td>
<td>0.848b</td>
</tr>
<tr>
<td>D</td>
<td>82 (29.4)</td>
<td>74 (29.5)</td>
<td>8 (28.6)</td>
<td></td>
</tr>
<tr>
<td>C or G</td>
<td>10 (3.6)</td>
<td>9 (3.6)</td>
<td>1 (3.6)</td>
<td></td>
</tr>
<tr>
<td>CRF or URF</td>
<td>37 (13.3)</td>
<td>32 (12.8)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as n (%) or median [interquartile range].

*Wilcoxon rank-sum test.

Chi-square test.

Some % may not add up to 100% because of missing data for some participants.

*Weight-for-age calculated for ages 0–10 years only, n = 231.

*Height-for-age, n = 265.

*CD4% in <5-year-olds, n = 135; CD4 count in ≥5-year-olds, n = 134; treatment threshold based on WHO 2010 guidelines.

*PMTCT consisted of single-dose nevirapine (n = 7), an extended course of nevirapine (n = 6), sometimes in combination with zidovudine (n = 2), or was unknown (n = 1).

*Fisher’s exact test.

ARV, antiretroviral; ART, antiretroviral therapy; CRF, circulating recombinant form; DRM, drug resistance mutation; PMTCT, prevention of mother-to-child transmission; SD, standard deviation; URF, unique recombinant form; WHO, World Health Organization.

mutations found were K103N (n = 11), Y181C (n = 9), and G190A (n = 6). Dual-class mutations were found among 9/279 (3.2%) patients.

Table 2 presents the demographic and clinical factors associated with HIVDR at initiation of first-line ART in a multivariate analysis. At multivariable level, factors, which significantly were associated with the presence of any DRMs, were previous ARV exposure, low CD4 count or percentage, breastfeeding status, and maternal ART status. Patients with prior PMTCT exposure were almost three times [adjusted OR (AOR): 2.6, 95% CI: 1.3–5.1] more likely, yet patients with unknown ART exposure were almost four times (AOR: 3.8, 95% CI: 1.1–13.5) more likely, to have any DRMs compared to those with no previous ART exposure, controlling for age, sex, CD4 count or percentage, breastfeeding status, and maternal ART status. Patients with CD4 count or percentages below treatment thresholds were twice (AOR: 2.2, 95% CI: 1.3–3.6) more likely to have any DRMs compared to those with CD4 count or percentages above treatment thresholds, adjusting for age, sex, their previous ARV exposure, breastfeeding status, and maternal ART status.
Compared to patients who had been breastfed in the past, patients who were still being breastfed at the time of study were seven times (AOR: 7.4, 95% CI: 2.6–21.0) more likely, yet patients whose breastfeeding status was unknown or who reported never to have breastfed were twice (AOR: 1.9, 95% CI: 1.5–2.3) more likely, to have any DRMs, after controlling for age, sex, their previous ARV exposure, CD4 count or percentage, and maternal ART status. Patients whose mothers were currently on ART were six times (AOR: 6.4, 95% CI: 3.4–11.9) more likely, whereas those whose mothers’ current ART status was unknown or whose mothers were reported to be deceased were 1.4 times (1.4, 95% CI: 0.8–2.3) more likely, to have any DRMs compared to those whose mothers reported not to be taking any ART, after controlling for age, sex, CD4 count or percentage, breastfeeding status, and previous ARV exposure.

**DISCUSSION**

This study assessed HIVDR mutations among Ugandan HIV-infected children <12 years of age initiating ART between January 2010 and August 2011. Given the study setup, the overall rate of HIVDR was 10.0% among all children and 15.2% among children <3 years old, with age significantly associated with having at least one DRM.
The HIVDR rates in this study are much higher than previously reported rates of transmitted drug resistance (TDR) in Uganda and Cameroon for similar age groups [27,28] and lower than recent rates shown in younger children in Kenya, South Africa, and Zimbabwe with much higher PMTCT exposure rates, although using HAART [11,14,29]. As in adults, the TDR rate is expected to increase over time with increasing coverage and uptake of PMTCT. The finding of lower rates of HIVDR in a limited number of other African pediatric cohorts is explained by the fact that our cohorts represent relatively low percentages of children with previous PMTCT exposure (sd-NVP). Moreover, HIVDR mutations might be archived [30–32] and not be detectable with the RNA-based technology of genotyping used since the average age of children

<table>
<thead>
<tr>
<th>Demographic and Clinical Factors Associated with HIVDR at Initiation of First-Line ART</th>
<th>No. of sequences</th>
<th>No. with any DRM</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5 years</td>
<td>138</td>
<td>9</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>141</td>
<td>19</td>
<td>2.2 (1.1–4.7)</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>139</td>
<td>14</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>140</td>
<td>14</td>
<td>1.0 (0.5–2.0)</td>
<td>0.983</td>
</tr>
<tr>
<td><strong>Previous ARV exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>233</td>
<td>18</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>PMTCT exposed</td>
<td>14</td>
<td>5</td>
<td>6.6 (1.0–42.1)</td>
<td>0.045</td>
</tr>
<tr>
<td>Unknown</td>
<td>32</td>
<td>5</td>
<td>2.2 (1.3–4.2)</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>CD4 count or %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above treatment threshold</td>
<td>106</td>
<td>7</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Below treatment threshold</td>
<td>165</td>
<td>19</td>
<td>1.9 (1.5–2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Breastfeeding</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>195</td>
<td>14</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Current</td>
<td>23</td>
<td>8</td>
<td>6.9 (1.7–27.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>None/unknown</td>
<td>61</td>
<td>6</td>
<td>1.4 (0.9–2.3)</td>
<td>0.168</td>
</tr>
<tr>
<td><strong>Maternal ART</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>86</td>
<td>6</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Current ART</td>
<td>79</td>
<td>15</td>
<td>3.1 (1.4–7.1)</td>
<td>0.007</td>
</tr>
<tr>
<td>Mother deceased/unknown</td>
<td>114</td>
<td>7</td>
<td>0.9 (0.5–1.4)</td>
<td>0.582</td>
</tr>
</tbody>
</table>

Multivariate logistic regression with robust standard errors. Data are given as odds ratio (95% CI). CD4 count or % treatment threshold based on WHO 2010 guidelines. Maternal previous ARV use, WHO clinical stage, weight-for-age, height-for-age, and HIV RNA load at ART initiation not associated in univariate analysis.

CI, confidence interval; HIVDR, HIV drug resistance.
included in our studies is higher. The decline in mutations is a function of time since ART exposure and reported rates may be an underestimation.

Overall, a trend of increasing HIVDR was detected in children who were reportedly ART naive (7.7%) versus those with unknown ART exposure (15.6%) versus PMTCT-exposed children (35.7%). Although higher rates were observed, similar trends were seen in children <3 years of age (92 of 279 children overall), with rates of 10.1%, 20%, and 38.5%, respectively. These data support classifying children with unknown PMTCT exposure as PMTCT exposed. In addition, children with unknown exposure were more likely to be orphans, and ascertainment of PMTCT exposure was difficult.

Children who were currently breastfeeding or whose mothers were currently on ART at the time of enrollment were more likely to have DRMs. Children with no/unknown history of breastfeeding had higher odds of having baseline resistance compared to past history of breastfeeding; hence, this group of children should be considered as high risk for DRMs. These data also underscore the fact that taking breastfeeding history is difficult and not so reliable, especially in cases where the mother is deceased (26.5%). The average age at study entry was 3 years for patients with at least one DRM versus 5 years for those without DRMs. This further supports classifying these categories of children as a broader definition of ART/PMTCT exposed as they are likely to respond better to PI-based regimens. The WHO ART guidelines 2013 [6] recommend lopinavir-based regimen for all children <3 years of age irrespective of PMTCT exposure. However, current Uganda guidelines [8] and other developing countries still have NNRTI-based regimen as the preferred first-line treatment for children aged <3 years and may be exposing many children younger than 3 years with known and unknown ARV exposure to suboptimal therapy.

HIVDR in ARV-naive children was higher among children <3 years, with overall and NNRTI-specific mutation rates of 10.1% and 7.3%, respectively, compared to 6.7% and 4.9% among children ≥3 years. This rate is much lower compared to other settings, where NNRTI mutations were up to 24% in ART-naive children [11]. High rates of NNRTI mutations were observed in children with unknown history of PMTCT, with 10% in children <3 years compared to 13.6% in children ≥3 years. Among all children not exposed to PMTCT (none and unknown), NNRTI mutations were detected among 7.59% of children <3 years compared to 5.91% of children ≥3 years who would be started on a suboptimal NNRTI-based ART regimen. These data provide more support for the WHO recommendations for empiric PI-based first-line ART in children <3 years.
While PI-based regimens are considered the most appropriate regimen for infants and young children by the WHO, providing these regimens to all infants and children <3 years in some resource-limited settings, such as Uganda, may be challenging. Currently, pediatric LPV/r is only available as a liquid formulation with a high alcohol content that tastes terrible with the potential for suboptimal adherence and requires cold chain until the point of dispensing. Pediatric HIV treatment is complicated, and dosing strategies change as the child grows from infancy to adolescence. Furthermore, LPV/r is costly, and administering this with TB treatment is complex. Efforts to overcome some of these obstacles include replacement of this liquid formulation with a more tolerable version that is palatable and does not require refrigeration. Possible formulations include sprinkles, minitabs, or granules that could be mixed with food or given with breast milk [33].

The most common mutation, which was present in 3.9% of the samples, was M184V, which confers resistance to the NRTI drugs lamivudine, emtricitabine, and abacavir but delays resistance to zidovudine and stavudine [34,35]. The most common NNRTI mutations were K103N (3.9%), Y181C (2.9%), and G190A (2.2%), which confer resistance to efavirenz, nevirapine, and delavirdine. However, 3.2% of the children had dual-class resistance. These mutations are similar to what has been observed from other studies [11,27,29,36]. These findings suggest that genotypic resistance is fairly common among HIV-infected children starting ART in Uganda and may adversely affect response to first-line treatment in addition to other risk factors, which are unique to children.

The study had some limitations. This study is different in approach from the newly recommended pediatric HIVDR surveys by the WHO [37] Specifically, the enrolled children were older (median age, 4.9 years) than recommended by the WHO (<18 months) and are a survivor cohort since the majority of HIV children will die by age 2 years if not treated with ARV drugs [38]. In addition, the majority of children recruited at this time were reportedly PMTCT unexposed (233/279, 83.5%), while this will be less likely the case in more recent pediatric HIV surveys when adoption of option B+ for PMTCT is more widespread. Given the age group and PMTCT exposure, caution should be applied when directly comparing these results with (future) pediatric HIVDR surveys according to the new WHO guidelines.

We recruited older children than most studies that have evaluated TDR in children, and the observed HIVDR may be an underestimate of the actual rates due to archived DRMs. More sensitive assays may be able to help address this issue. We were not able to determine drug resistance in the mothers to link the DRMs to the resistance patterns
in their children. With revised pediatric guidelines and expansion of EID programs, increasingly younger children will be recruited on treatment in the future. However, current ART access for children is only 35% in sub-Saharan Africa [39], and there are still many older children who are yet to be put on treatment in the coming years as per the 2013 WHO guidelines. These data are relevant for their management and selection of optimal ART regimens. The patients in the study were recruited before the era of intensive PMTCT, with the majority of women using either sdNVP or double therapy and limited access to HAART for their general care. However, the study was able to show that PMTCT, maternal ART, and breastfeeding are still important risk factors for TDR in this cohort.

The strength of the study is that this is an observational study that recruited participants who would normally be seeking for treatment at these ART centers, and therefore, these results are directly applicable to programs. Detailed demographic, clinical, and programmatic data were collected to determine TDR and related risk factors, and the study was conducted in at least three regions of the country making it more representative.

In conclusion, this study demonstrated that HIVDR is common, especially in HIV-infected children of younger age and those exposed to ART through PMTCT, maternal ART, and breastfeeding. Even children with unknown PMTCT exposure had high rates of DRMs. This implies that just taking history of PMTCT does not clearly delineate children at risk of having DRMs. The broader definition of PMTCT exposure needs to include those children with unknown history as well as those with mothers on ART and/or current breastfeeding who need to be prioritized to receive PI-based regimens according to the new WHO guidelines.

In our setting, children without risk factors may still respond well to regimens without PIs, which is more practical to implement than PI-based regimens. Follow-up data from this cohort will show what the true outcome is of these children.

This study was done before the shift to option B/B+, and our results cannot predict resistance in this group. However, with rollout of option B+, the total number of infections should decrease with proper taking of drugs, and breakthrough infections possibly due to nonadherence of the mother may have more DRMs. However, when vertical transmission takes place in the presence of ARV drugs, the risk of HIVDR increases, as shown in this study. This highlights the need for in-depth evaluation and more extensive surveys among the infected children in the era of expanded PMTCT.
The availability of these data at relevant times would significantly inform clinical management and promote improved quality of life.

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GenBank accession numbers: All HIV-1 pol sequences in this study have been deposited in the GenBank under the following accession numbers: KT347880–KT348210.

AUTHOR CONTRIBUTIONS

C.K. is the principal investigator. C.K., T.F.R.d.W., P.N.M., K.C.E.S., and J.C.J.C. designed the study and developed the protocol. C.K., L.N.K., E.K., V.M., A.M., and M.K. established the cohort and supervised data collection. J.K. and T.S.B. supervised data management. C.K., K.C.E.S., J.K., and T.F.R.d.W. analyzed the data, interpreted it, and drafted the article. All authors reviewed and approved the final version of the article.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.
REFERENCES


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High levels of HIV drug resistance in treatment-naïve children in Lagos, Nigeria: original data and a systematic review in sub-Saharan Africa.


* Both first and both senior authors contributed equally to the work.

Submitted.
Chapter 4

ABSTRACT

Background
HIV-infected children are at risk of HIV drug resistance (HIVDR) prior to antiretroviral treatment (ART) initiation. Data on pediatric HIVDR prevalence, especially from sub-Saharan Africa, are scarce.

Methods
HIV-1 infected antiretroviral drug (ARV)-naïve children ≤12 years were enrolled at the Lagos University Teaching Hospital, Nigeria. Pre-ART viral load and population based pol genotypic testing was performed. HIVDR mutations were identified using the World Health Organization list for transmitted drug resistance. We conducted a systematic review and meta-analysis on pre-treatment HIVDR prevalence in children in sub-Saharan Africa.

Results
Thirteen of 82 (15.9%) ARV-naïve Nigerian children had pre-treatment HIVDR. All 13 harbored non-nucleoside reverse-transcriptase inhibitor (NNRTI) mutations, of whom seven also had nucleoside reverse-transcriptase inhibitor (NRTI) resistance. No protease inhibitor (PI) mutations were detected. We included 16 studies from 11 different African countries (2,057 children) in a meta-analysis. The pooled pre-treatment HIVDR-prevalence among ARV-naïve children was 10.8% (95%CI: 4.4-17.1). Meta-regression showed an increase in prevalence from 0.6% (95%CI 0.6-5.4) in 2004 to 36.2% (95%CI 25.5-46.9) in 2011 (p=0.05).

Conclusion
One in six Nigerian children had HIVDR. This high rate corroborates with other African data, which indicate a significant pre-treatment HIVDR increase over the past decade. Our findings stress the importance of PI-based regimens in all children <3 years of age. Overcoming practical barriers to implement PI-based regimens, and introduction of a population-based HIVDR surveillance system among children should receive priority to ensure optimal treatment for HIV-infected children in sub-Saharan Africa.
INTRODUCTION

Since the roll-out of antiretroviral treatment (ART) in sub-Saharan Africa in the beginning of this millennium, ART coverage has increased substantially. Nearly 12 million people in low- and middle-income countries (LMIC) were receiving ART at the end of 2013, including 740,000 children [1]. Coverage of prevention of mother-to-child transmission (PMTCT) programs has also increased, and new PMTCT strategies, including lifelong ART for pregnant women (option B+), are currently being implemented [1].

The increased usage of ART, however, is likely to come at a cost, as the levels of HIV drug resistance (HIVDR) are expected to rise [2,3]. Due to the scale-up of PMTCT, the absolute number of children born with HIV will decrease. When PMTCT is unsuccessful, however, children are prone to develop HIVDR, because of intra-uterine or perinatal exposure to antiretroviral drugs (ARV), usually containing a non-nucleoside reverse transcriptase inhibitor (NNRTI) [4,5].

The most recent World Health Organization (WHO) ART guidelines recommend all children under three years of age to start protease inhibitor (PI)-based treatment, regardless of previous PMTCT exposure [6]. In Nigeria, as in many other LMIC, national guidelines recommend PI-based treatment only for children under three years who are known to be PMTCT-exposed, because of high costs and logistic barriers of PI-treatment. All other children are recommended to start NNRTI-based treatment [7]. Determining the rate of resistance against NNRTIs will help answering the question to what extent this policy is still defendable.

Monitoring pre-treatment HIVDR is especially important in children as they have fewer ART options than adults, and will require ART for more years. However, data on HIVDR in African children are scarce [8]. This study documents the prevalence of HIVDR before treatment initiation in ARV-naïve HIV-infected children in Lagos, Nigeria, the country with the second highest number of people living with HIV worldwide: 3.2 million, of whom 260,000 are children [9]. Additionally, we conducted a systematic review and meta-analysis of the literature on pre-treatment HIVDR in children in sub-Saharan Africa. The outcomes of this review put the findings of our study into perspective and estimate the extent and trends of HIVDR since large-scale implementation of ART in sub-Saharan Africa.
METHODS

Study design and population
This study forms part of the Monitoring Antiretroviral Resistance in Children (MARCH) cohort, an observational prospective cohort study conducted in Nigeria and Uganda. HIV-infected children, eligible for ART, were enrolled at the pediatric HIV clinic and through HIV screening at the emergency ward of the Lagos University Teaching Hospital, Nigeria.

Inclusion criteria were: age \( \leq 12 \) years, confirmed HIV-1 test (positive HIV antibody test if age >18 months, or positive HIV nucleic acid PCR if age \( \leq 18 \) months), eligibility for initiation of first-line ART according to national guidelines (all HIV-infected children <2 years of age, CD4 count <750 cells/m\(^3\) in children 2-5 years, and CD4 count <350 cells/ mm\(^3\) in children >5 years) [10], and written informed consent by the parent or guardian. If the child was eight years or older and had disclosed HIV status, assent was required. Exclusion criteria were: HIV-2 co-infection, anticipated non-compliance with the protocol, and current participation in another study or clinical trial. All children received routine care according to national pediatric HIV treatment guidelines [10]. Clinical and socio-demographic data of mother and child, and laboratory (hematology, immunology, virology) data of the child were collected on standardized case report forms. Previous PMTCT exposure was documented as reported in the child’s medical files or reported by the child’s caregiver. For the current analysis of our cohort study, we included only ARV-naïve children, that is, children without any prior exposure to ART or PMTCT. All data were source-data-verified by monitors and transferred to a study-specific database. Programmed queries were used to rule out common data errors and inconsistencies.

Laboratory methods
Before ART initiation, a study blood sample (6 mL EDTA tube) was collected for HIV viral load testing using the Roche Cobas AmpliPrep TaqMan® (Cobas Amplicor; Roche Diagnostics, Switzerland). If viral load was >1,000 cps/ml, population based sequencing of the HIV-1 pol gene was performed by the reference laboratory of the Institute of Human Virology in Abuja, Nigeria, using an in-house method and primers designed and optimized for subtype CFR02_AG and G. Genotypic sequence data were submitted to ViroScore® [11]. Major drug-resistance mutations were identified based on the 2009 WHO list for surveillance of transmitted resistance [12] using the Stanford Calibrated Population Resistance analysis tool[13]. Susceptibility of the prescribed ART regimen was determined through calculation of the genotypic sensitivity score (GSS) using the Stanford algorithm (Version 7.0) [14]. Reduced susceptibility to
the prescribed regimen was defined as GSS <3; i.e. <3 fully susceptible drugs. HIV-1 subtyping was performed using the REGA HIV-1 subtyping tool V3 [15]. The study has received ethical clearance from the Health Research & Ethics Committee of the Lagos University Teaching Hospital, and was conducted in compliance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. All laboratory procedures were conducted according to Good Laboratory Practice guidelines.

**Systematic review**

A literature review was conducted to identify relevant studies reporting on the prevalence of pediatric pre-treatment HIVDR in sub-Saharan Africa. A search was done in Medline through PubMed using the search terms ‘transmitted’, ‘pre-treatment’, ‘naïve’, or ‘primary’ in combination with ‘drug resistance’, ‘hiv’ and ‘child’ or ‘infant’. Additionally, the conference abstracts of the last two editions of the Conference on Retroviruses and Opportunistic Infections (2014-2015), the IAS (2013-2014), and the International Workshop on HIV Pediatrics (2013-2014) were electronically searched for relevant studies using the search term ‘resistance’. The references of retrieved studies were screened for any additional relevant articles using snowballing techniques. Article selection and data extraction was performed by two individual reviewers (RB and JC), and discrepancies were resolved by discussion between both reviewers.

We searched for original studies reporting pre-treatment HIVDR prevalence in children with a median age ≤12 years, in any country in sub-Saharan Africa [16]. We excluded articles in which only a selection of specific mutations were genotyped, articles reporting a very small sample size (<20 patients), and articles in which HIVDR was not reported separately for children and adults. The HIVDR prevalence was extracted separately for PMTCT-exposed and -unexposed children, and by drug class (NNRTI, NRTI or PI), if reported. If only the HIVDR prevalence per drug class was reported, we used the prevalence of NNRTI resistance as a conservative estimate of the total HIVDR prevalence.

**Statistical analysis**

Patient characteristics were summarized for children with and without pre-treatment HIVDR separately. Continuous variables were analyzed using a student’s t-test or Mann-Whitney U test, and categorical variables using a $\chi^2$ test or Fisher’s exact test; 95% confidence intervals were calculated using the exact method. Nutritional status was assessed using WHO Anthro (version 3.2.2, January 2011) for children <5 years and WHO Reference 2007 for children ≥5 years [17]. Weight-for-age z-scores and weight-for-height z-scores were only calculated for children <10 years and <5 years of age, respectively.
Univariate and multivariate logistic regression was performed to identify factors associated with pre-treatment HIVDR. Explanatory variables considered in the analysis were: age, sex, WHO clinical stage, nutritional status, hemoglobin level, immunodeficiency for age (defined as CD4 percentage<25% in children under five years of age, and CD4 count<350 cells/ml in children of five years and older), viral load, HIV-1

Table 1. Population characteristics of 90 included children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>No HIV drug resistance</th>
<th>HIV drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 90</td>
<td>%</td>
<td>N = 69</td>
</tr>
<tr>
<td>Age (years (median, IQR))</td>
<td>4.6 (1.8-8.4)</td>
<td>4.5 (1.7-8.7)</td>
<td>4.8 (2.5-6.3)</td>
</tr>
<tr>
<td>&lt;18 months</td>
<td>19/90</td>
<td>21.1</td>
<td>15/69</td>
</tr>
<tr>
<td>&lt;3 years</td>
<td>35/90</td>
<td>38.9</td>
<td>28/69</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>41/90</td>
<td>45.6</td>
</tr>
<tr>
<td>WHO clinical stage</td>
<td>III or IV</td>
<td>55/90</td>
<td>61.1</td>
</tr>
<tr>
<td>Nutritional status</td>
<td>Stunted, HAZ&lt;-2</td>
<td>21/72</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>Wasted, WHZ&lt;-2*</td>
<td>12/36</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>Underweight, WAZ&lt;-2**</td>
<td>23/78</td>
<td>29.5</td>
</tr>
<tr>
<td>Hemoglobin g/dL (mean, SD)</td>
<td>9.8 (1.5)</td>
<td>9.7 (1.3)</td>
<td>10.7 (2.2)</td>
</tr>
<tr>
<td>CD4+ cell percentage*</td>
<td>14.9 (8.1-26.1)</td>
<td>16.2 (8.5-26.9)</td>
<td>12.9 (7.9-25.1)</td>
</tr>
<tr>
<td>CD4+ cell count**</td>
<td>395 (137-618)</td>
<td>370 (137-662)</td>
<td>454 (289-587)</td>
</tr>
<tr>
<td>HIV RNA load log10/ml (median, IQR)</td>
<td>5.2 (4.7-5.9)</td>
<td>5.3 (4.8-5.9)</td>
<td>5.0 (4.4-5.6)</td>
</tr>
<tr>
<td>HIV-1 subtype</td>
<td>A</td>
<td>2/82</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2/82</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>31/82</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>CRF02_AG</td>
<td>31/82</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>16/82</td>
<td>19.5</td>
</tr>
<tr>
<td>Mother currently on ART</td>
<td>Yes</td>
<td>46/77</td>
<td>59.7</td>
</tr>
<tr>
<td>ART regimen child</td>
<td>AZT+3TC+EFV</td>
<td>4/90</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>AZT+3TC+NVP</td>
<td>78/90</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>ABC+3TC+NVP</td>
<td>7/90</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Genotypic data were available for 82/90 children. Drug resistance mutations were identified based on the 2009 WHO list for surveillance of transmitted drug resistance [12]. HIV-1 subtyping was performed using the REGA HIV-1 subtyping tool V3 [15]. Nutritional status was assessed using WHO Anthro (version 3.2.2, January 2011) for children <5 years and WHO Reference 2007 for children ≥5 years [17]. Results for hemoglobin, CD4 count, CD4 percentage, and HIV RNA load were available for 87, 40, 48, and 82 children, respectively.

* Only for children <5 years of age.

** Only for children <10 years of age.

*** Only for children ≥5 years of age.

ART, antiretroviral therapy; HAZ, Height for age z-score; WAZ, weight for age z-score; WHZ, weight for height z-score; WHO, World Health Organization.
sub-type, mother’s use of ART, current living situation of the child, and mother’s health status. If explanatory variables were associated with the outcome variables (p<0.10) in the univariate analysis, they were forwarded to the multivariate model using a step forward procedure.

Meta-analysis of studies of the systematic review was conducted to pool the reported HIVDR prevalence using a random-effects model, because of expected heterogeneity among studies. Random-effects meta-regression was used to assess the reported HIVDR in the included studies by year of ART initiation and for PMTCT-exposed versus -unexposed children. The variance of the raw proportions was stabilized using a Freeman-Tukey arcsine square root transformation, and was subsequently back-transformed to the original scale. A two-sided p-value of ≤0.05 was considered significant. Data were analyzed using Stata 12® (StataCorp LP, TX, USA).

RESULTS

Between March 2012 and October 2013, 100 children were enrolled in the MARCH-Nigeria cohort, of which 90 children were PMTCT-unexposed and were included in the analysis. Most (N=78; 86.7%) children started an ART regimen consisting of zidovudine/lamivudine/nevirapine (AZT+3TC+NVP) as a fixed-dose combination, and none started a PI-based regimen. The median age was 4.6 years (IQR 1.8-8.4), 46% were male, and 61% were classified as WHO clinical stage III or IV (Table 1). The child’s primary caregiver was the mother for 70 (78%) of the children. The mother was sick in six (7%) and deceased in 15 (17%) cases, including five children (6%) of whom both parents died. The median viral load before treatment initiation was 160,000 cps/ml (IQR 45,700-730,000); one child had a viral load <1,000 cps/ml. HIV-1 sequencing of the pol gene was successful in 82 of 89 children with viral load >1,000 cps/ml. The children with and without sequencing results did not differ significantly regarding sex, age, and clinical characteristics (data not shown).

Thirteen of 82 (15.9% [95%CI: 9.5-25.3]) children had HIVDR; all 13 children carried NNRTI mutations, and seven (8.5%) also had NRTI mutations. No PI mutations were identified. G190A/S (n=7) and M184V/I (n=6), were the most prevalent mutations (Figure 1). For all 13 children with HIVDR, the drug regimen prescribed was predicted to be only partially active (mean GSS=1.5, SD 0.18). All 13 had mutations associated with high NVP resistance. For all seven children with NRTI mutations, these mutations resulted in an only partially active regimen with regards to the NRTI backbone; five had mutations associated with 3TC resistance and three had thymidine analogue
mutations (TAMs). Children with HIVDR had a median of two mutations (range 1-7) per sequence. We did not find significant associations between sex, age, nutritional or immunological status, viral load, subtype, mother’s ART use, child’s living situation, or mother’s health status and the presence of HIVDR (data not shown).

**Systematic review sub-Saharan Africa**

We performed a literature search on pediatric pre-treatment HIVDR in sub-Saharan Africa. We retrieved 467 articles and 378 conference abstracts, of which we included 16 studies: 11 articles, four conference abstracts and our current study in Nigeria (Figure 2), representing 2,057 children. Studies were performed between 2003 and 2012, in 11 African countries (three in east Africa, four in southern Africa, and four in west/central Africa). The median age of the children included in the studies ranged from 3.7 months to 8 years (Table 2). All studies except one [18] reported a higher prevalence of HIVDR towards NNRTI compared to NRTI. In that study, mothers were randomized to receive either NVP- or nelfinavir (NFV)-based triple therapy for PMTCT. All HIV-infected children in the NFV-arm developed NRTI resistance, but no NNRTI or PI resistance [18]. Only four studies reported data on PI mutations which were found in less than 2% of the cases (Supplementary Table S1).

Random-effects meta-analysis yielded a pooled HIVDR prevalence of 28.1% (95%CI 18.5-37.7) among PMTCT-exposed and unexposed children, with a high level of between-study heterogeneity ($I^2 = 97.7\%$). (Figure 3). The HIVDR prevalence was almost a fourfold higher in PMTCT-exposed compared to PMTCT-unexposed children, 40.0% (95%CI 24.1-56.0) versus 10.3% (95%CI 4.2-16.4), respectively ($p=0.009$). In PMTCT-unexposed children, we found an increase in HIVDR prevalence by year of ART initiation from 0.6% (95%CI -1.8-3.0) in 2004 to 36.2% (95%CI 25.5-46.9) in 2011, $p=0.05$ (Figure 4).
HIV drug resistance in children in Lagos, Nigeria

Figure 1. Drug resistance mutations detected in this cohort (n=82).
NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Figure 2. Flow diagram of included articles and conference abstracts.
Table 2. Studies included in systematic review of pediatric pre-treatment HIV drug resistance in sub-Sahara Africa

<table>
<thead>
<tr>
<th>Year of study</th>
<th>Author, year of publication</th>
<th>Country</th>
<th>N</th>
<th>Age in months (median, IQR)</th>
<th>HIV drug resistance prevalence, overall</th>
<th>HIV drug resistance prevalence, in PMTCT unexposed children</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-2006</td>
<td>Towler, 2010</td>
<td>Uganda [2]</td>
<td>74</td>
<td>51.5 (7-148)</td>
<td>2/74 (2.7%)</td>
<td>0/39 (0%)</td>
</tr>
<tr>
<td>2007-2009</td>
<td>Van Zyl, 2010</td>
<td>South-Africa [4]</td>
<td>49</td>
<td>3.7 (range 1-17)</td>
<td>3/49 (6.1%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>2008</td>
<td>Meini, 2015</td>
<td>Tanzania [6]</td>
<td>46</td>
<td>96 (84-132)</td>
<td>32/46 (70.0%)</td>
<td>NA</td>
</tr>
<tr>
<td>2008-2010</td>
<td>Weigel, 2014</td>
<td>Malawi [7]</td>
<td>381</td>
<td>30 (14.8-73.4)</td>
<td>59/381 (15%)</td>
<td>NR</td>
</tr>
<tr>
<td>2009-2011</td>
<td>Fokam, 2011</td>
<td>Cameroon [9]</td>
<td>41</td>
<td>72 (3-144)</td>
<td>2/41 (4.9%)</td>
<td>1/40 (2.5%)</td>
</tr>
<tr>
<td>2010-2011</td>
<td>Kebe, 2014</td>
<td>Senegal [10]</td>
<td>25</td>
<td>5.5 (range 1.5-17)</td>
<td>8/25 (32%)</td>
<td>1/12 (8.3%)</td>
</tr>
<tr>
<td>2010-2011</td>
<td>Sigaloff, 2012</td>
<td>Uganda [11]</td>
<td>279</td>
<td>58.8 (27.6-108)</td>
<td>28/279 (10%)</td>
<td>18/233 (7.7%)</td>
</tr>
<tr>
<td>2011</td>
<td>Kuhn, 2014</td>
<td>South-Africa [12]</td>
<td>230</td>
<td>&lt;24</td>
<td>122/230 (53.0%)</td>
<td>27/75 (36.0%)</td>
</tr>
<tr>
<td>2012-2013</td>
<td>Boender &amp; Boerma (Current study)</td>
<td>Nigeria</td>
<td>90</td>
<td>51.6 (18-93.6)</td>
<td>13/82 (15.9%)</td>
<td>13/82 (15.9%)</td>
</tr>
</tbody>
</table>

PMTCT, prevention of mother-to-child transmission; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; IQR, interquartile range; NR, not reported; NA, not applicable.

* Absolute numbers not reported.
In this study we found a high rate of 15.9% HIVDR among ARV-naïve children in Lagos, Nigeria. This implies that, even in children that have never been exposed to any antiretroviral drug, almost one in six children carries HIVDR mutations. All 13 children with HIVDR consecutively received suboptimal treatment. Seven (8.5%) children had mutations associated with one of the NRTIs they were prescribed.

The high [19] pre-treatment HIVDR prevalence in this study could be due to direct transmission of drug resistant HIV from the mother to her child. The prevalence of pre-treatment HIVDR among women of child-bearing age at the same hospital in Lagos was 3.8% (95%CI 1.5-9.4%) in 2008-2009 in one of our previous cohorts [20].
and 2.9% in 2010-2012 in a study conducted in north central Nigeria [21]. We did not have data on HIVDR in the mothers of the children in our cohort, but it is possible that the rate of pre-treatment HIVDR among women of child-bearing age has risen over the past years [2,3].

Some of the children in our cohort who were reported to be ARV-naïve might actually have received PMTCT, as some mothers or caregivers might not recall perinatal PMTCT. This has been suggested before and confirms that caregiver’s report of PMTCT exposure might not be a reliable criterion to base treatment decisions on [6, 22]. Furthermore, in this cohort of relatively old children (range 0.2-12.7 years), it is possible that children have been exposed to other ARVs than those for PMTCT and, as such, are not entirely ARV-naïve. In a study among adults in seven African countries, ARVs were found in the samples of 22% of the participants who reported to be ARV-naïve [23].
Our systematic review confirms the high pre-treatment HIVDR prevalence among ARV-naive children, and identifies a significant increase in prevalence in ARV-naive children in sub-Saharan Africa over the past decade. Our findings are in contrast with a recent systematic review from 2014 on pediatric HIVDR worldwide, which identified only two studies conducted on the African continent, and reported a low prevalence of 0 to 5% pre-treatment HIVDR [8]. In adults, pre-treatment HIVDR has been shown to be increasing as well. Among almost 2,500 adults in six countries in sub-Saharan Africa, we previously reported that the risk of HIVDR rose by 38% each year after ART roll-out with the highest prevalence in Uganda, the country with the longest history of large-scale ART provision [2]. A meta-analysis of pre-treatment HIVDR on a global level (26,102 patients) confirmed these results, and modelled an increase in HIVDR prevalence of almost 30% per year in east Africa, 14% in southern Africa, and 3% in west and central Africa [3].

One of the limitations of our study is the fact that data on PMTCT exposure of the included children were collected retrospectively. However, we attempted to collect all available data on prior PMTCT, by interviewing the mother or caregiver and by searching the medical files of all mothers and children. Children were recruited at a single

Figure 4. Meta-regression plot of pre-treatment HIV drug resistance prevalence in PMTCT-unexposed sub-Saharan children by year of treatment initiation. Prevalence increased from 0.6% (95%CI 0.6-5.4) in 2004 to 36.2% (95%CI 25.5-46.9) in 2011 (p=0.05). Size of the circle represents the precision of each estimate. For this analysis, the HIV drug resistance prevalence was transformed using a Freeman-Tukey-type arcsine square root transformation. PMTCT, prevention of mother-to-child transmission.
site, and the children’s age ranged from 0 to 12 years which makes comparison of HIVDR prevalence with other studies harder, as mutations might be archived in older children [22, 24]. However, our cohort directly reflects the day-to-day practice of a clinician working in a pediatric HIV clinic, and the practical challenges of HIVDR in a resource-constraint setting. One of the limitations of our meta-analysis is the relatively low number of included studies with limited sample size and the large heterogeneity, which may have affected our pooled results and meta-regression model.

**Implications**

The high rate of NNRTI resistance we found in PMTCT-unexposed children implies that one in six HIV-infected children is receiving suboptimal treatment, as current ART Nigerian guidelines still recommend NNRTI-based first-line treatment for PMTCT-unexposed children [7]. Larger studies in Nigeria are urgently needed to draw more robust conclusions on a national level. In a country with ~260,000 HIV-infected children, a pretreatment HIVDR prevalence of ~16% would have catastrophic implications for pediatric HIV treatment in Nigeria. Children with pre-treatment HIVDR are at risk of early virological failure and subsequent treatment switch. More than half of the children with NNRTI mutations in our cohort also had resistance against NRTIs, which could limit the effectiveness of a second-line PI-based regimen with an NRTI backbone. However, various studies have reported that the presence of NRTI mutations was not a risk factor for virological failure in children and adults on second-line PI-based treatment [25–27].

The alarming increase in HIVDR we report in sub Saharan Africa, including the figures in Nigeria, stress the importance of implementing the WHO recommendations of PI-based regimens for children under three years and of considering extending this policy for children up to at least 12 years. Pediatric PIs are more costly than NNRTIs and used to be only available as a liquid that requires refrigeration. Recently, however, the United States Food and Drug Administration (FDA) has approved LPV/r in pellet form for pediatric usage [28]. This is an important step towards overcoming the barriers of implementing PI-based first-line treatment in resource-constraint countries. Solving these issues should be given priority in order to control pediatric HIVDR.

Furthermore, population-based HIVDR surveillance programs on a national level could provide valuable information on pre-treatment HIVDR prevalence. This is especially important in children as we identified a high prevalence of pediatric pre-treatment HIVDR, and second-line drugs options are limited in this population. Early detection of increasing levels of HIVDR in the pediatric population of a country can help policy makers to take informed decisions on national ART guidelines and on
the selection of first-line regimens. Individual genotypic resistance testing before treatment initiation as in high-income countries [29], is currently not recommended in resource constraint settings, mainly due to the high costs and the complexity of testing assays [6]. The current efforts to develop simplified tests against lower costs are a step towards population-based surveillance [30], and possibly even individualized testing in the future.

In summary, in this cohort of ARV-naïve HIV-infected children in Nigeria we found a high rate of 16% pre-treatment HIVDR. Our systematic review and meta-analysis confirmed and expanded these findings, showing a significant increase in HIVDR prevalence in ARV-naïve children in sub-Saharan Africa over the past decade. Implementation of PI-based regimens for children under three years of age in countries in sub-Saharan Africa should receive priority. Overcoming the barriers of PI-based treatment in LMIC and close monitoring of HIVDR through regular surveillance programs are essential in order to ensure optimal treatment for HIV-infected children in LMIC.

FUNDING

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DECLARATION OF INTEREST

We declare no competing interests.
REFERENCES


Part II

Long-term outcomes of first-line antiretroviral therapy
Virological suppression among HIV-infected children in low- and middle-income countries: a systematic review and meta-analysis.


Submitted.
SUMMARY

Background
This review and meta-analysis aims to summarize virological suppression rates in HIV-infected children <18 years, up to five years after initiation of non-nucleoside reverse transcriptase inhibitor (NNRTI)- or protease inhibitor (PI)-based first-line antiretroviral treatment (ART) in low- and middle-income countries (LMIC).

Methods
We searched Medline, Embase, the Cochrane Central Register for Controlled Trials (CENTRAL) and the Literatura Latino Americana de Ciencias de Salud (LILACS) for randomized controlled trials, cohort studies and cross-sectional studies. We extracted data on virological suppression six to 60 months after first-line treatment initiation and summarized the proportion of children with virological suppression at six-monthly time intervals using random-effects meta-analysis.

Findings
Seventy-two papers, reporting on 51,347 children, were included in the analysis. Summary estimates of virological suppression after six, 12, and 24 months of ART were 71.3% (95%CI 67.9-74.6), 69.6% (95%CI 66.3-72.9), and 78.3% (95%CI 75.0-81.6), respectively. For NNRTI-treated children these rates were 65.5% (95%CI 56.7-74.3), 64.6% (95%CI 57.0-72.2), and 76.8% (95%CI 71.7-82.0), and for PI-treated children 77.2% (95%CI 66.8-87.6), 70.3% (95%CI 61.0-79.6), 74.2% (95%CI 58.6-89.7), respectively.

Interpretation
In the most comprehensive literature overview of first-line ART in children in LMIC to date, virological suppression rates were 70-80%, which is lower compared to adults in LMIC and to children in high-income countries. Children in LMIC continue to be a vulnerable population. More attention should be given to strategies to increase virological suppression rates, including improved monitoring, increased access to and accelerated development of adequate and affordable first- and second-line ART.
INTRODUCTION

Treating HIV in early childhood is life-saving. However, children are vulnerable to developing virological failure. Variability in pharmacokinetics, limited pediatric treatment options, and lack of adherence support are among the potential reasons for this increased risk [1]. In addition, drug exposure as part of the prevention of mother-to-child transmission (PMTCT) can lead to the emergence of HIV drug resistance [2, 3], thus increasing the risk of virological failure.

The World Health Organization (WHO) recommends all children below three years of age to receive a PI-based regimen (lopinavir/ritonavir [LPV/r]), regardless of history of PMTCT exposure [4]. Unfortunately, despite these recommendations, the use of PIs for young children in low- and middle-income countries (LMIC) in routine programs is limited due to practical barriers. PIs are more costly than NNRTIs, and infant formulations were to date only available as a liquid that requires refrigeration, although new formulations are on their way [5–7].

Previous systematic reviews on virological suppression in children only focused on sub-Saharan Africa, only on young children, or only included randomized controlled trials (RCTs) in which circumstances are usually well controlled and less likely to represent ‘real life’ settings [8, 13–15]. This review and meta-analysis is the first attempt to analyze virological outcomes up to five years after treatment initiation, in children up to the age of 18 years, on three different continents, and including all types of study designs.

The goal of this comprehensive assessment of available data was to provide summary estimates of virological suppression rates in children retained on first-line ART six to 60 months after treatment initiation. In addition, we stratified results of children on NNRTI- or PI-based first-line ART and compared results of children with and without prior PMTCT exposure.

METHODS

Search strategy and selection criteria

This study was performed in accordance with the PRISMA statement for reporting systematic reviews and meta-analyses [8]. The systematic review protocol is provided in Supplement V. We systematically reviewed relevant literature in Medline (through PubMed), Embase, the Cochrane Central Register for Controlled Trials (CENTRAL)
and the Literatura Latino Americana de Ciencias de Salud (LILACS), using the search strategy as provided in Supplement I. In short, a search was conducted (by RB) to find articles on virological outcomes in HIV-infected children <18 years of age living in LMIC [9], including information about viral load outcomes in the first five years of first-line ART. We searched for articles published between January 2005 and May 2015, in English, French and Spanish. Additionally, online databases of the International AIDS Society 2012-2014, the Conference on Retroviruses and Opportunistic Infections 2012-2015, and the HIV pediatric workshop 2012-2014 were screened for relevant conference abstracts. Snowballing techniques were used to retrieve any additional articles.

**Study screening**

Eligible studies included prospective and retrospective cohort studies, cross-sectional studies and RCTs. Participants of the study had to be children (<18 years of age) with an HIV-1 infection, on first-line ART, and receiving treatment in LMIC. Both children with and without prior exposure to PMTCT were eligible for inclusion. We included studies reporting on pre-defined six-monthly time points up to 60 months after treatment initiation. For studies reporting a suppression rate at a median time after treatment initiation, we included these if the interquartile range was ≤12 months. The selection of studies was done independently by two reviewers (RB and TSB) and any disagreement was resolved by mutual discussion. Study selection was done first based on the title, subsequently on the abstract, and finally based on reading the full text of the article.

**Data extraction**

Data were extracted using a data extraction form designed for this purpose in MS Access 2013®. Data extraction (by RB and AB) was done separately for each pre-defined time point at which the proportion of participants with virological suppression was reported. The virological suppression threshold used in each study was extracted and was later pooled into three categories: <50 cps/ml, <400 cps/ml or <1000 cps/ml. The extraction was done separately for participants on PI- or NNRTI-based regimens, and for participants with and without PMTCT exposure, when reported. This yielded multiple ‘analysis cohorts’ extracted from a single study. The following additional data were extracted from each study: year of study; country of study; number of study sites; percentage female; median age; ART regimen. If needed, study authors were contacted to clarify or confirm the extracted data. Study quality and risk of bias was assessed by two independent reviewers (RB and AB) using an adapted version of the Newcastle Ottowa Scale [10] (Supplement II).
**Statistical analysis**

The primary analysis was done on an on-treatment basis, defined as the number of children with virological suppression divided by the number of children who were on treatment and had viral load results at that time point. The results were pooled for each pre-defined time point (6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 months after ART initiation). All analyses were done using a random effects model, because of the heterogenic nature of the included studies. Heterogeneity of outcomes was assessed through calculation of I²-statistics. The variance of the raw proportions was stabilized using a Freeman-Tukey arcsine square root transformation and was subsequently back-transformed to the original scale.

Subgroup analyses were performed among participants on NNRTI- or PI-based regimens and with or without PMTCT exposure at each time point. A meta-regression was performed to obtain p-values for comparison of proportions between NNRTI- and PI-treated children, and PMTCT-exposed and unexposed children. To test for the robustness of our data, we performed sensitivity analyses, which included, only: 1. studies performed after 2005; 2. studies conducted in sub-Saharan Africa; 3. studies in which the participants’ median age was <3 years; 4. studies yielding the maximum score on the Newcastle Ottowa Scale for quality assessment. In addition, we conducted an intention-to-treat analysis, in which we calculated the virological suppression rate by dividing the number of suppressed children at each time point by the number of children who had started treatment at baseline, thus considering all children with unknown viral load results, who stopped ART, had deceased or were lost to follow-up, as having virological failure. All data analyses were performed using Stata 12.1® (StataCorp. 2011. College Station, TX: StataCorp LP).

**RESULTS**

We retrieved 1,580 articles through our literature search and one article through snowballing. Additionally, we found 1,009 potentially relevant conference abstracts. Of the 1,581 retrieved papers, 855 were excluded based on the title, 345 after reading the abstract and 267 after reading the full text. After removal of 44 duplicate cohorts, 70 full-text papers were included in this review. Of the 1,009 conference abstracts found, 680 were excluded on title and 327 after reading the abstract. Two conference abstracts were included in this review (Figure 1).

We included 72 studies, reporting on a total of 51,347 children. Of the 72 included studies, we extracted 188 analysis cohorts, based on time after ART initiation; NNRTI
or PI regimen; and previous PMTCT exposure. A median of two cohorts were extracted per study (range 1-12). Fifty-two (72.2%) studies had a maximum score of six on the Newcastle Ottowa Scale. Further details are described in Table 1. A complete overview of all studies is provided in Supplement III.

Hundred and sixty six of 188 cohorts (88.3%) reported virological outcomes in the first two years after treatment initiation, i.e. at six, 12, 18 or 24 months. The summary estimates of the proportion of participants with virological suppression, using a threshold of virological suppression of <1000 cps/ml, were 71.3% (95%CI 67.9-74.6, n=18,730) at six months, 69.6% (95%CI 66.3-72.9, n=20,622) at 12 months, 68.2% (95%CI 58.8-77.7, n=3,778) at 18 months, and 78.3% (95%CI 75.0-81.6, n=6,262) at 24 months after ART initiation. Suppression rates using a virological suppression threshold of <1000 cps/ml, <400 cps/ml, and <50 cps/ml are reported in Table 2.

The intention-to-treat analysis yielded summary estimates of virological suppression of 53.8% (95%CI 45.2-62.4, n=33,288) at six months, 46.0% (95%CI 38.5-53.6, n=42,725)
Table 1. Characteristics of included studies and cohorts within included studies.

<table>
<thead>
<tr>
<th></th>
<th>Number of studies</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>72</td>
<td>100</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>58</td>
<td>80.6</td>
</tr>
<tr>
<td>Asia</td>
<td>12</td>
<td>16.7</td>
</tr>
<tr>
<td>Caribbean</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Latin-America</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Study design</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomized controlled trial</td>
<td>9</td>
<td>12.5</td>
</tr>
<tr>
<td>Retrospective cohort</td>
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<td>40.3</td>
</tr>
<tr>
<td>Prospective cohort</td>
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<td>44.4</td>
</tr>
<tr>
<td>Cross-sectional study</td>
<td>4</td>
<td>5.6</td>
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<tr>
<td><strong>Year of antiretroviral treatment initiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2006</td>
<td>30</td>
<td>41.7</td>
</tr>
<tr>
<td>≥ 2006</td>
<td>39</td>
<td>54.2</td>
</tr>
<tr>
<td>Not reported</td>
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<td>4.2</td>
</tr>
<tr>
<td><strong>Score on adapted Newcastle Ottawa Scale</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>72.2</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>26.4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.4</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number of cohorts</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>188</td>
<td>100</td>
</tr>
<tr>
<td><strong>ART regimen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusively PI-based</td>
<td>16</td>
<td>8.5</td>
</tr>
<tr>
<td>Exclusively NNRTI-based</td>
<td>89</td>
<td>47.3</td>
</tr>
<tr>
<td>Not specified or outcomes not reported separately</td>
<td>83</td>
<td>44.2</td>
</tr>
<tr>
<td><strong>Regimen contains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nevirapine</td>
<td>98</td>
<td>52.1</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>80</td>
<td>42.6</td>
</tr>
<tr>
<td>Lopinavir/ritonavir</td>
<td>32</td>
<td>17.0</td>
</tr>
<tr>
<td><strong>PMTCT exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>4.3</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>10.6</td>
</tr>
<tr>
<td><strong>Median age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 years</td>
<td>54</td>
<td>28.7</td>
</tr>
<tr>
<td>≥ 3 years</td>
<td>134</td>
<td>71.3</td>
</tr>
</tbody>
</table>

ART, antiretroviral treatment; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission.
at 12 months, 45.7% (95% CI 35.6-55.9, n=8,138) at 18 months, and 51.8% (95% CI 40.8-62.9, n=17,991) at 24 months (Figure 3 and Supplementary Table S1).

Twenty cohorts (10.6%) reported on virological suppression rates at 30-60 months after first-line ART initiation. Suppression rates fluctuated between 60.3% at 42 months (95% CI 34.6-86.0, n=162) and 79.6% at 60 months (95% CI 64.0-95.1, n=927) Virological suppression rates at other time points are reported in Table 2.

We stratified the studies according to the type of first-line regimen, NNRTI- or PI-based. In the NNRTI-cohorts virological suppression was achieved in 65.5% (95% CI 56.7-74.3, n=4,993) after six months; 64.6% (95% CI 57.0-72.2, n=6,253) after 12 months; and 76.8% (95% CI 71.7-82.0, n=1,712) after 24 months of ART. In PI-cohorts these rates were 77.2% (95% CI 66.8-87.6, n=3,426); 70.3% (95% CI 61.0-79.6, n=2,527); and 74.2% (95% CI 58.6-89.7, n=28), respectively (Figure 2 and Supplementary Table S2). Restricting these analyses to children under three years of age, who are recommended to receive PI-based treatment, yielded similar results (Supplementary Table S2).

At six months after ART initiation, 63.3% (95% CI 29.0-97.6, n=150) of PMTCT-exposed children had virological suppression compared to 76.9% (95% CI 66.2-87.6, n=501) of unexposed children, p=0.523. At 12 months, these proportions were 51.7% (95% CI -22.5-125.8, n=59) versus 70.6% (95% CI 59.4-81.8, n=460), p=0.954, and at 24 months 14.6% (95% CI -5.9-35.2, n=9) versus 66.6% (95% CI 50.3-82.8, n=107), p=0.867, for exposed and unexposed children, respectively (Supplementary Table S2). Exposed children were significantly younger than unexposed children (median age 0.7 versus 3.1 years, p=0.007). No significant difference in ART regimen was found between exposed and unexposed children. The limited amount of data did not allow for subgroup analyses.

Sensitivity analyses restricting the analysis to geographic location, calendar year, age, and study quality showed similar patterns of virological suppression rates of ~70-80% in the first two years after treatment initiation (Figure 3 and Supplementary Table S3).
Virological suppression is defined as viral load <50 cps/ml.

NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 2. Summary estimates of the proportion of children with virological suppression at 6 to 60 months after treatment initiation.

<table>
<thead>
<tr>
<th>Months</th>
<th>&lt;1000 cps/ml</th>
<th>&lt;400 cps/ml</th>
<th>&lt;50 cps/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>71.3</td>
<td>67.9-74.6</td>
</tr>
<tr>
<td>12</td>
<td>47</td>
<td>69.6</td>
<td>66.3-72.9</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>68.2</td>
<td>58.8-77.7</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td>78.3</td>
<td>75.0-81.6</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>73.6</td>
<td>64.8-82.4</td>
</tr>
<tr>
<td>36</td>
<td>7</td>
<td>78.7</td>
<td>69.4-87.9</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>60.3</td>
<td>34.6-86.0</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
<td>75.5</td>
<td>64.8-86.3</td>
</tr>
<tr>
<td>54</td>
<td>1</td>
<td>66.3</td>
<td>57.8-74.7</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>79.6</td>
<td>64.0-95.1</td>
</tr>
</tbody>
</table>

Virological suppression is defined as a viral load <50 cps/ml, <400 cps/ml, or <1000 cps/ml.

Data on virological suppression after 24 months were pooled using a threshold of <1000 cps/ml only, because of limited availability of data.

95% CI, 95% confidence interval; NA, not available.
In this systematic review and meta-analysis, including 72 studies reporting on 51,347 children, we show that virological suppression rates in HIV-infected children in LMIC are between 70 and 80% in the first 24 months, and 60 to 80% up to five years after first-line ART initiation. These outcomes are considerably lower than the rates of adults in LMIC, and of children in high-income countries [11–14]. For the first two years of treatment, suppression rates were sustained at 70-80%, and were robust when accounting for calendar year of treatment initiation, continent, age groups, and assessed study quality. The proportions we found are in accordance with the findings of previous systematic reviews with shorter follow-up periods, in which a rate of 46-81% at six months of treatment [15] and a rate of 70% [16], and 49-81% [15] at 12 months were reported. Proportions are lower, however, compared to a recent systematic review on virological suppression in adults in LMIC, which found suppression rates of approximately 85% in the first two years after first-line treatment initiation [11]. Outcomes are also poorer compared to the suppression rates of >90% found in HIV-infected children in high-income countries [12–14].
Our results indicate that HIV-infected children treated with ART in LMIC are a vulnerable population and, despite current efforts, are at high risk of treatment failure. Two other findings stress these disconcerting results: first, also in cohorts using more potent PI-based treatment, virological suppression rates were <80%; second, the intention-to-treat analysis showed suppression rates in children of only 45-55% in the first two years, while in adults these rates were 65-75% [11].

To improve virological suppression rates in children in LMIC, a better understanding of their poor outcomes as compared to adults and to children in high income countries is required. First of all, children may be more vulnerable to virological failure after exposure to failed PMTCT, which is associated with NNRTI resistance [17, 18]. In this review, PMTCT-exposed children had lower virological suppression rates than unexposed children, although differences were not significant. Using the currently recommended PI-based treatment, clinical outcomes in young children have been shown to be better compared to NNRTI-treated children, regardless of previous PMTCT exposure [19]. Second, treatment adherence is challenging in children who are dependent on their caregivers to receive their medication and to remain in care. Little is known about the long-term treatment outcomes of children on first-line ART, as we identified only nine studies reporting on virological suppression rates more than two years after ART initiation. Third, accurate drug dosing in children is difficult, because of changing pharmacokinetics in children of different weights, and the limited availability of pediatric formulations. This increases the risk of underdosing and subsequent HIV drug resistance. In order to improve treatment response in children in LMIC, various steps will need to be taken. Implementation of PI-based treatment for young children regardless of PMTCT exposure, as recommended by the WHO [4], is essential to achieve better treatment results in this vulnerable population who will need treatment for the rest of their lives. To be able to treat all children under three years with a PI, financial and logistic barriers for LMIC to implement this strategy need to be overcome. It has been suggested that PIs might be used only to achieve initial virological suppression, after which children can be switched to less costly NNRTI-based treatment [20]. However, this is only recommended in settings where viral load monitoring is available, which is often not the case in LMIC. Cost-reduction of ARVs for LMIC, the production of generics, and harmonization of treatment guidelines are important steps to increase access to PI-treatment. In addition, the development of new antiretroviral agents, in formulations appropriate for children, should receive priority. In children who fail PI-based first-line treatment, second-line options are very limited and outcomes are poor [21, 22]. New generation PIs such as darunavir could be a second-line option after failure of first-line LPV/r-based treatment in children
above three years of age. The new integrase inhibitor raltegravir has recently been approved for young infants. These new agents are, however, currently not available in LMIC [23]. As access to first-line PI regimens for children increases, evidence-based strategies for durable second-line options should be developed. Third, as we found that virological suppression rates in children are considerably lower than in adults, it is important that especially children are monitored closely during treatment in order to timely detect treatment failure. Implementation of viral load monitoring for this population in LMIC should be a priority. Finally, the specific aspects of pediatric HIV-care, such as blood sampling techniques, specific laboratory assays, and pediatric drug formulations, should receive more attention in the training of health workers in LMIC. Treatment protocols should be, when possible, simplified, in order to improve HIV-care for children in resource-limited settings.

Strengths of our study include the fact that it provides the largest and most comprehensive literature review of HIV treatment outcomes in children in LMIC to date, and that our findings were robust across several sensitivity analyses. In order to assess all available data on this topic, we chose to include both observational studies and RCTs. A limitation to this approach is the large heterogeneity among the studies, and care should be taken when comparing results across studies. We are aware of the fact that other factors apart from drug class and PMTCT exposure are likely to play a role in virological suppression rates in children, such as the specific drugs used within each drug class [24]. Very few studies reported the number of virologically suppressed children separately for each drug used, so we could not conduct separate analyses to correct for specific drugs used. As we did not have individual patient data of the included studies, but only study-level data, we did not correct for potentially relevant patient characteristics, such as age or clinical and immunological status. Including study averages of such data, such as mean age or mean CD4-count, would have introduced the risk of ecological bias in our analysis [25].

In conclusion, this systematic review and meta-analysis is the most comprehensive literature overview of response to first-line ART in children in LMIC to date. We show that rates of virological suppression in children are considerably lower than those found in adults and in children in high-income countries. Implementation of PI-based treatment, the development of new pediatric formulations against affordable costs, improved pediatric treatment monitoring, and specific pediatric training for health workers are necessary in order to achieve optimal treatment results for HIV-infected children in LMIC.
CONTRIBUTORS

RB did the initial search of published work, checked all full-text articles, extracted data from the full reports and conference abstracts, conceived and coordinated the analyses, and wrote the first draft of the paper. TSB also checked all full-text articles and abstracts, and AB also extracted data. KS conceived the systematic review, supervised the reviewing process and was available to resolve conflicts during data extraction. JC supervised the analysis process and interpreting the data. TRW and MBvH supervised KS and JC and assisted conceiving the study and interpreting the data. SB assisted interpreting the data. All authors participated in discussion of the results and in writing of the final paper.

DECLARATION OF INTEREST

We declare that we have no conflicts of interests.

ACKNOWLEDGMENTS

We thank Annette Sohn for critical review of the manuscript and Ingeborg Nagel and René Spijker for librarian assistance with the literature search. Some of the authors are staff members of the World Health Organization. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or stated policy of the World Health Organization.
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Long-term virological outcomes of first-line antiretroviral therapy for HIV-1 in low- and middle-income countries: a systematic review and meta-analysis.


Clinical Infectious Diseases
2015, 61 (9): 1453-1461.
ABSTRACT

Background
More than 11.7 million people are currently receiving antiretroviral therapy (ART) in low- and middle-income countries (LMICs), and focused efforts are needed to ensure high levels of adherence and to minimize treatment failure. Recently, international targets have emphasized the importance of long-term virological suppression as a key measure of program performance.

Methods
We systematically reviewed publications and conference abstracts published between January 2006 and May 2013 that reported virological outcomes among human immunodeficiency virus type 1 (HIV-1)–infected adults receiving first-line ART for up to 5 years in LMICs. Summary estimates of virological suppression after 6, 12, 24, 36, 48, and 60 months of ART were analyzed using random-effects meta-analysis. Intention-to-treat (ITT) analysis assumed all participants who were lost to follow-up, died, or stopped ART as having virological failure.

Results
Summary estimates of virological suppression remained >80% for up to 60 months of ART for all 184 included cohorts. ITT analysis yielded 74.7% (95% confidence interval [CI], 72.2–77.2) suppression after 6 months and 61.8% (95% CI, 44.0–79.7) suppression after 48 months on ART. Switches to second-line ART were reported scarcely.

Conclusions
Among individuals retained on ART, virological suppression rates during the first 5 years of ART were high (>80%) and stable. Suppression rates in ITT analysis declined during 4 years.
INTRODUCTION

The World Health Organization (WHO) estimated that nearly 11.7 million people living with human immunodeficiency virus (HIV) in low- and middle-income countries (LMICs) received antiretroviral therapy (ART) in 2013 [1]. Global ART scale-up has been made possible by the use of standardized and simplified treatment protocols and decentralized service delivery, with limited reliance on laboratory monitoring [2, 3]. In order to enhance treatment monitoring, WHO recommended in 2013 that viral load measurements among people receiving ART be performed 6 months after initiating ART and every 12 months thereafter [4, 5].

As the availability of viral load testing grows in LMICs, the percentage of patients with virological suppression can be an important measure of overall ART clinic and program success. The Joint United Nations Programme on HIV/AIDS 90-90-90 by 2020 initiative was established with the goal of achieving virological suppression in 90% of all people receiving ART by the year 2020 [6], emphasizing the need for robust data on short- and long-term programmatic levels of virological suppression against which program performance can be assessed.

In this context, population-level summary estimates of virological suppression measured at different time points are needed to guide ART program managers on the normative levels of population-level virological suppression. The objective of this systematic review was to determine summary estimates of virological suppression among HIV-infected people receiving first-line ART for up to 5 years in LMICs.

METHODS

Search Strategy

This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement and the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [7, 8]. We searched the electronic databases Ovid Medline (through PubMed), Embase, and the Cochrane Central Register of Controlled Trials for research papers published in English between 1 January 2006 and 14 May 2013 (Supplementary Material). The start date of 2006 was chosen to search for published virological outcomes from ART programs that followed the WHO public health approach [3]. We defined search terms pertaining to HIV/AIDS or ART combined with LMICs. Subsequently, studies were restricted to those matching search terms for HIV viral load and/or HIV drug resistance.
We combined this with search terms specifying time factors in order to select viral load measurements obtained from individuals on ART for 6 months and up to 5 years. Finally, we excluded studies of children (aged <13 years) from the search. Additionally, we manually searched conference abstracts of the International AIDS Society in 2011 and 2012 and the Conference on Retroviruses and Opportunistic Infections in 2012 and 2013. Finally, unpublished cohort data from the Pan-African Studies to Evaluate Resistance Monitoring (PASER-M) program and TREAT Asia (TAHOD [TREAT Asia HIV Observational Database] and TASER [TREAT Asia Studies to Evaluate Resistance]) programs were added as additional sources of data [9].

**Study Selection**

We included original research papers and conference abstracts that reported on virological outcomes among HIV type 1 (HIV-1)–infected adults on first-line combination ART for up to 5 years in LMICs. Studies reporting on both adults and adolescents (aged >13 years) were included if the majority of the population (>90%) was aged >18 years. Cross-sectional studies, cohort studies (prospective and retrospective), and clinical trials were eligible if they reported virological outcomes. Included studies had to report data on the proportion of participants below (or above) a threshold of HIV RNA in copies per milliliter at a specified duration of ART. LMICs were categorized by gross national income according to the World Bank [10]. Previous use of antiretroviral drugs for (pre- or post-exposure) prophylaxis, prevention of mother-to-child transmission, or treatment of HIV infection did not preclude inclusion.

We excluded studies in which participants were HIV-1/HIV-2 coinfected, were receiving mono- or duotherapy, had started second-line ART, were selected based on treatment failure, lived in high-income countries, or were children (aged <13 years or >10% adolescents aged 13–18 years). Studies reporting a median follow-up of <4 months or >5 years on ART only were not eligible for inclusion. Because we wanted to extract and compare summary estimates of virological suppression after 6, 12, 24, 36, 48, and 60 months of ART initiation, studies were excluded if the reported interquartile range (IQR) of follow-up duration extended beyond 2 time points. For example, a study reporting on persons with a median ART duration of 43 months (IQR 28–61) was excluded. When more than 1 study reported on the same cohort of patients, we included the publication that contained the most complete information. Two reviewers independently selected eligible papers and abstracts for inclusion in the analysis and removed duplicates. If the description of the study was unclear with respect to eligibility criteria, we contacted the authors for further information. Any disputes about inclusion were resolved by discussion between the 2 reviewers with help of a third reviewer.
Data Extraction
The following information was extracted from the included articles: location(s) and time frame of the study, study design, number of participants, participants’ characteristics at time of ART initiation, ART regimens (nonnucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor with nucleoside reverse transcriptase inhibitor (NRTI) backbone or triple NRTI regimen), time on first-line ART, number of switches to second-line ART, viral load threshold used to define virological suppression, number of participants with and without viral load results, number of participants with virological suppression, and categories of patient attrition (ie, lost to follow-up [LTFU], death, stop ART) as reported in the study. When 1 study reported virological outcomes at multiple time points and/or reported multiple thresholds to define virological suppression, all outcomes were extracted. When multiple definitions of virological suppression were provided, the study definition closest or equal to 1000 copies per milliliter was included in the analysis to fit the current WHO definition of virological failure and ART program benchmarks [4, 11, 12]. Studies reporting patient outcomes separately, for example, clinical trial arms, were considered to consist of different cohorts. Therefore, the number of cohorts reported in this review exceeds the number of studies included. We assessed quality of reporting using the STROBE checklist (Supplementary Material) [8].

Statistical Analyses
The proportion of study participants meeting the definition of virological suppression was determined for each cohort and for each duration of ART (ie, 6, 12, 24, 36, 48, or 60 months). The primary analysis for each time point was the on-treatment (OT) analysis, that is, the number of participants with virological suppression divided by the number of participants retained in care and with viral load results available at a given time point. The secondary analysis included only studies reporting attrition over time. This intention-to-treat (ITT) analysis included the number of people who were deceased, LTFU, and/or who stopped ART in the denominator of estimates of virological suppression. Switches to second-line ART were not considered virological failures in the OT and ITT analyses. Switch rates were calculated separately by dividing the number of switches to second-line ART by the number of participants at baseline.

Descriptive statistics were used to assess the proportion of virological suppression, followed by random-effects meta-analysis; we determined the summary estimates of people with virological suppression at each time point. The variance of the raw proportions was stabilized using a Freeman–Tukey arcsine square root transformation and subsequently back transformed to the original scale. Between-study heterogeneity was reported by means of the $\tau^2$ of the meta-analysis of the transformed proportions.
Three sensitivity and subgroup analyses were carried out to explore reasons for heterogeneity observed in the analyses. First, we explored the role of study design on the reported outcomes. We excluded trials and analyzed data from programmatic settings only in order to explore the effect of a controlled trial setting. Second, we only included studies in which at least 90% of the participants received NNRTI-based first-line ART in order to look at the effect of WHO-recommended first-line regimens [4]. Third, we explored the role of geographic location by analyzing regions separately. Subgroup differences were tested through $\chi^2$ test for heterogeneity. Statistical analyses were performed using Stata 12 [14].

RESULTS

A total of 2391 research papers and conference abstracts were deemed potentially eligible after duplicate papers were removed. On the basis of title and abstract, 1665 reports were excluded (Figure 1). Of the remaining 726 studies, 163 met the inclusion criteria, including 158 full-text research papers and 5 conference abstracts. In addition, we included unpublished outcome data from the PASER-M cohort (24 and 36 months of follow-up) and TREAT Asia cohort (6, 12, 24, 36, 48, and 60 months of follow-up).

In total, 184 cohorts from 35 countries were retrieved from 163 studies (Figure 2); 69.0% of the cohorts originated from sub-Saharan Africa. Almost half (48.4%) of the studies were prospective cohorts, 20.1% were trials, and the remaining were either cross-sectional or retrospective cohort designs. Almost three-quarters (73.4%) of the studies reported at least 90% of participants initiating NNRTI-based first-line ART. Fifty-four percent adhered to all STROBE guidelines. Study characteristics are summarized in Table 1, and a full list and characteristics of all included studies are provided in Supplementary Table. Sample sizes ranged from 8 to 27 516 study participants, and most studies report outcomes up to 2 years after ART initiation (Table 2). All outcomes beyond 2 years from ART initiation originated from Asia and sub-Saharan Africa, and 5-year outcomes were only available for OT outcomes.

OT summary estimates of virological suppression for all time points assessed were >80%. For cohorts on ART for 6, 12, and 24 months, OT summary estimates were 84.9% (95%CI, 83.5–86.3; N = 116 051), 85.6% (95% CI, 84.4–86.9; N = 103 632), and 84.4% (95% CI, 82.0–86.9; N = 39 694), respectively. After 3, 4, and 5 years of ART,
**Figure 1.** Flow chart of study selection.
An asterisk (*) indicates studies without original or virological data, studies with a design not meeting inclusion criteria or without specific data on duration of antiretroviral treatment (ART), studies outside low- and middle-income countries, and studies with only pediatric participants.
Abbreviations: CENTRAL, Cochrane Central Register of Controlled Trials; CROI, Conference on Retroviruses and Opportunistic Infections; HIV, human immunodeficiency virus; IAS, International AIDS Society; LMIC, low- and middle-income countries; PASER, Pan-African Studies to Evaluate Resistance; TREAT Asia, Therapeutics Research, Education, and AIDS Training in Asia.
Figure 2. World map indicating 35 countries reporting data included.

Table 1. Summary of Cohort Characteristics, by Analysis Type.

<table>
<thead>
<tr>
<th>Region</th>
<th>On-Treatment Analysis</th>
<th>Intention-to-Treat Analysis</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>127</td>
<td>69.0</td>
<td>61</td>
<td>65.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>38</td>
<td>20.7</td>
<td>23</td>
<td>24.7</td>
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<td></td>
</tr>
<tr>
<td>Latin-America and the Caribbean</td>
<td>14</td>
<td>7.6</td>
<td>7</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple regions</td>
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<td>2.2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prospective cohort</td>
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<td>48.4</td>
<td>48</td>
<td>51.6</td>
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<td></td>
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<tr>
<td>Retrospective cohort</td>
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<td>21</td>
<td>22.6</td>
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<td></td>
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<tr>
<td>Trial (any)</td>
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<td>20.1</td>
<td>23</td>
<td>24.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>19</td>
<td>10.3</td>
<td>1</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of antiretroviral therapy initiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before 2006</td>
<td>81</td>
<td>50.9</td>
<td>34</td>
<td>43.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 2006</td>
<td>78</td>
<td>49.1</td>
<td>44</td>
<td>56.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not reported</td>
<td>25</td>
<td>13.6</td>
<td>15</td>
<td>16.1</td>
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<td></td>
</tr>
<tr>
<td>ART regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nonnucleoside reverse transcriptase inhibitor–based</td>
<td>135</td>
<td>73.4</td>
<td>70</td>
<td>75.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protease inhibitor–based</td>
<td>7</td>
<td>3.8</td>
<td>6</td>
<td>6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other or unknown</td>
<td>42</td>
<td>22.8</td>
<td>17</td>
<td>18.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>100.0</td>
<td>93</td>
<td>50.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For a full list and characteristics of study cohorts included in the systematic review, see Supplementary Table.

a Studies reporting numbers of deaths and persons lost to follow-up were included in the intention-to-treat analysis.

b Nonnucleoside reverse transcriptase inhibitor/protease inhibitor (NNRTI/PI)-based regimen defined as >90% of participants receiving an NNRTI/PI as part of first-line antiretroviral therapy.

c Including triple nucleoside reverse transcriptase inhibitor regimens.
the summary estimates were 88.5% (95% CI, 85.5–91.4; N = 18 729), 88.6% (95% CI, 84.2–93.0; N = 4673), and 85.2% (95% CI, 76.6–93.9; N = 1414), respectively.

The ITT analysis included a subset of 93 (50.5%) cohorts reporting LTFU and mortality outcomes. Summary estimates of the ITT analysis after 6, 12, 24, 36, and 48 months of ART were 74.7% (95% CI, 72.2–77.2; N = 47 838), 67.3% (95% CI, 63.6–71.0; N = 40 938), 64.6% (95% CI, 60.8–68.4; N = 6332), 68.1% (95% CI, 58.0–78.2; N = 1411), and 61.8% (95% CI, 44.0–79.7; N = 504) virological suppression, respectively. Descriptive and summary estimates of OT and ITT virological suppression are presented in Table 2 and Figure 3; forest plots displaying individual and summary estimates can be found in Supplementary Figures.

Of 93 cohorts included in the ITT analysis, 46.4% reported the number of switches to second-line ART. Summary estimates of the reported switches were <2% during the first 2 years of ART: 1.7% (95% CI, 1.0–2.5; N = 24 451), 1.6 (95% CI, 1.1–2.1; N = 19 923), and 1.7% (95% CI, 1.7–2.6; N = 7100) after 6, 12, and 24 months of first-line ART, respectively. After 36 and 48 months, 5.4% (95% CI, 3.3–7.5; N = 2407) and 9.2% (95% CI, 4.8–13.6; N = 595) of participants switched (Table 3, Figure 4).

Sensitivity analyses excluding clinical trials and analyses excluding studies in which <90% of participants received NNRTI-based ART did not yield different summary estimates in either the OT or ITT analyses (Supplementary Tables). However, regional differences were found in subgroup analyses comparing studies conducted in sub-Saharan Africa to studies from Asia, as shown in Figure 3. At 2 time points, there was a statistically significant difference in OT virological suppression. At 6 months, the OT summary estimate was 89.6% (95% CI, 87.3–92.0; N = 3942) in Asia compared with 84.2% (95% CI, 82.5–85.9; N = 100 613) in sub-Saharan Africa (P = <.01). At 12 months, the OT summary estimate was 90.2% (95% CI, 87.7–92.6; N = 5997) in Asia compared with 84.3% (95% CI, 82.8–85.8; N = 93 415) in sub-Saharan Africa (P = <.01). Although summary estimates from Asia were generally higher compared with those from sub-Saharan Africa, no differences were observed at any of the other time points assessed. Statistically significant regional differences were also observed in the ITT analysis at months 12 and 24; summary estimates were approximately 12% higher in Asia compared with sub-Saharan Africa. At 12 months of ART, we found 76.4% (95% CI, 70.4–82.5; N = 2354) virological suppression in Asia vs 64.3% (95% CI, 60.0–68.7; N = 37 898) in sub-Saharan Africa (P = .01). At 24 months, we found 73.9% (95% CI, 69.2–78.5; N = 1634) virological suppression in Asia vs 62.3% (95% CI, 57.2–67.4; N = 4356) in sub-Saharan Africa (P = .01).
Table 2. Virological Suppression after 6 to 60 Months of First-Line Antiretroviral Therapy.

<table>
<thead>
<tr>
<th>Months on Antiretroviral Therapy</th>
<th>Cumulative N</th>
<th>Participants, N</th>
<th>Total Median</th>
<th>Interquartile Range</th>
<th>Interquartile Range</th>
<th>Summary Estimate</th>
<th>Low</th>
<th>High</th>
<th>τ²</th>
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</thead>
<tbody>
<tr>
<td>On-treatment analysis</td>
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<td></td>
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</tr>
<tr>
<td>6</td>
<td>100</td>
<td>116,051</td>
<td>173</td>
<td>84</td>
<td>453</td>
<td>87.2</td>
<td>78.7</td>
<td>91.3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>117</td>
<td>103,632</td>
<td>174</td>
<td>78</td>
<td>427</td>
<td>87.7</td>
<td>80.8</td>
<td>91.9</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>45</td>
<td>39,694</td>
<td>203</td>
<td>78</td>
<td>475</td>
<td>86.0</td>
<td>80.5</td>
<td>91.9</td>
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<tr>
<td>36</td>
<td>21</td>
<td>18,729</td>
<td>373</td>
<td>163</td>
<td>980</td>
<td>90.1</td>
<td>82.5</td>
<td>93.6</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>13</td>
<td>4,673</td>
<td>201</td>
<td>104</td>
<td>384</td>
<td>90.9</td>
<td>85.1</td>
<td>94.6</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>1,433</td>
<td>117</td>
<td>60</td>
<td>505</td>
<td>86.6</td>
<td>78.6</td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>Intention-to-treat analysis</td>
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<td></td>
<td></td>
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<tr>
<td>6</td>
<td>40</td>
<td>47,838</td>
<td>106</td>
<td>40</td>
<td>371</td>
<td>72.9</td>
<td>63.8</td>
<td>82.3</td>
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<tr>
<td>12</td>
<td>67</td>
<td>40,938</td>
<td>153</td>
<td>66</td>
<td>351</td>
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<td>24</td>
<td>23</td>
<td>6,332</td>
<td>142</td>
<td>66</td>
<td>321</td>
<td>65.0</td>
<td>52.4</td>
<td>72.2</td>
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<tr>
<td>36</td>
<td>4</td>
<td>1,411</td>
<td>309</td>
<td>160</td>
<td>546</td>
<td>63.0</td>
<td>61.3</td>
<td>75.1</td>
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<tr>
<td>48</td>
<td>4</td>
<td>504</td>
<td>130</td>
<td>66</td>
<td>187</td>
<td>59.0</td>
<td>51.4</td>
<td>72.4</td>
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</tr>
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</table>

*τ² is a measure of between-study heterogeneity that is less affected by the number of studies than other common measures.

Table 3. Switches to Second-Line Antiretroviral Therapy after 6 to 48 Months of First-Line Antiretroviral Therapy Descriptive.

<table>
<thead>
<tr>
<th>Months on Antiretroviral Therapy</th>
<th>Cumulative N</th>
<th>Participants, N (at baseline)</th>
<th>% of Studies from Intention-to-Treat Analysis</th>
<th>Total Median</th>
<th>Interquartile Range</th>
<th>Summary Estimate</th>
<th>Low</th>
<th>High</th>
<th>τ²</th>
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<tbody>
<tr>
<td>6</td>
<td>22</td>
<td>55.0</td>
<td>24,451</td>
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<td>27</td>
<td>382</td>
<td>1.7</td>
<td>1.0</td>
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<tr>
<td>12</td>
<td>25</td>
<td>37.5</td>
<td>19,923</td>
<td>230</td>
<td>61</td>
<td>596</td>
<td>0.0</td>
<td>0.0</td>
<td>1.4</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>52.2</td>
<td>7,100</td>
<td>283</td>
<td>104</td>
<td>701</td>
<td>1.7</td>
<td>0.7</td>
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<tr>
<td>36</td>
<td>3</td>
<td>75.0</td>
<td>2,407</td>
<td>1,009</td>
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</tr>
<tr>
<td>48</td>
<td>2</td>
<td>50.0</td>
<td>595</td>
<td>298</td>
<td>80</td>
<td>515</td>
<td>9.2</td>
<td>4.8</td>
<td>13.6</td>
</tr>
</tbody>
</table>

*τ² is a measure of between-study heterogeneity that is less affected by the number of studies than other common measures.
Long-term HIV suppression in LMICs

### A. On-treatment virological suppression

#### Figure 3. Summary estimates of virological suppression outcomes.

Note: All summary estimates are informed by a forest plot (see Supplementary Figures).

Full tables of the subgroup analysis are provided in Supplementary Tables.

<table>
<thead>
<tr>
<th>Months on antiretroviral therapy</th>
<th>All regions</th>
<th>Sub-Saharan Africa only</th>
<th>Asia only</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
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<tr>
<td>12</td>
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<td>60</td>
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</tr>
</tbody>
</table>

### B. Intention-to-treat virological suppression

#### Figure 4. Switches to second-line therapy.

Note: All summary estimates are informed by a forest plot (see Supplementary Figures).

Full tables of the subgroup analysis are provided in Supplementary Tables.
DISCUSSION

This review describes summary estimates of virological suppression for people receiving first-line ART for HIV in 35 LMICs for up to 5 years after treatment initiation. Reported virological outcomes for people who remained in care and on treatment were stable over time, with OT virological suppression rates remaining >80% for up to 5 years after ART initiation. In the ITT analysis, which considered all people who died, stopped ART, or were LTFU as having virological failure, the virological suppression rate declined to 62% after 4 years. The outcomes were consistent irrespective of study design and ART regimen. Early virological suppression rates were found to be significantly higher in Asia compared with sub-Saharan Africa, but differences tended to disappear over time.

Overall, OT estimates of virological suppression up to 36 months on ART were consistently high and informed by a large number of cohorts and study participants. Similar trends have been reported in resource-rich countries, where the proportion of patients with virological suppression has been shown to increase over time in the Netherlands and British Columbia [15, 16]. Stable rates of OT virological suppression may be partially explained by the inherent survivor bias of analyses limited to individuals on ART retained in care [17, 18]. Spontaneous viral resuppression and the impact of adherence interventions may also contribute to the maintenance of high levels of virological suppression among individuals on ART. However, the aggregate proportion of individuals who died, were LTFU, or stopped therapy increased over time, leading to decreasing rates of virological suppression in the ITT analysis. Interestingly, we did not observe an increase in the proportion of individuals with virological failure retained in care. This implies that those failing first-line ART either dropped out of care or were switched to a second-line regimen. Although the proportion of patients switching to second-line care did increase over time in the subset of studies with available data, such numbers were small and consistent with the very low uptake of second-line ART in LMICs in general, suggesting that the majority of individuals failing ART most likely dropped out of care before they could be identified [2].

In contrast to previous reviews [19–21], this review included data from all LMICs including Asia and Latin America and the Caribbean for up to 5 years of follow-up. A previously published review of 12-month outcomes across all LMICs reported 84% suppression at 12 months [20]. Two other reviews that reported on virological outcomes on ART in sub-Saharan Africa showed lower OT suppression rates compared with this review: 78%, 76%, 67% vs 75%, 67%, 65% after 6, 12, and 24 months of ART [19, 21]. One of these reviews (concerning sub-Saharan Africa only) also reported ITT
Long-term HIV suppression in LMICs

outcomes, which were in line with our findings: 78%, 69%, 63% after 6, 12, and 24 months of ART [19]. Early virological suppression rates were found to be significantly higher in Asia compared with sub-Saharan Africa, but differences tended to disappear over time. While this may indicate superior program performance in the early stages of ART in Asia, the paucity of data after 36 months prevents additional analyses.

This review has several limitations. First, it reflects findings from clinics where patients had access to virological monitoring or access to virological testing for research purposes, which likely represent well-resourced ART sites [22]. Therefore, estimates of virological suppression presented in this review may not be representative of actual program conditions that may be prevalent in other clinics and/or settings. ART programs or clinics that evaluated and reported viral loads may have greater resources and better clinical outcomes than those where viral loads were not evaluated and/or where virological outcomes were not reported. Second, in settings where viral loads were evaluated but where poor virological outcomes were observed, researchers may have chosen not to disseminate the results, potentially leading to publication bias. Estimates may therefore overestimate the mean frequency of virological suppression in the broader population receiving ART in LMICs. Third, we searched for studies reporting on virological outcomes of ART and not specifically on attrition. Therefore, the ITT analysis could report biased results as attrition rates could possibly be underreported in the included studies, potentially overestimating program efficacy. Conversely, the assumption that all those who died or were LTFU had detectable viral loads is likely to yield conservative estimates. Fourth, this review included studies reporting OT outcomes from cross-sectional analysis and it did not focus only on studies following cohorts longitudinally. One should take into account that the findings include cross-sectional outcomes from different studies at each time point, which could induce some bias in interpreting differences across time since these differences may be attributable to the studies included in the analysis rather than a true change in virological suppression over time. Fifth, this review might include overlap in data between studies. Because of thorough exclusion, we believe that the presence of duplicate data in our review is minimal.

Strengths of our review include its broad scope, encompassing studies from various ART programs across LMICs and complementary unpublished data from large African and Asian cohort studies. We were able to assemble a large dataset for meta-analysis, especially at the time points up to 3 years after the initiation of ART. Our results were consistent in different sensitivity and subgroup analyses, taking the study design and type of ART into account. This review included both longitudinal and cross-sectional studies from programmatic and clinical trial settings, yet excluding trials from the analysis did not affect summary estimates. Additionally, studies reporting both pro-
tease inhibitor- and NNRTI-based regimens were included, but subgroup analyses examining different regimens were comparable to the overall analysis.

In the context of lifelong treatment, data on long-term virological outcomes from LMICs are much needed [23]. This review found high and stable levels of virological suppression (>80%) among individuals retained in care during the first 5 years of first-line ART in LMICs. When accounting for individuals who were LTFU, died, or stopped therapy and if one assumes that all of them had virological failure, available data suggest that suppression rates declined during the first 4 years of ART. This review also highlights the need for more accurate data on retention to inform the development of a comprehensive benchmark framework to assess and monitor program performance. Further research is needed to better describe virological outcomes among individuals who are LFTU and among those who stop therapy, so that they can be taken into account in future estimates of population-level virological suppression.

SUPPLEMENTARY DATA

Supplementary materials are available at Clinical Infectious Diseases online (http://www.cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

NOTES

Acknowledgments

We thank Annette Sohn and Awachana Jiamsakul for providing further data on behalf of the TAHOD (TREAT Asia HIV Observational Database) (TREAT Asia) (TREAT Asia Studies to Evaluate Resistance [TASER]/TAHOD) program. Also, we thank Ingeborg Nagel for librarian assistance with the search and Caspar Roelofs and Vera van Rijn for their research assistance on literature selection and data extraction.

Author contributions

S. B. conceived the systematic review. T. S. B. did the initial search of published work, checked all full-text articles, extracted data from the full reports and conference abstracts, conceived and coordinated the analyses, and wrote the first draft of the paper. Literature selection and data extraction were performed with the help of 2 research
assistants. K. C. E. S. also checked all full-text articles and abstracts, was available to resolve conflicts during data extraction, and supervised the reviewing process. N. F. supported the statistical analyses. J. H. M., M. R. J., N. F., S. K., T. F. R. d. W., J. B., and S. B. participated in discussion of the results. All authors participated in writing of the final paper.

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**Potential conflicts of interest.**

S. B. and N. F. are staff members of WHO. J. B. is a consultant with the WHO. The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions or stated policies of the WHO. J. H. M. has a dual appointment with the Department of Public Health and Community Medicine, Tufts University School of Medicine, Boston, Massachusetts, and the Alfred Hospital, Melbourne, Australia. J. H. M.’s institution (Alfred Hospital) has received payment for consultancy to Gilead and Viiv Healthcare. M. R. J. has an appointment with the Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, Massachusetts, and is also a consultant for WHO. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
REFERENCES


Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa.


Clinical Infectious Diseases
ABSTRACT

Background
After the scale-up of antiretroviral therapy (ART) for human immunodeficiency virus (HIV) infection in Africa, increasing numbers of patients have pretreatment drug resistance.

Methods
In a large multicountry cohort of patients starting standard first-line ART in six African countries, pol genotyping was retrospectively performed if viral load (VL) \( \geq 1000 \) cps/mL. Pretreatment drug resistance was defined as a decreased susceptibility to \( \geq 1 \) prescribed drug. We assessed the effect of pretreatment drug resistance on all-cause mortality, new AIDS events and switch to second-line ART due to presumed treatment failure, using Cox models.

Results
Among 2579 participants for whom a pretreatment genotype was available, 5.5% had pretreatment drug resistance. Pretreatment drug resistance was associated with an increased risk of regimen switch (adjusted hazard ratio [aHR] 3.80; 95% confidence interval [CI], 1.49–9.68; \( P = .005 \)) but was not associated with mortality (aHR 0.75, 95% CI, .24–2.35; \( P = .617 \)) or new AIDS events (aHR 1.06, 95% CI, .68–1.64; \( P = .807 \)). During three years of follow up, 106 (4.1%) participants switched to second-line, of whom 18 (17.0%) switched with VL < 1000 cps/mL, 7 (6.6%) with VL \( \geq 1000 \) cps/mL and no drug resistance mutations (DRMs), 46 (43.4%) with VL \( \geq 1000 \) cps/mL and \( \geq 1 \) DRMs; no HIV RNA data was available for 32 (30.2%) participants.

Conclusions
Given rising pretreatment HIV drug resistance levels in sub-Saharan Africa, these findings underscore the need for expanded access to second-line ART. VL monitoring can improve the accuracy of failure detection and efficiency of switching practices.
INTRODUCTION

Nearly 12 million people living with human immunodeficiency virus (HIV) in low- and middle-income countries received antiretroviral therapy (ART) by the end of 2013. In the context of the ambitious UNAIDS targets set for 2020 and updated treatment guidelines aiming for earlier treatment initiation, it is anticipated that increasing numbers of people will start ART in the coming years [1, 2]. The expanding exposure to antiretroviral drugs at a population level will lead to increased transmission of drug-resistant viruses [3]. As a consequence, ART programs will be confronted with increasing numbers of patients that already carry drug resistant strains before starting standard first-line ART; pretreatment drug resistance (PDR). The rise in PDR is largely attributable to an increase in nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance [3, 4].

Previous studies from low- and middle-income [5–7] as well as high-income countries [8] have shown that individuals with PDR are at risk of a diminished virological response to first-line ART. Given the fact that in low- and middle-income countries viral load (VL) monitoring is very limited and genotypic resistance testing is largely absent, regimen switching is often guided by CD4+ cell counts and clinical criteria. This approach can lead to incorrect assumptions, risking continuation of a failing regimen on the one hand, and inappropriate switches to second-line ART (while the virus is suppressed) on the other hand [9, 10]. Even in the presence of VL monitoring, studies from Uganda and South Africa reported a delay in switching after the detection of virological failure, resulting in increased mortality relative to switching [11, 12]. Currently, access to second-line ART is restricted in many settings due to high costs; rates of switching to second-line ART have remained relatively low in resource-limited settings [13–15].

The consequences of rising levels of PDR in sub-Saharan Africa on long-term clinical outcomes on first-line ART have not been evaluated. In a large longitudinal multi-country study, we assessed the impact of PDR on switching from first- to second-line ART due to presumed therapy failure, and the occurrence of new AIDS-events and death following up to three years of first-line ART.
METHODS

Study Design and Population
The Pan-African Studies to Evaluate Resistance Monitoring (PASER-M), a prospective cohort study at 13 clinical sites in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe [16], enrolled patients who were HIV-1 infected, aged ≥18 years, and eligible to start first-line ART because of advanced immunodeficiency (CD4+ cell count <200 cells/µL) or HIV disease (World Health Organization [WHO] clinical stage 3 or 4) in accordance with national guidelines, between 2007 and 2009 [17]. Exclusion criteria were: pregnancy at enrolment, HIV-2 coinfection (in Nigeria), and previous use of ART ≤30 days before treatment initiation. Cohort characteristics and first year outcomes have been described previously [4, 5, 16]. For the current analysis, all participants receiving a standard first-line regimen consisting of one NNRTI drug with a dual nucleoside reverse transcriptase inhibitor (NRTI) backbone who had genotypic resistance test (GRT) results available at ART initiation were included. Participants provided written informed consent at enrolment. The study protocol was approved by national and local research ethics committees.

All participants received routine care according to local guidelines. A standard case-report form capturing data on demographics, clinical status, medication, adherence, and laboratory results was completed at enrolment, every 3 months thereafter, and at regimen switch. CD4+ cell counts recorded closest to the ART start date were used as pretreatment counts. Follow-up time was measured from ART initiation, and participants were followed up for 24 (all sites) or 36 (5 sites in Nigeria, Zimbabwe and Uganda) months on ART, or until discontinuation because of death, transfer-out, loss-to-follow-up (LTFU, when not seen for >180 days [18]) or switch to second-line ART.

Laboratory Methods
Plasma samples were collected before ART initiation, after 12, 24, and 36 months (if applicable) and at the time of regimen switch, for retrospective testing of HIV-RNA and GRT. VL was determined using NucliSens EasyQ real-time assay (version 2.0; bioMérieux, Lyon, France) or COBAS Ampliprep/COBAS Taqman assay (Roche, Branchburg, New Jersey). Virological suppression was defined as VL < 400 cps/mL. Subsequently, GRT of protease and partial reverse-transcriptase was performed if VL ≥ 1000 cps/mL, using in-house sequencing methods. HIV drug resistance was determined in 2 steps for each sequence. First, major drug resistance mutations (DRMs) were scored using the 2014 IAS-USA list [19]. Second, the genotype susceptibility scores (GSS) of the prescribed ART regimen were calculated using the Stanford algorithm (Version 7.0) [20]. Subsequently, participants were classified in 2 categories: “PDR”: presence of ≥1
HIV drug resistance increases ART switch

DRM associated with reduced susceptibility to the prescribed regimen (GSS < 3); and “no PDR”: no DRM or presence of DRMs not associated with the prescribed regimen (GSS = 3). Subtypes were determined using the REGA algorithm version 3 [21].

**Statistical Analysis**

We evaluated the effect of PDR on the incidence of clinical outcomes through a time-to-event analysis. Three outcomes were defined: (I) switch to second-line ART due to presumed treatment failure; (II) onset of new AIDS events (WHO stage 4 event [2]), that is, a first-ever event, a new condition on top of an ongoing event, or recurrence of the condition; and (III) all-cause mortality. Based on retrospective testing, unnecessary switches were defined as switch with VL < 1000 cps/mL or VL ≥ 1000 cps/mL without DRMs based on GRT [2].

Participants were censored when transferred out, or when they received a substitution with a protease inhibitor for reasons other than treatment failure; intra-class drug substitutions were ignored. Additional censoring occurred per analysis: at switch to second-line when investigating all-cause mortality and new AIDS-events; at death for the analysis of new AIDS-events and switch to second-line. The association between PDR and the 3 outcomes was modeled through Kaplan–Meier curves with a Tarone–Ware test and univariate Cox-models, and multivariable using Cox-models with age and sex as fixed-covariates, and CD4+ cell count and 30-day self-reported adherence (visual analogue score <95% or ≥95%) as time-varying covariates. Standard errors were adjusted for clustering within clinical site. The following fixed-variables were considered confounders and included in the model if ≥10% changes in the regression coefficient describing the association between PDR and all four outcomes: WHO clinical stage at baseline (1–3 vs 4), prior antiretroviral drug exposure (yes, no, unknown), year of ART initiation (2007, 2008, 2009), hemoglobin at baseline, and subtype (C, non-C). The proportional-hazard assumption was checked using log-log plots and testing the Schoenfeld residuals.

**Sensitivity Analyses**

First, we repeated the analyses using more specific outcomes: HIV-related mortality instead of all-cause mortality and “appropriate switches” (VL ≥ 1000 cps/mL and ≥1 DRM) instead of any switch. Second, we repeated the analyses for all sites up to 24 months, vs the 5 sites who had follow-up data available up to 36 months. Third, we repeated the analyses, only for participants who were antiretroviral drug-naive, excluding those who had any prior antiretroviral drug exposure. All analyses were performed using Stata 12 [22].
RESULTS

Of the 2737 participants initiating ART, 2579 (94.2%) had GRT results available and were included in our analysis (Figure 1, Table 1). In total, 357 (13.8%) had DRMs: 297 (11.5%) had NNRTI-mutations, 69 (2.7%) had NRTI-mutations, and 37 (1.4%) had dual-class resistance. Overall, 141/2579 (5.5%) participants were classified as having PDR. All pretreatment DRMs are listed in the Supplementary Material. After 12 and 24 months respectively, 83.4% and 72.2% of patients were still alive, retained in care and receiving first-line ART. In Nigeria, Uganda, and Zimbabwe, 64.6% of participants were still alive and retained on first-line ART after 36 months (Figure 1). LTFU was equal in participants with and without PDR. Most participants remained on the initial regimen; 728 (28.3%) received at least 1 drug substitution for reasons other than treatment failure (range 1–4); protease inhibitor-substitutions for reasons other than treatment failure occurred in 55 (0.02%) participants who were subsequently censored.

**Figure 1.** Study flow.
The flow shows the cumulative attrition and is calculated from the baseline number.
Abbreviations: ART, antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; PASERM, Pan-African Studies to Evaluate Resistance Monitoring.
HIV drug resistance increases ART switch

<p>| Table 1. Pan-African Studies to Evaluate Resistance Monitoring Population Characteristics at Antiretroviral Therapy Initiation, by Predicted Regimen Activity. |
|---|---|---|---|---|
| Total | No PDR | PDR |</p>
<table>
<thead>
<tr>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>PValue</th>
</tr>
</thead>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1,493</td>
<td>57.9</td>
<td>1,409</td>
<td>57.8</td>
<td>84</td>
<td>59.6</td>
</tr>
<tr>
<td>Male</td>
<td>1,086</td>
<td>42.1</td>
<td>1,029</td>
<td>42.2</td>
<td>57</td>
<td>40.4</td>
</tr>
<tr>
<td>Age (mean, SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-29</td>
<td>36.8</td>
<td>9.0</td>
<td>36.7</td>
<td>9.0</td>
<td>37.5</td>
<td>8.8</td>
</tr>
<tr>
<td>30-39</td>
<td>1,116</td>
<td>45.1</td>
<td>1,100</td>
<td>45.1</td>
<td>66</td>
<td>46.8</td>
</tr>
<tr>
<td>≥40</td>
<td>907</td>
<td>35.2</td>
<td>858</td>
<td>35.2</td>
<td>49</td>
<td>34.8</td>
</tr>
<tr>
<td>Country</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zambia</td>
<td>551</td>
<td>21.4</td>
<td>523</td>
<td>21.5</td>
<td>28</td>
<td>19.9</td>
</tr>
<tr>
<td>South Africa</td>
<td>601</td>
<td>23.3</td>
<td>574</td>
<td>23.5</td>
<td>27</td>
<td>19.2</td>
</tr>
<tr>
<td>Uganda</td>
<td>606</td>
<td>23.5</td>
<td>545</td>
<td>22.4</td>
<td>61</td>
<td>43.3</td>
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<tr>
<td>Kenya</td>
<td>424</td>
<td>16.4</td>
<td>411</td>
<td>16.9</td>
<td>13</td>
<td>9.2</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>204</td>
<td>7.9</td>
<td>198</td>
<td>8.1</td>
<td>6</td>
<td>4.3</td>
</tr>
<tr>
<td>Nigeria</td>
<td>193</td>
<td>7.5</td>
<td>187</td>
<td>7.7</td>
<td>6</td>
<td>4.3</td>
</tr>
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<td>Calendar year of initiation</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>619</td>
<td>24.0</td>
<td>590</td>
<td>24.2</td>
<td>29</td>
<td>20.6</td>
</tr>
<tr>
<td>2008</td>
<td>1,595</td>
<td>61.9</td>
<td>1,496</td>
<td>61.4</td>
<td>99</td>
<td>70.2</td>
</tr>
<tr>
<td>2009</td>
<td>365</td>
<td>14.2</td>
<td>352</td>
<td>14.4</td>
<td>13</td>
<td>0.2</td>
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<tr>
<td>WHO clinical stage at initiation</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>382</td>
<td>14.8</td>
<td>368</td>
<td>15.1</td>
<td>14</td>
<td>9.9</td>
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<tr>
<td>2</td>
<td>618</td>
<td>24.0</td>
<td>576</td>
<td>23.6</td>
<td>42</td>
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<tr>
<td>3</td>
<td>1,150</td>
<td>44.6</td>
<td>1,087</td>
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<tr>
<td>4</td>
<td>429</td>
<td>16.6</td>
<td>407</td>
<td>16.7</td>
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<td>15.6</td>
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<tr>
<td>Previous antiretroviral experience</td>
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<tr>
<td>No</td>
<td>2,442</td>
<td>94.7</td>
<td>2,333</td>
<td>95.7</td>
<td>109</td>
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<tr>
<td>Yes</td>
<td>115</td>
<td>4.5</td>
<td>86</td>
<td>3.5</td>
<td>29</td>
<td>20.6</td>
</tr>
<tr>
<td>Unknown</td>
<td>22</td>
<td>0.9</td>
<td>19</td>
<td>0.8</td>
<td>3</td>
<td>2.1</td>
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<tr>
<td>Type of initial NRTI backbone</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zidovudine-based</td>
<td>960</td>
<td>37.2</td>
<td>904</td>
<td>37.1</td>
<td>56</td>
<td>39.7</td>
</tr>
<tr>
<td>Stavudine-based</td>
<td>685</td>
<td>26.6</td>
<td>652</td>
<td>26.7</td>
<td>33</td>
<td>23.4</td>
</tr>
<tr>
<td>Tenofovir-based</td>
<td>870</td>
<td>33.7</td>
<td>821</td>
<td>33.7</td>
<td>49</td>
<td>34.8</td>
</tr>
<tr>
<td>Abacavir-based</td>
<td>64</td>
<td>2.5</td>
<td>61</td>
<td>2.5</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>Type of initial NNRTI</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Efavirenz</td>
<td>1,542</td>
<td>59.8</td>
<td>1,464</td>
<td>60.1</td>
<td>78</td>
<td>55.3</td>
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<tr>
<td>Nevirapine</td>
<td>1,037</td>
<td>40.2</td>
<td>974</td>
<td>39.9</td>
<td>63</td>
<td>44.7</td>
</tr>
</tbody>
</table>
Virological suppression was achieved in 89.8% (95% confidence interval [CI], 88.4–91.1), 89.8% (95% CI, 88.3–91.2), and 90.7% (95% CI, 88.1–92.8) of participants retained in care at 12, 24, and 36 months, respectively. Suppression rates were significantly lower in people with vs those without PDR: 76.2% (95% CI, 66.7–84.1), 78.8% (95% CI, 67.0–87.9) and 81.4% (95% CI, 66.6–91.6) vs 90.5% (95% CI, 89.1–91.8), 90.3% (95% CI, 88.7–91.6) and 91.3% (95% CI, 88.8–93.5) after 12, 24, and 36 months, respectively (P < .001; P = .003; and P = .030).

### Table 1. Pan-African Studies to Evaluate Resistance Monitoring Population Characteristics at Antiretroviral Therapy Initiation, by Predicted Regimen Activity. (continued)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
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<th>PDR</th>
<th>PValue</th>
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<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>BMI (median, IQR)</td>
<td>21.1</td>
<td>18.8-24.1</td>
<td>21.1</td>
<td>18.8-24.1</td>
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<tr>
<td>Haemoglobin g/dL (&lt;mean, SD)</td>
<td>11.4</td>
<td>2.2</td>
<td>11.4</td>
<td>4.2</td>
</tr>
<tr>
<td>CD4+ cell count (median, IQR) &lt;50</td>
<td>133</td>
<td>62-204</td>
<td>133</td>
<td>62-203</td>
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<tr>
<td>CD4+ cell count 50-200</td>
<td>1,342</td>
<td>52.2</td>
<td>1,269</td>
<td>52.2</td>
</tr>
<tr>
<td>CD4+ cell count 200-350</td>
<td>674</td>
<td>26.2</td>
<td>637</td>
<td>26.2</td>
</tr>
<tr>
<td>CD4+ cell count ≥350</td>
<td>21</td>
<td>0.8</td>
<td>20</td>
<td>0.8</td>
</tr>
<tr>
<td>HIV-RNA copies per ml, log10 (mean, SD)</td>
<td>5.0</td>
<td>0.8</td>
<td>5.0</td>
<td>0.8</td>
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<tr>
<td>HIV-1 subtype</td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>616</td>
<td>23.9</td>
<td>548</td>
<td>24.0</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>0.2</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>C</td>
<td>1,387</td>
<td>53.8</td>
<td>1,325</td>
<td>54.4</td>
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<td>CRF02_AG</td>
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<td>96</td>
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</tr>
<tr>
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<td>5</td>
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<td>1</td>
<td>0.04</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>D</td>
<td>237</td>
<td>9.2</td>
<td>208</td>
<td>8.5</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0.01</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>G</td>
<td>68</td>
<td>2.6</td>
<td>64</td>
<td>2.6</td>
</tr>
<tr>
<td>Recombinants</td>
<td>254</td>
<td>6.0</td>
<td>145</td>
<td>6.0</td>
</tr>
<tr>
<td>Unassigned</td>
<td>7</td>
<td>0.3</td>
<td>5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Subtypes were determined using the REGA version 3 [21].

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PDR, pretreatment drug resistance; SD, standard deviation; WHO, World Health Organization.

a Based on 2562 observations.
b Based on 2533 observations.
c Based on 2571 observations.
### Table 2. Virological and Drug Resistance Outcomes.

<table>
<thead>
<tr>
<th></th>
<th>12 mo</th>
<th></th>
<th>24 mo</th>
<th></th>
<th>36 mo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>No PDR</td>
<td>PDR</td>
<td>Total</td>
<td>No PDR</td>
<td>PDR</td>
</tr>
<tr>
<td><strong>Viral Load</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results available</td>
<td>1978</td>
<td>95.0</td>
<td>1877</td>
<td>94.9</td>
<td>101</td>
<td>96.2</td>
</tr>
<tr>
<td>&lt;1000 cps/mL</td>
<td>1803</td>
<td>91.2</td>
<td>1725</td>
<td>91.9</td>
<td>78</td>
<td>77.2</td>
</tr>
<tr>
<td>&lt;400 cps/mL</td>
<td>1776</td>
<td>89.8</td>
<td>1699</td>
<td>90.5</td>
<td>77</td>
<td>76.2</td>
</tr>
<tr>
<td>&lt;50 cps/mL</td>
<td>1638</td>
<td>82.8</td>
<td>1571</td>
<td>83.7</td>
<td>67</td>
<td>66.3</td>
</tr>
<tr>
<td><strong>Drug Resistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results available</td>
<td>149</td>
<td>85.1</td>
<td>129</td>
<td>84.9</td>
<td>20</td>
<td>87.0</td>
</tr>
<tr>
<td>Any mutation</td>
<td>114</td>
<td>76.5</td>
<td>94</td>
<td>72.9</td>
<td>20</td>
<td>100.0</td>
</tr>
<tr>
<td>NNRTI-resistance</td>
<td>101</td>
<td>67.8</td>
<td>81</td>
<td>62.8</td>
<td>20</td>
<td>100.0</td>
</tr>
<tr>
<td>NRTI-resistance</td>
<td>93</td>
<td>62.4</td>
<td>74</td>
<td>57.4</td>
<td>19</td>
<td>95.0</td>
</tr>
</tbody>
</table>

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PDR, pretreatment drug resistance.
Among participants with a VL ≥ 1000 cps/mL and successful GRT (88.2%) after 12, 24, and 36 months, DRMs were present in 75.6% (95% CI, 68.9–83.1), 80.9% (95% CI, 73.4–87.0), and 82.5% (95% CI, 67.2–92.7). DRM rates were higher in people with PDR vs those without PDR: 100.0% (95% CI, 83.2–100.0), 90.0% (95% CI, 55.–99.7), and 100.0% (95% CI, 59.0–100.0) vs 72.9% (95% CI, 64.3–80.3), 80.2% (95% CI, 72.3–86.6), and 78.8% (95% CI, 61.1–91.0), respectively (P = .008; P = .446; and P = .180) (Table 2).

**Switches to Second-line ART**

In total, 106 (4.1%) participants switched from first- to second-line ART due to presumed treatment failure; 21.8 per 1000 person-years (95% CI, 18.0–26.3) (Supplementary Material). The switch rates were much higher in the group with PDR (86.8 [95% CI, 57.1–131.8] per 1000 person-years), compared to the group without PDR (18.2 [95% CI, 14.7–22.5] per 1000 person-years). In adjusted analysis, PDR was strongly associated with switching to second-line: adjusted hazard ratio (aHR) 3.80 (95% CI, 1.49 9.68; P = .005, Table 3 and Figure 2).

Overall, clinicians reported using VL testing to confirm treatment failure for 75/106 (70.8%) switches, and clinical and/or immunological criteria for 23/106 (21.7%). For 8/106 (7.5%) switches no reason was reported. A retrospective study VL-test result at the time of switch was available for 74/106 (69.8%) participants: 18/74 (24.3%) participants had VL < 1000 cps/mL and 56/74 (75.7%) had a VL ≥ 1000 cps/mL. Of those with VL ≥ 1000 cps/mL, 53/56 (94.6%) had a GRT available: 7/53 (13.2%) harbored no DRM (wild-type virus) and in 46/53 (86.8%) ≥1 DRM was detected. Overall, 25/106 (23.6%) switched unnecessarily; 18/106 (17.0%) with VL < 1000 cps/mL and 7/106 (6.6%) VL ≥ 1000 cps/mL without DRM (Figure 3). Unnecessary switching also occurred in sites where VL monitoring was reported to be used to diagnose treatment failure. The incidence of appropriate switching was 9.85 per 1000 person-years (95% CI, 7.43–13.1) and was much higher in the group with PDR (51.3 [95% CI, 29.8–88.3] per 1000 person-years), compared to the group without PDR (7.6 [95% CI, 5.4–10.6] per 1000 person years): aHR 6.22 (95% CI, 2.02–19.09; P = .001) (Supplementary Material).

**New AIDS-event(s)**

Overall, 182 new AIDS-events were observed, with a rate of 39.1 (95% CI, 33.8–45.2) new events per 1000 person-years (Supplementary Material). The events were mainly extra-pulmonary tuberculosis (26.1%), HIV wasting (24.1%), chronic herpes simplex infection (12.3%), and esophageal candidiasis (8.4%) and are specified in the Supplementary Material. The incidence of AIDS-events did not differ significantly between participants with and without PDR in both unadjusted (Tarone–Ware P = .7380; Cox
Table 3. Cox Models: Association Between Pretreatment Drug Resistance and Switching and Clinical Outcomes on First-line Antiretroviral Therapy.

<table>
<thead>
<tr>
<th>Events</th>
<th>N</th>
<th>%</th>
<th>Crude Hazard Ratio</th>
<th>Adjusted Hazard Ratioa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switchesb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No PDR</td>
<td>84</td>
<td>3.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PDR</td>
<td>22</td>
<td>15.6</td>
<td>4.95</td>
<td>3.09–7.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>New AIDS event(s)</td>
<td></td>
<td></td>
<td>5.80</td>
<td>1.49–9.68</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No PDR</td>
<td>172</td>
<td>7.1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PDR</td>
<td>10</td>
<td>7.1</td>
<td>1.08</td>
<td>.57–2.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.822</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.68–1.64</td>
<td>.807</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No PDR</td>
<td>176</td>
<td>7.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PDR</td>
<td>12</td>
<td>8.5</td>
<td>1.16</td>
<td>.63–2.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.642</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.24–2.35</td>
<td>.617</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; PDR, pretreatment drug resistance.

a Adjusted model: Adjusted for sex and age (fixed), CD4+ cell count and adherence (time-varying), clustered for site. All-cause mortality and switches were also adjusted for previous antiretroviral use.

b Switches due to presumed treatment failure.
P = .822) and adjusted (aHR 1.06, 95% CI, .68–1.64; P = .807) analysis (Table 3 and Figure 2).

All-cause Mortality

Overall, 188 (7.3%) persons died during follow-up; the mortality-rate was 38.6 (95% CI, 33.4–44.5) per 1000 person-years and varied over time (Supplementary Material). Ninety-seven (51.6%) died <90 days of treatment with a rate of 436.1 (95% CI, 357.4–532.1) per 1000 person-years. All-cause mortality was not significantly different for participants with or without PDR, both in the unadjusted (Tarone–Ware P = .5746; Cox P = .642) and adjusted (aHR 0.75, 95% CI, .24–2.35; P = .617) analysis (Table 3 and Figure 2). The majority of deaths were reported to be HIV-related (N = 125, 66.5%); 7 (3.7%) were reported to be not HIV-related and for 56 (29.8%) the cause of death was unknown. Sensitivity analysis of confirmed HIV-related mortality yielded similar results (Supplementary Material).

Both sensitivity analyses excluding participants with previous antiretroviral drug use, and exploring different follow-up at the sites yielded similar results for all 3 outcomes (Supplementary Material).

DISCUSSION

In this large prospective study in sub-Saharan Africa, we demonstrated that participants harboring drug-resistant HIV before starting standard first-line ART (ie, PDR) resulted in a nearly 4-fold increase of switches to second-line ART. During the first 3 years of ART, PDR was not associated with new AIDS-related events or excess mortality. Our findings remained robust in several sensitivity analyses. To our knowledge, this is the first study that provides direct evidence that the rise in PDR levels after the ART scale-up in Africa will drive a significant growth in the demand for second-line ART in the region. This emerging threat to the effectiveness of national ART programs will have major public health implications in terms of budget requirements and program planning in the coming years. There is a critical need for the scale-up of second-line ART, which calls for augmented resources and training for clinicians.

This study found no significant differences in incidence of AIDS-events and mortality during the first three years on first-line ART among participants with PDR compared to those without. Although several studies have reported that PDR predicts a poor virological response to ART [5, 7, 8], the continuation of a failing regimen may still provide some clinical and immunological benefit because of residual drug activity and the
HIV drug resistance increases ART switch

HIV drug resistance increases ART switch

reduced fitness of the mutant virus [23]. Nonetheless, it is possible that the impact of PDR on the development of new AIDS-events and mortality will only become manifest on the long term, that is, >3 years of ART [24].

In our cohort, we documented an overall PDR prevalence of 5.5% in sub-Saharan Africa in 2007–2009. The most comprehensive global assessment to date suggested a significant increase in PDR over time since ART rollout, at an estimated annual rate of 29% for east Africa and 14% for southern Africa. The rise is driven by NNRTI-resistance, which is of particular concern as this drug class constitutes the foundation of current first-line ART regimens and prophylaxis for prevention of mother-to-child transmission. A mathematical model based on combined data on transmitted and acquired drug resistance from Kampala, Uganda, and Mombasa, Kenya, predicted that the continued ART scale-up may lead to increased prevalence of PDR in the coming 10 years, up to 19% [25].

Of note, the same model projected that future levels of NNRTI resistance could be diminished through better switching practices. By means of enhanced implementation of VL monitoring, accurate switching to second-line therapy can improve patient outcomes and reduce drug resistance [25, 26]. In the light of these trends, our data point toward substantial increases in the need for second-line antiretroviral drugs in the coming years. Increased use of second-line drugs could in turn enhance long-term treatment success and curb the emergence of drug-resistant HIV [25–27].

The switch rate in our study of 21.8 per 1000 person-years (95% CI, 18.0–26.3) was in line with the IeDEA cohort that reported 24 switches per 1000 person-years (95% CI, 22–26) [28]. By contrast, switch rates previously reported in MSF-run ART programs were much lower with 4.2 switches per 1000 person-years. The difference is possibly attributable to limited access to VL monitoring in the latter study [29]. In a recent meta-analysis of first-line ART outcomes, the current rate of switch to second-line in resource-limited settings was low, that is, <2% during the first 2 years on ART [15]. Long-term switch rates were estimated at 5.4% (95% CI, 3.3–7.5) and 9.2% (95% CI, 4.8–13.6) after 3 and 4 years, respectively [15]. Barriers for switching are the low availability of second-line drugs (including drug stock-outs), lack of virological monitoring, limited physician training and experience with switching, and reluctance to shift to the last available treatment option in low-resource settings [28, 30, 31].

Of concern, 24% of participant in this study who were switched to second-line ART either had VL < 1000 cps/mL, or VL ≥ 1000 cps/mL without presence of DRMs, which means they could still have benefited from continuing on first-line ART. Unnecessary
switching to more costly and toxic second-line ART, as reported in previous studies [10], impairs the efficiency in the use of scarce resources available in ART programs. Moreover, previous studies from Africa suggested that a considerable proportion of patients with detectable viremia can (re-)suppress on their first-line ART after adherence counselling [10, 32]. These findings support the current WHO recommendation that patients who have suspected virological failure should receive adherence counselling and a repeat VL test for confirmation of failure before considering a regimen switch [2]. Thus, increased access and uptake of available VL testing can avert unnecessary switches to second-line therapy [10].

Although VL monitoring is the preferred monitoring tool to diagnose and confirm treatment failure, access to VL testing followed by regimen switching remains challenging and costly [12, 33]. Given the fact that second-line regimens are currently at least 2.4 times more expensive than first-line ART (and third-line regimens 15 times), increased switching will inevitably lead to substantial increased expenditures of HIV treatment in the region [34, 35]. These expenditures will have to compete for resources for (first-line) ART scale-up. Mathematical modelling studies and economic analyses have evaluated different treatment monitoring strategies, and yielded conflicting results [26, 36, 37]. Cost-effectiveness, however, will improve as more affordable VL testing techniques (using dried blood spots) become available in resource-limited settings [27, 37, 38]. Furthermore, further price reductions of second-line drugs (protease inhibitors) will reduce treatment costs. Although the largest clinical benefit is expected to be achieved by VL monitoring, individual GRT after first-line failure might increase appropriate switching, and recent studies from South Africa have indicated that it is potentially cost effective in middle-income countries [27, 39, 40].

The main strengths of the study were its large size, longitudinal design with long-term follow-up, and the setting of large-scale, routine ART programs, which enhance the generalizability of the results. The reported attrition rates, including high early mortality, were substantial but in line with other studies from sub-Saharan Africa [41–43]. Our study adds to the current knowledge that the increase in PDR will necessitate expanded access to VL monitoring and second-line ART in order to sustain effective long-term HIV treatment in Africa [2, 28]. Further studies and mathematical modelling are needed to establish optimum strategies for the prevention of HIV drug resistance, the role of pre-ART genotyping, and use of protease inhibitor-based regimens for specific high-risk groups.

This study has some potential limitations. Although the PASER network includes mostly free-access, routine ART programs, nongovernment and urban sites were over-
HIV drug resistance increases ART switch

represented and rural sites underrepresented. Therefore, caution is warranted when extrapolating results to other settings where resource constraints might be even more substantial [16]. Second, participants LTFU may have introduced bias, but LTFU was equal among participants with and without PDR [44]. Third, there is potential for attrition bias because differential follow-up duration across the sites. Sensitivity analyses showed that such bias is expected to be limited. Last, population-based sequencing is not able to detect minority drug resistance; therefore, participants who had minority resistant strains could have been classified as having a “no PDR,” resulting in a potential underestimation of the effect of PDR on treatment outcomes.

In conclusion, our findings demonstrate that the presence of PDR diminishes the long-term effectiveness of first-line ART and is strongly associated with switching to second-line regimens. In view of rising PDR levels in Africa, these findings underscore the urgent need for increased implementation of VL monitoring and access to affordable second-line regimens to secure durable ART success in sub-Saharan Africa.

SUPPLEMENTARY DATA

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

NOTES

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GenBank accession numbers

All HIV-1 pol sequences in this study have been deposited in GenBank under the following accession numbers: HM119603–HM120150, HQ993572–HQ995497, JN630892–JN631033.

Author contributions

T. F. R. d. W. is the principal investigator. R. L. H. and T. F. R. d. W. designed the study and developed the protocol. R. L. H., K. C. E. S., and T. S. B. set up the study and trained and supervised study workers. M. W., M. S., E. E. F. L., C. M. K., A. S. A., K. M., and M. E. B. established the cohort and supervised data collection. T. S. B. and B. M. H. conceived and undertook the data analyses, and drafted the manuscript. K. C. E. S. checked and supervised the statistical analyses. T. S. B., B. M. H., K. C. E. S., P. O., R. L. H., M. W., A. S. A., and T. F. R. d. W. helped interpret the data and reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.
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Potential conflicts of interest
All authors: No potential conflicts of interest.
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
REFERENCES


Part III

Salvage drug options
Accumulation of HIV-1 drug resistance after continued virological failure on first-line antiretroviral therapy in adults and children in sub-Saharan Africa.


Journal of Antimicrobial Chemotherapy, accepted pending minor revisions.
SYNOPSIS

Background
Limited availability of viral load (VL) monitoring in HIV treatment programs in sub-Saharan Africa can delay switching to second-line antiretroviral therapy (ART), leading to the accumulation of drug resistance mutations.

Objectives
The current study evaluated the accumulation of resistance to reverse transcriptase inhibitors after continued virological failure on first-line ART, among adults and children in 13 sites in sub-Saharan Africa.

Methods
We included prospective cohort data from HIV-1-positive adults and children on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based first-line regimen in sub-Saharan Africa. Retrospective VL and, if VL $\geq 1,000$ cps/ml, pol genotypic testing was performed. Among participants with continued virological failure ($\geq 2$ VL $\geq 1,000$ cps/ml), drug resistance mutations were scored and predicted susceptibility was calculated.

Results
2,737 adults and 289 children received NNRTI-based first-line ART. Virological failure rates were higher in children compared to adults: 8.8% and 9.1% in adults, and 24.1% and 29.5% in children after 12 and 24 months, respectively (both p<0.001). At first virological failure, drug resistance mutation(s) were detected in 87% of participants. The predicted susceptibility declined significantly after continued virological failure for all reverse transcriptase inhibitors (all p<0.001). Acquired drug resistance patterns were similar in adults and children.

Conclusions
Virological failure rates on first-line ART are significantly higher in children compared to adults in sub-Saharan Africa, but drug resistance patterns are similar. This study provides field evidence for current ART guidelines. Improved VL monitoring to prevent accumulation of mutations, and new drug classes to construct fully-active regimens, are required in sub-Saharan Africa.
INTRODUCTION

Access to antiretroviral therapy (ART) for HIV is expected to further increase as treatment is now recommended by the World Health Organization directly after diagnosis, irrespective of clinical stage or CD4+ cell count [1]. Concerns about the development of HIV drug resistance persist both in resource-rich and resource-limited settings [2–5]. Due to limited availability of viral load (VL) monitoring and subsequent delayed switching to second line ART, the accumulation of drug resistance mutations (DRMs) is of concern in adults and children in sub-Saharan Africa [6].

The large majority of HIV-positive adults and children in sub-Saharan Africa receive standard first-line ART following the public health approach [7]. According to international guidelines, first-line ART for adults and older children consists of a non-nucleoside reverse transcriptase inhibitor (NNRTI), combined with two nucleoside reverse transcriptase inhibitors (NRTIs) [8–11]. Since 2013, a boosted protease inhibitor combined with two NRTIs is recommended for children under the age of three [8]. However, uptake of protease inhibitor-based first-line for children in routine programmes in sub-Saharan Africa is limited, due to higher costs and only recent development of oral pellets for children [12,13]. In adults, second-line ART after NNRTI-based first line failure consists of a boosted protease inhibitor, combined with two (previously unused) NRTIs to minimize cross-resistance. While NRTI resistance mutations can accumulate during prolonged first-line failure [6], new and recycled NRTIs in second-line regimen have shown substantial virological activity [14–16]. Recommended third-line drug options such (darunavir and integrase inhibitors) are not routinely available in the public sector in sub-Saharan Africa, and ~15 times more costly compared to first-line drugs [17].

The genetic barrier to the development of nevirapine and efavirenz resistance-associated mutations is low; the accumulation of resistance-associated mutations is rapid, often occurring within three months of virological failure [18,19]. Second generation NNRTIs, etravirine and rilpivirine, have a higher genetic barrier to resistance and confer only partial cross-resistance with nevirapine and efavirenz [20–22]. While currently unavailable, second-generation NNRTIs might become available in resource-limited settings as prices are falling [17]. In resource-limited settings it is yet unclear to what extent NNRTI resistance, accumulated during (prolonged) first-line failure, affects the susceptibility to second-generation NNRTIs [23,24]. In the context of the public health approach to ART, the question arises which role second-generation NNRTIs could potentially play in sequential use after nevirapine/efavirenz-based first-line ART for adults and children [23].
Chapter 8

The current study aimed to evaluate the accumulation of resistance and predicted drug susceptibility to NNRTIs and NRTIs after continued virological failure on first-line ART in sub-Saharan Africa. As harmonized ART guidelines for adults and older children are desired to facilitate the transition from paediatric to adult care, we compared drug resistance rates and patterns in adults and children. In the setting of limited or absent virological monitoring, we were able to examine the effect of ongoing virological replication on the rate of NNRTI as well as NRTI resistance accumulation.

METHODS

Study population
The current analysis is based on HIV-1 positive adults and children from two cohorts: the PanAfrican Studies to Evaluate Resistance Monitoring (PASER-M) and Monitoring Antiretroviral Resistance in Children (MARCH) which shared a harmonized protocol. PASER is a prospective cohort study at 13 clinical sites in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe, of HIV-1 positive adults (≥18 years), eligible to start first-line ART [25]. MARCH is a prospective cohort at 3 clinical sites in Uganda of HIV-1 infected children (≤12 years), eligible to start first-line ART [26]. Exclusion criteria for adults were: pregnancy at enrolment, HIV-2 co-infection (in Nigeria), and previous use of ART ≤30 days before treatment initiation. The exclusion criterion for children was previous use of ART for the purpose of therapy; antiretroviral prophylaxis was allowed. All participants received routine care according to local guidelines [11]. Participants provided written informed consent before enrolment. Children above the age of eight who were aware of their HIV status provided written informed assent. The study protocols were approved by national and local research ethics committees.

We included adults and children on standard NNRTI-based first-line ART, defined as one NNRTI, nevirapine or efavirenz, combined with a dual NRTI backbone. Participants were censored when they were no longer on nevirapine/efavirenz-based ART. For the current analysis, we selected participants who experienced continued virological failure (defined as ≥2 VL ≥1,000 cps/ml during follow-up) with a genotype test result available at virological failure.

Laboratory methods
In adults, plasma samples were collected at ART initiation, after 12 and 24 months for all sites, and after 36 months for sites in Uganda, Nigeria and Zimbabwe. In children, plasma samples were collected at ART initiation, and at 6, 12, 18 and 24 months. Plasma samples were used for retrospective testing of HIV-1 RNA plasma load (VL)
Acquired HIV drug resistance in adults and children

and resistance genotyping. VL was determined using NucliSens EasyQ real-time assay (version 2.0; bioMérieux, Lyon, France) or COBAS Ampliprep/COBAS Taqman assay (Roche, Branchburg, NJ, USA). Virological failure was defined as VL ≥1,000 cps/ml after at least 6 months of ART. If VL was ≥1,000 cps/ml, genotypic resistance testing of protease and partial reverse-transcriptase was performed using in-house sequencing methods [27–29] and, if needed, Virosel. Major DRMs were scored using the 2015 IAS-USA list [22]. To determine the susceptibility for NNRTIs (nevirapine, efavirenz, etravirine, rilpivirine) and NRTIs (lamivudine, emtricitabine, zidovudine, stavudine, didanosine, tenofovir, abacavir), the genotype susceptibility scores were calculated using the Stanford algorithm Version 7.0 [30]. Reduced susceptibility was defined as a genotype susceptibility score <1 for a particular drug. Resistance was assumed to be a cumulative outcome: DRMs identified at a first genotype were assumed to still be present at the second, third and fourth genotype, even if they were not detected at that time point. In other words, the genotype susceptibility scores calculated for one participant could only decrease over time. Subtypes were determined using the REGA algorithm version 3 [31].

**Statistical methods**

At first virological failure, patient characteristics of adults and children were compared, using Kruskall-Wallis and Pearson’s χ² tests. The prevalence of DRMs and genotype susceptibility scores were compared using a two-sample Wilcoxon rank-sum test. To evaluate the difference in drug susceptibility after first and continued virological failure, the genotype susceptibility scores of the first and last available genotype measurement were compared. After continued virological failure, newly acquired DRMs and changes in genotype susceptibility scores over time were compared using a Wilcoxon signed-rank test.

The individual accumulation of DRMs was calculated as a rate per year, to take into account the differences in time between repeated measurements. The rate was calculated using each available measurement: i.e. if a participant contributed >2 genotypes, the difference was tested between the first and second measurement, between the second and third measurement, and (if applicable) between the third and fourth measurement.
RESULTS

Cohort characteristics

Between January 2007 and September 2009, 2,737 adults initiated standard first-line NNRTI-based antiretroviral therapy and were enrolled in the PASER-M cohort. Between January 2010 and September 2011, 289 children initiated standard first-line NNRTI-based antiretroviral therapy and were enrolled in the MARCH cohort. At ART initiation, 13.9% of participants had ≥1 DRM: 13.8% of adults and 14.3% of children (p = 0.846). Pretreatment drug resistance patterns in both adults and children [32,33], and the effect of resistance on ART regimen switching, clinical and virological outcomes in adults [4,34], have been described previously.

During follow-up, virological failure was detected in 8.8% (95% CI: 7.6-10.1), 9.1% (95% CI: 7.8-10.6) and 6.0% (95% CI: 4.3-8.2) of adults, after 12, 24 and 36 months respectively. In children, virological failure was detected in 19.7% (95% CI: 14.7-25.6), 24.1% (95% CI: 18.8-30.0), 23.6% (95% CI: 18.2-29.8) and 29.5% (95% CI: 23.7-35.9) after 6, 12, 18 and 24 months, respectively. Virological failure rates were significantly higher in children compared to adults at month 12 and 24 (both p<0.001). Genotyping was successful in 84.5% of all samples with VL≥1,000 cps/ml; genotype success rates were similar in adults and children.

In total, 119 participants (63 adults and 56 children) had VL≥1,000 cps/mL twice and had a genotype test result available at both time points. Of 119 participants, 33 contributed a third genotype and 11 contributed a fourth genotype. Participant characteristics at the time of first virological failure are presented in Table 1. Overall, 65% of participants received nevirapine- and 35% received efavirenz-based ART. The NRTI backbone differed in adults compared to children (p<0.001). Adults received zidovudine (N=32, 63.5%), tenofovir (N=18, 28.6%) or stavudine (N=5, 7.9%) and children received zidovudine (N=32, 57.1%), stavudine (N=23, 41.1%) or abacavir (N=1, 1.8%), combined with emtricitabine or lamivudine. The predominant HIV-1 subtypes were A, D and C: subtype C was most common in adults (44.4%) and subtype A in children (57.1%).

The genotype at first virological failure was measured after a median of 366 days (IQR 232-412) of first-line ART. The median time between the first and subsequent genotype was 303 days (IQR 183-365). The first two genotypes were used in this descriptive analysis; the third and fourth genotypes were used in the analysis on acquired drug resistance. At first and continued failure, the median VL was log10 4.5 cps/ml (IQR 3.9-5.0) and log10 4.5 (IQR 3.8-5.1), and did not differ between adults and children.
Table 1. Characteristics of adults and children at first virological failure.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=119, 100.0%)</th>
<th>Adults (N=63, 52.9%)</th>
<th>Children (N=56, 47.1%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>54</td>
<td>45.4</td>
<td>29</td>
<td>46.0</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years (median, interquartile range)</td>
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<td>30.1-40.5</td>
<td>5.4</td>
<td>3.4-10.4</td>
</tr>
<tr>
<td><strong>Country of origin</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>12</td>
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<tr>
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<td>Zimbabwe</td>
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<td>6.4</td>
</tr>
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<td>Uganda (all sites)</td>
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<td>68.9</td>
<td>26</td>
<td>41.3</td>
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<td><strong>Antiretroviral therapy regimen</strong></td>
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<td></td>
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<td>Stavudine/lamivudine/nevirapine</td>
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<td>Zidovudine/lamivudine/nevirapine</td>
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<td>41.2</td>
<td>29</td>
<td>46.0</td>
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<td>Zidovudine/lamivudine/efavirenz</td>
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<td>23.5</td>
<td>11</td>
<td>17.5</td>
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<tr>
<td>Tenofovir/emtricitabine/nevirapine</td>
<td>8</td>
<td>6.7</td>
<td>8</td>
<td>12.7</td>
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<tr>
<td>Tenofovir/emtricitabine/efavirenz</td>
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<td>6.7</td>
<td>8</td>
<td>12.7</td>
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<td>0</td>
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<td><strong>Non-nucleoside reverse transcriptase inhibitor</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nevirapine-based</td>
<td>77</td>
<td>64.7</td>
<td>39</td>
<td>61.9</td>
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<td>Efavirenz-based</td>
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<td>35.3</td>
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<td></td>
<td></td>
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<td>Zidovudine-based</td>
<td>72</td>
<td>60.5</td>
<td>40</td>
<td>63.5</td>
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<td>Tenofovir-based</td>
<td>18</td>
<td>15.1</td>
<td>18</td>
<td>28.6</td>
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<tr>
<td>Stavudine-based</td>
<td>28</td>
<td>23.5</td>
<td>5</td>
<td>7.9</td>
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<tr>
<td>Abacavir-based</td>
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<td><strong>HIV-1 subtype</strong></td>
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<td></td>
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<td>20.6</td>
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<td>D</td>
<td>33</td>
<td>27.7</td>
<td>12</td>
<td>19.1</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recombinants, unassigned</td>
<td>10</td>
<td>8.4</td>
<td>10</td>
<td>15.9</td>
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<tr>
<td><strong>HIV-1 viral load</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log_{10} cps/ml (median, interquartile range)</td>
<td>4.5</td>
<td>3.9-5.0</td>
<td>4.4</td>
<td>3.8-5.0</td>
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<tr>
<td>CD4+ T cell count; cells/µl (median, interquartile range)</td>
<td>289</td>
<td>165-467</td>
<td>232</td>
<td>128-324</td>
</tr>
<tr>
<td>%</td>
<td>-</td>
<td>-</td>
<td>33</td>
<td>24-42</td>
</tr>
</tbody>
</table>

Difference in adults and children were tested using Kruskall-Wallis and χ² tests. Age and country of origin between the adult and children’s cohort are different by (study) design and were therefore not tested. CD4+ cell counts are not comparable in adults and children and was therefore not tested. CD4+ T cell count was determined in 56 adults and 28 children ≥5 years of age. CD4+ T cell percentage was calculated in 22 children <5 years of age.

- Combined with lamivudine or emtricitabine.
- * P <0.05.
In adults, the median CD4⁺ T cell count was 232 cells/µl (IQR 128-324) and 188 cells/µl (IQR 106-295) at first and continued failure, respectively. In children ≥5 years and older, the median CD4⁺ T cell count was 550 cells/µl (IQR 343-826) and 635 cells/µl (IQR 316-989) at first and continued failure, respectively. In children <5 years of age, the median CD4⁺ T cell percentage was 33% (IQR 24-42) and 33% (IQR 26-40) at first and continued failure, respectively. The change of CD4⁺ T cell count and percentage at first versus continued virological failure did not differ significantly, in both adults and children.

**Drug resistance mutations**

At first virological failure, ≥1 DRM was detected in 104 (87.4%) participants. Among these, 99 (83.2%) harboured NNRTI-resistance mutations and 87 (73.1%) harboured NRTI-resistance mutations (Table 2). The median number of DRMs detected at first failure was 2 (IQR 1-3): median 1 NNRTI resistance mutation (IQR 1-2) and median 1 (IQR 0-1) NRTI resistance mutation. The most frequent NNRTI mutations detected were: K103N (N=46, 38.7%), G190A (N=26, 21.8%), Y181C (N=24, 20.2%), V106M (N=10, 8.4%), K101E (N=10, 8.4%), any E138 (N=9, 7.6%) and V108I (N=9, 7.6%). The most frequent NRTI mutations detected were: M184V (N=83, 69.7%), any thymidine analogue mutation (N=11, 9.2%), K65R (N=7, 5.9%) and K70R (N=6, 5.0%).

New DRMs accumulated with an average rate of 1.45 (SD 2.07) DRM per year; 0.62 (SD 1.11) NNRTI resistance mutations per year and 0.84 (SD 1.38) NRTI resistance mutations per year. Most (N)NRTI mutations increased significantly over time (Table 2). The highest NNRTI mutation accumulation rates were found for V108I at 0.11 (SD 0.45) mutation per year, P225H at 0.08 (SD 0.35) mutation per year, K101E at 0.06 (SD 0.31), Y181C at 0.06 (SD 0.30) mutation per year, G190A at 0.06 (SD 0.30) mutation per year, and H221Y at 0.05 (SD 0.27) mutation per year; all p <0.001. The highest NRTI mutation accumulation rates were found for: any thymidine analogue mutations at 0.26 (SD 0.59) mutation per year, M184V at 0.20 (SD 0.57) mutation per year, K70R at 0.13 (SD 0.42) mutation per year, M41L at 0.10 (SD 0.40) mutation per year (all p<0.001).

At first virological failure, no significant differences were found in DRM prevalence and patterns between adults and children except for K65R, which was detected in 7 (11.1%) adults but not in children (p=0.010). The DRM accumulation rate did not differ between adults and children for all DRMs, NNRTI and NRTI resistance mutations (p=0.437, p=0.319 and p=0.414), respectively. The DRM at first virological failure and subsequent DRM accumulation rates are reported separately for adults and children in Supplemental Table 1.
**Table 2.** Drug resistance mutations at first virological failure with subsequent accumulation rates.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=119)</th>
<th>Accumulation rate (new mutations / year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
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<td>Any drug resistance mutation</td>
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<td>87.4</td>
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<tr>
<td>Non-nucleoside reverse transcriptase inhibitor mutation</td>
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<td>Any</td>
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<td>K101P</td>
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<td>K101E</td>
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<tr>
<td>K103S</td>
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<tr>
<td>K103N</td>
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<tr>
<td>V106A</td>
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<td>V106M</td>
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<td>V108I</td>
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<tr>
<td>Any E138</td>
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<td>Nucleoside reverse transcriptase inhibitor mutation</td>
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<td>L74V</td>
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Table 2. Drug resistance mutations at first virological failure with subsequent accumulation rates. (continued)

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Total (N=119)</th>
<th>Accumulation rate (new mutations / year)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
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<td>Y115F</td>
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</tr>
<tr>
<td>K219E</td>
<td>2</td>
<td>1.7</td>
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</table>

The difference in mutations between first and subsequent genotypes was tested using Wilcoxon signed-rank test. The following drug resistance mutations were not detected: E138G; E138R; V179L; Y181V; Y188H; M230I; K65E; K65N; F77L; Q151M.

* P <0.05.

Predicted drug susceptibility

While drug susceptibility was already reduced in many participants at first virological failure, the predicted susceptibility declined significantly between first and continued virological failure for all NNRTIs and NRTIs (all p<0.001). The number of participants with predicted full susceptibility to nevirapine and efavirenz dropped from 16.0% to 5.9% (p<0.001) and 16.8% to 6.7% (p<0.001), respectively. The predicted full susceptibility to etravirine and rilpivirine dropped from 47.9% to 37.0% (p<0.001) and from 44.5% to 31.9% (p<0.001), respectively.

At first virological failure, the predicted NRTI susceptibility was highest for zidovudine (91.6%), tenofovir (89.1%) and stavudine (86.6%). After continued failure the predicted susceptibility remained highest for tenofovir (69.7%) and was lowest for abacavir (9.2%) and lamivudine/emtricitabine (10.1%). The predicted susceptibility to all NNRTIs and NRTIs at first and continued virological failure are shown in Figure 1 and Supplemental Table 3.

At first virological failure, NNRTI susceptibility did not differ between adults and children. NRTI susceptibility was mostly similar in adults and children, but showed some differences (supplemental table 2). The loss of tenofovir susceptibility was significantly more substantial in adults (19.0%) as compared to children (1.8%, p=0.003).
Acquired HIV drug resistance in adults and children

**Figure 1.** Predicted drug susceptibility at first and continued virological failure.
The difference in genotype sensitivity score between measurements was compared within participants using a Wilcoxon signed rank-sum test. The susceptibility decreased significantly over time for all antiretroviral drugs: all $p<0.001$.

Predicted drug susceptibility (genotype susceptibility scores) were calculated using the Stanford algorithm Version 7.0. Based on 326 available genotypes of 119 participants.

Also see Supplemental Table 3.
Additionally, higher levels of stavudine resistance (19.0% versus 7.1%, p=0.050) and didanosine resistance (27.0% versus 14.3%, p=0.057) were detected in adults. The rate of decline in all NNRTI and NRTI susceptibility after continued failure was similar in adults and children (data not shown).

Drug resistance development by drug exposure

Nevirapine versus efavirenz

To assess if the type of NNRTI received was associated with resistance development, we compared patients on regimens containing efavirenz or nevirapine. Most participants stayed on the same NNRTI throughout virological failure. Three (1.8%) participants changed from efavirenz to nevirapine during virological failure, and were excluded from this sub-analysis.

<table>
<thead>
<tr>
<th>Table 3. Predicted drug susceptibility at first virological failure, by drug exposure. A. Nevirapine versus efavirenz</th>
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<tbody>
<tr>
<td>Predicted susceptibility (genotype sensitivity score)</td>
</tr>
<tr>
<td>Nevirapine susceptible (1)</td>
</tr>
<tr>
<td>Nevirapine intermediate resistance (0.25)</td>
</tr>
<tr>
<td>Nevirapine high-level resistance (0)</td>
</tr>
<tr>
<td>Efavirenz susceptible (1)</td>
</tr>
<tr>
<td>Efavirenz intermediate resistance (0.50)</td>
</tr>
<tr>
<td>Efavirenz intermediate resistance (0.25)</td>
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<td>Efavirenz high-level resistance (0)</td>
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<tr>
<td>Etravirine susceptible (1)</td>
</tr>
<tr>
<td>Etravirine intermediate resistance (0.50)</td>
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<td>Etravirine intermediate resistance (0.25)</td>
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<tr>
<td>Etravirine high-level resistance (0)</td>
</tr>
<tr>
<td>Rilpivirine susceptible (1)</td>
</tr>
<tr>
<td>Rilpivirine intermediate resistance (0.50)</td>
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<td>Rilpivirine intermediate resistance (0.25)</td>
</tr>
<tr>
<td>Rilpivirine high-level resistance (0)</td>
</tr>
</tbody>
</table>
Acquired HIV drug resistance in adults and children

At first virological failure, 77 (66.4%) participants received nevirapine-based and 39 (33.6%) received efavirenz-based ART. Participants receiving efavirenz-based ART, retained better susceptibility for second-line NNRTIs compared to those failing nevirapine-based ART: 64.1% compared to 40.3% retained susceptibility for etravirine (p=0.024), and 64.1% compared to 35.1% retained susceptibility to rilpivirine.

**Table 3.** Predicted drug susceptibility at first virological failure, by drug exposure. (continued)

**B. Tenofovir versus zidovudine**

<table>
<thead>
<tr>
<th>Predicted susceptibility (genotype sensitivity score)</th>
<th>Total (N= 55, 100.0%)</th>
<th>Tenofovir-based antiretroviral therapy (N= 17, 30.9%)</th>
<th>Zidovudine-based antiretroviral therapy (N=38, 69.1%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
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<tr>
<td>Lamivudine/emtricitabine</td>
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<tr>
<td>susceptible (1)</td>
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<td>high-level resistance (0)</td>
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<tr>
<td>Zidovudine</td>
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</tr>
<tr>
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<tr>
<td>high-level resistance (0)</td>
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<td>7.3</td>
<td>0</td>
</tr>
<tr>
<td>Stavudine</td>
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<tr>
<td>susceptible (1)</td>
<td>44</td>
<td>80.8</td>
<td>12</td>
</tr>
<tr>
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<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>intermediate resistance (0.25)</td>
<td>6</td>
<td>10.9</td>
<td>5</td>
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<tr>
<td>high-level resistance (0)</td>
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<td>0</td>
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<tr>
<td>Didanosine</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>susceptible (1)</td>
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<td>80.0</td>
<td>11</td>
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<tr>
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<td>7.3</td>
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<td>high-level resistance (0)</td>
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<td>10.9</td>
<td>5</td>
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<td>Abacavir</td>
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<tr>
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<tr>
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<tr>
<td>high-level resistance (0)</td>
<td>9</td>
<td>16.4</td>
<td>6</td>
</tr>
</tbody>
</table>

The genotype susceptibility scores were compared using a two-sample Wilcoxon rank-sum test.

* P <0.05.
(p=0.031), respectively (Table 3A). The subsequent DRM accumulation rate did not differ between participants failing nevirapine- or efavirenz-based ART. Therefore, the predicted drug susceptibility after continued failure was also in favour of those failing efavirenz-based ART.

**Tenofovir versus zidovudine**

To assess if the type of NRTI received was associated with resistance development, we compared adults on regimens containing tenofovir or zidovudine. Because children did not receive tenofovir they were excluded from this sub-analysis. At first failure, 40 (63.5%) adults received zidovudine, 18 (28.6%) received tenofovir and 5 (7.9%) received stavudine and most (90.5%) stayed on the same NRTI throughout virological failure. Overall, 6 (9.5%) adults changed their NRTI backbone throughout virological failure and were excluded from the sub-analysis.

Adults failing zidovudine-based ART retained better susceptibility to tenofovir compared to those failing tenofovir-based ART (86.8% versus 64.7%, p=0.036). Vice versa, adults failing tenofovir-based ART retained more susceptibility to zidovudine compared to those failing zidovudine-based ART (100% versus 84.2%, p=0.086). Predicted susceptibility to the remaining NRTIs did not differ significantly between those failing tenofovir-based compared to zidovudine-based ART (Table 3B). Overall, 80.0% retained full susceptibility to stavudine, 72.7% retained full susceptibility to didanosine, 30.9% retained full susceptibility to abacavir, and 32.7% retained full susceptibility to lamivudine/emtricitabine. The subsequent DRM accumulation rate did not differ between participants failing tenofovir- or zidovudine-based ART.

**DISCUSSION**

The current study showed that virological failure rates on NNRTI-based first-line ART were significantly higher in children compared to adults in sub-Saharan Africa. At 12 and 24 months, 9% of adults compared to 24 and 30% of children had VL≥1,000 cps/ml. NNRTI and NRTI susceptibility was already reduced extensively at first virological failure, and further declined with continued virological failure. At first and continued virological failure, the DRM patterns and predicted susceptibility to NNRTIs and NRTIs did not differ significantly between adults and children. With regard to drug resistance, our study provides field evidence that alignment of ART guidelines for adults and older children is feasible. New drug classes -- such as second-generation protease inhibitors and integrase inhibitors -- are required to construct fully-active second- and third-line regimens for adults and children in sub-Saharan Africa. The
role of second-generation NNRTIs is expected to be limited, supporting the latest ART guideline update [8,9].

Our findings were in line with recent meta-analyses on VL suppression on NNRTI-based first line ART in adults (>85%) and children (60-70%) in low- and middle-income countries [35,36]. In the present study, ≥1 DRM was detected in 87% of adults and children at first virological failure: 83% harboured NNRTI-resistance mutations and 73% harboured NRTI-resistance mutations. In the setting of limited or absent virological monitoring, we found that the predicted susceptibility to all NNRTIs and NRTIs decreased significantly after continued failure. New DRMs accumulated with an average rate of 0.62 (SD 1.11) NNRTI resistance mutations per year and 0.84 (SD 1.38) NRTI resistance mutations per year. The emergence of acquired DRMs was in line with other studies [18,19,37–40].

At first virological failure, the most common NNRTI-mutations were the K103N (38.7%) and G190A (21.8%) mutations, associated with nevirapine/efavirenz resistance. The Y181C mutation, associated with resistance to all NNRTIs, was detected regularly (20.2%). The highest NNRTI-mutation accumulation rate was found for K103N and V108I. Predicted full susceptibility to etravirine and rilpivirine was observed in 48% and 45% of all participants at first-time failure, and in 37% and 32% after continued virological failure, respectively. Our findings imply that second-generation NNRTIs will be of limited use in subsequent treatment regimens. Second-generation NNRTIs can be useful for some patients failing nevirapine/efavirenz-based ART, but only in a setting where resistance testing is available [41]. Third-line regimens should therefore ideally be guided by genotype resistance testing and include new drugs with minimal risk of cross-resistance to previously used regimens [9]. Of note, it is possible that NNRTI mutations that are selected during first-line ART might not be detected after second-line failure. Therefore it is likely that the activity levels of second-generation NNRTIs will be overestimated at the moment of switch to third-line ART.

Our data confirms that adults and children failing efavirenz-based ART retain significant better susceptibility to etravirine and rilpivirine compared to those failing nevirapine based-ART. This is in line with previous studies from resource-rich and resource-limited settings [24,42,43]. Our findings supports current guidance on the preferred use of efavirenz in first-line ART regimen [8,9,44].

The M184V (69.7%) mutation and thymidine analogue mutations (9.2%) were the most frequent NRTI mutations detected at first failure, and had the highest subsequent mutation accumulation rates. Due to the high levels of the M184V mutation, susceptibility
to emtricitabine/lamivudine was highly compromised. However, continued treatment with emtricitabine or lamivudine is not contraindicated as M184V impairs viral replication fitness, and increases tenofovir activity [45,46]. The K65R mutation, associated with tenofovir resistance, was detected in seven (11.1%) adults but not in children (p=0.010). This is likely because TDF was not prescribed to children. Stavudine, however, was frequently prescribed to children. The high retention of tenofovir susceptibility after virological failure in children support the World Health Organization’s recommendation for tenofovir use for children in sub-Saharan Africa [47].

There are currently no adequate alternatives in resource-limited settings for children failing (either first- or second-line) protease inhibitor containing regimens [48]. As drug options for children are very limited, second-generation NNRTIs could play an important role in future regimens. At this moment, etravirine is only approved for children >5 years of age [49]. Rilpivirine is currently only approved for adults, but a clinical trial in treatment-naïve adolescents is on its way [50]. Our findings show that the potential of second-generation NNRTIs in future regimens is limited.

Our findings should be interpreted with caution, taking the following factors into account. First, the data originated from two prospective cohort studies, using different measurement frequencies in adults and children. However, both cohorts were initiated by the same research group. In Uganda, data collection took place at the same clinical sites. Both cohorts used the same research logistics and infrastructure and VL testing and sequencing was performed at the same laboratories, which makes the cohorts highly compatible. Second, genotypic algorithms have shown to overestimate resistance to etravirine and rilpivirine in non-B subtypes, which could have led to an overestimation of the actual phenotypic resistance to second generation NNRTIs [51,52]. Clinical studies on phenotypic susceptibility of second-generation NNRTIs are needed.

Our findings underline the importance of virological monitoring in both adults and children receiving care in sub-Saharan Africa. Sequential resistance data from two large prospective cohorts allowed direct comparison of adults and children receiving NNRTI-based first-line ART in a programmatic setting. DRM accumulation is similar in adults and children failing first-line ART in sub-Saharan Africa. Uptake of virological monitoring and prompt switching to protease inhibitor based second-line ART can limit the accumulation of drug resistance [53], and can enable successful virological suppression [14,54]. Drug resistance testing and additional drug options, protease inhibitors and integrase inhibitors, are needed to construct active future drug regimens.
GENBANK ACCESSION NUMBERS

All HIV-1 pol sequences in this study have been deposited in GenBank: accession numbers are not yet available.

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TRANSPARENCY DECLARATIONS

None to declare.
REFERENCES


Acquired HIV drug resistance in adults and children


Protease inhibitor resistance in the first three years of second-line antiretroviral therapy for HIV-1 in sub-Saharan Africa.


Journal of Infectious Diseases, accepted pending minor revisions.
ABSTRACT

Background
As antiretroviral therapy (ART) programs in sub-Saharan Africa mature, increasing numbers of HIV-positive people will experience treatment failure, and require second- or third-line ART. Data on second-line failure and development of protease inhibitor (PI) resistance in sub-Saharan Africa are scarce.

Methods
HIV-1 positive adults were included if they received >180 days of PI-based second-line ART. We assessed risk factors for having a detectable viral load (VL, ≥400 cps/ml) using Cox models. If VL was ≥1,000 cps/ml, genotyping was performed.

Results
Of 227 included participants, 14.6%, 15.2% and 11.1% had VL≥400 cps/ml at 12, 24 and 36 months. Risk factors for a detectable VL were: exposure to non-standard non-nucleoside reverse transcriptase inhibitor (NNRTI)-based (HR 7.10; 95%CI: 3.40-14.83; p<0.001) or PI-based first-line regimen (HR 7.59; 95%CI: 3.02-19.07; p=0.001) compared to zidovudine/lamivudine/NNRTI, PI-resistance at switch (HR 6.69; 95%CI: 2.49-17.98; p<0.001) and suboptimal adherence (HR 3.05; 95%CI: 1.71-5.42; p=0.025). Among participants with VL ≥1,000 cps/ml, 22/32 (69%) harboured drug resistance mutation(s); 7/32 (22%) harboured PI resistance.

Conclusions
While VL suppression rates were high, PI resistance was detected in 22% of participants with VL ≥1,000 cps/ml. To ensure long-term ART success, intensified adherence support, virological monitoring and third-line drugs will be necessary.
BACKGROUND

Increasing numbers of HIV-positive people have access to antiretroviral therapy (ART) in sub-Saharan Africa [1]. As more people will experience treatment failure, the need for second-line boosted protease-inhibitor (PI) based ART, and eventually third-line ART, is rising [2]. The roll-out of routine viral load (VL) monitoring and availability of second-line drugs is expected to further increase the number of patients switched to second-line ART in the coming years [3–5]. The numbers of patients receiving second-line ART are expected to rise from an estimated 300,000 in 2013 to 2-4 million by 2030, comprising 12-17% of all patients on ART [3].

While the PIs lopinavir/ritonavir (LPV/r) and atazanavir/ritonavir (ATV/r) have become generically available and prices continue to fall, second-line regimens are still 2.4 times more expensive compared to first-line regimens in sub-Saharan Africa [6]. Darunavir/ritonavir (DRV/r) can be prescribed as an alternative PI in second-line, or as a third-line option [7–9]. However, DRV/r is not formulated as a heat-stable fixed-dose combination, is more costly, and is currently unavailable in sub-Saharan Africa.

The published rates of virological suppression on PI-based second-line ART in Africa vary, and data on long-term outcomes are scarce [10,11]. On the one hand, resistance to nucleotide reverse transcriptase inhibitors (NRTIs), acquired during first-line ART, seems to have a limited impact on the effectiveness of second-line ART [12–14]. Previous studies in resource-limited settings showed that virological failure on second-line ART was mainly associated with adherence, suggesting poor adherence rather than resistance as the cause of failure [10,15–18]. PIs generally provide a high genetic barrier to selection of antiretroviral resistant variants [19]. This suggests that after failure, re-suppression on a PI-based regimen could be achieved [20]. On the other hand, studies reported that if continuous virological failure on PI-based second-line ART occurs, PI-resistance mutations will emerge [21–23]. Since the majority of participants on second-line use LPV/r [9], substantial cross-resistance with alternative PIs can be expected. In these cases, third-line drug options like DRV/r, second-generation non-nucleotide reverse transcriptase inhibitors (NNRTIs) and integrase inhibitors are required. However, genotype resistance testing to construct an optimal third-line regimen based on the remaining few drug options is not routinely available in the public sector in sub-Saharan Africa.

Studies reporting long(er)-term outcomes on second-line ART in sub-Saharan Africa are scarce; it is yet unclear to what extent third-line ART regimens are required for patients in sub-Saharan Africa. This study aimed to determine the risk factors for having
a detectable VL among patients receiving PI-based second-line ART within the PanAfri-
can Studies to Evaluate Resistance Monitoring (PASER-M) cohort. Subsequently, we
established the rate and patterns of major PI mutations among those with VL ≥1,000
cps/ml after up to 3 years of second-line PI-based ART, and explored potential third-
line treatment options.

METHODS

Study design
The PASER-M study is a prospective cohort study at thirteen clinical sites in Kenya,
Nigeria, South Africa, Uganda, Zambia, and Zimbabwe [24]. HIV-1 positive adults (age
≥18 years) were enrolled at the time of switch to second-line boosted-PI containing
ART due to presumed first-line failure, diagnosed at the respective clinical site. Partici-
pants were included in the current analysis if they had received >180 days of second-
line booster-PI-based therapy. Participants provided written informed consent. The
study protocol was approved by the appropriate national and local research ethics
committees at all collaborating sites.

Cohort characteristics and first year outcomes have been described previously [25,26].
All participants were followed up in routine care according to national guidelines,
in line with WHO guidelines [7,8,27]. Pre-switch CD4+ T cell counts recorded closest
to the switch date were used as baseline counts. Information on first-line ART use
(specific ARVs and duration) was collected through patient self-report and their medi-
cal history. Clinical failure was defined as a new AIDS event (WHO stage 4) or death
(all-cause mortality), and immunological failure was defined as a decrease in CD4
count to the value before regimen switch or persistent CD4<100 cells/ml, as per WHO
guidelines [7,8,27]. Participants were followed up for 24 (all) or 36 (5 sites in Nigeria,
Zimbabwe and Uganda) months on second-line ART, or until discontinuation because
of death, transfer-out, loss-to-follow up (LTFU, unseen for >180 days) or switch to
third-line ART.

Plasma samples for retrospective testing were collected at switch, and at 12, 24 and 36
months (if applicable) of follow-up. HIV-1 RNA VL was determined using NucliSens
EasyQ real-time assay (version 2.0; bioMérieux, Lyon, France) or COBAS Ampliprep/
COBAS Taqman assay (Roche, Branchburg, NJ, USA). Genotypic resistance testing
was performed if VL ≥1,000 cps/ml. Population-based genotyping of protease and
partial reverse-transcriptase was done by in-house sequencing methods [28]. HIV drug
resistance was determined in two steps for each sequence. First, major drug resistance
mutations (DRMs) were scored using the 2015 IAS-USA drug mutation list [29]. Second, the genotype susceptibility scores (GSS) were calculated using the Stanford algorithm (Version 7.0) [30]. HIV subtypes were determined using the REGA version 3 [31].

**Statistical methods**

First, we identified risk factors for having a detectable VL ($\geq 400$ cps/ml) on second-line ART using Cox proportional hazard models, with adjusted standard-errors to account for clustering within sites. Sex and age (continuous) were included in the multivariate model as fixed variables due to biological plausibility, regardless of significance. We submitted all potential risk factors to the multivariate model, if $p<0.10$ in the univariate analysis: CD4$^+$ T cell count at switch ($\leq 50$; 51-199; 200-350; $>350$ cells/ml), first-line ART regimen at failure (zidovudine/lamivudine/NNRTI; stavudine/lamivudine/NNRTI; tenofovir/emtricitabine/NNRTI; non-standard NNRTI-based; triple NRTI; PI-based), duration of first-line regimen (years), VL at switch (log10 cps/mL), NRTI resistance at switch (GSS for prescribed NRTI backbone, $<2/\geq 2$), PI resistance at switch (GSS for PI prescribed, $<1/\geq 1$) as fixed variables, and 30-day self-reported adherence to second-line ART (visual-analogue score $<95%/\geq 95%$) as time-updated variable. Participants were censored when LTFU, transferred-out, or not on a PI-containing regimen anymore.

Second, we determined the rate and patterns of acquired DRMs among those with VL $\geq 1,000$ cps/ml and genotype available. Third, we explored potential third-line ART options for individual participants with major PI resistance mutations. When multiple genotypes were available for the same participant, the lowest susceptibility score measured was used (including genotypes at switch to second-line).

We performed several sensitivity analyses. First, to explore the impact of missing data, we considered participants with missing VL results, deceased and LTFU as having a detectable VL (missing equals failure). Second, we excluded all participants with VL $<1,000$ cps/ml at the time of switch to second-line ART, because a VL $\leq 1,000$ cps/ml at first-line failure (i.e. an inappropriate switch) could have influenced the rate of having a detectable VL on second-line. Third, to assess the effect of different durations of follow up in different settings, we repeated the analysis with follow up to 24 months for (all sites) and follow up to 36 months for 3 countries (5 sites). Fourth, to explore the effect of the VL threshold used, we used the detectable VL definition of $\geq 1000$ cps/ml instead of $\geq 400$ cps/ml. We used Stata 12 (TX: StataCorp LP) for all statistical analyses.
RESULTS

In total, 251 participants were enrolled: 24 were excluded from the current analysis because 2 (0.8%) switched to an NNRTI-based regimen and 22 (8.8%) participants were on second-line therapy for <180 days, due to early death (n=9, of which 8 HIV-related), transfer-out (n=3) or LTFU (n=10) (Supplemental Figure S1). Among the 227 included participants, the median age at switch was 38.3 years (IQR 34.3-44.9) and 50.7% were female. Participants had been on first-line ART for a median of 26 months (IQR 14-45) which commonly consisted of zidovudine or stavudine, combined with lamivudine, and nevirapine or efavirenz (N=188; 83.5%). Other NNRTI-based regimen were stavudine/didanosine/efavirenz (N=3; 1.3%), zidovudine/didanosine/nevirapine (N=1; 0.4%) and abacavir/lamivudine/nevirapine (N=1; 0.4%). Six (2.7%) participants reported previous PI use, all LPV/r based. Most participants were switched using targeted VL testing (N=191; 76.7%) and 58 (23.3%) were switched using clinical and immunological criteria only. Second-line regimen consisted of LPV/r combined with two NRTIs for 192 (84.6%) participants, 28 (12.3%) switched to a regimen of LPV/r with three NRTI drugs, and 7 (3.1%) switched to a non-preferred or double-PI regimen (Table 1).

Retrospective testing showed that 181 (80.7%) had VL≥1,000 cps/ml at the time of switch; 46 (20.3%) had VL≤1,000 cps/ml (i.e. an inappropriate switch). Among participants with VL≥1,000 cps/ml, genotype was available for 173 (95.6%) and 153 (88.4%) had a least one DRM; the specific DRMs are specified in Supplemental Table S1. Overall, 149 (86.1%) had NNRTI mutations, 147 (84.8%) had NRTI mutations and 143 (82.7%) had both NNRTI and NRTI mutations: 91 (52.6%) harboured at least one thymidine analogue mutation, 140 (80.9%) harboured M184V and 14 (8.1%) harboured K65R. Five (2.8%) participants harboured major PI mutation(s). Overall, 126 (72.8%) percent had reduced predicted susceptibility to their prescribed second-line regimen (mean GSS 2.0; SD 0.74).

During follow-up, 24 participants were LTFU (55.5 [95%CI: 37.2-82.7] per 1,000 person-years), 6 transferred-out (13.9 [95%CI: 6.2-30.9] per 1,000 person-years), 5 deceased (11.6 [95%CI: 4.8-27.8] per 1,000 person-years) and 3 switched to third-line (6.9 [95%CI: 2.2-21.5] per 1,000 person-years); see Supplemental Figure S1. Overall, 16/227 (7.1%, 39.3 [95%CI: 24.1-64.1] per 1,000 person-years) participants developed a new AIDS event, 3 of whom subsequently died. Seventy participants (30.8%) received at least one (range 1-4) intra-class drug substitution while on second-line ART. Thirty-day adherence was self-reported as >95% at all visits by 159 (70.0%) participants; 42 (18.5%) participants reported <95% adherence prior to one visit, and 26 (11.5%) prior to 2-5 visits.
**Table 1. Participant characteristics at switch.**

<table>
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<td>Stage 3</td>
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<td>CD4⁺ cell count</td>
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<tr>
<td>51-199</td>
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<td>200-350</td>
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<tr>
<td>&gt;350</td>
<td>16</td>
<td>7.1</td>
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<tr>
<td>Median duration of first-line regimen</td>
<td>26</td>
<td>14-45</td>
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<tr>
<td>ART regimen at failure</td>
<td></td>
<td></td>
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<tr>
<td>ZDV/3TC/NNRTI</td>
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<tr>
<td>d4T/3TC/NNRTI</td>
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<tr>
<td>NNRTI-based, other</td>
<td>5</td>
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</tr>
<tr>
<td>Triple NRTI</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>PI-based</td>
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<tr>
<td>Second-line ART regimen</td>
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<td></td>
</tr>
<tr>
<td>LPV/r + 2NRTI, incl 3TC/FTC</td>
<td>130</td>
<td>57.3</td>
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<tr>
<td>LPV/r + 2NRTI, other</td>
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<td>LPV/r + 3NRTI</td>
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<td>Non preferred PI, double PI</td>
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<td>Criteria used for switch</td>
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<tr>
<td>Clinico-immunological only</td>
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<tr>
<td>Targeted viral load testing</td>
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<td>Country of origin</td>
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<tr>
<td>Zambia</td>
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<td>South Africa</td>
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<td>Kenya</td>
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<td>Zimbabwe</td>
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<td>4.4</td>
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<td>Nigeria</td>
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<td>12.3</td>
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<td>HIV-1 RNA load at switch</td>
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<tr>
<td>10log cps/mL (median, IQR)</td>
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<td>3.4-5.0</td>
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<tr>
<td>&lt;1,000 cps/ml</td>
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<tr>
<td>≥1,000 cps/ml</td>
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<td>80.4</td>
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<tr>
<td>Drug resistance</td>
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<td></td>
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<tr>
<td>≥1 mutation</td>
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</tr>
<tr>
<td>Resistance to prescribed NRTI</td>
<td>122</td>
<td>71.8</td>
</tr>
<tr>
<td>Resistance to prescribed PI</td>
<td>5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

---

**Notes:**
- N=180;  b N=226;  c N=224;  d N=225;  e NNRTI-based, other: d4T/ddI/EFV (N=3), ZDV/ddI/NVP (N=1), ABC/3TC/NVP (N=1);  f Triple NRTI-TDF/3TC/ABC (N=1), ZDV/3TC/ABC (N=1), TDF/3TC/ZDV (N=1);  g PI-based; d4T/3TC/LPV/r (N=1), ZDV/3TC/LPV/r (N=1), ZDV/ddI/LPV/r (N=1), TDF/ddI/LPV/r (N=1);  h Non preferred PI, double PI: ABC/3TC/NNRTI (N=1), ZDV/3TC/NNRTI (N=1), TDF/FTC/NNRTI (N=1), SQV/r/LPV/r (N=1), LPV/r/ATV (N=1), TDF/FTC/LPV/r/NNRTI (N=1), LPV/r/NNRTI (N=1);  i N=225;  j Drug resistance was assessed among participants with HIV-1 RNA ≥1,000 cps/ml and genotype available (N=171).

Drug resistance mutations were scored using the 2015 IAS-USA drug mutation list, and resistance against the prescribed NRTIs and PIs was determined using the Stanford algorithm Version 7.0.

ART, antiretroviral therapy; IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; WHO, World Health Organization.
VL results were available for 205 (97.2%), 177 (94.2%) and 90 (93.8%) participants who were still alive and on PI-based ART after 12, 24 and 36 months, respectively. Among these, 14.6%, 15.2% and 11.1% had a detectable VL (i.e., VL ≥400) cps/ml at 12, 24 and 36 months (Table 2). Of participants with VL results available, 25.0% (N=54/216) had a detectable VL at some point during follow-up at a rate of 138.9 failures (95%CI: 106.4-181.3) per 1,000 person-years. For 41/54 (75.9%) participants with a detectable VL at some point during follow-up, a subsequent visit was available: 16/41 (39.0%) experienced viral re-suppression (VL <400 cps/ml) one year later, 14/41 (25.9%) still had a detectable VL one year later, 5/41 (9.3%) were LTFU, 3/41 (5.6%) died, 1/41 (1.9%) switched to third-line ART and 2 remained alive and in care but did not have VL data available.

### Table 2. Virological outcomes.

<table>
<thead>
<tr>
<th></th>
<th>12 months</th>
<th></th>
<th>24 months</th>
<th></th>
<th>36 months</th>
<th></th>
<th>Total follow up</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Viral load</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Results available</td>
<td>205</td>
<td>97.2</td>
<td>177</td>
<td>94.2</td>
<td>90</td>
<td>93.8</td>
<td>216</td>
<td>95.2</td>
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<tr>
<td>&lt;1,000 cps/ml</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>a</td>
<td>184</td>
<td>89.8</td>
<td>151</td>
<td>85.3</td>
<td>82</td>
<td>91.1</td>
<td>173</td>
<td>80.1</td>
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<tr>
<td>&lt;400 cps/ml</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>175</td>
<td>85.4</td>
<td>150</td>
<td>84.8</td>
<td>80</td>
<td>88.9</td>
<td>162</td>
<td>75.0</td>
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<td><strong>Drug resistance</strong></td>
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<td></td>
<td></td>
<td></td>
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<td>Genotypic test results available</td>
<td>17</td>
<td>81.0</td>
<td>21</td>
<td>80.8</td>
<td>3</td>
<td>37.5</td>
<td>32</td>
<td>76.2</td>
</tr>
<tr>
<td>Any mutation c</td>
<td>9</td>
<td>52.9</td>
<td>16</td>
<td>76.2</td>
<td>3</td>
<td>100.0</td>
<td>22</td>
<td>68.8</td>
</tr>
<tr>
<td>NRTI-resistance</td>
<td>7</td>
<td>41.2</td>
<td>12</td>
<td>57.1</td>
<td>3</td>
<td>100.0</td>
<td>17</td>
<td>56.3</td>
</tr>
<tr>
<td>PI-resistance</td>
<td>2</td>
<td>11.8</td>
<td>6</td>
<td>28.6</td>
<td>2</td>
<td>66.7</td>
<td>7</td>
<td>21.9</td>
</tr>
<tr>
<td>PI- &amp; NRTI-resistance</td>
<td>2</td>
<td>11.8</td>
<td>6</td>
<td>28.6</td>
<td>2</td>
<td>66.7</td>
<td>7</td>
<td>21.9</td>
</tr>
</tbody>
</table>

a of those with viral load results available;

b of those with viral load > 1,000 cps/ml;

c of those with genotypic test results available, using the 2015 IAS-USA list.

NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

### Risk factors for a detectable VL

In multivariable analysis, the following risk factors for a detectable VL on second-line ART were identified: exposure to a non-standard NNRTI-based first-line regimen (HR 7.10; 95%CI: 3.40-14.83; p<0.001) or PI-based first-line regimen (HR 7.59; 95%CI: 3.02-19.07; p=0.001) compared to zidovudine/lamivudine/NNRTI, PI-resistance at switch (HR 6.69; 95%CI: 2.49-17.98; p<0.001) and <95% self-reported adherence to second-line ART (HR 3.05; 95%CI: 1.71-5.42; p=0.025) (Table 3). Sex, age, CD4+ cell count at switch, duration of first-line regimen, VL at switch and NRTI-resistance at switch were
not associated with having a detectable VL on second-line ART. The results of our sensitivity analyses were in line with the main analysis; see Supplemental Table S2.

**Drug resistance**

For 32/43 (74.4%) participants with VL≥1,000 cps/ml during follow-up, genotypic data was available (Table 2). DRMs were found in 22/32 (68.8%) participants: 17 (56.3%) harboured NRTI resistance mutations and 7 (21.9%) harboured major PI resistance mutations (of which all 7 also had NRTI mutations). The average number of DRMs per sequence was 1 (range 0-10), 3 (range 0-7) and 4 (range 3-5) after 12, 24 and 36 months of follow up, respectively (Figure 1). Of the 32 participants with a genotype result at second-line failure, 27 (84.4%) also had a genotype result at first-line failure, prior to switch to second-line (the remaining 5 had VL<1,000 cps/ml at switch). At second-line failure, 42 NRTI resistance mutations were detected, of which 11 (26.8%) were newly acquired; 16 major PI resistance mutations were detected, of which 13 (81.3%) were newly acquired.

Among the 7 participants with major PI resistance at second-line failure, the most common PI mutations detected were: M46I (N=6), associated with resistance to indinavir/ritonavir and V82A (N=5), associated with resistance to indinavir/ritonavir, LPV/r, and tipranavir/ritonavir, and the L76V mutation (N=3), associated with DRV/r, LPV/r, and

![Figure 1. Proportion of sequences with drug resistance mutations detected.](image)
Proportion of the total number of sequences: 17, 21 and 3 after 12, 24 and 36 months, respectively.
Table 3. Risk factors for virological failure on second-line protease inhibitor-based antiretroviral therapy.

<table>
<thead>
<tr>
<th>Incidence of virological failure</th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate (per 1,000 person-years)</td>
<td>HR [95% CI]</td>
<td>HR [95% CI]</td>
</tr>
<tr>
<td>No Person-time (person-years)</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>No</th>
<th>Person-time (person-years)</th>
<th>Rate [95% CI]</th>
<th>HR [95% CI]</th>
<th>P</th>
<th>HR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>17</td>
<td>75,490</td>
<td>82.25 51.13 132.31</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>37</td>
<td>66,527</td>
<td>203.14 147.18 280.37</td>
<td>2.58 1.26 5.26</td>
<td>0.009</td>
<td>2.36</td>
<td>0.82 6.75</td>
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</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>No</th>
<th>Person-time (person-years)</th>
<th>Rate [95% CI]</th>
<th>HR [95% CI]</th>
<th>P</th>
<th>HR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>per year (continuous)</td>
<td>1.01</td>
<td>0.97 1.04</td>
<td>0.758</td>
<td>0.98</td>
<td>0.93 1.05</td>
<td>0.397</td>
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<tr>
<td>18-29 years</td>
<td>6</td>
<td>12,978</td>
<td>168.86 75.86 375.87</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39 years</td>
<td>23</td>
<td>62,165</td>
<td>135.14 89.80 203.36</td>
<td>0.79 0.30 2.09</td>
<td>0.634</td>
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<tr>
<td>≥40 years</td>
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<td>63,218</td>
<td>144.44 97.60 213.76</td>
<td>0.85 0.28 2.57</td>
<td>0.774</td>
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<table>
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<tr>
<th>CD4+ cell count at switch</th>
<th>No</th>
<th>Person-time (person-years)</th>
<th>Rate [95% CI]</th>
<th>HR [95% CI]</th>
<th>P</th>
<th>HR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤50</td>
<td>16</td>
<td>32,355</td>
<td>180.62 110.65 294.83</td>
<td>1.00</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>51-199</td>
<td>25</td>
<td>70,432</td>
<td>129.65 87.60 191.87</td>
<td>0.72 0.36 1.45</td>
<td>0.357</td>
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<td></td>
</tr>
<tr>
<td>200-350</td>
<td>7</td>
<td>28,072</td>
<td>91.08 43.42 191.05</td>
<td>0.50 0.24 1.07</td>
<td>0.075</td>
<td></td>
<td></td>
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<tr>
<td>&gt;350</td>
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<td>10,895</td>
<td>201.15 90.37 447.73</td>
<td>1.08 0.57 2.06</td>
<td>0.805</td>
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<table>
<thead>
<tr>
<th>First-line ART regimen</th>
<th>No</th>
<th>Person-time (person-years)</th>
<th>Rate [95% CI]</th>
<th>HR [95% CI]</th>
<th>P</th>
<th>HR [95% CI]</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>ZDV, 3TC, NNRTI</td>
<td>19</td>
<td>69,849</td>
<td>99.35 63.37 155.76</td>
<td>1.00</td>
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<td></td>
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<tr>
<td>D4T, 3TC, NNRTI</td>
<td>21</td>
<td>51,174</td>
<td>149.89 97.75 229.88</td>
<td>1.51 0.64 3.56</td>
<td>0.343</td>
<td>1.49</td>
<td>0.65 3.44</td>
</tr>
<tr>
<td>TDF, FTC, NNRTI</td>
<td>5</td>
<td>13,380</td>
<td>136.49 56.81 327.92</td>
<td>1.35 0.74 2.46</td>
<td>0.334</td>
<td>1.22</td>
<td>0.70 2.13</td>
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<tr>
<td>Non-standard NNRTI-based</td>
<td>3</td>
<td>2,610</td>
<td>419.83 135.40 1301.70</td>
<td>3.65 1.08 12.15</td>
<td>0.037</td>
<td>7.10</td>
<td>3.40 14.83</td>
</tr>
<tr>
<td>Triple NRTI</td>
<td>1</td>
<td>2,097</td>
<td>174.18 24.54 1236.50</td>
<td>1.59 0.20 12.38</td>
<td>0.659</td>
<td>1.77</td>
<td>0.19 16.89</td>
</tr>
<tr>
<td>PI-based</td>
<td>4</td>
<td>2,185</td>
<td>668.65 250.96 1781.56</td>
<td>7.59 3.02 19.07</td>
<td>&lt;0.001</td>
<td>6.83</td>
<td>2.12 22.02</td>
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</table>
Table 3. Risk factors for virological failure on second-line protease inhibitor-based antiretroviral therapy. (continued)

<table>
<thead>
<tr>
<th>Duration of first-line ART</th>
<th>Incidence of virological failure</th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Person-time (person-years)</td>
<td>Rate (per 1,000 person-years)</td>
<td>HR [95% CI]</td>
<td>P</td>
</tr>
<tr>
<td>0.90</td>
<td>0.78</td>
<td>1.04</td>
<td>0.140</td>
</tr>
</tbody>
</table>

HIV-1 RNA load at switch per 10log

| <1,000 cps/ml | 9 | 25,865 | 127.09 | 66.13 | 244.26 | 1.00 |
| ≥1,000 cps/ml | 45 | 115,046 | 142.87 | 106.67 | 191.35 | 1.15 | 0.60 | 2.19 | 0.670 |

NRTI resistance at switch GSS ≥ 2 15 | 29,298 | 187.00 | 112.74 | 310.19 | 1.00 |
| GSS < 2 | 30 | 82,697 | 132.50 | 92.64 | 189.51 | 0.70 | 0.35 | 1.40 | 0.309 |

PI resistance at switch GSS ≥ 1 42 | 111,839 | 137.17 | 101.37 | 185.60 | 1.00 |
| GSS < 1 | 4 | 1,996 | 731.96 | 274.72 | 1950.25 | 6.69 | 2.49 | 17.98 | <0.001 |

Adherence to second-line ART (time-updated)

| ≥95% | 44 | 131,966 | 121.78 | 90.63 | 163.65 | 1.00 |
| <95% | 9 | 8,961 | 366.84 | 190.87 | 705.03 | 3.05 | 1.71 | 5.42 | <0.001 |

Risk factors for virological failure (defined as HIV-1 RNA > 400 cps/ml) on second-line ART were assessed using Cox proportional hazard models, with adjusted standard-errors to account for clustering within sites. The multivariate analysis was performed by submitting all potential risk factors for virological failure with \( p < 0.10 \) in the univariate analysis.

Non-standard NNRTI-based: d4T/ddI/EFV (N=3), ZDV/ddI/NVP (N=1), ABC/3TC/NVP (N=1); Triple NRTI: TDF/3TC/ABC (N=1), ZDV/3TC/ABC (N=1), TDF/3TC/ZDV (N=1); PI-based: d4T/3TC/LPV/r (N=1), ZDV/3TC/LPV/r (N=1), ZDV/ddI/LPV/r (N=1), TDF/ddI/LPV/r (N=1), ABC/LPV/r (N=1), 3TC/LPV/r (N=1).

ART, antiretroviral therapy; GSS, genotypic sensitivity score; HR, hazard ratio; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
Table 4. Protease inhibitor resistance on second-line antiretroviral therapy: 7 cases.

<table>
<thead>
<tr>
<th>Time on 2nd line (months)</th>
<th>ART regimen</th>
<th>CD4+ cell count (cells/µl)</th>
<th>Viral load (cps/ml)</th>
<th>NRTI major mutations</th>
<th>PI major &amp; minor mutations</th>
<th>Remaining PI options</th>
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<tbody>
<tr>
<td></td>
<td>12 -</td>
<td>287</td>
<td>266</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td></td>
<td>24 -</td>
<td>513</td>
<td>2,050</td>
<td>K65KR,L74LV</td>
<td>Major: M46I,M,V84I,M84V</td>
<td>Minor: L10F</td>
</tr>
<tr>
<td></td>
<td>12 -</td>
<td>204</td>
<td>515</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td></td>
<td>24 -</td>
<td>172</td>
<td>5,956</td>
<td>M184V</td>
<td>Major: V82A Minor: L10I</td>
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</tr>
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<td></td>
<td>36 -</td>
<td>264</td>
<td>1,150</td>
<td>M184V</td>
<td>Major: M46I,M101V,V82AFSV Minor: L10I</td>
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</tr>
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<td></td>
<td>12 FTC substituted by 3TC</td>
<td>195</td>
<td>33,882</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 -</td>
<td>19</td>
<td>1,124,415</td>
<td>T69N,M184V</td>
<td>Major: M46I,M154V,V82A Minor: L10I,L24I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 ZDV substituted by TDF</td>
<td>36</td>
<td>275</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Time on 2nd line (months)</td>
<td>ART regimen</td>
<td>CD4+ cell count (cells/µl)</td>
<td>Viral load (cps/ml)</td>
<td>NRTI major mutations</td>
<td>PI major &amp; minor mutations</td>
<td>Remaining PI options</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>---------------------------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>--------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>34</td>
<td>5th line: RAV/ETR/DRV/r</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>S: ATV/r, SQV/r, TPV/r, IDV/r, LPV/r, NFV</td>
</tr>
<tr>
<td>V - 62 year old male from Zambia, subtype C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 1st line: D4T/3TC/NNRTI, 2nd line: TDF/FTC/LPV/r</td>
<td>67</td>
<td>67,000</td>
<td>D67DN, M184V, L210I/W, T215Y</td>
<td>None.</td>
<td></td>
<td>S: ATV/r, SQV/r, TPV/r, IDV/r, LPV/r, NFV</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>146</td>
<td>&lt;25</td>
<td>NA</td>
<td>NA</td>
<td>R: FPV/r.</td>
</tr>
<tr>
<td>VI - 52 year old male from Zambia, subtype C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII - 44 year old male from Zambia, subtype C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 LPV/r substituted by EFV at month 3, changed to ABC/ddI/LPV/r at 6 months, then LPV/r substituted by NFV at month 12.</td>
<td>14</td>
<td>89,000</td>
<td>M41L, M184V, T215FY</td>
<td>Major: M46I, L54V, V82A, Minor: L10I, K20I, L24I, T74S</td>
<td></td>
<td>R: ATV/r, FPV/r, IDV/r, LPV/r, NFV</td>
</tr>
<tr>
<td>24 -</td>
<td>11</td>
<td>20,000</td>
<td>M41LM, V75IMV, M184MV, T215NSTY</td>
<td>Major: M46I, L154V, L76LV, V82AV</td>
<td></td>
<td>Minor: L10I, T74S</td>
</tr>
</tbody>
</table>

Subtype was determined using the REGA subtyping tool V3.

Drug resistance mutations were detected using the Stanford algorithm version 7.0: susceptible (S) = susceptible/potential low-level resistance, intermediate (I) = low-level/intermediate resistance and resistant (R) = high-level resistance.
tipranavir/ritonavir resistance. Furthermore, I50V, associated with ATV/r, DRV/r and fosamprenavir/ritonavir resistance, and L90M mutation, associated with nelfinavir and saquinavir/ritonavir resistance, were both detected once. The acquired PI resistance mutations conveyed reduced susceptibility to all PIs and remained highest for DRV/r; 81.3% remained susceptible for DRV/r (Figure 2).

After 2-3 years of follow-up, 5 out of 7 participants with PI resistance were still alive and still on second-line ART (Table 4), although 3 of those experienced immunological and clinical failure; 1 participant was switched to a third-line regimen (DRV/r/raltegravir/etravirine, participant IV) and one passed away due to an HIV-related disease after experiencing clinical, immunological and virological failure (participant VI). Two participants experienced virological failure after 24 months without clinical or immunological failure. All but one (participant VII) had good self-reported adherence. Two participants had major PI resistance mutation(s) at the time of switch to second-line (participant III and VI); one (participant VI) received a PI as part of first-line.

**DISCUSSION**

This study found high VL suppression rates on second-line PI-based ART in sub-Saharan Africa (≥85%). However, one out of four participants had a detectable VL (≥400 cps/ml) at any time point during 2-3 years of follow-up, and one out of five experienced a VL≥1,000 cps/ml. In our cohort, having a VL≥400 cps/ml on second-line ART was associated with non-standard first-line regimens, PI-resistance at switch, and poor adherence to treatment. NRTI-resistance at switch, accumulated after first-line failure, was not a risk factor for failing second-line ART. We detected major PI resistance mutations in 7 out of 32 (22%) participants with VL≥1,000 cps/ml. DRV/r was the PI with the highest remaining susceptibility after second-line failure. These findings indicate that future treatment of individuals failing second-line ART requires third-line drug options (i.e. DRV/r, dolutegravir, and/or raltegravir), which are currently unavailable and/or unaffordable in the public sector in sub-Saharan Africa [2,32]. To ensure long-term ART success, availability of these third-line drug options will be essential.

A systematic review on treatment outcomes on second-line ART in resource-limited settings reported high virological failure rates: 23.1%, 26.7% and 38.0% had VL≥400 cps/ml at 12, 24 and 36 months, respectively [33]. In addition, poor adherence rather than NRTI-resistance was associated with virological failure but information was limited. More recent studies from Asia [34] and South Africa [35] also suggested that suboptimal adherence is the main driver of early second-line failure, rather than drug
Figure 2. Antiretroviral drug resistance at second-line failure.

Among 32 participants with HIV RNA ≥1,000 cps/ml and genotype available during follow up. When multiple genotypes were available for the same participant, the most conservative susceptibility score measured was used (including genotypes at switch to second-line).

Predicted susceptibility (genotype sensitivity score) was calculated using the Stanford algorithm version 7.0 [30]. For participants with multiple genotypes, the most conservative susceptibility is plotted (i.e. the highest level of resistance).

Abbreviations: ABC, abacavir; dDI, didanosine; FTC, emtricitabine; 3TC, lamivudine; d4T, stavudine; TDF, tenofovir; AZT, zidovudine; LPV, lopinavir; ATV, atazanavir; NFV, nelfinavir; FPV, fosamprenavir; IDV, indinavir; SQV, saquinavir; TPV, tipranavir; DRV, darunavir; /r, ritonavir (booster).
resistance. In our study, we found that non-standard first-line regimen and PI-resistance at switch significantly contribute to a (5-fold) increase in having a detectable VL on second-line ART; this confirms the WHO recommended standardized first- and second-line ART regimens for optimal treatment outcomes [8]. In addition, our study confirmed poor adherence as a risk factor for failure. In line with the EARNEST trial, we found that NRTI-resistance accumulated after first-line failure was not a risk factors for second-line failure [13].

The arrival of multi-class (i.e. NRTI and PI) resistant HIV has been expected, and the first cases have been reported in South Africa, Nigeria and Uganda [15,21,22,36]. In our prospective cohort, we found that 22% of participants who failed second-line therapy in a routine care setting in sub-Saharan Africa had triple-class resistance. In other words, in 3% (7 out of 227) of people initiating second-line ART major PI mutations developed at failure after 24-36 months. This is the first study showing that an estimated 3% of people switched to second-line ART in sub-Saharan Africa may acquire multi-class resistant HIV within 2-3 years.

Little is known about the impact of multi-class resistant HIV on treatment outcomes and the need for third-line ART in resource limited settings. However, even in a resource-rich setting such as the UK, people with multi-drug resistant HIV were reported to have a 3-fold increase in mortality risk compared to the overall risk for HIV-infected individuals [37]. It can be reasonably anticipated that the emergence of multi-class resistance in settings with serious limitations in drug options and in the ability to monitor participants for therapy effectiveness, will have a major impact on survival and well-being.

In this study, most participants experiencing virological failure with drug resistance had preserved susceptibility to DRV/r (81%) and less than half had preserved susceptibility to etravirine (47%). Individualized genotype resistance testing enables optimization of treatment regimen in experienced patients. Despite efforts to develop affordable assays, individualized resistance testing is currently not feasible in sub-Saharan Africa due to high costs and logistical challenges. There are currently no WHO-prequalified generic versions of third-line options such as raltegravir, dolutegravir or DRV/r, and prices remain extremely high. The lowest possible price for a third-line regimen is around US$2,000/year in low-income countries; almost 18 times more than the lowest price for first-line regimens [6,38]. However, recent agreements with pharmaceutical manufacturers will increase the availability of a generic dolutegravir (for US$44 per patient per year) and heat-stable DRV/r in resource-limited settings by the end of 2016 [39]. By 2030, 2-4 million people will receive second-line ART in sub-Saharan
Africa, comprising 12-17% of all patients on ART [3]. Based on these numbers and our findings, it can be speculated that 62,000-124,000 of them may develop PI-resistant HIV and will be in need on third-line ART.

Without the option to switch to third-line ART, continuation of the PI-based second-line regimen is expected have some residual activity. First, intensified adherence support through intensified VL monitoring could lead to re-suppression on second-line ART despite drug resistance. This is supported by 39% re-suppression in the current study, and as shown in a small study by MSF in Khaelitsha, South Africa [20]. Second, the continuation of ART with residual drug activity can preserve immune function, and thereby prevent clinical progression [40]. The replicative capacity and pathogenicity of drug-resistant virus is often diminished [41]. However, continuing a suboptimal regimen remains inferior to fully-active ART.

The following limitations of our study require consideration. First, although this multi-centre study includes data from routine ART programs in sub-Saharan, non-government and urban sites were over-represented and rural sites under-represented [24]. In a rural setting with limited resources, the provision of second-line HIV care is challenged by drug stock-outs and limited treatment monitoring tools and may be worse. We previously accessed the WHO early warning indicators among the PASER sites [42]. Site-associated differences that might affect VL outcomes and the development of drug resistance were beyond the scope of the current study.

Second, not all participants were appropriately switched to second-line ART; some had suppressed virus or high VL with wild-type virus at the time of switch. These participants might have still benefitted from first-line ART and are perhaps less likely to experience virological failure. Sensitivity analysis excluding participants with VL<1,000 cps/ml at switch, however, yielded similar outcomes. Furthermore, the participants with a reported PI as part of their first-line regimen were not the same participants with PI-resistance mutations at the time of switch to second-line: one out of seven participants with PI resistance at second-line failure had PI as part of first-line, and one out of six participants with PI-based first-line had PI-resistance at switch. This reflects the real-life situation in sub-Saharan Africa where regimen switches are not always guided by virological monitoring and rarely by drug resistance testing. Last, population-based sequencing is not able to detect minority variants, which may have underestimated the rate of drug resistance. Our genotypic analysis was limited to the pol region. There are indications of polymorphic mutations associated with PI-resistance in the gag cleavage site and in env [43,44], which have not been measured.
in our study. Their potential contribution to PI-failure is not captured in conventional resistance testing, and understudied.

Strengths of the study were the prospective data collection based on a large multi-country cohort [24]. Other studies reporting on second-line ART outcomes were based on national outcomes, or are collected in a controlled trial setting. This study provides insight in second-line ART outcomes from a routine HIV care setting in sub-Saharan Africa, following the WHO public health approach [45]. While previous studies have been limited to first year outcomes of second-line ART, or cross-sectional reports at the time of treatment failure, this study reports on the outcomes after up to 3 years of second-line ART.

In conclusion, our data show that the majority of patients receiving PI-based second-line ART in HIV treatment programs in sub-Saharan Africa achieve virological suppression. However, a small but substantial proportion of patients failing PI-based second-line ART acquires PI resistance within 2-3 years. This affects drugs that may be considered for constructing a next-line regimen such as DRV/r. As more people initiate ART and VL monitoring will be expanded, the number in need of second- and third-line regimens is expected to increase [3]. There is a real and growing need for expanded access to third-line drug options, ideally guided by genotypic resistance testing, to ensure life-long HIV treatment success in sub-Saharan Africa. In anticipation, increased efforts must be made to achieve price reduction of currently unaffordable third-line drugs, including DRV/r and integrase inhibitors.

NOTES

Potential conflicts of interest
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. All authors declared no conflicts of interest.

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Presented
Part of this work was presented at the Conference on Retroviruses and Opportunistic Infections (3-6 March 2014) in Boston, Massachusetts USA. This work was presented in part at the Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment on November 18th 2015. This work has been accepted for presentation at the International HIV Drug Resistance Workshop (20-21 February 2016), and the Conference on Retroviruses and Opportunistic Infections (22-25 February 2016), both in Boston, Massachusetts USA.

GenBank accession numbers
All HIV-1 pol sequences in this study have been deposited in GenBank under the following accession numbers: JN132214-JN132396, JN393292-JN393306.

Contributors
TFRdW is the principal investigator. RLH and TFRdW designed the study and developed the protocol. RLH, KCES and TSB set up the study and trained and supervised study workers. MW, MS, EL, CK, ASA, KM, and MEB established the cohort and supervised data collection. TSB conceived and undertook the data analyses, and drafted the manuscript. KCES checked and supervised the statistical analyses. TSB, KCES, RLH, PO and TFRdW helped interpret the data and reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.

PASER collaborating sites
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of the University of Amsterdam, Amsterdam Institute for Global Health and Development, Amsterdam, the Netherlands (K C E Sigaloff, R I Hamers, P Ondoa, T S Boender, C Manting-de Vries, D Lathouwers, N Pakker, B Prins, E Straatsma, P van Leeuwen, F W Wit, M van Vugt, J M Lange, T F Rinke de Wit).

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REFERENCES


Epilogue
Research in action: from AIDS to global health to impact.
A symposium in recognition of the scientific contributions of Professor Joep Lange.

*Primary author. Subsequent authors listed alphabetically.

Antiviral Therapy
SUMMARY

On Tuesday 14 October 2014, 850 family members, friends, colleagues, prominent scientists and dignitaries from all over the world gathered in Amsterdam to pay tribute to the lives and legacies of Joep Lange and Jacqueline van Tongeren. The remembrance was held at the Amsterdam Medical Centre (AMC) where Joep and Jacqueline met and worked together for many years. The day was organized by the AMC, the Amsterdam Institute for Global Health and Development (AIGHD) and PharmAccess Foundation. The latter two were both founded by Joep. A morning symposium titled ‘Research in action: from AIDS to global health to impact’ highlighted Joep’s scientific legacy (Figure 1). During the remembrance in the afternoon, a range of speakers shared memories of Joep and Jacqueline. As was the case during their lives, the personal and the professional were closely intertwined throughout the day. As Prof Peter Piot said, Joep and Jacqueline shared a common perspective on life: ‘La folie suprême est de voir la vie comme elle est et non comme elle devrait être.’ If there was one thing that defined them both, it was indeed that they saw life – and lived it – not as it was, but as it should be.

Figure 1. Morning symposium.
Photo by Rebke Klokke.
‘Joep’s place in history is really as the visionary architect of combination therapy,’ Prof Piot stated, adding that ‘it cannot be stressed enough that he was ahead of his time, a true innovator.’ Joep’s contribution didn’t stop at science. Dr Khama Rogo of the World Bank explained that ‘it’s not enough to be a doctor or a researcher if you’re not also an activist.’ Joep fully understood the importance of translating research into action and generating impact for people. Prof Marcel Levi, chairman of the AMC, summarized the enormity of the impact Joep had on the world with the words ‘it’s rare to know someone who has saved millions of lives.’

The scientific symposium traced Joep’s career, starting in the early eighties with the treatment of the first AIDS patients and the design of antiretroviral therapy, moving towards the emerging field of global health and ending with his most recent focus: using knowledge derived from scientific research to improve access to quality health care in real-world settings. From Prof Françoise Barré-Sinoussi, who won the Nobel Prize for the discovery of HIV, to Prof Michael Merson, who founded Duke University’s Global Health Institute, the list of presenters reads like a who’s who of people involved at key moments in the history of HIV and global health (Figure 2). ‘And Joep,’ as Barré-Sinoussi said, ‘contributed to all eras of HIV.’

More memories of Joep and Jacqueline shared throughout the day are available at http://www.joepandjacqueline.org/remembrance/.
RESEARCH IN ACTION: FROM AIDS TO GLOBAL HEALTH TO IMPACT.

The morning symposium served as a platform to recognize Joep Lange’s scientific achievements and to pay tribute to his activism in the field of HIV treatment and global health. It was also an occasion for colleagues to share their feelings and personal anecdotes about working with Joep.

Joep’s friend and colleague Prof Peter Reiss of the University of Amsterdam opened the symposium, welcoming attendees and encouraging the audience to once again be inspired by Joep’s work and to continue his legacy into the future.

A word from the co-chairs
Peter Reiss introduced the two co-chairs of the symposium, Prof Michel Kazatchkine and Dr Debrework Zewdie.

Prof Michel Kazatchkine shared his memories of Joep as a man who ‘was convinced of the power of science to build knowledge and then translate it into action.’ Kazatchkine emphasized Joep’s generosity, compassion and tolerance, all qualities that are considered essential for a good doctor. Qualities, Kazatchkine added, that Joep fully shared with Jacqueline. While Joep was a tolerant man, he would never accept a policy that wasn’t evidence-based. Joep fought for equity, for the right of everyone to access and enjoy the advancements of science.

Dr Debrework Zewdie began by saying that ‘Joep was born a Dutchman, but became a global citizen who felt that the fruits of science should be shared by everyone.’ For example, the yearly International Workshop on HIV Treatment, Pathogenesis and Prevention Research in Resource-Poor Settings (INTEREST), nicknamed ‘the African CROI’, provides young African researchers with the opportunity to present their data in the presence of established scientific leaders and to learn from this experience. ‘I was amazed by Joep and Jacqueline’s work in building African scientists and shepherding the INTEREST group. Now, it is up to us to step in and make sure to continue what they started.’

The co-chairs introduced the subsequent speakers, who each touched upon different aspects of Joep’s career as a scientist and activist.
Chapter 10

Peter Reiss is Professor of Medicine at the AMC in Amsterdam in the Division of Infectious Diseases, the Department of Global Health and the AIGHD. He was recently appointed Director of the Netherlands HIV Monitoring Foundation, and serves on several Scientific Advisory Boards.

Debrework Zewdie is a clinical immunologist by training and has spent the last 30 years on research and development work including managing development programmes at country, regional and global levels. She worked for the World Bank and the Global Fund.

Francoise Barré-Sinoussi was awarded the Nobel Prize for Medicine 2008 and is Director of the Regulation of Retroviral Infections Unit, Department of Virology, Institut Pasteur. She is involved in retrovirology research and is particularly recognized for the discovery of HIV.

Michel Kazatchkine is the UN Secretary General’s special Envoy on HIV/AIDS in Eastern Europe and Central Asia. Previously, he was Head of the French Agence Nationale de Recherches sur le Sida, France’s Ambassador on AIDS and Executive Director of the Global Fund to Fight AIDS, Tuberculosis and Malaria.

David Cooper is Scientia Professor of Medicine at the University of New South Wales, a Fellow of the Australian Academy of Science (FAA), and Director of the Kirby Institute for Infection and Immunity in Society based at UNSW Australia.

Menno de Jong is Professor of clinical virology and head of the Department of Medical Microbiology at the AMC. Joep Lange recruited him as a trial physician and supervised his PhD research on the causes and implications of HIV treatment failure in 1996.

Michael Merson is the founding director of the Duke Global Health Institute. He is Vice President and Vice Provost of Global Strategy and Programs and Vice Chancellor for Duke-National University of Singapore Affairs at Duke University.

Eric Goosby served in the US State Department as Ambassador-at-Large and US Global AIDS Coordinator, overseeing the implementation of the President's Emergency Plan for AIDS Relief (PEPFAR). He is Director at the Institute for Global Health Delivery and Diplomacy, University of California, San Francisco.

John Simon has been US Ambassador to the African Union and the Executive VP of the Overseas Private Investment Corporation. He served on the National Security Council staff, White House, and as Deputy Assistant Administrator, USA Agency for International Development. He is a Founder/Managing Partner of Total Impact Advisors.

Fola Laoye is Chair of Hygeia Nigeria Limited. She has an MBA from Harvard Business School and qualified as an Associate member of the Institute of Chartered Accountants of England and Wales in 1995 and of the Institute of Chartered Accountants of Nigeria in 1997.

Khama Rogo is Lead Health Sector Specialist with the World Bank and Head of the World Bank Group’s Health in Africa Initiative. Prior to this, he taught Obstetrics and Gynaecology at the University of Nairobi and was President of Medical Affairs Africa for Ipas.

Onno Schellekens is the Managing Director of PharmAccess, an organization founded by Joep Lange, which is dedicated to improving access to affordable quality health care for people in sub-Saharan Africa.

Figure 2. Speakers and chairs.
ARV therapy: past, present and future

*It is hard to imagine robust economic growth where so many adults are dying in their productive prime, leaving the very young and the very old to cope alone.*

_Economist, 12 August 1999._

Prof David Cooper took the audience back to the 1980s when he, like Joep in the Netherlands, was confronted with the first AIDS cases in Australia [1–6]. Cooper detailed the important findings made by Joep that plasma levels of viral protein P24 (a then proxy for viral load) were predictive of the stage of HIV infection, with low P24 in the absence of symptoms and high P24 in symptomatic AIDS patients [7]. In addition, Joep was involved in important research on syncytium-inducing versus non-syncytium-inducing viruses, with the latter being more virulent [8].

Later on, the molecular basis for this phenomenon was found with the discovery of the secondary receptors for HIV – the chemokine receptors. Non-syncytium-inducing viruses are CCR5-tropic while syncytium-inducing viruses are CXCR4-tropic, with implications for prognosis [9]. Cooper provided examples of the first clinical trials of antiretroviral therapy that failed due to a transient response related to the development of drug resistance: AZT monotherapy, AZT+3TC dual therapy and NVP-AZT alternating therapy [10–12]. These trials convinced Joep that only triple therapy would be the way forward to prevent drug resistance, as revealed by the successful INCAS trial that tested the triple therapy combination of AZT, ddI and NVP, published in _JAMA_ in 1998 [13]. Triple therapy remains the cornerstone of HIV treatment today and is now scaled-up to provide increasingly early treatment which reduces the risk of ongoing HIV transmission [14–17].

Together with Prof Cooper and Prof Praphan Phanuphak, Joep initiated the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) in Bangkok in 1996 to build research capacity in Thailand in the field of HIV treatment. To date, HIV-NAT encompasses 112 staff and runs long-term studies involving over 2,000 adults and children on antiretroviral therapy.

Cooper observed that Joep was lately returning to his original passion and was involved with various efforts toward a functional cure for HIV. Referring to the continued spread of HIV, Cooper emphasized the thoughts of Joep’s favourite economist JF Rischard that “we are facing a problem of immense complexity, which is getting out of hand in an exponential way. The traditional way of working along a linear time scale
will not be able to cope with this.’ In addition, he sketched how Joep understood that HIV/AIDS seriously hampers economic growth in many developing nations, and the ensuing need for a concerted global effort, a broad coalition of the public and private sectors, civil society and academia, with clear divisions of tasks and accountability. In his conviction that ‘what works’ should prevail over the notion of what constitutes an ‘ideal society’, Joep often quoted Martin Wolf: ‘The sight of the affluent young of the west wishing to protect the poor of the world from the processes that delivered their own remarkable prosperity is unutterably depressing.’ He saw sustainable financing of health care for the masses, not necessarily through the public sector, as a prerequisite for a future without donor dependence.

Cooper ended his talk with what he described as Joep’s legacy: ‘As there is no vaccine or cure right now, antiretroviral therapy is our major intervention. It would be a tragedy if we fail to get enough people on treatment to reduce incidence and we need to persuade civil society that flat lining of funds is just plain unacceptable. We MUST finish the job.’


**Towards an HIV cure**

*Joep contributed to all eras of HIV.*

Prof Françoise Barré-Sinoussi.

Subsequently, Nobel Prize laureate Prof Françoise Barré-Sinoussi, co-discoverer of HIV [18], acknowledged Joep’s long-term commitment to find a cure and referred to how he was also an advocate for a global scientific strategy: joining forces in an international working group to define the main research priorities and coordinate research efforts. ‘The time to accelerate HIV research is now.’ Prof Barré-Sinoussi emphasized the need for basic science for HIV vaccine development and HIV cure. She highlighted that the formation of viral reservoirs immediately after primary infection is very rapid: even if only one out of 1.7 billion CD4 T-cells is infected, a viral rebound is possible [19]. In order to work towards a cure, it is important to learn more about the properties of latently HIV-infected cells and how viral re-activation pathways can be influenced [20].

In her presentation, Barré-Sinoussi considered complete eradication of HIV in an infected patient (sterilizing cure) less likely than putting HIV into remission (functional cure) [21]. Working towards a functional cure was one of Joep’s most recent topics.
of research in Amsterdam. New research that was taking place at AIGHD under his supervision may lead to a potential breakthrough in the treatment of people with HIV. New insights suggest that immediate treatment of a newly contracted HIV infection can be so effective that it may be possible to effect a functional cure. This would mean that after some time, treatment can cease and the virus will no longer be detectable in the patient’s blood. The virus, which would still be present in certain parts of the body, would be so weakened that disease progression would come to a halt and HIV would no longer be transmissible.


From HIV to emerging infectious diseases

‘Be creative and think big to tackle the real problems.’

Prof Menno de Jong recalls how Joep taught him to approach his research. Prof Menno de Jong started his presentation by recalling how Joep had been his mentor, friend and inspiring colleague. One of the things that Joep felt strongly about was that there was no point in research for research’s sake, but that it should always have the aim to generate impact in real-world settings. De Jong then drew a parallel between HIV and his current professional field, explaining that ‘we can apply the lessons learnt from HIV to other emerging infectious diseases like influenza and Ebola.’ Mirroring aspects of HIV treatment, the concept of triple therapy has proved promising against H1N1 influenza infection in immune-compromised children [22]. He provided an overview of the important emerging infectious diseases during the past decade, all of which are transmitted from animals to humans (2003 SARS-CoV, 2004 H5N1 influenza, 2009 H1N1 influenza, 2012 MERS-CoV, 2013 H7N9 influenza and 2014 Ebola virus).

One common denominator with HIV is that the emergence of new pathogens usually takes place in regions of the world where the infrastructure and the human capacity to recognize and contain such outbreaks are the lowest [23]. De Jong explained that the early recognition and containment of infectious diseases requires, among other things, laboratory and research capacity. While the importance of building laboratory capacity in Africa is being increasingly recognized, there is much room for improvement. For example, more optimal use could be made of the opportunities for cross-fertilization between research and laboratory capacity in Africa, both in terms of HIV and other important infectious diseases.
According to De Jong, clinical research responses to infectious disease outbreaks are usually fragmented and often too late. There are global regulatory hurdles for timely clinical research during epidemics and as such, important opportunities for gathering essential data in the early stages of an outbreak are often missed. The average time that passes between the development of a clinical research protocol and the recruitment of the first patient, De Jong said, is far too long. Needless to say, an epidemic doesn’t wait for paperwork. He argued that it is crucial to develop pre-approved standardized open-access clinical research protocols, translated in many languages, to react as swiftly as possible in case of disease outbreaks. ‘We need a new paradigm, we need to be prepared and ready to act.’


**From HIV to global health**

*Joep leaves behind a legacy of leadership, vision and always striving for a healthier tomorrow.*

Prof Michael Merson.

In the early 1990s, Prof Michael Merson hired Joep as Chief of Clinical Research and Drug Development for the World Health Organization’s Global Programme on AIDS. It was in this capacity that Joep made his first trip to Africa, which proved to be a life-changing event for him. In the course of their careers, both Merson and Joep moved from HIV research to global health, which has been defined as ‘the area of study, research and practice that places a priority on improving health and achieving equity in health for all people worldwide.’

In his presentation, Merson outlined five key ways in which HIV research contributed to the relatively new discipline of global health [24]. First of all, HIV was the first post-modern pandemic that affected populations globally in both low- and high-income settings. Second, HIV fostered new collaborative approaches, such as the treatment–prevention model. This method highlighted the importance of multidisciplinary approaches to prevention and care, which has become a hallmark of global health research. Third, HIV led to a global advocacy movement in which scientists joined forces with human rights and other activists to fight stigma and demand better treatment. This alliance of biomedical research and activist communities has served as a model for advocacy around other global issues such as breast cancer, tobacco control and access to essential medicines, including medications for non-communicable
Research in action: from AIDS to global health to impact

diseases. Fourth, the HIV pandemic secured enormous international funding (Global Fund, World Bank, PEPFAR) that is increasingly moving from ‘vertical’ (disease-specific) to ‘horizontal’ (general health system) approaches [25]. When Joep founded PharmAccess in 2000, his primary concern was to bring life-saving ARVs to people in Africa. Soon, he realized that true equity in health required a systems approach, after which PharmAccess pioneered an integrated demand and supply side approach to improving access to affordable and quality health care for all. Finally, HIV inspired increased international academic engagement and leadership, something that Merson described as ‘dear to Joep’s heart.’ Today, global health is a discipline in over 100 universities in the United States of America alone.

Just a few days before the symposium in Amsterdam, the extensive international media coverage on the recent Ebola outbreak often included CDC director Tom Frieden’s remark that: ‘In my 30 years in public health, the only thing that has been like this is AIDS. We have to work now so that this is not the world’s next AIDS.’

Merson reflected on the parallels between the early days of HIV and the Ebola virus outbreak, which include the stigma involved, the limited knowledge of the disease, the fact that it was originally presumed to be deadly in all cases, the lack of effective treatment and the inadequate global response. As such, he underscored the importance of learning from the consequences of the slow initial response to HIV. Ebola should also be considered a global security threat which requires a prompt and proactive response.


**From research to action**

‘Programs finish, and whether they fail or succeed they are discontinued… there is scant attention paid to carefully embedding successful programs into sustainable national programs. And no one is outraged.’

Prof Eric Goosby.

Prof Eric Goosby first met Joep in San Francisco in 1984, where Goosby was treating the first AIDS patients at San Francisco General Hospital. ‘Joep had a laser focus on the individual, on honouring the link between the physician and the patient. His authentic
way of living continues to reverberate with me and I am honoured to be a part of this collective acknowledgement of Joep’s contribution.’

Goosby’s presentation focused on global health delivery, diplomacy and the long road to sustainable health-care delivery systems. He addressed the challenge of equity in health and called for the prioritization of the principles of health-care quality, just as Joep had done. Goosby advocated that there are many diseases we know how to prevent, diagnose and treat effectively, yet efforts are falling short. ‘We know what works but we are not delivering. The fact that we are not doing so is impacting millions of lives and costing billions of dollars around the world. How can we encourage and promote development when tuberculosis and HIV are still ravaging the young and productive core of many African societies?’

According to Goosby, science has given us the tools, but most development efforts take an inordinate amount of time to implement. ‘We need to provide countries with external funding, without creating parallel systems of care. We cannot afford to be in a constant state of emergency when dealing with epidemics.’ Goosby referred to the Ebola outbreak as the canary in the cage to identify weaknesses in the medical delivery system and called for stronger partnerships. ‘Health systems need all four legs of the “delivery stool”: academia (rigor), the private sector (efficiency), the community (ownership) and the national and local government (management).’


**How public policy can deliver health results**

‘With PharmAccess and the Health Insurance Fund, Joep improved healthcare quality and created health insurance schemes that ensured that people are no longer a disease or a mosquito bite away from complete destitution.’

> Ambassador John Simon.

Ambassador John Simon underscored his appreciation for Joep’s pragmatic approach. ‘We know what to do, yet it’s not getting done. Joep found this unacceptable. He knew that in order to achieve results, you need to influence public policy and to make a difference on the ground, not from behind your desk.’

In many developing countries, Ambassador Simon explained, the limited functioning of the state and its institutions hampers the development of health care and thus
universal access to quality health services. Most of these countries are ruled by a power elite that maintains a state that is designed to serve a limited elite as opposed to overall society. Building on the theoretical framework of Nobel Prize for Economics laureate Douglass North, Simon took the audience through the historic developments of governments moving from being extractive (limited access orders) to becoming inclusive institutions (open access orders).

He continued to describe the PharmAccess approach of simultaneous strengthening of demand and supply to turn the vicious cycle of malfunctioning health systems into a virtuous cycle. By combining interventions such as standards for quality improvement, loans for health-care providers, health insurance plans and mHealth, PharmAccess builds trust in the health-care system.

**The private sector and global health goals**

*‘Joep realized the importance and potential impact of market dynamics and how that can be catalytic in health.’*

Fola Laoye.

‘Meeting Joep was a life-changing experience for us at Hygeia,’ said Ms Fola Laoye, chair of the Board of Hygeia, Nigeria’s largest health maintenance organization. She commended Joep’s continuous commitment to involve the private sector in health and health-care financing. Elaborating on what she called the African contradiction, Laoye explained that when it comes to health care, a good deal of spending comes from private pockets. ‘The private sector has a huge role to play in public health. While we are making progress, being here today and listening to everyone’s presentations makes me realize that our work is not done.’

Laoye shared several examples from the shared PharmAccess and Hygeia shop floor. Focusing on the Kwara State Health Insurance Program, set up in partnership with the Kwara State Government, she spoke of how this programme has improved the quality of health care at clinics and increased use of modern health-care providers, as well as improved health outcomes. ‘In areas like non-communicable diseases, malaria and maternal health, we have seen indices like we never expected.’

The programme, which was recently named as one of the finalists in the OECD DAC Prize for Taking Development Innovation to Scale, has had a significant impact on the rural populations of Kwara, one of the poorest states in Nigeria. In-depth impact evaluations conducted by the AIGHD have resulted in many publications, including a
paper in JAMA Internal Medicine [26] showing the positive effect of health insurance and facility quality improvement on blood pressure in adults with hypertension.


**Panel discussion**

Dr Khama Rogo shared with the audience that he saw Joep as a health revolutionary, referring to him as the Che Guevara of global health. ‘By nature,’ he said, ‘revolutionaries ask questions that are uncomfortable,’ and in this tradition Dr Rogo led the panel of eminent speakers in an animated discussion on global health and possible solutions to today’s biggest challenges. After stirring up the discussion with statements such as ‘Of all the ills that kill the poor, none is as lethal as bad government’, Rogo ended the session with the shared conclusion that we must all work to make sure that health is no longer a footnote in budget discussions and public policies. After all, as he proclaimed, ‘It’s not enough to be a doctor or a researcher, if you’re not also an activist.’

**Closing remarks**

PharmAccess Managing Director Onno Schellekens reflected on Joep’s conviction to drive results, whether the road to such results was politically convenient or not. ‘He started with mother-to-child transmission of HIV studies in 1995, at a time when no one wanted to finance such studies because finding a solution brought with it the responsibility of addressing the problem. He pushed through and insisted on doing the trials and developing treatment, even though the political buy-in was not yet there.’

When antiretroviral therapy became affordable and the regimens less complex, Joep founded PharmAccess to increase access to treatment for those who needed it most. To Joep, the fact that HIV/AIDS treatment was not available in Africa was – at best – a lack of will mixed with stupidity. At worst, it was pure racism. ‘Joep was always one step ahead,’ Schellekens said. ‘He taught us that doctors who can talk economics can change the world. While working to change policies at governmental level, Joep remained determined to deliver care all the way to the local last mile.’

Wrapping up the symposium, chairs Dr Zewdie and Prof Kazatchkine summarized the day as follows: From the first case of AIDS Joep Lange saw in 1983 and his first trip to the African continent in 1992 to the first treatment successes in 1996 in providing access to antiretroviral therapy in resource-poor settings, Joep advocated to treat all
HIV-affected persons equally. From the public sector to the private sector, from activists to pharmaceutical companies, from scientists to patients, Joep involved all parties.

Commemorating Joep’s awe-inspiring achievements towards universal access to HIV treatment, global health and universal health coverage, we continue to be inspired by him today. Let us be determined doctors, humanitarians, scientists, activists, economists, provocateurs and health revolutionaries who get things done in the field of HIV and global health. Let us continue Joep’s legacy.

ACKNOWLEDGEMENTS

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Summary & General discussion
LONG-TERM EFFECTS OF HIV TREATMENT IN SUB-SAHARAN AFRICA: FROM ACCESS TO QUALITY.

Since the early 2000s, international partnerships, major donor funding, political commitment and community activism have transformed the global HIV/AIDS epidemic [1]. As of June 2015, 15.8 million people had access to antiretroviral therapy (ART), of whom over two-third reside in sub-Saharan Africa [2]. This has led to large reductions in HIV-related morbidity and mortality, and improved national life-expectancy. Furthermore, access to ART has reverted the HIV epidemic and reduced transmission. Increasing numbers of HIV-positive pregnant women receive antiretroviral (ARV) drugs, which protects both themselves against HIV progression and prevents vertical transmission of HIV to their babies. Since 2000, there has been a reduction of 35% in all new HIV infections, with a 58% decrease in new infections among children [3]. At the same time, the epidemic is still ongoing with an estimated 2 million people newly infected with HIV and the death of 1.2 million in 2014 due to AIDS-related disease [3].

The recent international 90-90-90 targets are aiming for continued expansion of access to and lifelong treatment with ART [4]. According to these targets, 90% of all people living with HIV should know their HIV status; 90% of all people with diagnosed HIV infection should receive sustained ART; and 90% of all people receiving ART should have viral suppression, by 2020. If these targets are reached, 73% of all people living with HIV worldwide will achieve suppression. Modelling suggests that if these targets are met by 2020, it will be possible to end the AIDS epidemic by 2030. This will be possible through the prevention of AIDS and onward transmission of HIV as a results of durable HIV suppression. While the global response to the HIV epidemic has been a major effort with promising results, the job is certainly not yet complete. Scale-up must continue and new challenges lie ahead to secure long-term HIV treatment success in all steps of the cascade of HIV care. Without scale-up, the HIV/AIDS epidemic will continue to outrun the response, increasing the long-term need for HIV treatment and increasing future costs [5].

One of the challenges of long term ART success is durable viral suppression, in the context of increasing HIV drug resistance in sub-Saharan Africa [6]. The studies presented in this thesis evaluate the effect of HIV treatment programmes and the emergence of HIV drug resistance in adults and children, in sub-Saharan Africa. First, we described pretreatment challenges for HIV-infected children in sub-Saharan Africa. Second, we described the long-term virological suppression rates in HIV-infected children and adults receiving first-line ART in low- and middle-income countries. Third, we described the future ARV drug options for people who are failing standard
first- and second-line ART. This chapter provides a summary and general discussion of the study findings, followed by future perspectives.

Part I - Starting HIV treatment: pretreatment challenges

Chapter 1 provides a general introduction of this thesis. In Chapter 2, we studied the barriers to ART initiation for 306 HIV-infected children aged ≤12 years, in three clinics in Uganda. We found that the children reported very late for treatment: 72% of the children were diagnosed with advanced HIV disease at ART initiation (i.e. WHO clinical stage 3 or 4). The risk factors for late presentation were: being younger than two years of age, living without parents, caregiver unemployment, lack of prevention of mother-to-child transmission (PMTCT) services, and high transportation costs to the clinic. We confirmed these risk factors through interviews with both health workers and caregivers of children attending the clinics. In addition, these interviews revealed that inconsistent referral from perinatal care, caregivers’ lack of awareness of HIV symptoms, fear and stigma also contributed to late presentation.

It is important to start ART early, especially in children. To improve access to (paediatric) HIV care, there is a need for better psychosocial support for mothers, community and orphanage outreach programs, and linkage of antenatal care systems to ART providers. Our study added insight into the challenges of identifying HIV-infected infants directly after birth and recruiting them into care. Knowledge of these factors and their potential solutions is important to help health workers and ART program planners to create interventions that reach HIV-infected infants as early as possible. This will in turn avoid preventable child morbidity and mortality [7]. Children are not only at higher risk when HIV-infected, they are also taking longer to get diagnosed and require special attention to reach the first 90-90-90 target (90% of all people living with HIV knowing their status). In addition, an improvement in HIV diagnosis in children could also increase the number of HIV diagnosis in adults (the mother and father), preventing future infant infections.

In Chapter 3 and 4, we assessed the prevalence of HIV drug resistance mutations (DRMs) before first-line ART initiation among children in Uganda and Nigeria. In the same cohort of children in Uganda as described in chapter 2, we found that 28 out of 279 (10%) children initiating first-line ART had DRMs (Chapter 3). Previous exposure to ARV drugs for PMTCT was a significant contributor to the presence of DRMs: 8% of ARV-naïve children versus 36% of ARV-exposed children had DRMs. Interestingly, 16% of children with unknown ARV exposure also had DRMs. Further significant risk factors for HIV drug resistance were younger age, maternal ART use and breastfeed-
ing. In Chapter 4, we found that 13 out of 82 (16%) ARV-naïve children aged ≤12 years in Lagos, Nigeria initiating first-line ART had DRMs.

To put these findings into context, we systematically reviewed all published literature on the prevalence of DRM in children in sub-Saharan Africa (Chapter 4). We included 16 studies, including our own studies, from 11 African countries (2,057 children). In a meta-analysis, we found an overall prevalence of 28% of DRMs among children in sub-Saharan Africa. The prevalence of children harbouring at least one DRM was four times as high in children who received PMTCT: 40% in ARV-exposed children versus 10% in ARV-naïve children, respectively. Among ARV-naïve children, the prevalence has increased over the past decade.

For the survival of HIV-infected children, early identification of HIV infection in infancy and prompt initiation of effective ART is critical [7,8]. Our studies (Chapter 3 and 4) indicate that knowledge of ARV-exposure (ie, PMTCT) is not enough to clearly rule-out children at risk of harbouring DRMs. The high and increasing prevalence of HIV drug resistance underlines the need for protease inhibitor (PI) based, rather than non-nucleoside reverse transcriptase (NNRTI) based first-line ART regimens for children in sub-Saharan Africa, irrespective of ARV exposure through PMTCT [9]. Since 2013, the WHO recommends initiation of PI-based first-line ART for children under 3 years of age [10,11]. However, implementation of PI-based ART for children is not always feasible due to the higher costs, and the limited availability of paediatric formulations which require a cold-chain [12,13]. Efforts should focus on making paediatric formulations for PIs available for Africa. Surveillance of pretreatment drug resistance remains essential in the optimization of first-line ART regimen, especially in children.

**Part II - Long-term outcomes of first-line antiretroviral therapy**

In Chapter 5 and 6, we systematically reviewed all published literature and conference abstracts reporting on virological outcomes among HIV-positive adults and children on first-line ART, in low- and middle-income countries. We investigated the proportion of adults and children reaching virological suppression from 6 months up to five years after ART initiation. In total, we included 72 paediatric and 165 adult studies in these meta-analyses.

In Chapter 5, we found that 70-80% of children on first-line ART in low- and middle-income countries reached virological suppression after 6-24 months. After up to five years, 60-80% remained virologically suppressed, but few studies reported long-term outcomes. When using an intention to treat approach (accounting for children who were lost to follow-up, died, or stopped therapy and assuming that these children
experienced virological failure) suppression rates were much lower, at 45-55% after 6-24 months. To assess the guideline change from NNRTI-based to PI-based first-line ART, we compared children receiving an NNRTI- versus PI-based first-line ART regimen. Our findings indicated that PI-based regimens are favourable compared to NNRTI-based regimen for children in a programmatic setting. Furthermore, virological suppression rates at 6-12 months were significantly better in children without PMTCT exposure.

In Chapter 6, we found high and stable levels of virological suppression (>80%) among adults retained in care during the first five years of first-line ART in low- and middle-income countries. When accounting for adults who were lost to follow-up, died, or stopped therapy and assuming all of them had virological failure, available data suggest that suppression rates declined from 75% to 62% during the first four years of ART. Early virological suppression rates (i.e. after 6-12 months) were significantly higher in Asia compared with sub-Saharan Africa, but differences tended to disappear over time (24-60 months).

In these two large meta-analyses (Chapter 5 and 6), we showed that rates of virological suppression in children were slightly lower (70-80%) than those found in adults (>80%). In general, satisfactory rates of virological suppression are possible in low- and middle-income countries. Children remain a vulnerable population, at increased risk for treatment failure. While high (>90%) virological suppression rates have been achieved in children in high-income countries [14,15], this was not the case in low- and middle-income countries. In order to achieve optimal treatment results for HIV-infected children in low- and middle-income countries, practical and affordable paediatric formulations and regimens (i.e. PIs) and improved treatment monitoring is needed. Further research is needed to better describe virological outcomes among adults and children who are lost to follow-up and those who stop therapy, so that these results can be taken into account in future estimates of population-level virological suppression. These retention data are needed to inform a comprehensive benchmark framework, and to assess and monitor program performance, including the 90-90-90-targets [4].

In Chapter 7, we assessed the effect of pretreatment HIV drug resistance on mortality, the development of AIDS and switches to second-line ART in adults on first-line ART in sub-Saharan Africa. We included 2579 adults on NNRTI-based first-line ART with genotypic test results available from Uganda, Nigeria, Zimbabwe, Kenya, Zambia, Zimbabwe and South Africa. Before ART initiation, 14% had DRMs which were mostly NNRTI-associated. Overall, 6% of participants had DRMs associated with reduced
susceptibility to the prescribed regimen (i.e. pretreatment drug resistance). We found that among participants with pretreatment drug resistance, switching to second-line ART was nearly 4-times as common compared to those without. During the first 3 years on ART, pretreatment drug resistance was not associated with the development of new AIDS-related events or excess mortality. We found that at 3-year follow-up, 4% of patients had switched to second-line ART. Of concern, 24% of participant in this study who were switched to second-line ART either had VL < 1000 cps/mL, or VL ≥ 1000 cps/mL without presence of DRMs, which means they could still have benefited from continuing on first-line ART.

This study showed that the presence of pretreatment drug resistance diminishes the long-term effectiveness of first-line ART, and is strongly associated with switching to second-line ART (Chapter 7). The study extends previous findings of the PASER cohort, that pretreatment drug resistance is associated with increased risk of virological failure, reduced CD4 recovery and increased accumulation of additional drug resistance after one year of first-line ART [16]. Increased access and uptake of available viral load testing could avert unnecessary switches to second-line ART. These switches to more costly and toxic second-line ART impair the efficiency in the use of scarce resources available in ART programs. Increased implementation of viral load monitoring and access to affordable second-line ART is urgently needed to secure long-term virological suppression in sub-Saharan Africa.

**Part III - Salvage drug options**

In Chapter 8 and 9, we described the prevalence and patterns of acquired drug resistance and future ARV drug options for people who are failing standard first- and second-line ART. In the setting of limited or absent virological monitoring we were able to investigate the effect of ongoing viral replication on the accumulation rate and patters of DRMs as well as predict susceptibility of future ARV drug regimens. First, we described the remaining drug susceptibility after continued virological failure on first-line ART in both adults and children (Chapter 8). Next, we described the risk of virological failure on second-line ART in adults and explored the level of PI-resistance after failure (Chapter 9).

In Chapter 8, we evaluated the virological outcomes of 2,737 adults and 289 children on NNRTI-based first-line ART in sub-Saharan Africa. We found that virological failure rates were approximately three times higher in children compared to adults: 24 and 30% versus 9% after 1 and 2 years. We included both adults (N=63) and children (N=56) with continued virological failure and a genotype test result at at least two follow-up points. At first virological failure, ≥1 DRM was detected in 87% of participants: 83%
harboured NNRTI-resistance mutations and 73% harboured NRTI-resistance mutations. While drug susceptibility was already reduced in many participants at first-time failure, the predicted susceptibility declined significantly after continued virological failure for all NNRTIs and NRTIs. Whereas virological failure rates were significantly higher in children compared to adults, we found no significant differences in the patterns and accumulation rate of DRMs.

In Chapter 9, we found that among 227 adults on PI-based second-line ART in sub-Saharan Africa, \( \geq 85\% \) reached virological suppression at any time point during 2-3 years of follow-up. The risk factors for having a detectable VL were non-standard or PI-based first-line ART, PI-resistance at switch and poor adherence. While we found high rates of virological suppression, major PI resistance was detected in 22% of those participants failing second-line ART. After virological failure, most participants retained susceptibility to darunavir (81%), and less than half retained susceptibility to second-generation NNRTIs (etravirine 47%, rilpivirine 31%).

High rates of acquired drug resistance after failure of NNRTI-based first-line treatment were common in both adults and children, requiring second-line PI-based ART. After virological failure on PI-based second-line ART, 1 out of 5 adults required third-line ARV drugs due to acquired PI resistance which are currently unavailable and/or unaffordable in the public sector sub-Saharan Africa. According to our findings, new drug classes (i.e. second-generation PIs and integrase inhibitors) are required to construct fully-active second- and third-line ART regimens for Africa. The role of second-generation NNRTIs in both second- or third-line regimens is expected to be limited. To ensure long-term ART success, intensified adherence support, virological monitoring and third-line drug options are urgently needed.

**Epilogue – from AIDS to global health impact**

In the epilogue (Chapter 10), we paid a tribute to the late Professor Joep Lange and Jacqueline van Tongeren. We reported on the symposium held on October 14th 2014 at the Academic Medical Center of the University of Amsterdam, titled ‘Research in action: from AIDS to global health to impact. A symposium in recognition of the scientific contributions of Professor Joep Lange’. The scientific symposium traced Joep’s career, starting in the early eighties with the treatment of the first AIDS patients and the design of ART, moving towards the emerging field of global health and ending with his most recent focus: using knowledge derived from scientific research to improve access to quality health care in real-world settings.
As discussed in this thesis, the future of good quality HIV care is only feasible if it forms part of a high quality sustainable healthcare system in sub-Saharan Africa. Access to care for all, long-term retention in care, continuous annual viral load monitoring and adherence to treatment are important topics for durable HIV treatment programmes. Sustainable healthcare delivery systems and healthcare financing are essential to ensure long-term (HIV) care of high quality in sub-Saharan Africa.

**FUTURE PERSPECTIVES**

**HIV diagnosis and treatment initiation**

Immediate ART initiation after HIV diagnosis is recommended in adults and children, due to both the proven clinical and epidemiological benefits [11]. Therefore, the first two 90-90-90 targets entail increased HIV testing (90% of all people living with HIV should know their HIV status) and prompt ART initiation (90% of all people with diagnosed HIV infection should receive sustained ART) to prevent AIDS and onward HIV transmission [4]. Results from randomized clinical trials have shown that early initiation of ART in asymptomatic individuals with high CD4 counts provides a large clinical benefit, effectively reducing morbidity and mortality [17,18]. Besides the clinical benefit, this ‘test & treat’ approach also simplifies the process of ART initiation, as the moment of treatment initiation no longer depends on a clinical assessment or CD4 count testing. Point-of-care tests for HIV diagnosis are a good and cost-effective tool to improve diagnosis and linkage to care in adults [19,20].

The effects of early treatment initiation on morbidity and mortality are particularly important in young children [7]. However, diagnosis of children through ‘early infant diagnosis programs’ remains challenging [20]. This is due to a high rate of loss to follow up as well as reliance on DNA PCR for children under 18 months of age [21,22]. While 41% [38-46%] of all people living with HIV in sub-Saharan Africa had access to ART, only 30% [28-32%] of children received ART in 2014. The proportion of children living with HIV who receive ART more than doubled from 14% [13–15%] in 2010 to 32% [30–34%] in 2014. Community support, and improved access to and knowledge of early infant diagnosis services are needed to increase paediatric HIV diagnosis and uptake of paediatric HIV services [23–25].

**Antiretroviral therapy in sub-Saharan Africa**

The public health approach has been very successful in the administration of standard NNRTI-based first-line and PI-based second-line ART regimen in resource-limited settings [3,26,27]. Access to affordable first-line NNRTI-based ART has led to major
success in the prevention of HIV-related morbidity and mortality, and will continue to be important to reach the target of providing ART to 90% of all people with an HIV diagnosis. This was confirmed by our studies, which showed that the large majority of adults and children are reaching good levels of virological suppression on first- and second-line ART (Chapter 5 and 6) [28,29].

Since the introduction of ART in 2001, safer and more efficacious ARV drugs are becoming available, and new drug classes are becoming more affordable in resource-limited settings. To ensure the future success of the public health approach for ART in sub-Saharan Africa, it is important to know which ARV drugs are cost-effective. Generic NNRTI-based first-line ART is now available in sub-Saharan Africa for US$115 per person per year. Prices of second-line PI-based ART have also fallen to US$330 per person per year but third-line drug options cost at least $1500 per person per year, and are therefore not affordable for most HIV-positive people in sub-Saharan Africa. While prices of generic ARV drugs have declined by 90% over 15 years, further price reductions and sustainable healthcare financing are essential for long-term HIV treatment in sub-Saharan Africa [1]. Continued global advocacy and community activism are needed to reduce the price of ARVs and associated healthcare in resource-limited settings [13,30].

Since the end of 2015, the integrase inhibitor dolutegravir is recommended by the WHO as an alternative first-line drug for adults [11]. This follows ART guidelines from resource-rich settings, where integrase inhibitors are increasingly being recommended and implemented because of the favourable toxicity profile, once-daily dosing and high barrier for drug resistance [31–34]. While NNRTI-based ART is much cheaper and effective for most people in sub-Saharan Africa, the introduction of integrase inhibitors could become appropriate in regions with high levels of pretreatment drug resistance. It is not yet concluded however, what level of pretreatment drug resistance should constitute a change in recommended first-line regimen within national guidelines. Mathematical modelling and economic analysis showed that, with increasing levels of transmitted drug resistance, recommending viral load testing 6 months after ART initiation will be preferred over changing to a PI-based first-line regimen [35]. As shown in Chapter 7, timely detection of virological failure on NNRTI-based first-line regimen, followed by prompt switching to second-line, could precede HIV-related morbidity and mortality [29]. This is reflected in current HIV treatment guidelines which recommend access to ART as a priority, followed by access to viral load monitoring [10,11].

While the absolute number of children becoming newly infected with HIV is decreasing because of PMTCT success, infants and children who do get infected have a
high risk of pretreatment drug resistance (Chapter 3 and 4) [9,26]. This supports the recommended use of protease-inhibitors (PIs) in first-line regimen for children under 3 years of age. Where feasible, it is recommended to extend the initiation of PI-based first-line ART to children $\geqslant 3$ years of age [36,37]. The limited availability of paediatric formulations of PIs is of concern. Considering high rates of virological failure in children, next-line regimens (i.e. integrase inhibitors) are urgently needed for children [38].

Laboratory monitoring
To monitor the response to ART, viral load testing is the preferred approach to detect and confirm treatment failure [10,11]. Sustainable virological suppression on ART is also the third target of the 90-90-90 targets (90% of all people receiving ART should have viral suppression). Viral load monitoring can also be used as an adherence enhancement tool, leading to viral (re)suppression after targeted adherence counselling [39,40]. Early detection of treatment failure, followed by switching to second-line ART if needed, can enable re-suppression and prevent the accumulation of drug resistance mutations [41].

At current prices of viral load assays and second-line ARV drugs, cost-effectiveness analyses shows that viral load monitoring should be considered only after high ART coverage has been achieved [42]. Viral load monitoring strategies could be improved however and costs should be reduced, for example by means of centralized viral load testing using dried blood spots, which has been found to be a cost-effective strategy to improve treatment monitoring [43,44]. The logistics used for centralized viral load testing could pave the way for drug resistance testing which will become cheaper and therefore more accessible in the future.

The implementation of viral load monitoring requires more than the availability of viral load technology; uptake of viral load test results [45,46]. When viral load testing is available, the turnaround times of viral load results from the lab to the clinician need to be optimized [47]. Logistical challenges need to be overcome to effectively integrate viral load testing within HIV care. Subsequently, uptake of viral load results in clinical decision making can improved, as switching to second-line regimens is currently limited. Availability of affordable second-line and eventually third-line ARV drugs could reduce the reluctance to switch to different regimen.

The scale up of ART is not uniform within each country. The prevalence of transmitted drug resistance varies across geographical regions, and is associated with the year of ART roll-out [6]. National and regional surveys of HIV drug resistance are required
in both populations starting ART (pretreatment drug resistance) and receiving ART (acquired drug resistance) to inform national guidelines on preferred ART regimen [48–51]. The implementation of prospective surveys has been challenging, especially in areas of concentrated or low prevalence HIV epidemics, and where service delivery is decentralized. Since 2012, the World Health Organization changed strategies moving from prospective surveys to cross-sectional surveys among adults and children initiating ART and among those receiving ART [52]. As international efforts are concentrating more on key populations and locations [2], national representative surveys remain important to benchmark the success of focussed interventions. Personalized genotyping at first- or second-line treatment failure is challenging and underlines the importance of monitoring and surveillance. Only few African laboratories are accredited for genotypic resistance testing and costs are high. After virological failure on second-line PI-based ART, individual genotype resistance testing is recommended to distinguish drug resistance from poor adherence. Like with viral load testing, the rollout of genotype resistance testing requires implementation strategies to yield optimal results in clinical practice.

Challenges for laboratory strengthening are numerous: specimen transport, equipment breakdown, shortage in trained personnel, and weak laboratory information management systems and laboratory infrastructure [53,54]. Improvements to laboratory capacity are vital for HIV treatment and the benefits incurred will extend beyond this specific field. The rising levels of antimicrobial resistance have reached the international security agenda, because of its threat to global health as well as the associated costs [55]. In the long term, national surveillance of pretreatment and acquired HIV drug resistance should be part of systematic global surveillance systems, to track the spread of both infectious diseases and antimicrobial resistance.

**Long-term HIV care**

There is currently no HIV vaccine available and there is no cure [56]. ARVs are a powerful weapon, but also the only weapon against HIV. Therefore, ARVs should be used wisely, to keep the development of drug resistance to a minimum and sensibly target drug resistant HIV with new drugs. The demand for second- and third-line ART will increase as access to viral load monitoring improves and first-line ART continues to be scaled up [57].

ARV drugs are also very effectively used in the prevention of HIV transmission. Successful viral suppression reduces the risk of HIV transmission between serodiscordant couples, and prevention of mother-to-child transmission (PMTCT) during pregnancy, labour and breastfeeding. In order to eliminate HIV/AIDS, we must protect children
from becoming infected with HIV through optimal use of PMTCT interventions. With increased ART use during pregnancy (PMTCT option B+), the number of HIV-infections among infants is decreasing [26]. Still, 2.3 [2.2-2.5] million children were living with HIV in sub-Saharan Africa in 2015 [26]. Even with continued progress in prevention of mother-to-child transmission, the WHO and UNICEF project that 1.9 million children will require HIV treatment in 2020 [4]. Additionally, ARV drugs can protect HIV-negative people with a high risk of HIV acquisition through pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) [58–60]. Strong adherence to PrEP and periodic HIV testing are required however, to maximize its protective effect and prevent emergence of drug resistance [61]. Future research should evaluate the impact of PrEP on new HIV-infections and drug resistance in a real-life, programmatic setting [62].

Historically, healthcare systems in sub-Saharan Africa have not been designed to provide chronic care. Currently, international HIV treatment targets need to shift from access to ART to long-term, high-quality HIV care. Continued international funding and political commitment remain essential in the HIV response [63]. To optimize HIV diagnosis, direct ART initiation and durable viral suppression, healthcare services need improved referral systems, good patient retention in care, and an efficient clinic-laboratory interface. To combat rising levels of HIV drug resistance, efforts are needed to provide quality HIV care for those failing standard treatment regimen. Prevention of drug resistance development in a proportion of the population will limit overall levels of HIV drug resistance. Laboratory capacity and the availability of affordable second- and third-line drugs for adults and children are needed to achieve long-term virological suppression in sub-Saharan Africa.
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Addendum
Nederlandse samenvatting
(Summary in Dutch)
LANGETERMIJNEFFECTEN VAN HIV-BEHANDELINGEN IN AFRIKA TEN ZUIDEN VAN DE SAHARA: VAN TOEGANG NAAR KWALITEIT.


HIV-behandeling

HIV vermenigvuldigt zich in het menselijk lichaam in een hoog tempo: ieder virus-deeltje produceert circa 100.000.000.000 nieuwe virussen per dag. Verschillende virale enzymen zorgen voor de vermenigvuldiging van HIV, namelijk: reverse-transcriptase, voor de omzetting van viraal RNA naar DNA; integrase voor de innesteling van het virale DNA in het DNA van de gastheer (de mens); en protease, die er voor zorgt dat de virusdeeltjes de cel weer kunnen verlaten. De replicatie van HIV is echter erg foutgevoelig. In iedere levenscyclus ontstaat er ten minste één willekeurige (random) mutatie in het virus. Dit zorgt er voor dat er talloze verschillende versies van het virus ontstaan, wat het ontwikkelen van medicatie of een vaccin tegen HIV ernstig bemoeilijkt. In 1987 kwam de eerste HIV-remmer op de markt: azidothymidine (AZT), een nucleosideanaloog reverse-transcriptaseremmer (nucleoside reverse transcriptase inhibitor, NRTI). HIV-patiënten die het middel gebruikten zagen hun levensverwachting indrukwekkend verbeteren. Helaas was het effect van korte duur: HIV ontwikkelde resistentie tegen AZT. Om diezelfde reden verloren ook nieuwe HIV-remmers, die op de markt kwamen als mono- of duotherapie, hun effectiviteit.

In 1996 bracht de introductie van combinatietherapie wel langdurige verbetering van de levensverwachting. Nieuwe klassen van HIV-remmers, non-nucleoside analoog reverse-transcriptaseremmers (non-nucleoside reverse transcriptase inhibitors, NNRTIs) en proteaseremmers (protease inhibitors, PIs), werden ontwikkeld, die ieder ingrijpen in een andere fase van de vermenigvuldigingscyclus van het virus. Combina-
tietherapie bestaat uit een mix van minimaal drie HIV-remmers uit twee verschillende klassen. De combinatietherapie is zo in staat om de replicatie van HIV langdurig te onderdrukken, met minimale ontwikkeling van resistentie. We noemen dit fenomeen virologische suppressie. Na de ontwikkeling van NRTIs, NNRTIs en PIs, zijn er nieuwe klassen van HIV-remmers bijgekomen, namelijk: integraseremmers, fusieremmers en maturatieremmers.

Kinderen met HIV


HIV-behandeling in Afrika – de volksgezondheidsbenadering

Het jaar 1996 was voor HIV-patiënten in de westerse landen een omslagpunt, echter voor Afrika gold dat geen enkel vooradvies. Combinatietherapie was daar niet beschikbaar en ook niet betaalbaar. Eind 2001 waren er 40 miljoen mensen HIV-positief, waarvan er 26 miljoen in Afrika woonden. Drie miljoen mensen stierven aan AIDS datzelfde jaar. De impact van de epidemie zorgden voor een achteruitgang in de maatschappelijke ontwikkeling in Afrika, omdat het aantal nieuwe HIV-infecties toenam en de levensverwachting scherf daalde.

HIV-behandelrichtlijnen waren in die tijd gebaseerd op een westerse setting. Artsen konden alle beschikbare HIV-remmers voorschrijven en de behandeling vervolgens nauwkeurig monitoren met geavanceerde laboratoriumtechnieken. Deze individuele, op maat gesneden behandelplannen waren in Afrika echter niet haalbaar. De capaciteit van artsen en laboratoria was – en is – daarvoor ontoereikend. Daarom besloot de Wereldgezondheidsorganisatie (World Health Organization, WHO) in 2002 tot een nieuwe aanpak in Afrika. Deze volksgezondheidsbenadering (public health approach) voorzag in het verstrekken van een gestandaardiseerde, generieke
combinatietherapie, met beperkte laboratoriummonitoring en het verschuiven van zorgverlening naar lager geschoolde gezondheidswerkers, met name van artsen naar verpleegkundigen.

De standaard HIV-behandeling in ontwikkelingslanden bestaat sindsdien uit een eerstelijnscombinatietherapie van twee NRTIs in combinatie met een NNRTI. Mocht de therapie falen, dan kan worden overgegaan tot een zogenaamde switch. De patiënt krijgt nu een tweedelijnscombinatietherapie toegediend. Deze bestaat weer uit twee NRTIs, nu met een PI, die de aanmaak van nieuwe virussen remt.

De volksgezondheidsbenadering heeft het mogelijk gemaakt om de HIV-zorg snel en efficiënt op te schalen en miljoenen mensen van combinatietherapie te voorzien. Echter, vanwege de beperkte laboratoriummonitoring kan het zijn dat therapiefalen lang onopgemerkt blijft, waardoor er toch resistentieontwikkeling plaats vindt. Een tweede switch, van tweede- naar derdelijnscombinatietherapie, is op dit moment in de Afrikaanse publieke gezondheidszorg niet beschikbaar en onbetaalbaar.

Monitoren van de behandeling
Het ziektebeloop van HIV en de respons op combinatietherapie kan worden gemeten door middel van klinische, immunologische en virologische monitoring. Klinisch monitoren is de evaluatie van klinische symptomen. Eenvoudig gezegd het onderzoeken van een patiënt naar uiterlijk herkenbare symptomen, bijvoorbeeld koorts, diarree, extreme vermoeidheid en gewichtsverlies zonder duidelijke reden. Immunologisch monitoren is de evaluatie van het immuunsysteem aan de hand van het aantal CD4+ T-cellen in het bloedplasma. CD4+ T-cellen zijn witte bloedcellen die een essentiële rol spelen in ons immuunsysteem. HIV infecteert en vermenigvuldigd zich in de CD4+ T-cellen en vernietigt de cel. Wanneer het aantal CD4+ T-cellen per mm³ bloedplasma onder een bepaald niveau daalt, wijst dit op een niet effectieve HIV-behandeling. Bij virologisch monitoren wordt gekeken naar de virale lading; de hoeveelheid virusdeeltjes in het bloedplasma. Het primaire doel van combinatietherapie is het bewerkstelligen van een zo laag mogelijke virale lading. Over het algemeen is een hoge virale lading (virologisch falen) het eerste teken van therapiefalen. Bij een hoge virale lading wordt het immuunsysteem aangevallen en daalt vervolgens het aantal CD4+ T-cellen (immunologisch falen). Door het aangetaste immuunsysteem kunnen eenvoudige infecties niet meer worden overwonnen en zullen er klinische symptomen optreden (klinisch falen). Er is sprake van AIDS bij een ernstige vorm van klinisch falen.

De volksgezondheidsbenadering gaat uit van het klinisch en – indien mogelijk – immunologisch monitoren van de HIV-behandeling. De middelen om virologisch te
monitoren zijn in Afrika slechts zelden voorhanden. Omdat virologisch falen op die manier vaak pas laat kan worden ontdekt, kunnen complexe resistentie mutaties accumuleren; HIV zal steeds resisteren worden tegen de HIV-remmers.

**Resistentieontwikkeling**

Bij resiste HIV kan het virus zich, ondanks het gebruik van HIV-remmers, blijven vermenigvuldigen. We onderscheiden verworven en overgedragen resistentie. Verworven HIV-resistentie kan ontstaan wanneer iemand wel HIV-remmers gebruikt, maar niet in toereikende mate, bijvoorbeeld vanwege slechte therapietrouw. Door een proces van selectie en mutatie ontwikkelt HIV vervolgens een resistente vorm. Overgedragen HIV-resistentie treedt op wanneer deze resistente virussen worden overgedragen naar een HIV-negatief persoon. We spreken van pre-therapie HIV-resistentie bij mensen die al een resistente vorm van HIV bij zich dragen als ze met combinatietherapie starten.

**IN DIT PROEFSCHRIFT**

In Afrika wordt resistente HIV in toenemende mate waargenomen en vormt het een bedreiging voor het succes van HIV-behandelprogramma’s. In dit proefschrift onderzoeken we welke factoren kunnen bijdragen aan de volgende fase van HIV-behandeling in Afrika: van toegang tot combinatietherapie naar kwalitatieve HIV-zorg voor volwassenen en kinderen.


**Deel I – Starten met HIV-behandeling**

Na de algemene introductie (*Hoofdstuk 1*), behandelden we in deel I de uitdagingen bij het starten met eerstelijns combinatietherapie bij HIV-geïnfecteerde kinderen. In *Hoofdstuk 2* onderzochten we de barrières die HIV-geïnfecteerde kinderen ervaren
om tijdig te starten met combinatietherapie, in drie klinieken in Oeganda. Voor het verdere ziektebeloop en hun overlevingskansen is een snelle start essentieel. We vonden dat 72% van de kinderen pas op latere leeftijd en met vergevorderde ziekteverschijnselen startten. We zagen dat deze kinderen ook vaker wees waren en minder vaak HIV-remmers hebben ontvangen voor de preventie van moeder-kind transmissie van HIV. Ook hadden zij hogere reiskosten en vervoersproblemen naar de kliniek. Dit werd bevestigd in interviews met ouders/verzorgers en gezondheidswerkers. Gevraagd naar mogelijke verbeteringen werden genoemd: een meer consistente doorverwijzing vanuit de perinatale zorg, een betere voorlichting aan ouders en verzorgers ten einde HIV-symptomen snel te herkennen en psychosociale steun om angst en stigmatisering te voorkomen.

Vervolgens onderzochten we de pre-therapie resistentiepatronen bij HIV-geïnfecteerde kinderen in Oeganda en Nigeria. We vonden resistente HIV bij 10% van de kinderen die startten met eerstelijnscombinatietherapie in Oeganda (Hoofdstuk 3). Eerder gebruik van HIV-remmers voor de preventie van moeder-kind transmissie bleek een belangrijke voorspeller voor resistentie. Ook bij kinderen van wie men niet wist of ze eerder HIV-remmers hadden gebruikt, bleek het risico verhoogd.

In Nigeria vonden we resistente HIV bij 16% van de kinderen die, zonder eerder gebruik van HIV-remmers, startten met combinatietherapie (Hoofdstuk 4). Vervolgens hebben we door middel van literatuurstudie en meta-analyse de pre-therapie HIV-resistentie bij kinderen in heel Afrika in kaart gebracht (Hoofdstuk 4). Op basis van zestien studies in elf Afrikaanse landen vonden we dit soort HIV-resistentie bij 28% van de kinderen. Bij kinderen die eerder HIV-remmers hadden gekregen, vonden we vier keer zo veel resistentie: 10% versus 40%. Ten slotte zagen we dat het aantal kinderen met pre-therapie HIV resistentie het afgelopen decennium is toegenomen, ook als niet eerder medicatie was ontvangen.

**Deel II – Langtermijnuitkomsten van eerstelijnscombinatietherapie**

In deel II beschrijven we de langtermijnuitkomsten van de eerstelijnscombinatietherapie in ontwikkeldelanden. Op basis van literatuurstudie en meta-analyse onderzochten we virologische suppressie na een half tot vijf jaar therapie bij volwassenen (Hoofdstuk 5) en kinderen (Hoofdstuk 6). We baseerden onze meta-analyses op 72 studies bij kinderen en 165 studies bij volwassenen. We vonden dat eerstelijnscombinatietherapie over het algemeen goede virologische suppressie geeft, maar minder bij kinderen (70-80%) dan bij volwassenen (>85%).
In **Hoofdstuk 7** stellen we vast dat pre-therapie HIV resistentie bij volwassenen de effectiviteit van standaard eerstelijns combinatietherapie verminderde. Zij hadden, in vergelijking met volwassenen zonder deze vorm van resistentie, vier keer zo veel kans om binnen twee tot drie jaar te moeten switchen naar tweedelijns combinatietherapie. We zagen geen verschillen in de aantallen nieuwe AIDS-gevallen of overlijdens. Tevens zagen we dat een kwart van de patiënten onnodig switchte naar tweedelijnscombinatietherapie, omdat er sprake was van virale suppressie dan wel virologisch falen zonder resistentie.

**Deel III – Latste medicatie opties**

In deel III beschrijven we het optreden en de patronen van verworven HIV-resistentie na het falen van standaard eerste- en tweedelijnscombinatietherapie. In **Hoofdstuk 8** onderzochten we het virologisch falen bij kinderen en volwassenen bij standaard eerstelijnscombinatietherapie. We zagen dat kinderen tot drie keer zo vaak virologisch falen vertonen als volwassenen: 24% en 30% versus 9% na respectievelijk één en twee jaar. We zagen geen verschillen tussen de beide groepen in de patronen en de snelheid van resistentieontwikkeling. Hoewel de verworven resistentie al bij 87% van de volwassenen en kinderen te meten was na de eerste keer virologisch falen, zagen we dat resistentie na langdurig virologisch falen significant toenam bij het gebruik van NNRTIs en NRTIs.

In **Hoofdstuk 9** constateren we goede langdurige virologische suppressie (≥85%) bij volwassenen op standaard tweedelijnscombinatietherapie. Risicofactoren voor virologisch falen waren een niet-standaard voorgeschreven samenstelling van de eerstelijnscombinatietherapie, PI-resistentie bij switch naar tweedelijnstherapie en slechte therapietrouw. Ook al vonden we goede virologische suppressie, 22% van de patiënten die virologisch faalden op tweedelijnscombinatietherapie had verworven PI-resistentie. Dit betekent dat één op de vijf patiënten na virologisch falen op tweedelijnscombinatietherapie derdelijns HIV-remmers nodig heeft, die echter op dit moment in de publieke sector in Afrika beschikbaar noch betaalbaar zijn.

**Epiloog - van AIDS naar wereldwijde impact op de volksgezondheid**

In het epiloog (**Hoofdstuk 10**) brengen we een eerbetoon uit naar de overleden hoogleraar Joep Lange en Jacqueline van Tongeren. We brengen verslag uit over het symposium ‘Research in action: from AIDS to global health to impact. A symposium in recognition of the scientific contributions of Professor Joep Lange’ gehouden op 14 oktober 2014 in het Academisch Medisch Centrum van de Universiteit van Amsterdam.
**Toekomstperspectief**

De beschikbaarheid van standaard eerstelijns- en tweedelijnscombinatietherapie heeft het afgelopen decennium miljoenen levens gered. Voor voortgaand succes bij de bestrijding van HIV in Afrika is extra aandacht nodig voor kinderen met HIV. Het gaat hier om een zeer kwetsbare groep met hoge kans op pre-therapie HIV-resistentie en virologisch falen. Surveillance van pre-therapie- en verworven HIV-resistentie zijn van essentieel belang om een optimale standaardcombinatietherapie te bepalen en, indien nodig, aan te passen. Virologisch monitoren en toegang tot betaalbare tweede- en derdelijnscombinatietherapie zijn in Afrika cruciaal voor langdurige virologische suppressie bij volwassenen en kinderen met HIV.
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Pascale Ondoa, thank you for the great scientific support and good company. I enjoyed how we could laugh about the next hurdle we ran into; for example when we
Addendum

were shipping samples or −80°C freezers across the Nigerian border. Thank you for making me feel like a grown-up scientist!

Corry Manting, Else van Schijndel, Julien Schrijver en Marloes Nijboer, dankzij jullie lopen de projecten en rapportages als een zonnetje. Ondanks dat alles altijd in verandering is, weten jullie altijd de projecten te maximaliseren. Corry, bedankt voor je vele secure werk, intensieve samenwerking en toewijding voor de studies; soms leek ik bijna deel uit te maken van je gezin!

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I am grateful for the creative energies. Moses Supercharger, a.k.a. PASER-MARCH ambassador, thank you so much for your music, community support, education and advocacy. Tenford Chitanana, MaFreezits Dance Group, Tinashe and Alban, thank you for the great video and dance adventures in Harare. Jjuuko Hoods, thank you for capturing contemporary Kampala on the cover of this thesis.
Special thanks to the study doctors, nurses, counsellors, phlebotomists, laboratory workers, study coordinators and data managers who have took care of all people included in the studies and beyond. Thank you for your major efforts and dedication, which started years before I joined the team, and thank you so much for the hospitality during my visits. The PASER and MARCH studies would not have taken place without the sites and contributors from Nigeria, Uganda, Zambia, Zimbabwe, Kenya and South Africa:

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Professor Akanmu, special thanks for your active involvement in both the practical as well as the academic aspects of the PASER and MARCH studies, and thank you for taking part of the doctorate committee.

The Institute of Human Virology; Abuja, Alash’lee Abimiku, and Nicaise Ndemi.

**Uganda**


Cissy, thank you for the opportunity to conduct field work during my MSc internship, which became the starting point of our collaboration and this PhD thesis. Your dedication to the HIV epidemic since the early nineties is impressive - all the best for the finalization of your PhD!

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Addendum

Zimbabwe
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Kenya
Coast Province General Hospital, International Centre for Reproductive Health Kenya, Mombasa; Saade Abdallah, Lily Baya, Irene Jao, Hope Kachila, Stanley Luchters, Kishor Mandaliya, Anne Mwangemi, Millicent Olulo Orera, and Francis Otieno.
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Coptic Hospital, Lusaka, Rose Chipala, Paul Chirwa, Youssef Edward, Evans Kabechani, Moheb Labib, Eman Labib Maksimos, Humiliana Mulenga and Sahar Nagib.

South Africa
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Department of Molecular Medicine and Haematology, University of the Witwatersrand, Johannesburg; Michelle Bronze, Esrom Letsoalo, Kim Steegen, Wendy Stevens, and Carole Wallis.
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Addendum

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biostatistiek op U4. Jullie hebben de basis gelegd voor mijn interesse in en kennis 
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Paranimf en grote zus Bella, je bent altijd een geweldige rots in de branding en aller- 
liefste grote zus.

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## PHD PORTFOLIO

<table>
<thead>
<tr>
<th>Name of PhD student</th>
<th>T.S. Boender</th>
</tr>
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<tbody>
<tr>
<td>PhD period</td>
<td>February 2013 – January 2016</td>
</tr>
<tr>
<td>Name PhD supervisors</td>
<td>Prof. dr.T.F. Rinke de Wit &amp; Prof. dr. M. Boele van Hensbroek</td>
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### PhD Training

<table>
<thead>
<tr>
<th>General courses</th>
<th>Year</th>
<th>Workload Hours/ECTS</th>
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<tbody>
<tr>
<td>Academic Medical Center Graduate School, Amsterdam, The Netherlands.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Systematic Reviews (Cochrane Collaboration)</td>
<td>2013</td>
<td>20 / 0.7</td>
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<tr>
<td>- BROK course and exam for Good Clinical Practice (passed with 88%)</td>
<td>2013</td>
<td>21 / 0.9</td>
</tr>
<tr>
<td>- Scientific Writing in English for Publication</td>
<td>2015</td>
<td>42 / 1.5</td>
</tr>
<tr>
<td>- Embase/Medline via OVID</td>
<td>2015</td>
<td>2.5 / 0.1</td>
</tr>
<tr>
<td>- Citation Analysis and Impact Factors</td>
<td>2015</td>
<td>2.5 / 0.1</td>
</tr>
<tr>
<td><strong>Specific courses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic Medical Center Graduate School, Amsterdam, The Netherlands.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DNA technology</td>
<td>2013</td>
<td>60 / 2.1</td>
</tr>
<tr>
<td>- Infectious Diseases</td>
<td>2014</td>
<td>30 / 1.0</td>
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<tr>
<td>- Computing in R</td>
<td>2014</td>
<td>12 / 0.4</td>
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<td>- Crash Course</td>
<td>2015</td>
<td>6 / 0.2</td>
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<tr>
<td>- Advanced Topics in Biostatistics</td>
<td>2015</td>
<td>60 / 2.1</td>
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<tr>
<td>Erasmus University summer programme, Rotterdam, The Netherlands.</td>
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<tr>
<td>- Survival analysis [ESP28], by Patrick Heagerty.</td>
<td>2014</td>
<td>40 / 1.4</td>
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<tr>
<td>- Joint Models for Longitudinal and Survival Data [ESP72], by Dimitris Rizopoulos.</td>
<td>2015</td>
<td>15 / 0.5</td>
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<tr>
<td>- Causal Inference [ESP48], by Miguel Hernán.</td>
<td>2015</td>
<td>15 / 0.5</td>
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<tr>
<td><strong>Seminars, workshops and master classes</strong></td>
<td></td>
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<tr>
<td>- Southern African HIV &amp; TB Drug Resistance and Treatment Monitoring Workshop, University of the Free State, November 2013, Bloemfontein, South Africa.</td>
<td>2013</td>
<td>24 / 0.9</td>
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<tr>
<td>- INTEREST workshop, Lusaka, Zambia.</td>
<td>2014</td>
<td>40 / 1.4</td>
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<tr>
<td>- International Symposium: Research in Action – From AIDS to Global Health to Impact, Academic Medical Center Amsterdam, The Netherlands.</td>
<td>2014</td>
<td>8 / 0.3</td>
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<tr>
<td>- HIV Masterclass, Global Child Health Group, Academic Medical Center Amsterdam, The Netherlands.</td>
<td>2013</td>
<td>8 / 0.3</td>
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<tr>
<td>- APROVE workshops, AMC Amsterdam, The Netherlands.</td>
<td>2014-2015</td>
<td>8 / 0.3</td>
</tr>
<tr>
<td>- Weekly Amsterdam Institute for Global Health and Development research meeting, Amsterdam, The Netherlands.</td>
<td>2013-2016</td>
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### Presentations

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<tr>
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<td>2014</td>
<td>96 / 3.4</td>
</tr>
<tr>
<td>2014</td>
<td>32 / 1.1</td>
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<tr>
<td>2014-2015</td>
<td>16 / 0.6</td>
</tr>
<tr>
<td>2014-2016</td>
<td>32 / 1.1</td>
</tr>
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#### Scientific conferences

*(for details, see list of publications)*

- Poster presentation at the Conference on Retroviruses and Opportunistic Infections February 2014, Boston, MA, USA.
- Oral presentation at the 2014 African Society for Laboratory Medicine Conference, Cape Town, South Africa.
- Oral and poster presentations at Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment, Amsterdam, The Netherlands.
- Oral and poster presentations at the International HIV Drug Resistance Workshop, Seattle, WA, and Boston, MA, USA.

#### Other

- Oral presentation at the National Uganda Virus Research Institute Stakeholders Workshop, Kampala, Uganda.

### Parameters of esteem

<table>
<thead>
<tr>
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<td>2015 &amp; 2016</td>
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- Young Investigator Award, Conference on Retroviruses and Opportunistic Infections (US$ 2000).

### Teaching

#### Lecturing

- Guest lecture on HIV drug resistance in adults and children in sub-Saharan Africa, Bachelors Health Sciences, VU University Amsterdam.
- Guest lecture on beta career choice and HIV drug resistance, 5VWO class Jac. P. Thijssen College Castricum.

#### Supervising

- Bernice Hoenderboom, MSc student Health Sciences - Infectious Diseases.
- Marieke de Pundert, MSc student Health Sciences - International Public Health.
- Rimke Bijker, MSc student Global Health.
- Ben van Nieuwenhuizen, MSc student Global Health.
Addendum

LIST OF PUBLICATIONS

Journal articles


Conference proceedings

Boender TS, Boerma RS, Sigaloff KCE, Rinke de Wit TF, Boele van Hensbroek M, Ndemb N, Adeyemo T, Temiy EO, Osibogun A, Ondoa P, Calis JC, Akanmu AS. High levels of HIV drug resistance in treatment-naïve children in Lagos, Nigeria: original data and a systematic review in sub-Saharan Africa. Netherlands Conference on HIV


CURRICULUM VITAE

Sonia Boender was born in Breda, the Netherlands, in 1988. After completing her secondary school (VWO) at the Onze Lieve Vrouwe Lyceum in 2006, she moved to Amsterdam to study Health Sciences at the VU University Amsterdam. During her bachelor’s, her interest in infectious diseases was triggered during her internship at the parasitology department of the Leids Universitair Medisch Centrum, analysing immune responses of Gabonese school children, in relation with soil-transmitted helminths and tuberculosis.

In 2011, she received her master’s in Health Science with a specialization in Public Health and Infectious Diseases at the VU University Amsterdam. Sonia wrote her master thesis during her research internship at PharmAccess Foundation, which became the starting point of her PhD research.

To further specialize in the field of global health, Sonia continued her postgraduate education at the Liverpool School of Tropical Medicine, UK, where she obtained a postgraduate-certificate (with distinction) in International Public Health in 2012. Subsequently, Sonia worked as a junior lecturer at the VU University at the Department of Health Science, teaching and coordinating courses on infectious diseases, epidemiology and applied biostatistics.

Sonia started her PhD research in February 2013 at the Amsterdam Institute for Global Health and Development and the Global Child Health Group of the Academic Medical Centre/Emma Kinderziekenhuis of the University of Amsterdam. Since February 2016, Sonia is working as a researcher at the analysis department of the Dutch HIV Monitoring Foundation.