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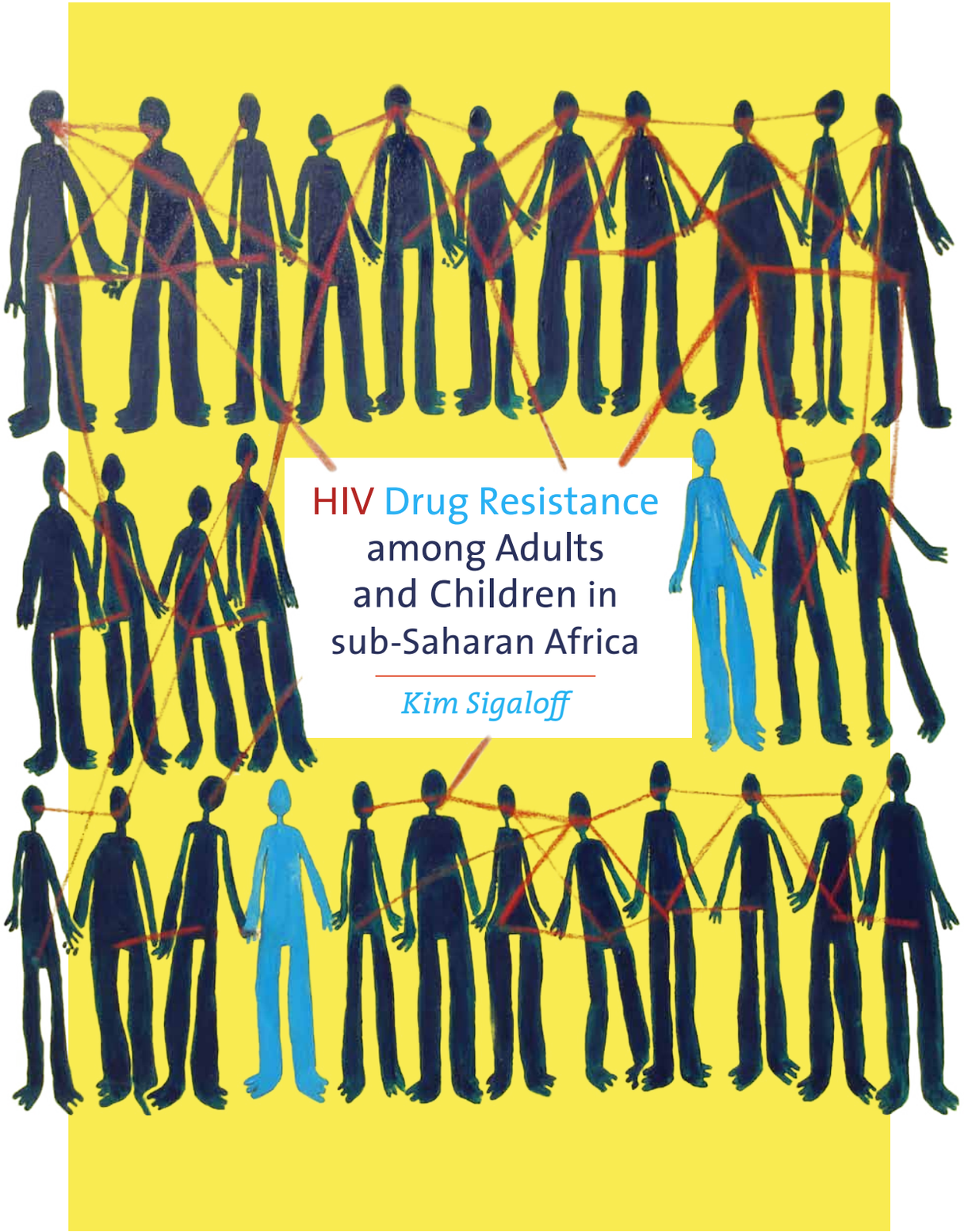
HIV Drug Resistance among Adults and Children in sub-Saharan Africa

One of the biggest medical accomplishments of the beginning of this century has been the expanded access to life-saving antiretroviral treatment for HIV-infected people living in sub-Saharan Africa. A potential downside of the large-scale exposure to antiretroviral drugs is the emergence of HIV drug resistance. This risk is increased by the weak health systems in many African countries, characterized by poor infrastructure, intermittent drug supply, and shortage of skilled staff. In order to evaluate the extent of HIV drug resistance in Africa, the PharmAccess African Studies to Evaluate Resistance (PASER) network was established in 2006. In six countries - Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe – over fifteen clinics participated in observational studies among HIV-infected adults. In Uganda, a pediatric study was initiated to investigate the development of HIV drug resistance among children. The studies included in this thesis were conducted as part of the PASER adult and pediatric programs.

HIV Drug Resistance among Adults and Children in sub-Saharan Africa

Kim Sigaloff



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Kim Sigaloff

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HIV drug resistance among adults and children in sub-Saharan Africa
Thesis, University of Amsterdam, The Netherlands

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HIV drug resistance among adults and children in sub-Saharan Africa

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Voor Diederik en Lisa

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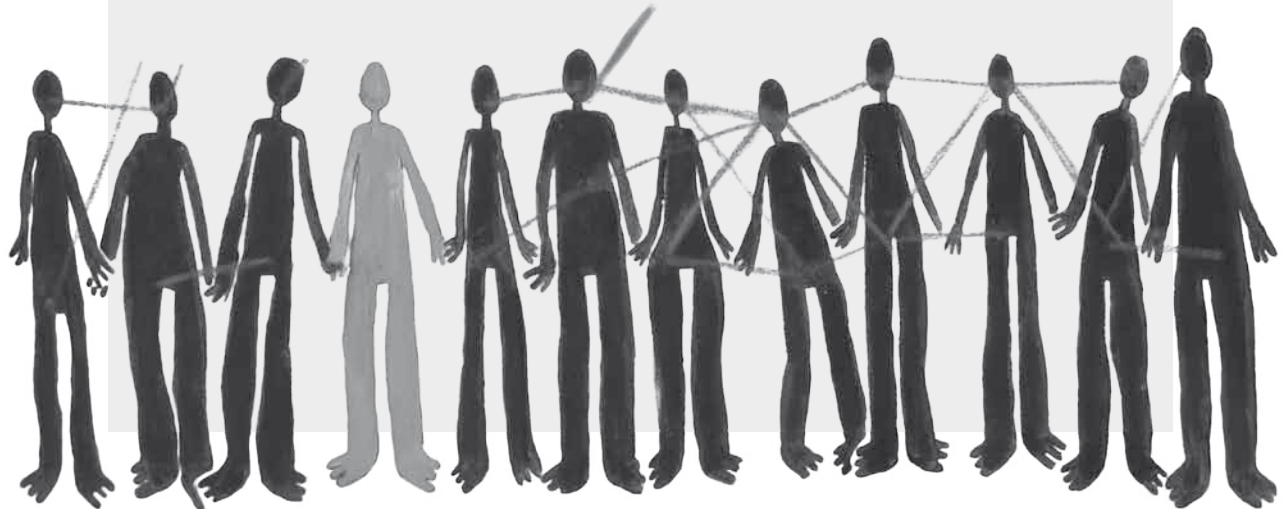
ABBREVIATIONS

3TC	lamivudine
ABC	abacavir
AIDS	acquired immunodeficiency syndrome
AIGHD	Amsterdam Institute for Global Health and Development
ART	combination antiretroviral therapy
ART-A	Affordable Resistance Test for Africa
ARV	antiretroviral (drug)
ATV	atazanavir
AZT	zidovudine
bPI	ritonavir-boosted protease inhibitor
CRF	circulating recombinant form; case report form
d4T	stavudine
DNA	deoxyribonucleic acid
DRM	drug resistance mutation
EFV	efavirenz
ETR	etravirine
EWI	early warning indicator
FTC	emtricitabine
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HIVDR	HIV drug resistance
HIVResNet	Global HIV Drug Resistance Network
HIV-RNA	HIV viral load
IAS-USA	International Antiviral Society–USA
LAASER	Linking African and Asian Societies for an Enhanced Response to HIV/AIDS
LPV	lopinavir
MARCH	Monitoring Antiretroviral Resistance in Children
MPR	medication possession ratio
NNRTI	non-nucleoside reverse transcription inhibitor
NRTI	nucleoside reverse transcription inhibitor
NVP	nevirapine
PASER	PharmAccess African Studies to Evaluate Resistance
PASER-M	PASER-Monitoring
PASER-S	PASER-Surveillance
PCR	polymerase chain reaction
PI	protease inhibitor
PMTCT	prevention of mother-to-child transmission of HIV
PR	protease
RNA	ribonucleic acid
RT	reverse transcriptase
SDRM	surveillance drug resistance mutation
sd-NVP	single-dose nevirapine
TAM	thymidine analogue mutation
TAQAS	TREAT Asia Quality Assessment Scheme
TASER	TREAT Asia Studies to Evaluate Resistance
TDF	tenofovir
TDR	transmitted drug resistance
VL	HIV viral load
WHO	World Health Organization

Chapter 1
General introduction



HIV IN SUB-SAHARAN AFRICA

The human immunodeficiency virus (HIV) epidemic remains a major global public health challenge, with a total of 34 million people infected with the virus worldwide.[1] Sub-Saharan Africa bears an inordinate share of the global HIV burden. Although the rate of new HIV infections is decreasing, the total number of people living with HIV in this region continues to rise. In 2010, that number reached 23 million, 68% of the global total.[1] Unlike most areas, the epidemic in Africa disproportionally affects women, and the virus is mainly transmitted during unprotected heterosexual intercourse. This sets the African epidemic apart from other regions where HIV transmission is largely limited to particular risk groups, such as men who have sex with men. With a high prevalence of HIV among women of reproductive age, onward transmission of HIV to newborns and breastfed babies is unfortunately also common in Africa. The estimated total number of children living with HIV is 3.4 million, of which over 90% reside in Africa.[1] Although the overall growth of the epidemic appears to have stabilized, HIV continues to have a profound impact on many aspects of African society. The epidemic has affected millions of households, reversed life expectancy in many countries, overburdened health care systems, and severely hampered productivity and economic development.

VIRAL CHARACTERISTICS

HIV is a retrovirus encasing in its core single-stranded RNA, structural proteins and enzymes. The pol gene of HIV codes for the enzymes reverse transcriptase, integrase and protease. Reverse transcriptase converts the single-stranded RNA of HIV to double-stranded DNA that can be incorporated in the chromosomal DNA of the host. Integrase participates in the insertion of viral genome into the cell genome. Protease breaks down proteins into small pieces for placement into new virions.

HIV has several intrinsic mechanisms that ensure rapid viral evolution. First, the reverse transcriptase of HIV lacks proofreading activity and is therefore error-prone, introducing on average one mutation for each viral genome transcribed.[2] Second, HIV infection is characterized by high viral turnover with a production of approximately 10^{10} virions per day.[3] Third, HIV recombination can lead to further viral diversity and occurs when one person is co-infected with two separate strains of the virus.[4,5] The production of millions of viral variants results in genetically diverse populations of viral species in each infected individual, a phenomenon termed 'quasispecies'.

Phylogenetic analysis of HIV samples has led to the classification of two serotypes: HIV-1, the most common throughout the world, and HIV-2, a less pathogenic variant which is concentrated in West Africa.[6] HIV-1 is further classified into three genetic groups: M (major), O (outlier) and N (non-M, non-O). Over 95% of people throughout the world are infected with HIV-1 belonging to group M, which consists of at least nine subtypes, designated by the letters A–D, F–H, J and K.[7] Advances in full-genome sequencing of HIV have led to the identification of circulating and unique recombinant forms (CRFs and URFs, respectively), which are the result of recombination between subtypes within a dually infected person. The different subtypes and recombinant forms have distinct global distribution patterns, with the highest diversity found in Africa.[8] While subtype B is most predominant in developed countries, the majority of infections worldwide are with the subtype C virus.

NATURAL HISTORY

HIV is transmitted through sexual contact, sharing of injection paraphernalia, transfusion of blood products, or from mother to child during pregnancy, delivery or breastfeeding. HIV targets, infects and replicates in CD4 T lymphocytes and CD4-positive monocytes and macrophages. The subsequent gradual depletion of CD4 T lymphocytes renders the host increasingly susceptible to opportunistic infections and malignancies. Following acute infection, which may be accompanied by a flu-like syndrome, most patients enter a clinically latent phase which may last from several months to more than 15 years. With continued viral replication and progressive decline of CD4 T lymphocytes, the immune system eventually collapses, leading to the acquired immunodeficiency syndrome (AIDS) and death, if untreated.

ANTIRETROVIRAL THERAPY

Since its introduction over 15 years ago, treatment with combination antiretroviral therapy (ART), also referred to as highly active antiretroviral therapy (HAART), has increased the life-expectancy of HIV-infected people. The goal of therapy is to suppress the HIV viral load, allow immunological recovery and prevent the occurrence of opportunistic infections. The classification of HIV medication is based on where in the viral infection or replication cycle the drug acts. Nucleoside reverse transcription inhibitors (NRTIs) were the first drugs approved for the treatment of HIV infection in 1987. In 1996, the non-nucleoside reverse transcription inhibitors (NNRTIs) and protease inhibitors (PIs) were introduced, resulting in major advances when clinicians started treating patients with

potent combinations of three or more drugs. From 2002 onward, an enormous scale-up of access to ART in Africa has been achieved, owing to high-level political commitment and substantial international funding. Today, more than five million HIV-infected Africans are estimated to be receiving ART, impacting nearly 50% of those in need (Figure 1).[1]

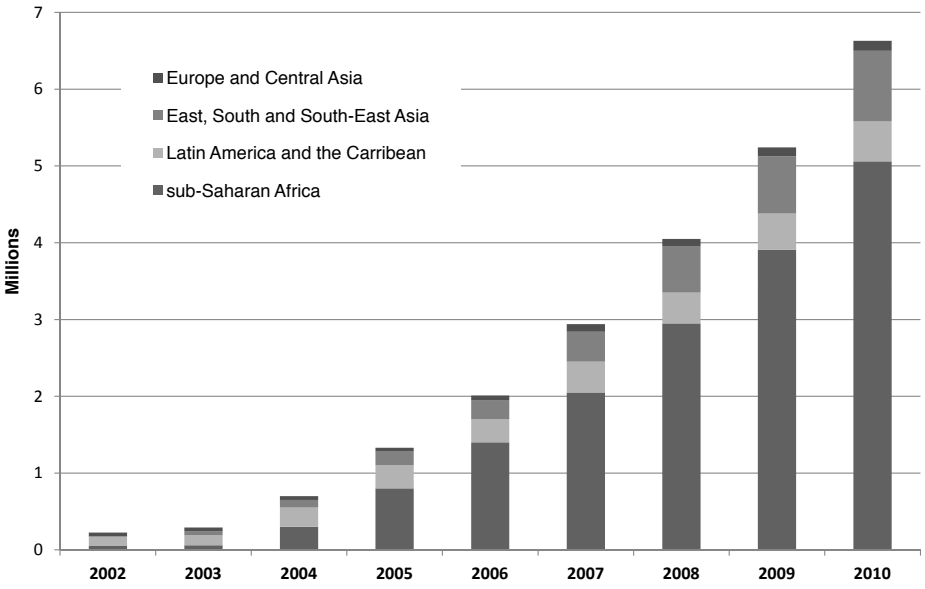


Figure 1. Number of people receiving antiretroviral therapy in low- and middle-income countries, by region, 2002–2010. Adapted from the WHO/UNAIDS/UNICEF progress report 2011.[1]

To support the establishment of treatment programs that reach as many people as possible, the World Health Organization (WHO) has developed a public health approach to ART delivery.[10, 11] This is in contrast to the individualized, specialist care in developed countries, that includes routine plasma viral load monitoring, drug resistance testing and a wide choice of antiretroviral drugs.[12, 13] Low-income countries are encouraged to use simplified treatment guidelines and a decentralized service delivery model. Standard first-line regimens consist of an NNRTI and a dual NRTI backbone, available in some countries as generic fixed-dose combinations. In the absence of routine virological monitoring, the diagnosis of treatment failure is generally based on clinical status and/or CD4 count. In case the decision is made to switch to second-line therapy, recommended regimens combine a ritonavir-boosted PI with two previously unused and/or recycled NRTIs.[11]

As part of the strategy to prevent mother to child transmission of HIV and eliminate pediatric infection, the WHO recommends administrating ART to women who need

treatment for their own health, and a short course of antiretroviral prophylaxis for their infants.[14] Women with higher CD4 counts who do not require treatment for their own health should receive antiretroviral prophylaxis, consisting of mono-, dual- and/or triple-therapy, to reduce transmission during the perinatal period and while breastfeeding.[14] These interventions for the prevention of mother to child transmission (PMTCT) are highly effective if used appropriately.[15, 16] The use of a peripartum single-dose of nevirapine, which is easily administrated to both mother and child but much less effective than longer prophylactic regimens, is no longer recommended. Nevertheless, it is still widely used in resource-limited settings.

Despite the major advances in scaling-up ART, most countries are still far from achieving universal access goals. Obstacles include weak health care infrastructure, a critical shortage of human resources and lack of sustainable, long-term funding. These deficiencies can lead to suboptimal drug prescribing practices, interrupted drug access and high patient attrition rates. Furthermore, treatment monitoring strategies, i.e. viral load measurements, are not routinely integrated in ART delivery programs. All factors mentioned above threaten the long-term success of HIV treatment programs and could create conditions for the accelerated development of HIV resistance to antiretroviral drugs.

HIV DRUG RESISTANCE

In the absence of therapy, the many genetic variants of HIV present in each infected person exist as minor populations only. However, under the selective pressure of antiretroviral drugs, certain variants may have an evolutionary advantage, thus overgrowing the wild-type virus. The speed of this process depends on the level of selective advantage conferred by the mutation, the prevalence of the mutant and the potency of the antiretroviral regimen.[17] In patients receiving antiretroviral drugs, the emergence of viral resistance is possible only if HIV continues to replicate in the presence of drug levels that are insufficient to completely block viral replication but sufficient to exert a selective pressure on variants with decreased drug susceptibility (Figure 2).

In resource-rich settings, patterns of HIV drug resistance to the different classes of antiretroviral drugs have been well documented. Soon after the availability of the NRTI zidovudine, it was noted that mutations in HIV could lead to antiretroviral resistance and failure.[18] Since the advent of combination ART and the availability of potent and well-tolerated regimens, rates of virological failure and antiretroviral resistance in the developed world appear to be falling.[19] However, in patients who have previously been treated with mono-nucleoside and dual-nucleoside therapies, higher rates of resistance

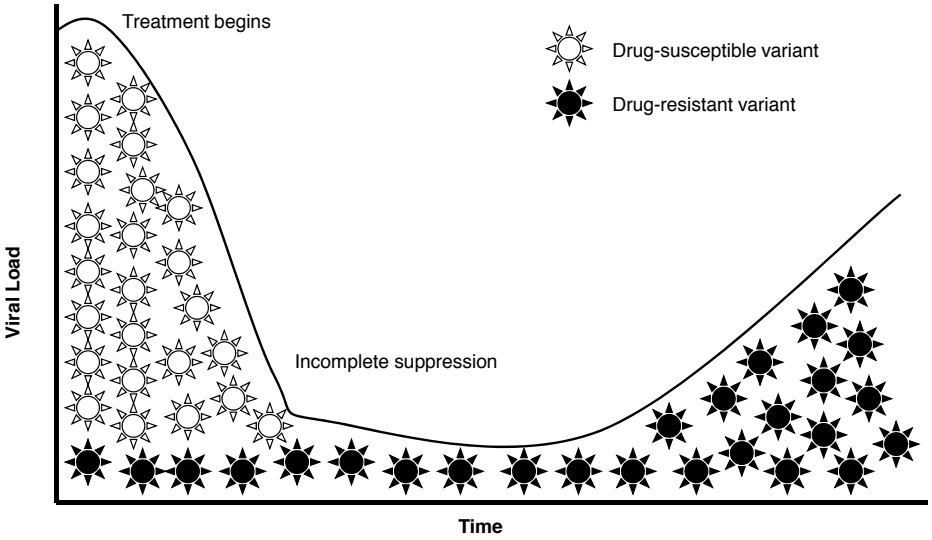


Figure 2. The selection of drug resistant variants.

will persist. HIV drug resistance which has developed in persons receiving ART is called acquired or secondary drug resistance. When drug resistant HIV is subsequently spread to newly infected individuals, this is referred to as transmitted or primary drug resistance.

AIM OF THE THESIS

It has been hypothesized that in resource-limited settings, where few patients have been previously exposed to suboptimal drug regimens, HIV drug resistance is less likely to emerge than in the developed world.[20] However, the weak health systems in many African countries may accelerate the development of resistance due to ART interruptions, poor adherence, lack of viral-load monitoring and limited drug choices.[21-23] Furthermore, the continued widespread use of peripartum single-dose nevirapine, which rapidly selects for resistance, increases the chance of virological failure when followed by NNRTI-based antiretroviral therapy for women and children.[24,25] Lastly, HIV drug resistance has been less well studied in subtypes other than subtype B and controversy remains over whether subtype diversity impacts treatment response due to different patterns of resistance-conferring mutations.[26] The aim of this thesis is to assess the extent and pattern of HIV drug resistance among treatment-naive and treatment-experienced adults and children, and to determine the effect of HIV drug resistance on the response to first- and second-line ART in routine ART programs in sub-Saharan Africa.

RESEARCH SETTING

The PharmAccess African Studies to Evaluate Resistance (PASER) network was established in 2006 as a partnership of clinical sites, laboratories and research groups in Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe (Figure 3).[27] PASER, together with its counterpart program TREAT Asia Studies to Evaluate Resistance (TASER) in Asia, constitutes a bi-regional capacity development program called LAASER (Linking African and Asian Societies for an Enhanced Response to HIV/AIDS; http://www.laaser-hivaids.org). From 2006 to 2011, this program was funded by The Netherlands Ministry of Foreign

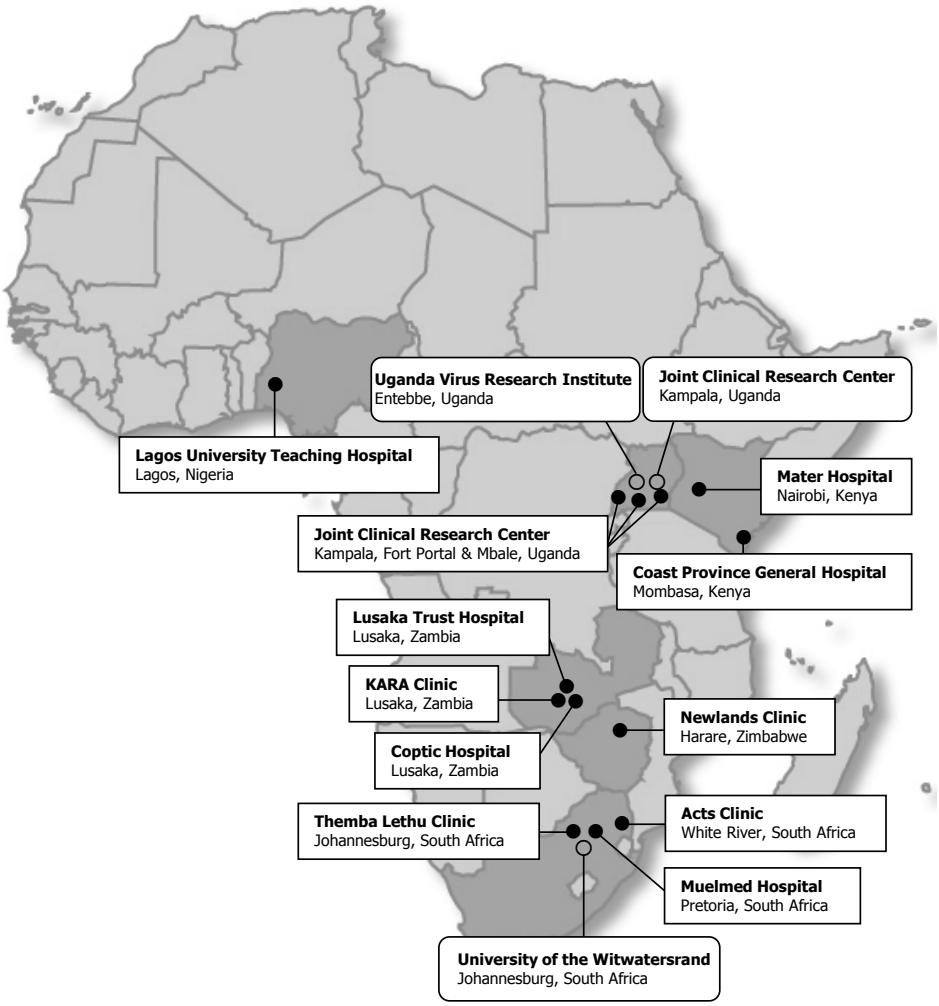


Figure 3. Geographical location of PASER sites. Closed circles represent clinical sites; open circles are reference laboratories. Reproduced from the PASER-M Cohort Profile.[30]

Affairs in partnership with the Aids Fonds. Laboratories providing genotyping results for PASER and TASER participated in the TREAT Asia Quality Assurance Scheme (TAQAS), an assessment program to build genotyping laboratory capacity.[28]

The PASER network is coordinated by the PharmAccess Foundation, a non-profit organization dedicated to the strengthening of health systems and improvement of access to quality basic health care in Africa (http://www.pharmaccess.org), in collaboration with the Amsterdam Institute for Global Health and Development (http://www.aighd.org). The program contributes to the Global HIV Drug Resistance Network (HIVResNet), developed by the WHO.[29] PASER study protocols incorporate the assessment of baseline and acquired drug resistance in adults receiving first- or second-line ART (PASER-Monitoring) and transmitted drug resistance in recently infected individuals (PASER-Surveillance).

The thirteen participating clinical sites represent a variety of clinic types in terms of available resources, administration (public, nongovernmental, private or faith based), and the level of experience with HIV treatment and research, which has been described in the study's cohort profile.[30] PASER developed a number of spin-off projects and studies related to HIV drug resistance. One of these is the MARCH (Monitoring Antiretroviral Resistance in Children) program, a pediatric cohort study in Uganda.

STUDY POPULATION

The PASER-Monitoring observational cohort recruited HIV-1 positive adults who were eligible for the initiation of a first-line ART regimen or switch to second-line ART after first-line failure, in accordance with national guidelines. Patients re-initiating a first-line regimen <30 days after stopping the previous three-drug regimen were excluded; other previous use of antiretroviral medication was allowed. Pregnancy at enrolment and HIV-2 infection (tested in endemic countries only) were additional exclusion criteria. Thirteen clinical sites collaborated on PASER-Monitoring in Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe. The site specific target was 240 participants and this was achieved in 12 out of 13 sites, enrolling a total of nearly 3000 participants.

PASER-Surveillance studies were conducted in Kampala and Mombasa in 2009-2010 and recruited newly-diagnosed HIV-1 infected persons who were either below 25 years of age or had laboratory evidence of recent infection (i.e. documented seroconversion in the past 12 months). Exclusion criteria were previous use of antiretroviral drugs, the presence of an AIDS-defining illness and previous pregnancy in females. The target sample size was 85 persons per geographic setting.

The MARCH study, conducted in three sites in Uganda was initiated in 2010. It aimed to enroll HIV-1 positive children who were eligible to start first-line ART or switch to second-line ART. For children initiating a first-line regimen, previous three-drug ART was an exclusion criterion; previous use of antiretroviral drugs for the prevention of mother to child transmission was allowed. The three sites jointly recruited over 360 children.

OUTLINE OF THE THESIS

Part 1 describes the epidemiology of transmitted drug resistance in East-Africa. We conducted two cross-sectional PASER-S surveys in Kampala, Uganda and Mombasa, Kenya (Chapters 2 and 3). Additionally, we used a mathematical model based on data from both PASER-M and PASER-S studies to predict future trends of transmitted drug resistance in the region (Chapter 4).

In Part 2, mutational patterns after first-line ART failure are characterized. In a cross-sectional study, we assessed the diagnostic accuracy of clinical and/or immunological criteria for ART failure as well as drug resistance patterns present at time of switch to second-line ART (Chapter 5). As part of a prospective study, we analyzed mutational patterns detected during routine viral load measurement after 12 months of therapy (Chapter 6). Finally, the rate of mutation accumulation was assessed in patients with prolonged failure and repeated viral load measurements on first-line ART (Chapter 7).

In Part 3 we investigated the effectiveness of ART in preventing HIV drug resistance or suppressing HIV once resistance has emerged. The WHO-defined site-level Early Warning Indicators for HIV drug resistance were assessed and evaluated within the PASER-M network (Chapter 8). The impact of drug resistance mutations on the clinical outcomes of patients receiving either first- or second-line ART were prospectively investigated in Chapters 9 and 10, respectively.

Part 4 is dedicated to pediatric studies. First, we summarized the literature on acquired drug resistance in children after therapy failure in a systematic review (Chapter 11). As part of the MARCH study, mutational patterns in children experiencing first-line ART failure are described (Chapter 12). Lastly, a qualitative investigation was performed evaluating the barriers to access to care for HIV-infected children and their caregivers in Uganda (Chapter 13).

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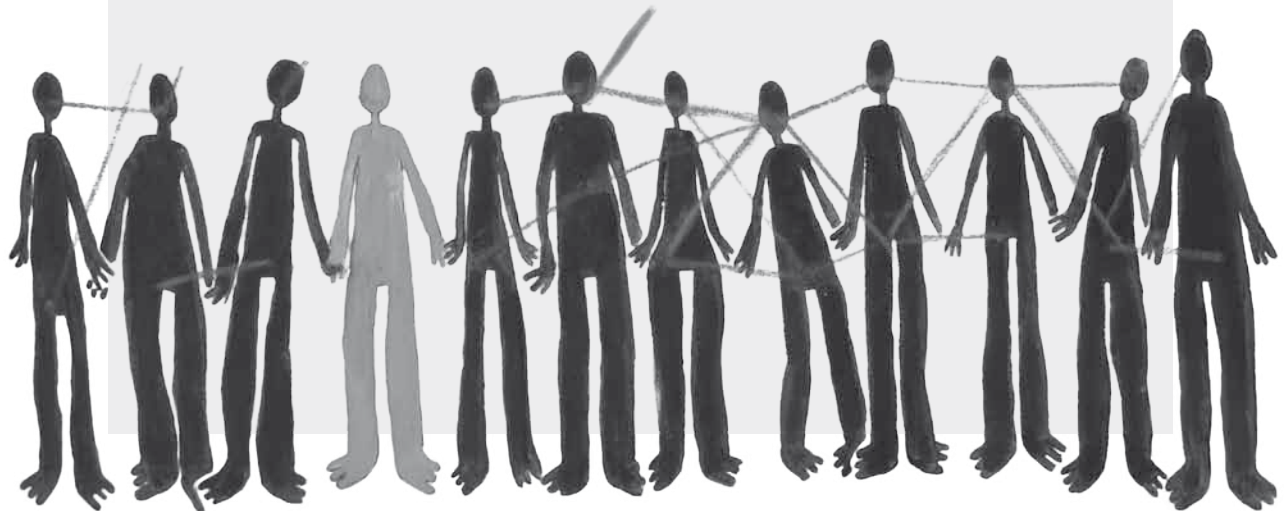
Part 1

Epidemiology of transmitted HIV drug resistance



Chapter 2

Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala

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** These authors equally contributed to this manuscript and share first authorship.*

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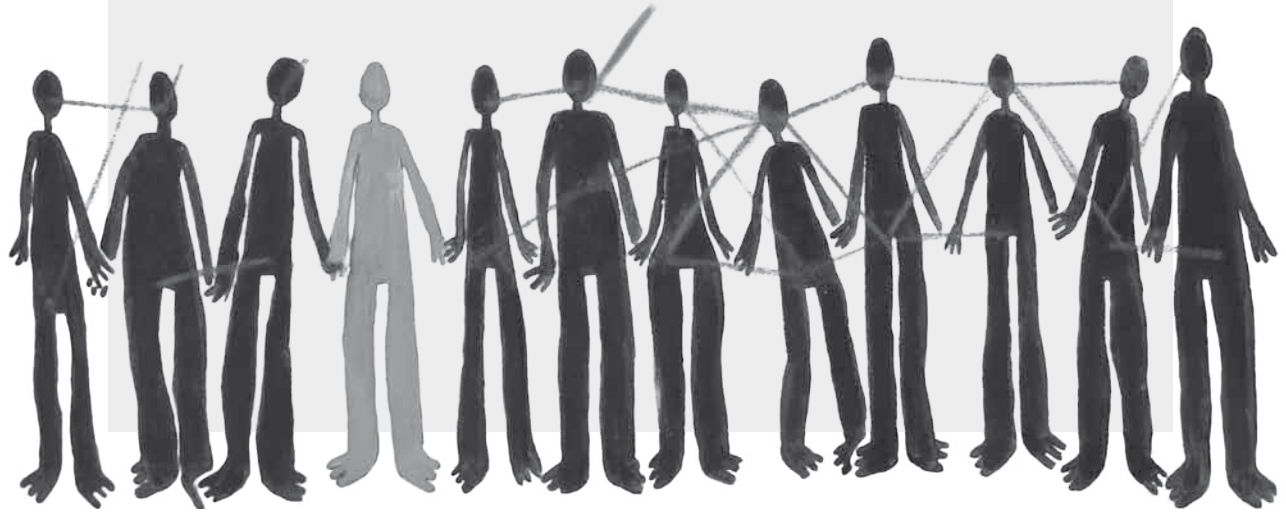

ABSTRACT

Objective

To assess the emergence of transmitted HIV-1 drug resistance (TDR) in Kampala, Uganda, ten years after the scale-up of antiretroviral treatment (ART), and to compare with a previous survey among antenatal clinic attendees in 2007 (reporting 0% TDR).

Design

A cross-sectional survey was conducted among newly HIV-1 diagnosed, antiretroviral-naive young adults attending two large voluntary counseling and testing centers within the geographic area of Kampala.

Methods

Proxy criteria for recent HIV-1 infection were used as defined by the World Health Organization. Population sequencing of the *pol* gene was performed on plasma samples with HIV-1 RNA ≥1000 copies/mL. Drug resistance mutations (SDRMs) were identified according to the 2009 World Health Organization list for surveillance of TDR. HIV-1 subtypes were designated using maximum likelihood phylogenetic reconstruction.

Results

Genotypic test results were obtained for 70 of 77 (90.9%) participants. SDRMs were identified in six samples yielding a prevalence of TDR of 8.6% (95% confidence interval 3.2% to 17.7%). Two had SDRMs to nucleoside reverse-transcriptase inhibitors (D67G, L201W), three had SDRMs to non-NNRTIs (G190A, G190S, K101E), and one had SDRMs to protease inhibitors (N88D). Frequencies of HIV-1 subtypes were: A (36/70, 51.4%), C (2/70; 2.9%), D (23/70, 32.9%) and unique recombinant forms (9/70, 12.9%).

Conclusions

This repeated survey suggests an increase of TDR in Kampala, compared with a previous survey. This finding justifies increased vigilance with respect to surveillance of TDR in areas in Africa where ART programs are rolled-out.

INTRODUCTION

Expanded access to combination antiretroviral therapy (ART) in many countries in sub-Saharan Africa during the past decade[1] has remarkably improved the prognosis of HIV-1 infected individuals.[2] Important deficiencies in health systems, such as lack of virological monitoring and intermittent drug supply, have raised concerns about the rapid emergence and spread of drug resistant HIV-1 strains in Africa.[3,4] Increasing levels of transmitted drug resistant HIV-1 variants (TDR) could compromise the effectiveness of standard first-line ART regimens,[5,6] which has severe public health consequences in areas where treatment options are limited. With the wider use of ART in industrialized countries, TDR to non-nucleoside reverse transcriptase inhibitors (NNRTIs) in newly infected individuals steadily increased, in San Francisco from 0% in 1996-1997 to 13.2% in 2000-2001,[5] and in Europe from 2.3% in 1996–1998 to 9.2% in 2001–2002.[6] Genotypic resistance to two or more classes of antiretroviral (ARV) drugs increased from 2.5% to 13.2%.[5]

Uganda was among the first African countries to distribute life-saving ARV medication. By the end of September 2009, nationwide an estimated 200,413 patients were receiving ART, reaching 39% of those in need.[1] In the capital city of Kampala the massive scale-up of ART was initiated in the year 2000, following limited-scale distribution since the mid 1990s. A survey performed in Entebbe, situated in the greater Kampala area, in 2006-2007 did not detect any significant drug resistance mutations among 47 newly HIV-1 diagnosed pregnant women with CD4 count > 350 cells/μL attending an antenatal clinic.[7]

We report the results of a subsequent survey in 2009-2010 that evaluated the prevalence of TDR among newly HIV-1 diagnosed young individuals attending voluntary counseling and testing (VCT) sites in Kampala, Uganda.

METHODS

Study design and population

A cross-sectional survey was conducted among clients attending two large free-access, non-governmental VCT sites in Kampala, Uganda: AIDS Information Centre (AIC), situated in Mengo area, and Naguru Teenage Health Information Centre (NTC), situated in Bugolobi area. The institutional review boards at the Academic Medical Center and the Uganda Virus Research Institute approved the study. Mandatory eligibility criteria, as defined by the World Health Organization (WHO), were used to identify individuals who were likely to have been recently infected: newly diagnosed with HIV-1 and aged

between 18 and 25 years, or laboratory evidence of recent HIV-1 infection (defined as a confirmed HIV-1 positive antibody test with a negative HIV-1 antibody test within the past 12 months, or an indeterminate/negative HIV-1 antibody test with detectable HIV-1 RNA or positive p24 antigen).[9] Exclusion criteria were any previous ARV use (also for the prevention of mother-to-child transmission of HIV-1), documented WHO clinical stage 4 event and previous pregnancy (parity).[9] All participants provided written informed consent prior to study enrolment. During the enrolment period of maximum 12 months, the VCT clients were all screened and sequentially enrolled. A case report form was completed and a blood draw was performed in all participants.

Laboratory procedures

Plasma was separated within two hours from blood draw and stored immediately at –80°C. HIV-1 RNA was tested with the Amplicor MONITOR 1.5 (Roche, Roche Molecular Systems, NJ, USA). HIV-1 RNA was extracted from 140µl of blood plasma using the Qiamp viral RNA mini kit (Qiagen Inc, Chatsworth, CA). Polymerase gene-specific primers were used for reverse transcriptase, followed by nested PCR to amplify a 1030-base pair *pol* gene encompassing amino acids 1–99 of protease and 1–242 of reverse transcriptase. The PCR products were then purified with a QIA-quick PCR purification kit (Qiagen, Valencia, CA) and sequenced in the sense and antisense direction with a set of nested primers.[10] To ensure the quality of the data set, each sequence was checked before inclusion using ViroScore Suite v8.1 (ABL, France).

Genotypic Resistance and Phylogenetic Analysis

Samples were sequentially genotyped and TDR was analyzed. Drug resistance mutations (SDRMs) were identified according to the 2009 WHO list for surveillance of genotypic TDR updated in 2009, which excludes polymorphisms.[11] For SDRM analysis, the Stanford calibrated population resistance analysis tool version 5.0 beta was used.[12] *Pol* region subtype classification and recombinant patterns were determined using the REGA subtyping tool[13] and the SCUEAL application[14], further confirmed using phylogenetic analysis. We performed maximum likelihood phylogenetic reconstruction using PhyML based on the General Time-Reversible model with gamma distributed rate variation among nucleotide sites.

Statistical methods

The survey sample size was estimated from the hypothesis that the prevalence of TDR in the target population was initially low (estimated at 2%) and increased with time (estimated at 10%). To detect such increase with 80% power using a two-sided significance level of 0.05, the required number of HIV-1 sequences per geographic area was 78. Assuming 10% amplification failure, the target sample was 85 individuals. The proportions

of sequences containing ≥1 SDRM were calculated overall and by each of the three main drug classes, i.e. protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs. TDR prevalence was estimated with a 95% confidence interval (CI) based on the binomial distribution. As a secondary analysis, the WHO-recommended truncated sampling technique was used to categorize TDR prevalence as low (<5%), moderate (5–15%), or high (>15%) for each of the three drug classes, based on the testing of the first £ 47 sequences.[15] Categorical data were compared using Chi-square test. Continuous data were investigated using Kruskal-Wallis or Student t-test. All analyses were performed using Stata version 10 (StataCorp LP, TX).

RESULTS

Patient characteristics

Study enrolment took place from February 2009 to February 2010 at AIC and from May 2009 to May 2010 at NTC. A total of 884 individuals were screened, of whom 81 (9.2%) met the eligibility criteria. Excluding four individuals due to protocol violations (i.e. 3 did not meet the age criterion, 1 had a previous pregnancy), 77 participants (43 from AIC and 34 from NTC) were included in the analysis (table 1). Seventy-six participants qualified based on the age criterion and one participant had a new confirmed HIV-1 diagnosis after a recent negative test. The mean age was 21.6 years (standard deviation, SD 2.1). Females comprised 70.1% (n=54). The mean age was lower for females (21.1 years, SD 2.0), compared to males (22.7 years, SD 1.9, p=0.0017). The median CD4 count was 417 cells/μL (interquartile range (IQR): 318.5-551.5 cells/μL) and the median HIV-1 RNA load was 4.49 $\log_{10}$ copies/ml (IQR: 3.96-5.28 $\log_{10}$ copies/ml). 94.8% (n=73) of participants were Ugandan nationals. Nearly all (73, 94.8%) participants reported sexual encounters with the opposite sex, whereas other exposures were uncommon. The median age at sexual debut was 18 years, with a range between 14 and 27 years. During the three years prior, 71 (92.2%) participants reported to have engaged in unprotected sex, with an average of 2.1 (SD 1.8) sexual partners, and 23 (29.9%) reported a first episode of a sexually transmitted infection. Among participants who had a steady sexual partner, 68.8% was unaware of their partner's HIV-1 status. Baseline characteristics, except for mean age, did not differ between sites (table 1).

Genotypic profiles

70 samples were successfully genotyped and seven samples failed to amplify or had no valid genotype. One or more SDRMs were identified in six of the 70 valid sequences, yielding an estimated TDR prevalence of 8.6% with a 95% CI 3.2% to 17.7%. The proportion of sequences with SDRMs associated with NRTIs, NNRTIs and PIs was 2.9% (2/70),

Table 1. Patient characteristics, by study site.

	Total	Aids Information Centre (AIC)	Naguru Teenage Health Information Centre (NTC)	p-value
Patients	77	43	34	
Sex				0.085
Female	54 (70.1)	27 (65.9)	27 (79.4)	
Male	23 (29.9)	16 (39.0)	7 (20.6)	
Age – mean yrs (sd)	21.6 (2.1)	22.4 (1.9)	20.7 (1.9)	<0.001
Ugandan nationality	73 (94.8)	41 (95.4)	32 (94.1)	0.809
Marital status				0.075
Now married/cohabiting	14 (18.2)	5 (11.6)	9 (26.5)	
Divorced/separated	0	0	0	
Widowed	4 (5.2)	4 (9.3)	0	
Never married/single	58 (75.3)	34 (79.1)	24 (70.6)	
Other	1 (1.3)	0	1 (2.9)	
Education level				0.134
None/illiterate	2 (2.6)	1 (2.3)	1 (2.9)	
Primary school	24 (31.2)	9 (20.9)	15 (44.1)	
Secondary school	30 (39.0)	18 (41.9)	12 (35.3)	
Higher education	21 (27.3)	15 (34.9)	6 (17.7)	
Main occupation				0.014
None/at home	24 (31.2)	7 (16.3)	17 (50.0)	
Student	16 (20.8)	9 (20.9)	7 (20.6)	
Employed	35 (46.7)	25 (61.0)	10 (29.4)	
Hemoglobin – median g/dL (IQR) [a]	12.6 (11.3-14.3)	13.1 (11.4-14.8)	12.4 (10.8-14.0)	0.1284
CD4 cell count –- median cells/µL (IQR) [b]	417 (318.5-551.5)	377.5 (236-519)	418.5 (264-573)	0.1159
HIV RNA – median $\log_{10}$ c/ml (IQR)	4.49 (3.96- 5.28)	4.47 (3.90-5.03)	4.55 (3.83-5.27)	0.4447

Data represent n (%) unless otherwise specified; [a] Data available for n=73; [b] Data available for n=76

4.3% (3/70) and 1.4% (1/70), respectively. We observed six different SDRMs: D67G, K101E, G190S, G190A and L210W in reverse-transcriptase and N88D in protease. TDR was confined to a single drug-class in all six sequences. Table 2 summarizes the demographic and virological characteristics of the six participants who harbored an SDRM.

Using the WHO-recommended truncated sequential sampling technique, four of the first 47 sequences harbored an SDRM (moderate prevalence category), of which two were NRTI-associated (low prevalence category), one PI-associated (low prevalence category); and one NNRTI-associated. HIV-1 subtype frequencies were: A (36/70, 51.4%), C (2/70; 2.9%), D (23/70, 32.8%), A1/D recombinants (9/70, 12.9%).

Table 2. Demographic and virological characteristics of the six participants who harboured a drug resistance mutation.

#	Date of enrolment	ID	Age (years)	Sex	Estimated year of infection	Estimated country of infection	CD4 count (cells/µl)	HIV-1 RNA load (log_{10} c/ml)	Viral subtype	Surveillance Drug Resistance Mutations		
										NRTI	NNRTI	PI
1	19-Mar-09	33	23	Female	Unknown	Unknown	615	3.84	D		G190S	
2	21-May-09	702	20	Female	2008	Uganda	348	5.88	A (A1)	L210W		
3	05-Jun-09	705	20	Male	2008	Uganda	706	4.60	A	D67G		
4	27-Jul-09	248	23	Male	Unknown	Uganda	457	5.55	A1D			N88D
5	06-Jan-10	438	24	Male	Unknown	Uganda	270	5.47	A (A1)		G190A	
6	25-Mar-10	728	24	Male	2009	Uganda	495	5.88	D		K101E	

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

DISCUSSION

This survey among 70 newly HIV-1 diagnosed young VCT clients in Kampala demonstrated an estimated prevalence of TDR of 8.6%, which is likely to represent an increase compared to the previous survey in 2006-2007 that did not detect any SDRMs among 47 pregnant women from the greater Kampala area.[7] Identified SDRMs were associated with NNRTIs (3), NRTIs (2) and PIs (1), but in each sequence TDR was confined to a single drug class. This study is among the first to suggest an increase of TDR between repeated surveys within the same geographic area in Africa, although the subsequent surveys targeted different subpopulations.

Most studies from Africa that were conducted during the early scale-up of ART have reported low levels of TDR.[7-9] In Botswana, the 2007 threshold survey indicated that five years following the countrywide ART roll-out, TDR was still less than 5%.[16] The IAVI cohort, however, of newly HIV-1 infected individuals in east and southern Africa reported a 5% overall prevalence of TDR, with an increase from 3% (4/157) in 2005-2006 to 7% (12/169) in 2007-2008.[17] The proportions of participants who harbored TDR was particularly high in Entebbe (4/17, 23.5%) and Kigali (8/68, 11.8%).[17] Consistent with this report, our study supports the hypothesis that increasing ARV drug exposure in African populations, following the roll-out of ARVs for treatment and prevention of mother-to-child transmission, may cause a rise in TDR and thereby new public health challenges.

In this study the categorization of TDR using prevalence (6/70) corresponded with the WHO-recommended truncated sequential sampling technique (4/47), i.e. "moderate" overall and "low" for each drug class separately. It should, however, be noted that the small sample sizes resulted in a wide confidence interval, warranting caution in interpreting and extrapolating the results.

This study has several limitations. Given the challenges, especially in resource-limited settings, in identifying individuals during acute or recent HIV-1 infection, WHO recommends the use of proxy criteria for the surveillance of TDR. A recent study in Botswana, however, found poor agreement between the WHO criteria and two laboratory-based methods to detect new infection.[16] The WHO approach could therefore lead to the inclusion of individuals with established infection, during which drug resistant mutants may have reverted to wild-type virus,[20, 21] thereby possibly underestimating the true current prevalence of resistance transmission. Although the study specifically selected newly diagnosed, ARV-naïve individuals, it cannot be completely ruled out that some participants had unknown or undisclosed prior exposure to ARV therapy and/or prophylaxis.

In conclusion, ten years following the ART scale-up in Kampala, Uganda, this repeated survey demonstrated that 8.6% of newly HIV-1 diagnosed youth harbored TDR, which is likely to represent an increase compared to the previous survey. The study findings should trigger public health action in performing additional surveys in the upcoming years to evaluate the evolution of TDR in the country and can provide guidance to drug resistance prevention strategies. This is especially urgent since current options for first-line therapy in Uganda are limited and access to second-line therapy is not widely available.

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Chapter 3

High prevalence of transmitted antiretroviral drug resistance among newly HIV-1 diagnosed adults in Mombasa, Kenya

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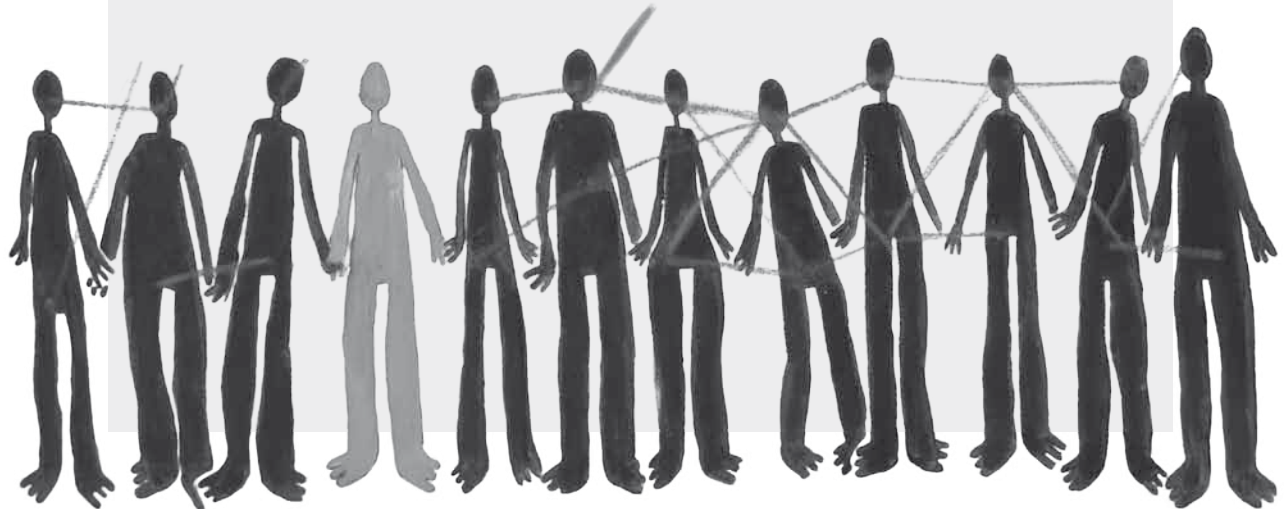

ABSTRACT

In view of the recent antiretroviral therapy (ART) scale-up in Kenya, surveillance of transmitted HIV drug resistance (TDR) is important. A cross-sectional survey was conducted among newly HIV-1 diagnosed, antiretroviral-naive adults in Mombasa, Kenya. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list. HIV-1 subtypes were determined using REGA and SCUEAL subtyping tools. Genotypic test results were obtained for 68 of 81 participants, and SDRMs were identified in 9 samples. Resistance to non-nucleoside reverse transcriptase inhibitors (K103N) occurred in five participants, yielding a TDR prevalence of 7.4% (95% confidence interval 2.4–16.3%). Frequencies of HIV-1 subtypes were A (70.6%), C (5.9%), D (2.9%), and unique recombinant forms (20.6%). The TDR prevalence found in this survey is higher than previously reported in different regions in Kenya. These findings justify increased vigilance with respect to TDR surveillance in African regions where ART programs are scaled-up in order to inform treatment guidelines.

INTRODUCTION

With expanded access to combination antiretroviral therapy (ART) in many countries in sub-Saharan Africa, the prevalence of HIV drug resistance is likely to increase. Viral resistance can develop through selective pressure while receiving ART and can subsequently spread to newly infected individuals. The latter is referred to as primary or transmitted drug resistance (TDR).[1,2] TDR increases the risk of virological failure,[3,4] which may have a substantial impact in areas where options for first-line therapy are limited. Studies from industrialized countries have shown an increased incidence of TDR with time after the introduction of ART.[5–7] In Africa, studies conducted during the early scale-up of ART reported low levels of TDR.[8–10] Recently accruing evidence, however, points to increasing TDR among individuals with recent HIV infection in East Africa.[11–13]

In Kenya, national ART roll-out started in late 2003, and by 2010 an estimated 50% of adults in need of ART were receiving treatment, which is among the higher coverage rates for sub-Sahara African countries.[14] Data on TDR from Kenya are limited, pointing toward levels of 0–10% in different regions and time periods.[11,15,16] Because the prevalence of transmitted resistance mutations can influence policy and guide treatment choices in the absence of individual resistance testing, the World Health Organization (WHO) has provided guidelines for TDR surveillance in developing countries.[1,9] We report the results of a survey evaluating the prevalence of TDR among newly HIV-1 diagnosed individuals attending voluntary counseling and testing (VCT) sites in Mombasa, Kenya.

MATERIALS AND METHODS

Study design and population

As part of the PharmAccess African Studies to Evaluate Resistance-Surveillance (PASER-S) program, a cross-sectional survey was conducted among clients attending four VCT sites in Mombasa, Kenya: Coast Provincial General Hospital, Likoni Sub-District Hospital, Kisauni Health Centre, and Magongo Health Centre. The institutional review boards at the Kenyatta National Hospital and the Academic Medical Center of the University of Amsterdam approved the study. Eligibility criteria as defined by the WHO were used to identify individuals who were likely to have been recently infected: newly diagnosed with HIV-1 and aged between 18 and 25 years, or laboratory evidence of recent HIV-1 infection (defined as a confirmed positive antibody test with a negative antibody test in the past 12 months, or an indeterminate/ negative antibody test with detectable HIV-RNA or p24 antigen).[9] Exclusion criteria were any previous antiretroviral use, WHO clinical stage 4 event, or previous pregnancy. All participants provided written informed

consent prior to enrollment. During the enrollment period of maximum 12 months, the VCT clients were screened and sequentially enrolled.

Population genotype analysis

HIV-RNA and genotypic testing was performed as previously described.[12] Final sequences were checked before inclusion using ViroScore Suite v8.1 (ABL, Paris, France). Neighbor-joining phylogenetic trees were constructed using MEGA 5.05,[17] in order to rule out cross-contamination. TDR was analyzed in sequences with > 1020 base pairs. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list for surveillance of TDR,[1] using the Stanford calibrated population resistance analysis tool (version 5.0).[18] Subtype classification and recombinant patterns were determined using REGA[19] and SCUEAL[20] subtyping tools.

Statistical methods

The survey sample size was estimated from the hypothesis that TDR prevalence increased with time (from 0 to 10%). To detect such an increase with 80% power using a two-sided significance level of 0.05, the required number of HIV sequences per geographic area was 71. Assuming 20% assay failures, the target sample was 85 individuals. The proportions of sequences containing at least one SDRM were calculated by each of three drug classes: nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs, and protease inhibitors (PIs). TDR prevalence was estimated with a 95% confidence interval (CI) based on the binomial distribution. As a secondary analysis, the WHO-recommended truncated sampling technique was used to categorize TDR prevalence as low (< 5%), moderate (5–15%), or high (> 15%) for each of the three drug classes, based on the testing of the first 47 sequences.[21] Categorical data were compared using the chi-square test and continuous data with the Wilcoxon rank-sum or Student's t-test. All analyses were performed using Stata version 10 (StataCorp LP, College Station, TX).

RESULTS

Participant characteristics

Study enrollment took place from May 2009 to March 2010 at four VCT centers in Mombasa, Kenya. A total of 241 individuals were screened, of whom 93 (38.6%) were enrolled. Protocol violations were noted in 12 individuals (8 had a previous pregnancy and 4 had no laboratory evidence of recent infection). Finally, 81 participants (11 from Coast Provincial General Hospital, 51 from Likoni Sub-District Hospital, 11 from Kisauni Health Centre, and 8 from Magongo Health Centre AIC) were included in the analysis. Sixty-one participants were included based on the age criterion and 20 participants had

a new confirmed HIV-1 diagnosis after a recent negative test (median 6.7 months ago, range 1.5–12 months). The median age was 23.4 years (IQR 21.6–24.9). Except for age, participant characteristics were similar for those included based on the age criterion and those with laboratory evidence of recent infection (Table 1). The median age at sexual debut was 16 years (IQR 14–18); all participants reported that they had engaged in unprotected sex in the 3 years prior to study enrolment. Among participants with a steady sexual partner, 40 (49.4%) were unaware of their partner's HIV status.

Genotypic profiles

Of 76 specimens with HIV-RNA > 1000 copies/ml, 72 were successfully genotyped and 4 failed to amplify or generated a poor sequence. Additionally, four sequences were excluded from analysis because they contained less than 1020 base pairs. An SDRM was

Table 1. Participant characteristics by criteria to identify recent infection

	Total	Age 18-24	Lab evidence	*p*
Participants	81	61	20	
Sex				0.678
Female	71 (87.7)	54 (88.5)	17 (85.0)	
Male	10 (12.4)	7 (11.5)	3 (15.0)	
Age - median yrs (IQR)	23.4 (21.6-24.9)	21.4 (22.6-23.5)	28.2 (31.5-34.2)	<0.001
Kenyan nationality	79 (97.5)	60 (98.4)	19 (95.0)	0.401
Marital status				0.210
Married/cohabiting	65 (80.3)	48 (78.7)	17 (85.0)	
Divorced/separated	3 (3.7)	2 (3.3)	1 (5.0)	
Widowed	4 (4.9)	2 (3.3)	2 (10.0)	
Never married/single	9 (11.1)	9 (14.8)	-	
Education level				0.518
None/illiterate	3 (3.8)	2 (3.3)	1 (5.3)	
Primary school	46 (57.5)	35 (57.4)	11 (57.9)	
Secondary school	25 (31.3)	18 (29.5)	7 (36.8)	
Higher education	6 (7.5)	6 (9.8)	-	
Main occupation				0.860
None/at home	48 (59.3)	35 (57.4)	13 (65.0)	
Employed	31 (38.3)	24 (39.3)	7 (35.0)	
Student	2 (2.5)	2 (3.3)	-	
Hemoglobin – median g/dL (IQR)	10.9 (8.7-12.3)	10.9 (8.7-12.3)	10.9 (9.2-12.3)	0.705
CD4 cell count – median cells/μL (IQR)	400 (239-564)	404 (246-589)	325.5 (230-550)	0.547
HIV RNA – median $\log_{10}$ c/ml (IQR)	4.6 (4.2- 5.1)	4.6 (4.3-5.1)	4.6 (3.5-5.1)	0.440

Data represent n (%) unless otherwise specified. IQR, interquartile range.

identified in 9 (13.2%, 95% CI 6.2-23.6%) of the 68 valid sequences. The respective TDR prevalence was 1.5% (95% CI 0.04–7.9%) for NRTIs, 7.4% (95% CI 2.4–16.3%) for NNRTIs, and 4.4% (95% CI 0.9–12.4%) for PIs. We observed five different SDRMs: K70R and K103N in reverse transcriptase and I85V, N88D and L90M in protease. TDR was confined to a single drug class in all sequences. Table 2 summarizes the characteristics of the nine participants who harbored an SDRM. Using the WHO-recommended truncated sequential sampling technique, 5 of the first 47 sequences harbored any SDRM (moderate prevalence), of which 3 were NNRTI-associated (moderate prevalence), 2 were protease inhibitor-associated (low prevalence), and none was NRTI-associated (low prevalence). HIV-1 subtype A (n = 48, 70.6%) was observed most frequently, followed by unique recombinant forms (n = 14, 20.6%), C (n = 4, 5.9%), and D (n = 2, 2.9%).

DISCUSSION

This survey among 68 newly HIV diagnosed VCT clients in Mombasa, Kenya, demonstrated an estimated TDR prevalence of 7.4% for NNRTIs, 4.4% for PIs, and 1.5% for NRTIs; no multiclass resistance was detected. The TDR prevalence corresponded with the WHO-recommended truncated sampling technique, yielding "moderate" prevalence overall for the NNRTI drug class and "low" prevalence for the NRTI and PI drug classes. To our knowledge, our survey is among the first to systematically quantify TDR in the coastal region of Kenya. Our findings support the hypothesis that a rise in TDR is occurring with increasing antiretroviral drug exposure.

Few studies of primary HIV drug resistance have been conducted in Kenya. The PASER-Monitoring study among chronically infected, ARV-naive adults revealed an HIV drug resistance prevalence of 4.9% in Mombasa and 4.5% in Nairobi.[16] A study conducted in Nairobi revealed reverse transcriptase mutations in 7.5% of 53 ARV-naive patients initiating ART.[15] The International AIDS Vaccine Initiative cohort of newly infected individuals found a TDR prevalence of 3.1% among 64 participants tested at three sites in Kenya (Nairobi, Mtwapa, and Kilifi).[11] A trend toward increasing prevalence over time was found, with a prevalence of 10% in 2009, but numbers of participants per time period were too small to draw significant conclusions.

To date, most surveys conducted in sub-Saharan Africa using WHO methodology have reported low levels of TDR.[8–10,22,23] By contrast, recent surveys conducted in Kampala,[12] Dar es Salaam,[13] and Yaounde[24] detected higher TDR rates (9–12%) compared to earlier estimates in the same regions, suggesting a trend of increasing TDR. Our study could unfortunately not be compared to an earlier survey in Mombasa. However, in the context

Table 2. Characteristics of the participants who harbored a drug resistance mutation.

ID	Date of enrolment	Inclusion criterion*	Age (years)	Sex	CD4 count (cells/µl)	HIV-1 RNA load (log_{10} c/ml)	Viral subtype	DRMs		
								NRTI	NNRTI	PI
KECGP041	29-01-2010	Lab evidence	35	M	369	4.6	A1			I85V
KEKSN408	23-10-2009	Lab evidence	33	M	508	2.7	A1/D		K103N	
KELKN209	25-01-2010	Age	21	F	476	4.5	A1		K103N	
KELKN227	23-02-2010	Age	23	M	87	6.1	A1//C/D			N88D
KELKN252	05-03-2010	Lab evidence	30	M	564	4.9	A1/D	K70R		
KELKN256	11-03-2010	Lab evidence	36	F	814	3.6	A/D			L90M
KELKN260	11-03-2010	Lab evidence	32	F	497	4.4	A1		K103N	
KELKN262	12-03-2010	Age	23	F	487	5.3	A2/D		K103N	
KEMGO602	02-06-2009	Age	24	F	374	4.6	A1		K103N	

DRM, drug resistance mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. *Study eligibility was based on age (newly diagnosed with HIV and aged between 18 and 25 years) or on laboratory evidence of recent HIV infection (defined as a confirmed positive antibody test with a negative antibody test in the past 12 months, or an indeterminate/negative antibody test with detectable HIV-RNA or p24 antigen).

of prior studies in East-Africa and the available data from chronically infected patients, our data likely represent a rise in TDR among newly infected individuals.

Subtype analysis revealed a predominance of HIV subtype A (70.6%), but also many unique recombinant forms (20.6%). This corroborates a recent study reporting high subtype recombination in a coastal town in Kenya.[25] Our study participants were recruited from the urban setting of Mombasa, a major port with worldwide shipping links, and therefore importation of new HIV-1 strains into the area is likely. These specific characteristics of Mombasa should also be taken into account when interpreting the observed TDR rate, as it may not be extrapolated to more rural, isolated settings in Kenya.

The presence of transmitted resistance mutations has important implications for clinical management of patients in whom ART is indicated, as their risk of virological failure is increased.[3,4] In our study, NNRTI-associated mutations were most common, which is consistent with the widespread use of this drug class for first-line ART and for prophylaxis to prevent vertical transmission. NNRTIs have a low genetic barrier for the development of resistance[26] and mutations may persist for a long time.[27] As NNRTIs are the cornerstone of standard first-line ART in sub-Saharan Africa, reduced susceptibility to this drug class is especially worrisome in view of the limited availability of alternative drug options.

Our study has the following limitations. The WHO-recommended proxy criteria for recent infection were used, which have been suggested to agree poorly with laboratory-based methods to detect recent infection.[22] However, our study included a relatively high number of participants with laboratory evidence of seroconversion and their characteristics, apart from age, did not differ significantly with the participants selected by proxy criteria. Furthermore, although the study specifically selected ARV-naive participants, any unknown or undisclosed prior exposure cannot be ruled out completely.

In conclusion, 7 years after large-scale ART introduction in Kenya, this survey demonstrated a high rate of TDR among newly HIV diagnosed persons in Mombasa. These findings have important clinical consequences as TDR can impair the response to standard first-line ART. Increased vigilance is warranted and TDR surveys should be repeated at regular intervals in order to inform treatment guidelines and drug choices. Surveillance of TDR will help to avoid unnecessary costs of using less-effective first-line regimens and consequent regimen failure. The implementation of an affordable, simplified HIV drug resistance test could help to make TDR threshold surveys more routinely applicable in field conditions in African countries.

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Chapter 4

Benefits of early antiretroviral treatment initiation outweigh risk of transmitted HIV drug resistance: a modeling study of two East African settings

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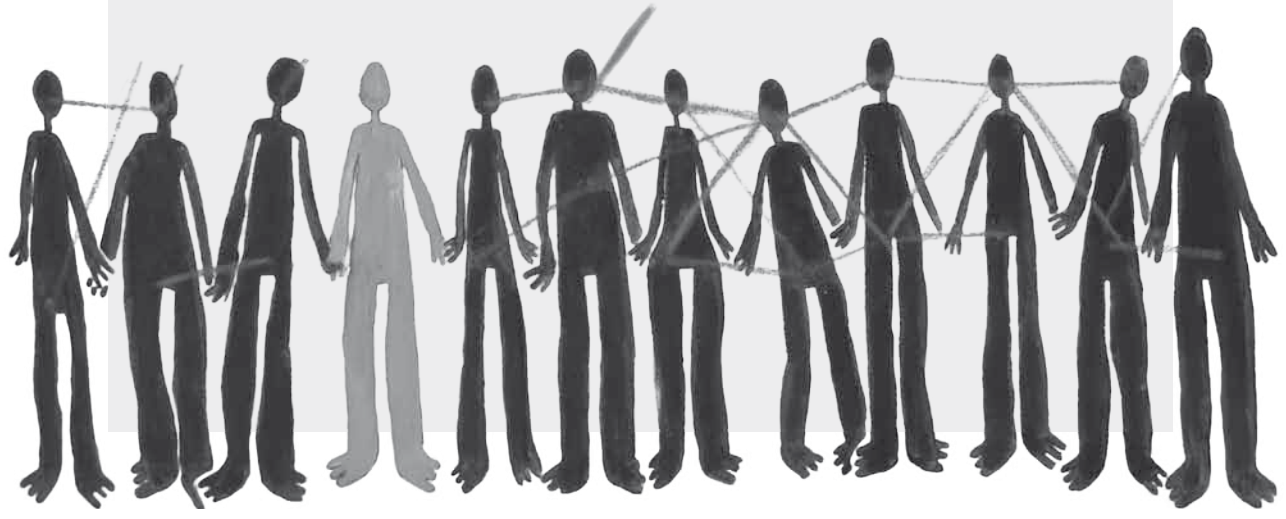
Submitted



ABSTRACT

Background

Earlier antiretroviral therapy (ART) initiation can reduce the number of incident HIV infections in a population. This benefit can, however, be offset by increased transmitted drug resistance (TDR). We compared the preventative benefits of reducing incident infections with the potential increases in TDR in East Africa.

Methods

A compartmental deterministic model was constructed and parameters were chosen to represent Kampala, Uganda, and Mombasa, Kenya. We predicted the effect of initiating at different immunological thresholds (<200, <350, <500 cells/μL) and increasing access to second-line therapy on the overall and mutation-specific TDR prevalence, as well as infections averted, over 10 years.

Findings

When initiating treatment at CD4 <350 cells per μL we predict 18 and 46 infections averted for each incident case of TDR in Kampala and Mombasa, respectively, and 22 and 32 infections averted when initiating at <500. Between 2012 and 2022, TDR prevalence will increase from 8.3% and 12.3%, to 9.4% and 11.1% when ART is initiated at CD4 <200 cells per μL, to 11.6% and 13.4% when ART is initiated at <350 and to 17.8 % and 18.7% when initiated at <500 in Kampala and Mombasa, respectively. Non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations will continue to dominate the future prevalence of TDR. However, when 80-100% of individuals with virological failure are switched to second-line, TDR is predicted to decline for all immunological thresholds.

Interpretation

The absolute number of incident HIV infections is predicted to be strongly reduced by early ART initiation. This outweighs the risk of the predicted increase in TDR. The effectiveness of first-line NNRTI-based therapy can be preserved by improving switching practices to second-line therapy.

INTRODUCTION

Since 2010, the World Health Organization (WHO) recommends earlier initiation of combination antiretroviral therapy (ART) for HIV-infected persons in resource-limited countries. This guideline change, from initiation at <200 to <350 CD4 cells per µL, has resulted in a substantial increase in the number of people eligible to initiate ART.[1] In addition to the individual clinical benefits,[2] earlier initiation of ART may reduce HIV incidence rates and the concept of treatment as prevention has attracted attention as a means to reduce the global HIV epidemic.[2]

As access to ART is expanded in resource-limited countries, concerns surrounding increasing numbers of patient failing treatment and the consequent emergence of drug resistant viruses will become increasingly important. Resistant virus selected for during treatment may subsequently be transmitted to newly infected individuals, undermining the effectiveness of currently recommended or available first-line ART.[3] A recent comprehensive assessment of transmitted drug resistance (TDR) reported a significant rise in prevalence of predominantly non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated resistance since ART rollout in east and southern Africa.[4] The highest TDR levels (9-13%) have been detected in East Africa.[5-6] As NNRTIs are the cornerstone of standard first-line ART in sub-Saharan Africa, reduced susceptibility to this drug class is especially worrisome in view of the limited availability of alternative first-line drug options.

Mathematical modeling is an important tool to inform policy makers about the potential consequences in terms of HIV drug resistance as a result of increased ART coverage in sub-Saharan Africa. Models of HIV transmission have been used to predict emergence of TDR,[7-10] but these models have not examined the impact of earlier treatment initiation. In this study we used a compartmental mathematical model to predict whether initiation of ART at different immunological thresholds and the availability of second-line ART have an effect on transmission of drug-class specific resistance in East Africa. Specifically, we examined whether the number of new HIV infections averted by early ART initiation offsets a potential rise in TDR.

METHODS

Study design and population

To predict time trends of TDR our model included resistance data from PharmAccess African Studies to Evaluate Resistance (PASER). This comprised two distinct observational studies on transmitted (PASER-Surveillance) and acquired (PASER-Monitoring) drug

resistance conducted in Kampala, Uganda and Mombasa, Kenya (Table 1). PASER-Monitoring used a prospective cohort method with a follow-up of 24 months that assessed HIV drug resistance in individuals initiating first-line ART in 2007-2008.[11] Viral load and drug resistance testing were not used to inform clinical decisions. PASER-Surveillance comprised two cross-sectional surveys among newly HIV-1 diagnosed, antiretroviral-naïve individuals attending voluntary counseling and testing sites in Kampala and Mombasa in 2009-2010.[5-6] Specimens collected in the PASER studies were genotyped in reference laboratories in the *pol* gene in two Uganda, which participated in quality assessment schemes for genotypic drug resistance testing.

Model and calibration

A compartmental deterministic mathematical model was constructed which stratified disease progression into an acute stage, three chronic stages, and two AIDS stages (Figure 1). Three chronic stages were included to indicate previous (ART initiation at CD4 <200 cells per μL[12]),[1] current (CD4 <350 cells per μL),[1] and potential future treatment guidelines (CD4 <500 cells per μL).[13] Infectivity varied by stage of infection.[14-15] Other key model parameters are summarized in Table 2. TDR was defined as the number of individuals infected with drug resistant HIV over total number of HIV infected treatment-naïve individuals.

The model identified four sexual activity groups ranging in the number of new sexual partners per year.[16] Using Monte Carlo filtering techniques,[17] we parameterized the different sexual activity groups and only accepted the simulations that were associated with distinct HIV prevalence from WHO data[18-19] and TDR prevalence in the two PASER-Surveillance sites. For Kampala, this resulted in 1017 simulations with HIV prevalence between 7.1-8.4% between 2005 and 2009 and TDR prevalence between 7.1-10% in 2009. For Mombasa, this resulted in 1247 simulations with an HIV prevalence of 5.8-8.2% between 2006 and 2010, and TDR prevalence between 11.9-14.9% in 2009.

Data and Additional Parameters

The PASER-Monitoring data from initially ARV-naïve patients were used as parameters in the model regarding regimens used and resistance patterns. In 2008-9, approximately 40% of individuals from PASER-Monitoring in Kampala were receiving tenofovir-containing regimens, and 60% zidovudine-containing regimens, both combined with emtricitabine or lamivudine and efavirenz or nevirapine. In Mombasa, just 1% of individuals were on tenofovir-containing regimens, and all others were on zidovudine or stavudine-containing regimens (Table 1). We assumed that stavudine would be phased out and replaced by zidovudine in Mombasa. We also evaluated the impact of instead replacing stavudine by tenofovir in a sensitivity analysis. Specific drug resistance mutations were assumed to be selected for by tenofovir (i.e. K65R) and zidovudine (i.e.

Table 1. Characteristics of patients included in the PASER studies

	Kampala, Uganda	Mombasa, Kenya
PASER-Surveillance cross-sectional survey among recently infected individuals		
Number of participants	77	81
Year of enrolment	2009-2010	2009-2010
Site Type	Voluntary Counselling and Testing	Voluntary Counselling and Testing
Age, years	22.0 (20.0-23.0)	23.4 (21.6-24.9)
Sex, female	54 (70.1)	71 (87.7)
CD4 cells per µL	417 (318.5-551.5)	400 (239-564)
Viral load, $\log_{10}$ c/ml	4.49 (3.96-5.28)	4.6 (4.2-5.1)
Any DRM	6 (8.6)[a]	9 (13.2)[b]
NRTI mutation	2 (2.9)	1 (1.5)
NNRTI mutation	3 (4.3)	5 (7.4)
PI mutation	1 (1.4)	3 (4.4)
PASER-Monitoring longitudinal study among patients receiving first-line ART		
Number of participants	203	221
Year of enrolment	2007	2007-2008
Site Type	Government HIV clinic	Government general hospital
Age, yrs	36.0 (29.2-40.9)	36.4 (31.2-42.8)
Sex, female	113 (55.7)	131 (59.3)
Initial ART regimens		
Zidovudine-containing	122 (60.1)	171 (77.4)
Tenofovir-containing	81 (39.9)	3 (1.3)
Stavudine-containing	0	47 (21.3)
CD4 count at ART initiation, cells per µL	136 (40-206)	128 (63-197)
Viral load at baseline, $\log_{10}$ c/ml	5.4 (5.0-5.9) $\log_{10}$ c/ml	4.6 (4.1-5.4)
Previous ARV exposure	15 (7.4)	10 (4.5)
VL > 400 cps/ml at 12 months	25 (14.3)[c]	21 (11.6)[d]
Any DRM at 12 months	17 (77.3)[e]	6 (46.2)[f]
NRTI mutation	12 (54.6)	4 (30.8)
NNRTI mutation	14 (63.6)	4 (30.8)
PI mutation	1 (4.5)	1 (7.7)
VL > 400 cps/ml at 24 months	25 (15.5)[g]	20 (13.6)[h]
Any DRM at 24 months	15 (75.0)[i]	12 (70.6)[j]
NRTI mutation	11 (55.0)	10 (58.8)
NNRTI mutation	14 (70.0)	12 (70.6)
PI mutation	1 (5.0)	0

Data are presented as n (%) or median (IQR). [a]For n=70 with available genotype; [b]For n=68 with available genotype; [c]For n=175 retained in care; [d]For n=181 retained in care; [e]For n=22 with available genotype; [f]For n=13 with available genotype; [g]For n=161 retained in care; [h]For n=147 retained in care; [i]For n= 20 with available genotype; [j]For n=17 with available genotype. PASER, PharmAccess African Studies to Evaluate Resistance; ART, antiretroviral therapy; ARV, antiretroviral; DRM, drug resistance mutations; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

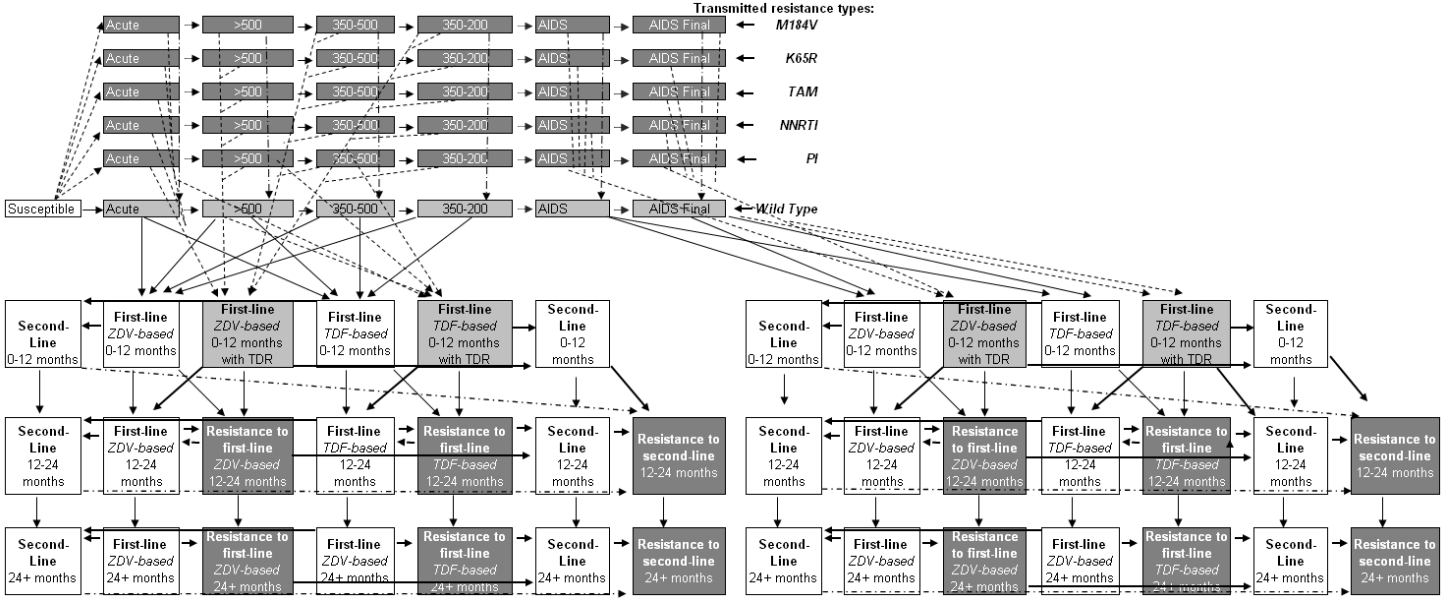


Figure 1. Structure of the compartmental deterministic model. Without treatment, infected patients progress through six stages: the acute stage, the chronic stage (divided into CD4 counts of >500, 350-500, and 200-350 cells per µL), an AIDS stage in which patients have a limited level of sexual activity and the final AIDS stage in which patients have no sexual activity. Individuals in the dark grey boxes are carrying a particular drug resistant virus and can pass on that virus to a susceptible individual. Individuals in the light grey boxes (before treatment) can pass on a wild-type virus to a susceptible individual. Individuals on treatment have a reduced infectiousness and move through three different periods defined by the time since start of antiretroviral therapy and the disease stage in which treatment was started. All boxes have different mortality rates.

Table 2. Additional Model Parameters

Description	Estimate or Range*	Reference
Disease stages duration		25-26
Acute stage	10-16 weeks	
Chronic stage >500 cells per µL	0.87-1 year	
Chronic stage 350-500 cells per µL	2.9-3.1 years	
Chronic stage 200-350 cells per µL	3.6-3.9 years	
AIDS stage**	6-12 months	
Final AIDS stage**	7-13 months	
Infectivity		14-15
Acute stage	27-43 x that of chronic stage	
Chronic stage (all)	10% per year	
AIDS stage**	3-5 x higher than chronic stage	
Final AIDS stage**	0%	
Proportion of people in sexual risk groups		Model Calibration
Highest	1.5-2.5%	
2nd	10-20%	
3rd	20-30%	
Lowest	47.5-68.5%	
Number of partners per year in each sexual risk group		Model Calibration
Highest	9-14	
2nd	1.7-3	
3rd	0.12-0.22	
Lowest	0.04-0.06	
Mortality rates per year		27
Population	0.02	
Chronic HIV stage	0.098	
AIDS stage	0.63	
On treatment during chronic stage, first year	0.02-0.098	
On treatment during chronic stage, 12+ months	0.02-0.05	
On treatment during AIDS stage, first year	0.03-0.3	
On treatment during AIDS stage, 12+ months	0.03-0.06	
HIV Test Rate		
Baseline	10-30%	Model Calibration
Rate of being tested in the acute stage of HIV	Half of the test rate	Assumption***
Rate of being tested in the chronic stage of HIV	test rate	Model Calibration
Rate of being tested in the AIDS stage	test rate + 10%	
Linkage to care from test to treat	75-100%	Model Calibration
Reduction in transmissibility of those patients on treatment	90-100%	2, 28-29
Reduction in transmissibility of those patients on treatment with a transmitted drug resistance virus	80-100%	Assumption

Table 2 (continued)

Description	Estimate or Range*	Reference
Percentage of people that go to second line after continued virological failure Kampala, Uganda, on zidovudine-based regimen on tenofovir-based regimen Mombasa, Kenya, on zidovudine/stavudine-based regimen	33-50% 33-66% 15-30%	PASER-Monitoring, Kampala PASER-Monitoring, Mombasa
Percentage of those who go onto second-line not due to resistance in the first 12 months	1.5-3%	30

*All ranges are uniformly distributed
**Two AIDS stages were included because during the final months before death, patients have limited sexual activity
*** Due to window phase of antibody-based test

thymidine analogue mutations [TAMs]), for lamivudine and emtricitabine (i.e. M184V), and as well as efavirenz and nevirapine (i.e. NNRTI-specific mutations). Individuals were classified as receiving either a zidovudine-containing or tenofovir-containing regimens, and within these regimens, there were different probabilities of acquiring the signature mutations of zidovudine/tenofovir as well as the M184V or NNRTI-specific mutations. The model also included second-line treatment which consisted of a ritonavir-boosted protease inhibitor (PI) in combination with two nucleoside reverse transcriptase inhibitors (NRTIs). Each different regimen (zidovudine-, tenofovir-, or PI-based) was assumed to have a different likelihood of transmitting acquired drug resistant mutations onwards to a susceptible individual.[20] In our model, those with an acquired drug resistant virus could either pass on a NRTI mutation (divided further into a TAM, M184V, or K65R), NNRTI mutation, or PI mutation. A specific fitness cost, estimated by a reduction in replication capacity, was included for each individual mutation.[20] Reversion to wild-type after infection with a drug resistant virus was also considered.

As a baseline scenario, we calculated the TDR prevalence for ART initiation at <200 CD4 cells per µL (including 20% of patients with CD4 200-350 cells per µL). We then predicted the effect on TDR prevalence of ART initiation at CD4 <350 and <500 cells per µL. The total number of people accessing treatment depends on the HIV testing and retention rate, both of which were calibrated in the model. We also investigated the impact of increasing access to second-line therapy. We expected an increased availability of second line regimens and viral load testing in the future. Accordingly, we increased the proportions of people who switch to second-line therapy after continued virological failure in Kampala and Mombasa from 33-66% and 15-30%, respectively, to 80-100%. We then determined the overall and mutation-specific TDR prevalence over 10 years for all immunological ART initiation cut-offs.

In order to investigate whether the prevention of new infections by earlier initiation of ART offsets rising TDR, we calculated the total number of new HIV infections averted for each additional case infected with drug resistant HIV.

RESULTS

Impact of ART on the HIV epidemic

Figure 2A shows the effect of earlier first-line ART initiation on estimated HIV prevalence in Kampala and Mombasa. Although the HIV prevalence remains relatively stable when treatment is initiated at <200 or <350 CD4 cells per µL due to reduced mortality of infected individuals, a decline in HIV prevalence is expected when treatment is initiated at <500 CD4 cells per µL. HIV incidence is expected to drop in both cities when treatment is initiated at <350 and <500 CD4 cells per µL (Figure 2B). The decrease in HIV incidence is also reflected in the proportion of infections that can be averted at particular immunological

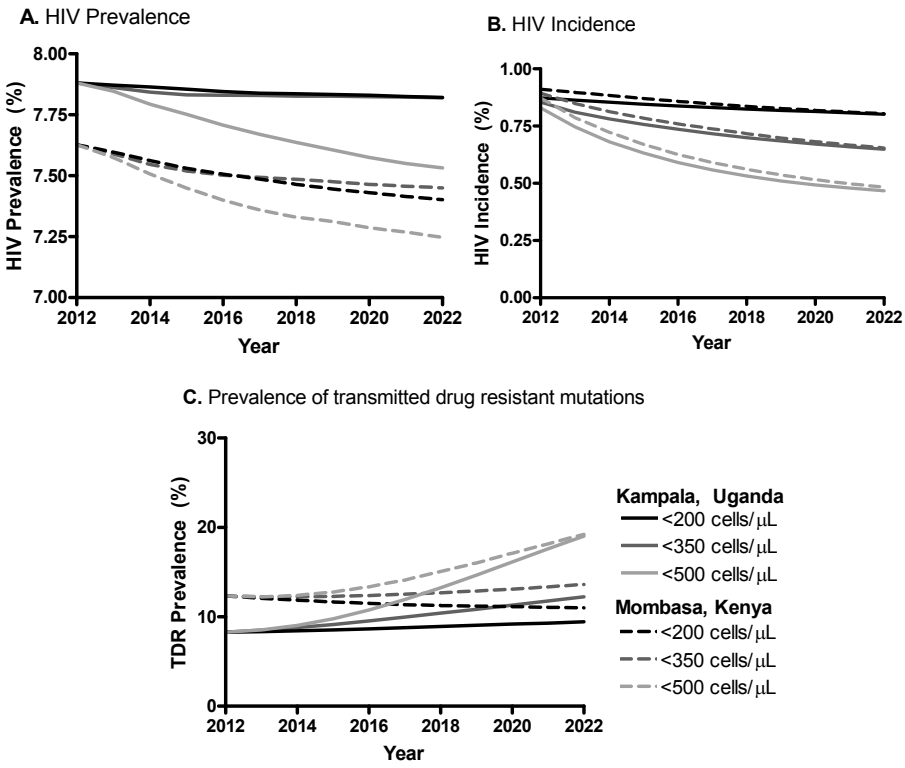


Figure 2. HIV Prevalence, incidence, and overall transmitted drug resistance prevalence from 2012-2022 when initiating treatment at CD4<200 cells per µL, <350 cells per µL, and <500 cells per µL in Kampala, Uganda, and Mombasa, Kenya.

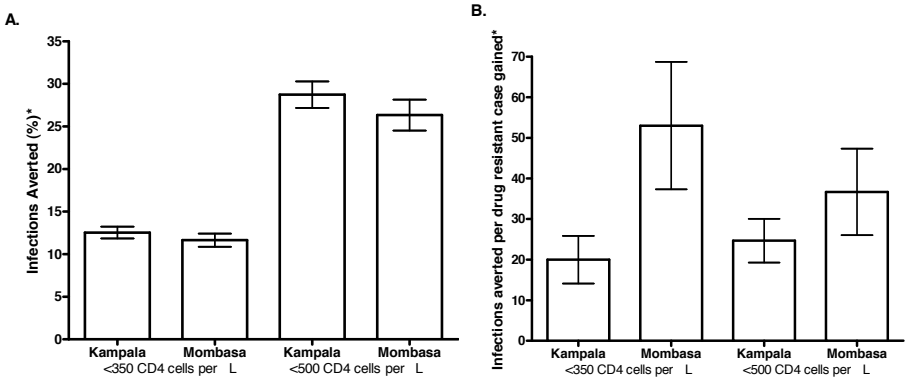


Figure 3. (A) Infections averted (%), in Kampala, Uganda, and Mombasa, Kenya compared to initiating treatment at CD4 <200 cells per µL. (B) Infections averted per additional drug resistant case in Kampala, Uganda, and Mombasa, Kenya compared to initiating treatment at CD4 <200 cells per µL.
*Error bars represent the inter-quartile range.

thresholds of ART initiation. Compared to initiating ART at CD4 <200 cells per µL, initiating ART at CD4 <350 cells per µL averts 12.6% (Inter-quartile range (IQR) 11.3%-13.7%) of infections over 10 years in Kampala and averts 11.6% (IQR 10.3%-13.0%) of infections in Mombasa. Initiating ART at CD4 <500 cells per µL averts 28.8% (IQR 26.0%-31.4%) and 26.3% (IQR 23.2%-29.5%) in Kampala and Mombasa, respectively (Figure 3A).

Prevention of new infections and increase of TDR

When treatment is initiated at CD4 <350 cells per µL, 18 (IQR 11-31) infections in Kampala and 46 (30-83) infections in Mombasa will be averted for every additional case infected with drug resistant virus. Similarly, when treatment is initiated at CD4 <500 cells per µL, the estimated number of infections averted per additional case of TDR will be 22 (17-35) in Kampala and 32 (IQR 21-57) in Mombasa (Figure 3B). The larger number of infections averted in Mombasa as compared to Kampala is in line with the smaller TDR increase predicted in Mombasa.

Evolution of overall TDR prevalence

Figure 2C shows that expanding access to ART by initiating treatment at higher CD4-counts is expected to increase TDR prevalence in both Kampala and Mombasa. Between 2012 and 2022, the estimated TDR prevalence in Kampala increases from 8.3% (IQR 7.7%-9.0%) to 9.4% (IQR 8.4%-10.5%), 12.2% (IQR 10.9%-13.8%) and to 19.0 % (IQR 16.5%-21.8%) when initiating ART at CD4<200, <350 or <500 cells per µL, respectively. During the same period, the estimated TDR prevalence in Mombasa remains 12.3% (IQR 11.7%-13.1%) when starting ART at CD4 <200 cells per µL, but increases to 13.6% (IQR 12.5%-14.9%) when starting at CD4 <350 cells per µL and to 19.2% (IQR 17.1%-21.5%) when starting at CD4 <500 cells per µL.

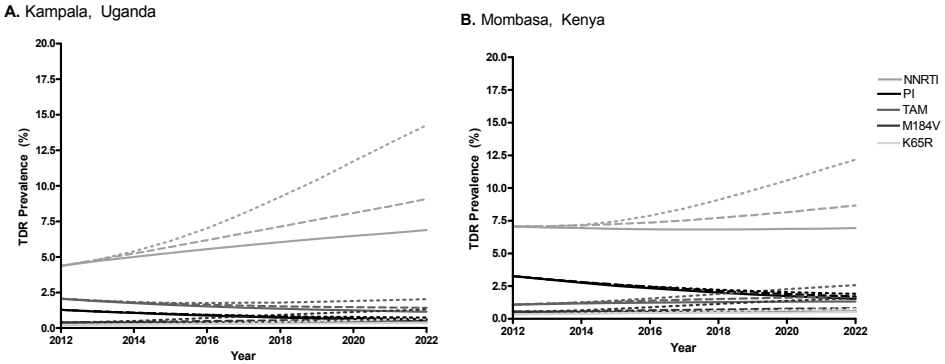


Figure 4. Transmitted drug resistant mutation prevalence by mutation, from 2012-2022 when starting treatment at CD4<200 cells per µL, <350 cells per µL, and <500 cells per µL in Kampala, Uganda, and Mombasa, Kenya. Solid line: treatment initiated at CD4 <200 cells/µL. Dashed line: treatment initiated at **CD4 <350** cells/µL. Dotted line: treatment initiated at CD4 <500 cells/µL.

Drug Resistance by drug class and mutation

In both settings, current TDR is predominantly characterized by resistance to NNRTIs. According to our modeling results, NNRTI mutations are predicted to continue to dominate the future prevalence of TDR (Figure 4A and B). In Kampala, the prevalence of transmitted NNRTI resistance is estimated to rise over the next ten years from 4.4% (IQR 3.8%-4.9%) to 6.9% (IQR 6.0%-7.9%) when initiating at CD4 <200 cells per µL. An increase to 9.1% (IQR 7.8%-10.5%) when initiating treatment at CD4 <350 cells per µL is predicted, and to 14.3% (IQR 11.9%-16.6%) when initiating at CD4 <500 cells per µL. In Mombasa, NNRTI resistance is estimated to remain stable (7.1%; IQR 6.3%-7.7% in 2012 to 6.9%; IQR 6.0%-7.9% in 2022) when initiating ART at CD4 <200 cells per µL, but to increase to 8.7% (IQR 7.6%-9.7%) when initiating at a CD4 count of <350 and to 12.2% (IQR 10.9%-13.7%) when starting at <500. With respect to the NRTI class, in Kampala, estimated prevalence of TAMs decrease between 2012 and 2022, regardless of the immunological threshold used. In contrast, the TAM prevalence in Mombasa is estimated to slightly increase over the same period. A sensitivity analysis in which stavudine was replaced by tenofovir instead of zidovudine yielded similar results (data not shown). Transmitted M184V increases in both Kampala and Mombasa when treatment is initiated at CD4 <500 cells per µL, although the prevalence was predicted to remain <2% in both areas. In both cities, PI resistance decreases over time, irrespective of immunological threshold used to initiate therapy. Transmitted K65R will increase slightly at all immunological thresholds but will remain <1% between 2012 and 2022 in both settings.

Increasing access to second-line

If access to virological monitoring and second-line treatment increases in parallel to the further scale-up of first-line ART, TDR is not expected to increase at any of the immuno-

logical thresholds used for ART initiation. In the scenario where 80-100% of individuals with continued virological failure are appropriately switched to second-line boosted PI therapy, the level of TDR is expected to decline in both locations at all immunological thresholds to below the 2012 TDR level. Increasing access to boosted PIs will either reduce or stabilize TDR for all drug classes (Figure 5A-D).

DISCUSSION

We have modeled the impact of ART initiation at different immunological thresholds on the prevalence of TDR in two East African settings over the next ten years. This is the first model to show that the benefits of early treatment initiation outweigh the risk of increased TDR. We predict that the number of infections that will be averted by earlier ART initiation will far exceed the number of infections with a drug resistant virus.

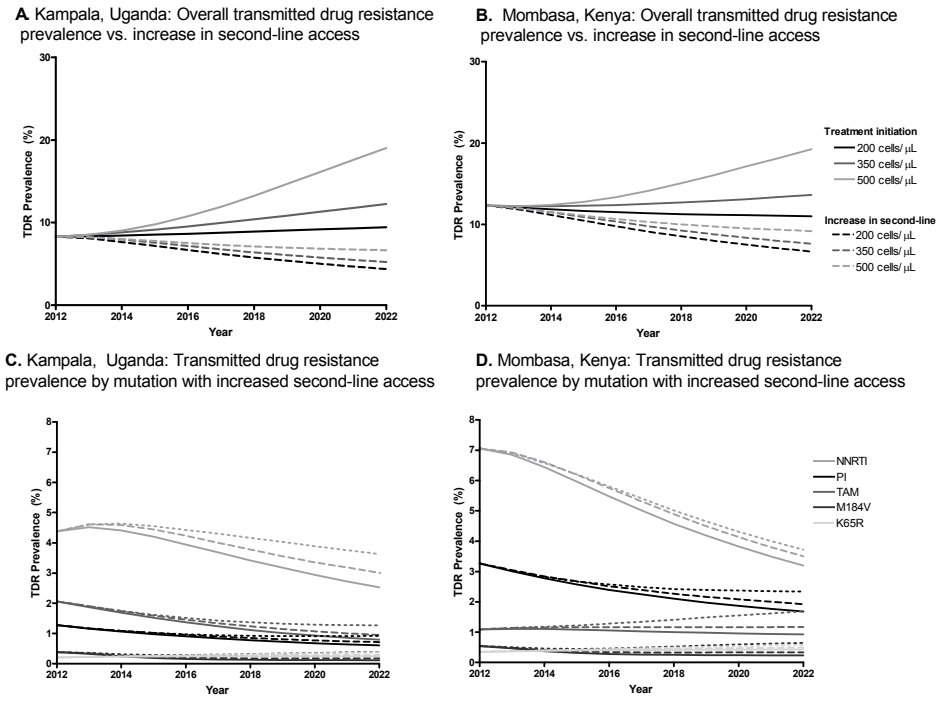


Figure 5. Increasing access to second-line treatment for those with continued virological failure to 80-100% from 2012-2022 when starting treatment at CD4<200 cells per µL, <350 cells per µL, and <500 cells per µL in Kampala, Uganda, and Mombasa, Kenya. Solid line: treatment initiated at CD4 <200 cells/µL. Dashed line: treatment initiated at CD4 <350 cells/µL. Dotted line: treatment initiated at CD4 <500 cells/µL.

When the current WHO treatment guidelines of ART initiation at CD4<350 cells per µL are implemented, TDR prevalence is expected to increase slightly. Expanding treatment by initiating ART at CD4 <500 cells per µL will lead to an increasing TDR prevalence. TDR mutations associated with the NNRTI drug class, the cornerstone of current first-line regimens,[21] are expected to drive the TDR rise. Importantly, if switches from first- to second-line occur for all patients necessitating a switch, then the overall TDR prevalence, including NNRTI resistance, will decrease in the next ten years. This implies that wider access to virological monitoring and boosted PIs for second-line therapy will preserve the effectiveness of NNRTI-based first-line treatment in all scenarios.

Current and predicted TDR is predominantly due to NNRTI resistance. This finding corroborates a recent meta-analysis of TDR which estimated an increase of predominantly NNRTI-related resistance of 29% per year in East Africa.[4] This can be explained by the low genetic barrier of NNRTIs for HIV drug resistance as only a single amino acid substitution is sufficient for high level resistance.[22] In addition, transmitted NNRTI-associated mutations persist for a prolonged period of time.[23] Transmission of NNRTI resistance can have important clinical ramifications as their presence is associated with an increased risk of virological failure of standard first-line treatment and for further selection of drug resistance after treatment initiation.[3] Our modeling study suggests that this can be prevented by increasing access to boosted PIs in second-line ART. Timely switches to second-line regimens are only possibly when routine virological monitoring, i.e. at 6- or 12-monthly intervals, is implemented. In agreement, a recent model of HIV transmission predicted that routine virological monitoring in patients on ART can reduce TDR.[7] For this purpose, cheap point-of-care viral load assays should be developed.[4, 7]

Our mathematical model has several strengths. To our knowledge, our model is the first to examine the impact of initiating ART at different immunological thresholds, including at CD4 <500 cells per µL, on the prevalence of TDR in sub-Saharan Africa. This is particularly relevant in light of increased interest for early initiation of ART as a means to prevent new infections. We have demonstrated that the number of new infections prevented by earlier ART initiation far outweighs the expected number of infections with drug resistant virus. Second, our analysis predicts future levels of HIV drug resistance in sub-Saharan Africa in terms of the presence of specific mutations to particular drugs, accounting for variation in transmissibility between individual mutations. A small number of mathematical HIV transmission models have examined the impact of antiretroviral drugs on transmitted drug resistance in sub-Saharan Africa.[7-10] Almost all previous models used overall TDR rates to describe HIV drug resistance.[8-10] One model used a classification of HIV drug resistance similar to ours, but only reported overall TDR.[7] Lastly, this model combines data on transmitted and acquired HIV drug resistance from the

same geographic areas and time period, collected within the same research project. This is important as resistance acquired during treatment constitutes the pool of variants that can be transmitted within a population. The TDR predictions were largely similar for the two distinct geographic settings even though these two settings have a different history of antiretroviral roll-out. In Uganda, antiretroviral drugs became available at least five years ahead of neighboring countries, including limited-scale distribution of mono and dual NRTI-based therapies.[24] This may account for the higher initial rate of TAMs observed in Kampala, but our analysis shows that it is unlikely to impact future rates of TDR.

This study has some potential limitations. First, the data on acquired resistance do not exceed 24 months of follow-up. Data on HIV drug resistance beyond 24 months of ART in resource-limited settings is scarce. Nonetheless, the PASER-Monitoring study provides the most accurate empirical data on acquired resistance patterns currently available in Africa. Second, we did not incorporate the type of ART monitoring to guide switching, i.e. clinical, immunological or virological, as a variable in our model. Instead, based on PASER-Monitoring data we noted that in Kampala 33-66% and in Mombasa 15-30% of patients with virological failure were appropriately switched to second-line. Third, baseline HIV test rates were assumed to be 10-30% of the populations. Increasing HIV testing uptake is likely to lead to greater numbers of people initiating ART at higher CD4 counts, although this poses logistical challenges for implementation. Fourth, we assumed that drug regimen use would remain constant for the next ten years, as it is difficult to make predictions about future drug substitutions. We did, however, account for the fact that stavudine is likely to be phased out. We assumed that stavudine would be replaced by zidovudine in Mombasa, or instead by tenofovir in a sensitivity analysis, with comparable results.

In conclusion, earlier ART initiation will lead to increased TDR, but this is not expected to offset the preventative benefits in controlling the HIV epidemic. Transmitted NNRTI resistance can potentially have a profound impact on the effectiveness of first-line treatment. Importantly, we have demonstrated that further increase of NNRTI resistance can be avoided by increasing access to second-line boosted PI regimens. Therefore, as ART is scaled-up, efforts should be made to make virological monitoring and effective second-line therapy available. Transmitted drug resistance should not be a reason to withhold early initiation of ART and the use of treatment as prevention in sub-Saharan Africa as the benefits of antiretroviral drugs are predicted to far outweigh the risk of increased transmitted resistance.

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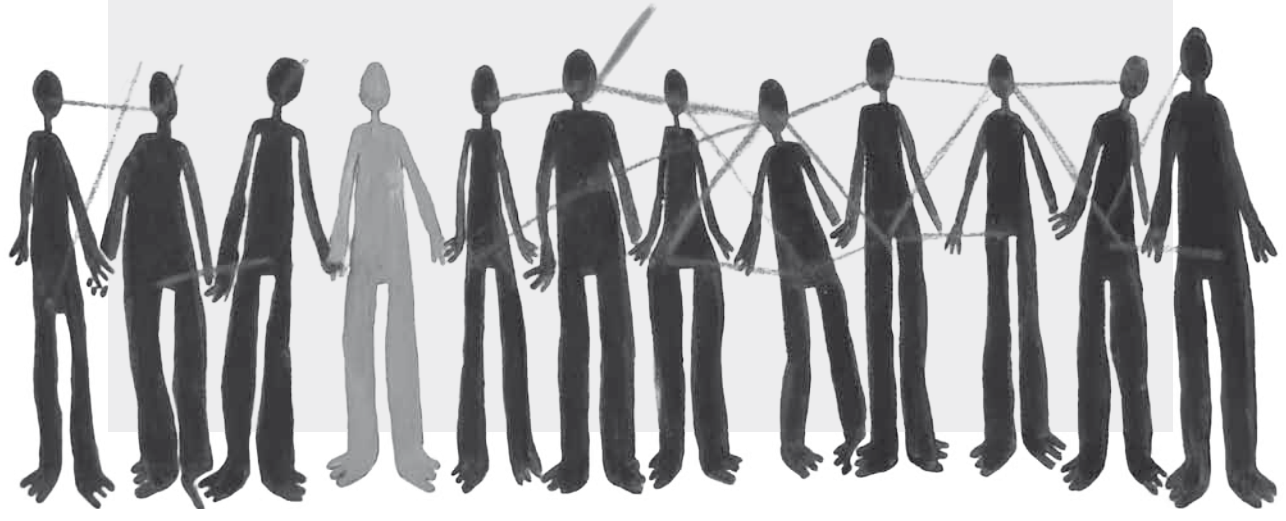
Part 2

Acquired HIV drug resistance in persons failing first-line antiretroviral therapy



Chapter 5

Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa

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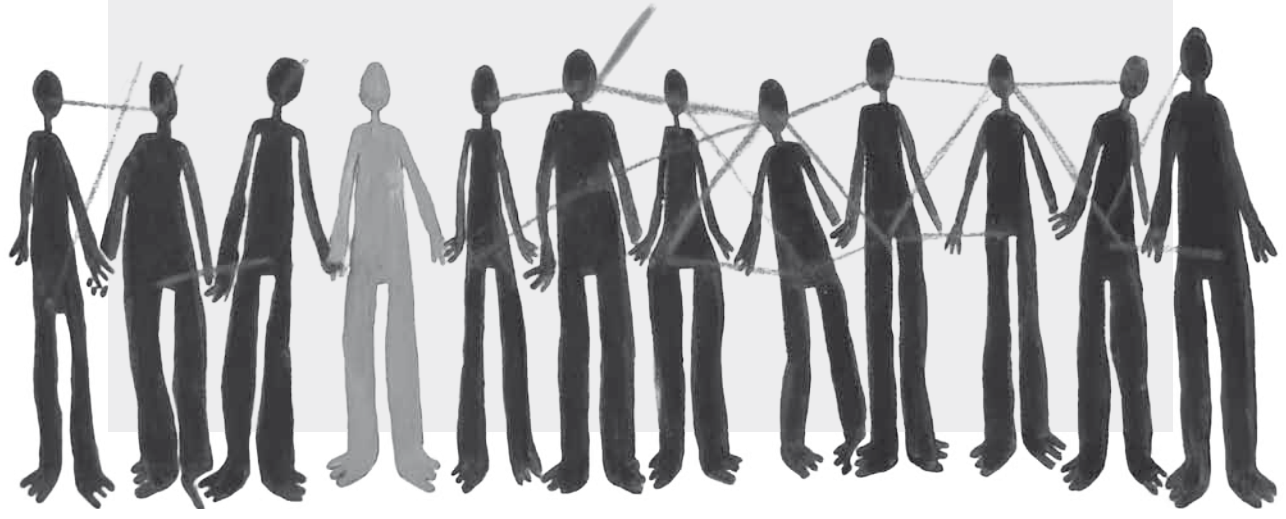
Journal of Acquired Immune Deficiency Syndromes 2011; 58(1): 23–31



ABSTRACT

Objectives

This study aimed to investigate the consequences of using clinico-immunological criteria to detect antiretroviral treatment (ART) failure and guide regimen switches in HIV-infected adults in sub-Saharan Africa. Frequencies of unnecessary switches, patterns of HIV drug resistance, and risk factors for the accumulation of nucleoside reverse transcriptase inhibitor (NRTI)–associated mutations were evaluated.

Methods

Cross-sectional analysis of adults switching ART regimens at 13 clinical sites in 6 African countries was performed. Two types of failure identification were compared: diagnosis of clinico-immunological failure without viral load testing (CIF only) or CIF with local targeted viral load testing (targeted VL). After study enrollment, reference HIV RNA and genotype were determined retrospectively. Logistic regression assessed factors associated with multiple thymidine analogue mutations (TAMs) and NRTI cross-resistance (≥2 TAMs or Q151M or K65R/K70E).

Results

Of 250 patients with CIF switching to second-line ART, targeted VL was performed in 186. Unnecessary switch at reference HIV RNA <1000 copies per milliliter occurred in 46.9% of CIF only patients versus 12.4% of patients with targeted VL ($P < 0.001$). NRTI cross-resistance was observed in 48.0% of 183 specimens available for genotypic analysis, comprising ≥2 TAMs (37.7%), K65R (7.1%), K70E (3.3%), or Q151M (3.3%). The presence of NRTI cross-resistance was associated with the duration of ART exposure and zidovudine use.

Conclusions

Clinico-immunological monitoring without viral load testing resulted in frequent unnecessary regimen switches. Prolonged treatment failure was indicated by extensive NRTI cross-resistance. Access to virological monitoring should be expanded to prevent inappropriate switches, enable early failure detection and preserve second-line treatment options in Africa.

INTRODUCTION

Over the past decade, there has been an unparalleled effort to provide access to antiretroviral treatment (ART) for HIV-infected individuals in sub-Saharan Africa, the region with the highest HIV burden.[1] Although short-term outcomes of first-line ART have been favorable,[2] extended follow-up data are still limited. A proportion of patients receiving ART will inevitably experience treatment failure, putting them at increased risk of HIV-related morbidity and mortality. Recent guidance from the World Health Organization (WHO) recommends viral load determination, if feasible, to improve the identification of treatment failure.[3] Due to financial and logistical constraints in resource-limited settings, however, access to this expensive and technically demanding test is limited. Therefore, as a substitute, WHO-recommended clinical criteria and CD4 cell counts are commonly used by clinicians to diagnose ART failure and guide switches to second-line regimens.

Several studies in African countries, however, have shown poor association of clinico-immunological criteria with virological failure in patients on first-line ART.[4–7] In absence of viral load testing, incorrectly diagnosed or presumed virological failure may result in inappropriate switches to more expensive and toxic second-line regimens. Additionally, delayed failure detection and continuation of a failing regimen can result in the selection of viruses with extensive resistance to antiretroviral (ARV) drugs.[8] In particular, the accumulation of mutations associated with cross-resistance within the nucleoside reverse transcriptase inhibitor (NRTI) drug class may compromise the effectiveness of standard second-line regimens, which are based on a dual backbone of new or recycled NRTIs and a ritonavir-boosted protease inhibitor (PI).

This multicenter study, conducted in a collaborative network of 13 ART sites in 6 African countries,[9] aimed to investigate the consequences of using clinico-immunological criteria to detect treatment failure and guide regimen switching. To this end, we sought to evaluate frequencies of unnecessary switches to second-line regimens, patterns of HIV drug resistance in patients failing first-line ART, and risk factors for the accumulation of NRTI-associated mutations.

METHODS

Study Design and Population

The PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) study is a multicenter prospective observational cohort of HIV-1–infected adults who receive ART in routine circumstances at 13 clinical sites in Kenya, Nigeria, South Africa, Uganda,

Zambia, and Zimbabwe. Collaborating sites were selected to represent a variety of ART programs in terms of site administration (government, nongovernment, and private), ART experience, and geography. Sites were considered eligible to collaborate in PASER-M if they had accruing ART programs and minimum standards of administration and laboratory capacity. Eight sites had access to viral load testing, whereas 5 sites used clinico-immunological monitoring only. Cohort and site characteristics have been profiled previously.[9] The study was approved by local Ethics Committees and the Academic Medical Center Institutional Review Board. Written informed consent was obtained from all participants before the start of study procedures.

For the current cross-sectional analysis, PASER-M study participants were included if they were switched to second-line ART by their treating clinician, regardless of whether clinical, immunological, and/or virological criteria had been used to diagnose treatment failure. Exclusion criteria were a positive pregnancy test at study screening, and, in Nigeria, HIV-2 co-infection. We compared patients based on the type of failure identification used by the treating clinician: diagnosis of CIF in absence of viral load testing ("CIF only" group) or CIF with local targeted viral load (VL) testing ("targeted VL" group). In the CIF only group, the clinician's decision to switch was based on a new WHO clinical stage 3 or 4 condition or immunological deterioration, as defined by a CD4 cell count fall to pretreatment value, CD4 cell count decrease of 50% or persistent CD4 levels <100 cells per cubic millimeter. In the targeted VL group, treating clinicians had access to a local real-time HIV RNA test result to confirm suspected treatment failure based on clinical and immunological information. Drug changes because of side effects or toxicity were not considered regimen switches and were excluded from analysis. Demographic and clinical information were collected using standard case report forms.

Laboratory Procedures

Routine laboratory results including CD4 cell count and HIV RNA were obtained from local laboratory records. Before switch to second-line ART, an additional phlebotomy was performed and EDTA anticoagulated plasma specimens were stored at -80°C and batch-shipped to 2 reference laboratories in South Africa (Genotyping Unit, Department of Molecular Medicine and Hematology, University of the Witwatersrand) and Uganda (Genotyping Laboratory, Joint Clinical Research Centre) for retrospective determination of reference HIV RNA and genotypic resistance testing on all specimens with HIV RNA >1000 copies per milliliter. The Ugandan laboratory performed testing for the Ugandan sites and the South African laboratory performed testing for all other sites. Both laboratories participated in external quality assessment schemes for HIV RNA and genotypic testing. Tests performed at the reference laboratory are denoted "reference HIV-RNA" throughout this report, and results obtained from the clinic are referred to as "local HIV

RNA". For patients in the targeted VL group, both local and reference HIV RNA results were available. The reference HIV RNA was considered the gold standard.

The South African laboratory used the NucliSens EasyQ real-time assay version 2.0 (bioMérieux, Lyon, France) for reference HIV RNA determination and an in-house sequencing method encompassing the whole of protease and codons 1–300 of reverse transcriptase with an ABI Prism 3730 Genetic Analyzer Genetic Analyzer (Applied Biosystems, Foster City, CA).[10] Sequences were assembled and manually edited using Sequencher v4.8 (Genecodes, Ann Arbor, MI). The Ugandan laboratory used the COBAS Ampliprep/COBAS Taqman HIV-1 test (Roche, Branchburg, NJ) for HIV RNA determination and 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).[11] Sequences were assembled and manually edited using BioEdit version 7.0.9.0. All final sequences were submitted to the ViroScore database (Advanced Biological Laboratories SA, France) for quality control and data storage. Drug resistance mutations were scored according to the 2009 International AIDS Society–USA list of December 2009.[12] HIV with genetic mixtures of wild-type and mutant sequences at amino acid sites that code for drug resistance mutations were considered resistant. Subtypes were determined using the REGA HIV-1 subtyping algorithm version 2.0[13] and additional STAR genotype analysis if required.[14]

Statistical Analysis

An unnecessary switch to second-line ART was defined using 3 reference HIV RNA cutoffs: <400 copies per milliliter, <1000 copies per milliliter, and the WHO-recommended threshold of <5000 copies per milliliter.[3] The sensitivity, specificity, positive predictive ratio (PPV) and negative predictive values of the type of failure identification used (CIF only vs. targeted VL) were calculated with 95% confidence intervals using 2 ´ 2 contingency tables, compared with the reference HIV RNA load. The positive and negative likelihood ratios were calculated from the sensitivity and specificity. Patient characteristics were compared using the χ^2 test for categorical data and the Wilcoxon rank-sum test for continuous data. NRTI cross-resistance was defined as the presence of ≥2 thymidine analogue mutations (TAMs), the tenofovir (TDF)-associated mutations K65R or K70E, or the Q151M complex. Univariate and multivariate logistic regression was performed to identify factors associated with the following outcomes: ≥2 TAMs, NRTI cross-resistance, or selected single mutations. Explanatory variables included in the analysis were sex, age, type of failure identification (CIF only vs. targeted VL), WHO clinical stage, CD4 cell count, and HIV-RNA load at time of treatment failure, HIV-1 subtype, total duration of previous ARV exposure and type of prior non-nucleoside reverse transcriptase inhibitors (NNRTIs) or NRTIs. Explanatory variables that were associated with the outcome vari-

ables ($P < 0.10$) in univariate analysis were forwarded to a multivariate prediction model, using a step forward procedure. Results were expressed as odds ratios (ORs) with 95% confidence intervals and P values with $P < 0.05$ regarded statistically significant. Analyses were performed using the statistical software package Stata version 10 (StataCorp LP, TX).

RESULTS

Patient Characteristics

Between March 2007 and September 2009, 250 patients with CIF were switched to a second-line regimen (Figure 1). The treating clinician had diagnosed CIF only in 64 (25.6%) patients and used targeted VL testing in 186 (74.4%) patients. Patients originated from

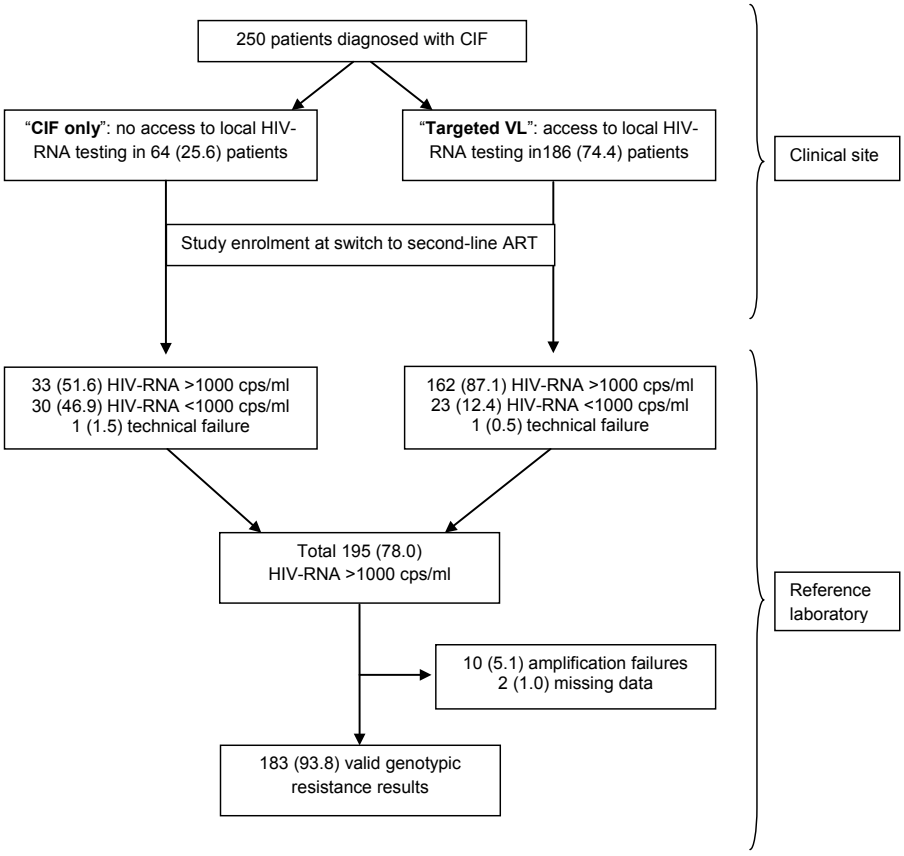


Figure 1. Flow chart of type of failure identification, study enrollment, and laboratory testing. Data are n (%) of patients. CIF, clinico-immunological failure; VL, viral load; ART, antiretroviral treatment.

Uganda (n = 78, 31.2%), South Africa (n = 66, 26.4%), Kenya (n = 37, 14.8%), Nigeria (n = 32, 12.8%), Zambia (n = 27, 10.8%), and Zimbabwe (n = 10, 4%). Patient characteristics at switch to second-line ART are summarized in Table 1. Advanced HIV disease (WHO clinical stage 3 or 4) and severe immunodeficiency (CD4 count <100 cells/mm^3) was more frequently observed in patients with CIF only (P = 0.007 and P = 0.021, respectively).

Table 1. Patient Characteristics at Switch to Second-Line Treatment, by Type of Identification of Failure

	Overall (n = 250)	CIF Only (n = 64)	Targeted VL (n = 186)	P*
Women (%)	125 (50)	36 (56.3)	89 (47.8)	0.246
Median age (IQR) (yrs)	38.4 (34.2–45.0)	39.5 (34.9–45.9)	38.0 (33.8–44.9)	0.305
Median duration of prior ART regimen (IQR) (months)	26.1 (14.3–43.7)	28.3 (17.0–47.4)	25.25 (13.9–43.2)	0.310
ART regimen at failure (%)				
ZDV + 3TC + NVP	67 (26.8)	15 (23.4)	52 (28.0)	0.016
ZDV + 3TC + EFV	40 (16.0)	9 (14.1)	31 (16.7)	
D4T + 3TC + NVP	52 (20.8)	22 (34.4)	30 (16.1)	
D4T + 3TC + EFV	47 (18.8)	10 (15.6)	37 (19.9)	
TDF + FTC + NVP	12 (4.8)	1 (1.6)	11 (5.9)	
TDF + FTC + EFV	12 (4.8)	0	12 (6.5)	
NNRTI-based, other	6 (2.4)	1 (1.6)	5 (2.7)	
Triple NRTI	4 (1.6)	3 (4.8)	1 (0.5)	
PI-based	7 (2.8)	1 (1.6)	6 (3.2)	
WHO clinical stage 3 (%)	78 (31.2)	27 (42.9)	51 (27.4)	0.031
WHO clinical stage 4 (%)	40 (16.0)	12 (19.1)	28 (15.1)	
Median CD4 cell count (IQR) (cells/mm^3)	124 (59.5–200)	119 (58–177)	125.5 (61.5–215)	0.195
<100 cells/mm^3 (%)	103 (41.5)	30 (46.8)	73 (39.7)	0.021
100–200 cells/mm^3 (%)	82 (33.1)	26 (40.6)	56 (30.4)	
>200 cells/mm^3 (%)	63 (25.4)	8 (12.5)	55 (29.9)	
Median HIV-1 RNA (IQR) ($\log_{10}$ copies/mL)	4.2 (3.3–5.0)	3.3 (1.4–4.4)	4.4 (3.7–5.0)	<0.001
<1000 copies/mL (%)	53 (21.2)	30 (46.9)	23 (12.4)	<0.001
1000–5000 copies/mL (%)	25 (10.0)	2 (3.1)	23 (12.4)	
5000–10000 copies/mL (%)	19 (7.6)	3 (4.7)	16 (8.6)	
>10000 copies/mL (%)	151 (60.4)	28 (43.8)	123 (66.1)	

Percentages are shown in parentheses and IQRs in square brackets.
*Categorical data were compared using the χ^2 test; continuous were compared using the Wilcoxon rank-sum test. 3TC, lamivudine; FTC, emtricitabine.

Table 2. Performance of CIF only* and CIF with targeted VL† criteria to identify virological failure at switch to second-line treatment

	Sensitivity (%)‡§	Specificity (%)‡§	PPV (%)‡§	NPV (%)‡§	PLR	NLR
Compared With WHO Definition for Regimen Switch (HIV RNA > 5000 Copies/mL)						
CIF only	18.2 (12.7 to 24.9) [31/170]	59.0 (47.3 to 70.0) [46/78]	49.2 (36.4 to 62.1) [31/63]	24.9 (18.8 to 31.7) [46/185]	0.4	1.4
Targeted VL	81.8 (75.1 to 87.3) [139/170]	41.0 (30.0 to 52.7) [32/78]	75.1 (68.3 to 81.2) [139/185]	50.8 (37.9 to 63.6) [32/63]	1.4	0.4
Compared With Reference Standard (HIV RNA > 1000 Copies/mL)						
CIF only	16.9 (11.9 to 22.9) [33/195]	43.4 (29.8 to 57.7) [23/53]	52.4 (39.4 to 65.1) [33/63]	12.4 (8.0 to 18.1) [23/185]	0.3	1.9
Targeted VL	83.1 (77.1 to 88.1) [162/195]	56.6 (42.3 to 70.2) [30/53]	87.6 (81.9 to 92.0) [162/185]	47.6 (34.9 to 60.6) [30/63]	1.9	0.3
Compared With stricter definition for virological failure (HIV RNA >400 Copies/mL)						
CIF only	16.7 (11.9 to 22.6) [34/203]	35.6 (21.9 to 51.2) [16/45]	54.0 (40.9 to 66.7) [34/63]	8.6 (5.0 to 13.7) [16/185]	0.3	2.3
Targeted VL	83.3 (77.4 to 88.1) [169/203]	64.4 (48.8 to 78.1) [29/45]	91.4 (86.3 to 95.0) [169/185]	46.0 (33.4 to 59.1) [29/63]	2.3	0.3

*CIF only: CIF diagnosis based on clinical (WHO clinical staging) and/or immunological (CD4+ cell count) parameters. †Targeted VL: targeted viral load testing used by treating clinician in decision to switch to second-line treatment. ‡Numbers in parentheses refer to the 95% confidence interval.
§Numbers in square brackets refer to the numerator and denominator from which the relevant percentages are calculated. PLR, positive likelihood ratio; NPV, negative predictive value; NLR, negative likelihood ratio; PPV, positive predictive value.

The median reference HIV RNA $\log_{10}$ level was 3.3 [interquartile range (IQR): 1.4–4.4] in patients with CIF only and 4.4 (IQR: 3.7–5.0) in patients with targeted VL ($P < 0.001$).

The median duration of the first-line ART was 28.3 months in patients with CIF only and 25.3 months in patients with targeted VL ($P = 0.310$). Most patients (n = 236, 95.5%) used a NNRTI triple regimen at time of treatment failure. Others had received PI-based regimens (n = 7, 2.8%) or triple nucleoside regimens (n = 4, 1.6%). The most frequently used NRTI was zidovudine (ZDV, n = 107, 43.3%), followed by stavudine (D4T, n = 99, 40.1% and TDF (n = 24, 9.7%). The prior use of D4T was more common in patients with CIF only ($P = 0.049$), and TDF was used more frequently in patients with targeted VL ($P = 0.011$). In addition to first-line ART, 9 (4.8%) patients with targeted VL had a history of non-suppressive ARV use, either as monotherapy (n = 2) or dual therapy (n = 3) or for the prevention of mother to child transmission (n = 4).

Diagnostic Performance of Treatment Failure Criteria

The median difference between the local and reference HIV RNA was 0.032 $\log_{10}$ copies (IQR: -0.23 to 0.52, $P = 0.0101$). Table 2 summarizes the performance of CIF only and targeted VL criteria to identify virological failure. Using the HIV RNA cut-off of <5000

copies per milliliter, unnecessary switches occurred in 78 (31.2%) patients: 32 (50.0%) in the CIF only group versus 46 (24.7%) in the targeted VL group ($P < 0.001$). CIF only criteria had a sensitivity of 18.2%, a specificity of 59%, and a PPV of 49.2%. Using the HIV RNA cut-off of <1000 copies per milliliter, unnecessary switches occurred in 53 (21.2%) patients: 30 (46.9%) in the CIF only group versus 23 (12.4%) in the targeted VL group ($P < 0.001$). The CIF only criteria had a sensitivity of 16.9%, specificity 43.3%, and PPV of 52.4%. Applying a more stringent HIV RNA cut-off of <400 copies per milliliter, unnecessary switches occurred in 45 (18.1%) patients: 29 (46.0%) in the CIF only group versus 16 (8.6%) the targeted VL group ($P < 0.001$).

Genotypic Analysis

Of the 195 specimens with HIV-1 RNA >1000 copies per milliliter, 183 (93.8%) valid genotypic test results were available (Figure 1). The HIV-1 subtype distribution was C (n = 82, 44.8%), A (n = 45, 24.6%), D (n = 28, 15.3%), G (n = 14, 7.7%), CRF02_AG (n = 12, 6.6%), and other (n = 2, 1.2%). Resistance profiles are listed in Table 3. At least 1 drug resistance mutation was present in 161 of 183 (88.0%) specimens; 149 (81.4%) harbored dual-class and 3 (1.6%) triple-class resistance. Wild-type virus was detected in 7 (21.9%) patients in the CIF only group and 15 (9.9%) patients in the targeted VL group ($P = 0.059$). The most frequently observed mutation was the M184V/I (n = 150, 82.0%), followed by TAMs (n = 100, 54.6%). At least 2 TAMs were present in 69 (37.7%) specimens and 3 or more in 44 (24%). Both TAM pathways 1 and 2 were observed; the M41L was present in 40 (21.9%) specimens and the D67N in 46 (25.1%) specimens. The M184V/I mutation was combined with TAMs in 97 (53.0%) sequences. The K65R and K70E mutations were observed in 13 (7.1%) and 6 (3.3%) specimens, respectively. Three (1.5%) specimens harbored both the K65R and TAM(s). Six (3.3%) specimens harbored the Q151M complex, of which 3 with TAM(s) and 3 with the K65R. Overall, NRTI cross-resistance mutations were observed in 87 (48.0%) specimens. NRTI-associated mutational patterns did not differ by type of identification of failure (CIF only vs. targeted VL). The most frequent mutation conferring resistance to NNRTIs was the K103N (n = 73, 39.9%), followed by the Y181C/V (n = 57, 31.1%) and the G190A/S (n = 50, 27.3%). Major PI mutations occurred in 6 (3.3%) patients.

Factors Associated With Accumulation of Drug Resistance Mutations

Table 4 summarizes factors associated with the presence of ≥2 TAMs and NRTI cross-resistance. In univariate analysis, the presence of ≥2 TAMs was associated with HIV-1 subtype, the total duration of previous ARV exposure, a history of ZDV use in failing and/or previous regimens and ≥2 different NRTIs. In multivariate analysis, the association persisted for ZDV use (OR: 3.49, 95% CI: 1.46 to 8.32, $P = 0.005$) and the duration of previous ARV exposure (OR for >24 months 2.90, 95% CI: 1.05 to 8.00, $P = 0.040$; OR for >36 months 4.47, 95% CI: 1.89 to 10.59, $P = 0.001$). The presence of multiple TAMs was not

Table 3. Resistance patterns, by type of identification of failure

Resistance Pattern	Overall (n = 183)	CIF Only (n = 32)	Targeted VL (n = 151)	*P*
Any resistance mutation	161 (88.0)	25 (78.1)	136 (90.1)	0.059
NRTI and NNRTI mutations	149 (81.4)	23 (71.8)	126 (83.4)	0.126
NRTI, NNRTI, and PI mutations	3 (1.6)	1 (3.1)	2 (1.3)	0.440
Any NRTI mutation	155 (84.7)	24 (75.0)	131 (86.8)	0.093
M184V/I	150 (82.0)	23 (71.9)	127 (84.1)	0.102
M184V/I only	0			n/a
M184V/I + TAM	97 (53.0)	18 (56.3)	79 (52.3)	0.686
TAM-containing virus*	100 (54.6)	19 (59.4)	81 (53.6)	0.554
≥2 TAMs	69 (37.7)	14 (43.8)	55 (36.4)	0.437
≥3 TAMs	44 (24.0)	12 (37.5)	32 (21.2)	0.050
K65R	13 (7.1)	3 (9.4)	10 (6.6)	0.404
K65R + TAM	3 (1.5)	1 (3.0)	2 (1.2)	0.428
K70E	6 (3.3)	0	6 (4.0)	n/a
Q151M	6 (3.3)	2 (6.3)	4 (2.7)	0.282
Q151M + TAM	3 (1.5)	0	3 (1.9)	n/a
Q151M + K65R	3 (1.5)	2 (6.0)	1 (0.6)	0.075
NRTI cross-resistance‡	87 (48)	17 (53.1)	70 (46.4)	0.486
Any NNRTI mutation	155 (84.7)	24 (75.0)	131 (86.8)	0.093
K103N	73 (39.9)	6 (18.8)	67 (44.4)	0.007
V106A/M	17 (9.3)	0	17 (11.3)	n/a
Y181C/V	57 (31.1)	13 (40.6)	44 (29.1)	0.202
G190A/S	50 (27.3)	11 (34.4)	39 (25.8)	0.324
Any PI mutation	6 (3.3)	2 (6.3)	4 (2.7)	0.282

Data are n (%) of patients. ‡NRTI cross-resistance, defined as ≥2 TAMs or Q151M or K65R/K70E.
*Frequencies of individual TAMs: M41L, 40 (21.9); D67N, 46 (25.1); K70R, 39 (20.6); L210W, 17 (9.3); T215Y/F, 64 (34.9); K219Q/E, 37 (20.2). n/a, not applicable.

associated with sex, age, type of failure identification, WHO clinical stage, CD4 count, HIV RNA load, or TDF use.

The presence of NRTI cross-resistance was univariately associated with HIV RNA load, duration of ARV exposure, ≥2 different NRTIs, ZDV and TDF use, and CD4 count. Multivariate analysis showed that the risk of NRTI cross-resistance was significantly increased for longer duration of previous ARV exposure (OR: for >36 months 3.95, 95% CI: 1.58 to 9.85, $P = 0.003$), ZDV use (OR: 2.66, 95% CI: 1.12 to 6.28, $P = 0.026$) and TDF use (OR: 5.00, 95% CI: 1.67 to 14.94, $P = 0.004$). The association with HIV RNA load was close to significance (OR: 1.57, 95% CI: 1.00 to 2.47, $P = 0.052$). NRTI cross-resistance was not associated with sex, age, type of failure identification, WHO clinical stage, or HIV-1 subtype.

Table 4. Factors associated with the accumulation of drug resistance mutations among patients failing first-line ART

	≥2 TAMs		NRTI Cross-Resistance	
	Univariate	Multivariate	Univariate	Multivariate
Sex				
Female	1.0	—	1.0	—
Male	1.17 (0.64 to 2.13)	—	1.16 (0.65 to 2.08)	—
Age (yrs)	1.02 (0.99 to 1.05)	—	1.01 (0.97 to 1.04)	—
Type of failure identification				
Targeted VL	1.0	—	1.0	—
CIF only	1.36 (0.63 to 2.94)	—	1.31 (0.61 to 2.82)	—
WHO clinical stage				
Stage I/II	1.0	—	1.0	—
Stage III/IV	0.99 (0.54 to 1.80)	—	1.23 (0.69 to 2.21)	—
CD4 count (cells/mm3)*	0.80 (0.61 to 1.06)	—	0.69 (0.53 to 0.92)	—
HIV RNA (log_{10})	1.09 (0.73 to 1.60)	—	1.60 (1.08 to 2.38)	1.57 (1.00 to 2.47)
HIV-1 subtype				
C	1.0	—	1.0	—
A	1.72 (0.81 to 3.65)	—	1.86 (0.89 to 3.87)	—
D	2.49 (1.03 to 5.97)	—	2.29 (0.95 to 5.52)	—
Other†	0.86 (0.34 to 2.21)	—	1.11 (0.47 to 2.66)	—
Duration of ARV use				
<24 months	1.0	1.0	1.0	1.0
24–36 months	2.97 (1.11 to 7.94)	2.90 (1.05 to 8.00)	1.6 (0.66 to 3.85)	1.99 (0.71 to 5.52)
>36 months	4.47 (1.95 to 10.23)	4.47 (1.89 to 10.59)	2.6 (1.27 to 5.31)	3.95 (1.58 to 9.85)
Thymidine analogue				
D4T	1.0	1.0	1.0	1.0
ZDV	3.02 (1.34 to 6.80)	3.49 (1.46 to 8.32)	2.43 (1.12 to 5.24)	2.66 (1.12 to 6.28)
TDF use				
No	1.0	—	1.0	1.0
Yes	0.75 (0.30 to 1.84)	—	4.19 (1.59 to 11.06)	5.00 (1.67 to 14.94)
Number of NRTIs used‡				
1	1.0	—	1.0	1.0
2 or more	2.71 (1.46 to 5.01)	—	2.83 (1.55 to 5.18)	1.09 (0.45 to 2.69)

Data are given as odds ratio (95% confidence interval). Outcomes include the emergence of ≥2 TAMs or NRTI cross-resistance, defined as: ≥ 2 TAMs or the Q151M complex or TDF-associated mutations (K65R/K70E). *OR for 100-cell increase of CD4 count. †Includes subtype G and circulating recombinant forms. ‡Excluding lamivudine or emtricitabine.

In multivariate analysis, the K65R mutation was associated with TDF use (OR: 14.33, 95% CI: 2.92 to 70.31, $P = 0.001$) and HIV RNA load (OR: 2.27, 95% CI: 1.02 to 5.08, $P = 0.045$), but not with D4T or HIV-1 subtype.

The NNRTI mutational profiles differed for patients failing efavirenz (EFV) versus nevirapine (NVP)-containing regimens. In univariate analysis, the use of EFV was associated with the K103N and V106A/M mutations, and NVP was associated with the Y181C/V. Multivariate analysis showed that EFV remained associated with the V106A/M (OR: 11.05, 95% CI: 2.39 to 50.98, $P = 0.002$) and NVP with the Y181C/V (OR: 28.5, 95% CI: 6.5 to 123.8, $P < 0.001$).

DISCUSSION

This multicenter observational study in 6 African countries investigated consequences of the common practice of using clinico-immunological criteria to detect ART failure and subsequently guide regimen switches. Without access to viral load testing, 47% of patients was switched to second-line ART inappropriately, at an HIV RNA below 1000 copies per milliliter. Targeted viral load testing to confirm treatment failure reduced unnecessary switches nearly 4-fold. A high frequency (88%) of clinically significant mutations was observed after first-line failure, suggesting late failure detection. Mutations associated with cross-resistance to NRTIs were observed in 48% of patients, comprising multiple TAMs (37.7%), K65R (7.1%), K70E (3.3%), or Q151M (3.3%). Accumulation of TAMs and NRTI cross-resistance were both associated with the duration of previous ARV exposure and ZDV use, and NRTI cross-resistance was additionally associated with TDF use.

Our data provide evidence that the use of clinico-immunological criteria to guide regimen switching has far-reaching public health implications. Treatment switches in patients who do not experience virological failure will result in mounting treatment costs and exhaust drug options. Additional concerns are the potential drug toxicity associated with second-line drugs and the costs of monitoring for adverse effects. This has particularly serious consequences in African countries where access to second-line regimens is limited. Moreover, continuation of treatment regimens in patients with virological failure will compromise their immunological and clinical status and, because of ongoing viral replication, result in the selection of viruses with accumulated resistance mutations. The effectiveness of future regimens, especially of those including NRTI backbones, is likely to be impaired.

An important strength of the study was the inclusion of a large international sample of patients diagnosed with treatment failure at different types of clinics, representative of the current clinical practice in many African ART programs. A limitation related to the cross-sectional design is that the prevalent cases identified in this study may not be representative of all incident cases with virological and/or CIF. For example, individuals who died before the study or in whom failure was not yet diagnosed could not be included. Therefore, care should be taken when extrapolating the study results. Unfortunately, information about the duration of treatment failure or possible previous periods of clinical immunological of virological failure was not available. Additionally, historical information about prior ARV use was collected retrospectively and might have been incomplete due to limitations in recall.

Detection of Treatment Failure

The poor diagnostic performance of clinical and immunological criteria for the identification of virological failure observed in this study confirms previous reports.[4–7] The PPV of CIF only criteria was somewhat higher in this study, which is explained by the fact that the PPV is dependent on the prevalence of treatment failure in the sampled population,[15] which was considerably higher in our study sample that consisted of patients who were switched to second-line ART, compared with the general population of patients receiving first-line ART. Our study underscores the importance of targeted viral load testing to maximize the clinical benefits of first-line regimens and prevent unnecessary switches to expensive second-line ART. This approach is in line with new WHO guidelines[3] and also corroborates findings of a recent study in India in which targeted viral load testing prevented unnecessary switch, at an HIV RNA <400 copies per milliliter, in nearly 25% of patients.[16] Taking into account that the cost of second-line regimens is currently up to 10 times higher than the cost of first-line ART, and that in our study approximately half of individuals with CIF only were truly failing, confirmation of failure by a viral load test before switching is likely to be cost saving. As simplified and more affordable methods of HIV RNA determination are being developed, the cost-effectiveness of this strategy is expected to increase further.

Despite access to local HIV RNA testing, 12% of patients were switched to second-line ART at a reference HIV RNA below 1000 copies per milliliter. The local HIV RNA had higher average values than the reference HIV RNA. This could be explained by differences between HIV RNA assays[17,18] and factors-related specimen storage and shipment which may account for the variation in results. Another reason for the disagreement might be due to reinforcement of drug adherence although awaiting the requested test results, resulting in a suppression of HIV RNA by the continued first-line ART regimen. A previous study has shown that resuppression can occur in up to 41% of patients with

viremia on NNRTI-based regimens.[19] Unfortunately, information on adherence or the time-lag between local HIV RNA testing and switch to second-line ART was not available in our study.

Drug Resistance Patterns and Predictors

The most frequently observed mutation in our study, the M184V, causes resistance to lamivudine and emtricitabine, but delays the emergence of mutations associated with ZDV and D4T.[20,21] Despite this increased susceptibility, we found high frequencies of multiple TAMs and 53% of patients harbored TAMs in combination with the M184V/I mutation. The rate of TAMs found in our study was higher than has previously been reported in similar population.[22] Furthermore, the considerable frequencies of K65R, K70E, and Q151M mutations observed are also likely to reflect a longer duration of treatment failure. Particularly, the Q151M complex usually requires a lengthy period of time to develop and confers broad NRTI cross-resistance.[23] K65R selection has been associated with higher levels of viral replication and lower CD4 cell counts, related to longer duration of virological failure.[24] TDF, and less frequently D4T, select for K65R[25–27], and in vitro studies have demonstrated its preferential acquisition in subtype C.[28,29] Our analyses indeed showed that K65R was significantly associated with HIV RNA load and TDF use but did not demonstrate an association with D4T use or HIV-1 subtype.

The presence of ≥2 TAMs was associated with ZDV but not with D4T use. This is in accordance with findings of a previous study from South Africa,[26] but differs from previous reports in subtype B.[30] Apart from genetic diversity, a possible explanation might be that short-term drug intolerance or toxicity is more frequently reported in relation to ZDV use, possibly leading to suboptimal adherence and prompting drug substitutions more often than in patients starting a D4T-based regimen.[31] Both TAM pathways 1 and 2 were observed, with the latter being slightly more common, which is congruent with previous reports from South Africa.[26,32]

Patterns of NRTI-associated mutations did not differ significantly according to the type of failure identification used. This is due to the fact that patients in the targeted VL group were also diagnosed with CIF and, consequently, failure was detected at a late stage. Indirect evidence for the accumulation of mutations due to late failure detection is provided by the significant associations of ≥2 TAMs and NRTI cross-resistance with the duration of previous ARV exposure. Also, HIV RNA load was marginally associated with NRTI cross-resistance.

NNRTI mutational patterns were in agreement with previous investigations[26,33] and varied depending on prior EFV or NVP use. EFV was associated with the V106A/M mutation,

whereas NVP selected for the Y181C/V mutation. Because only the Y181C/V reduces susceptibility to etravirine,[34] this finding is relevant if etravirine would be considered for future salvage therapy and would encourage the use of EFV over NVP as part of first-line regimens.

Implications for Clinical Management

The high frequency of mutations conferring NRTI cross-resistance detected in our study may have consequences for the effectiveness of the currently available second-line regimens in African countries. Although favorable initial response to second-line ART have been described in resource-limited settings despite resistance at time of regimen switch,[35–38] the development of NRTI cross-resistance will result in limited or no additional effect of the NRTI backbone, and standard second-line regimens will therefore primarily offer the benefit of the boosted-PI. Patients will thus receive functional monotherapy as second-line, which lowers the barrier for selection of PI resistance.[39] As a conditional recommendation, the WHO now advocates to perform routine viral load testing to detect virological failure early.[3] This strategy can trigger adherence interventions or changes in therapy that will limit ongoing viral replications, reducing the risk of accumulation of resistance mutations, and protect susceptibility to second-line and subsequent therapies.

In conclusion, this study demonstrated that, in the absence of viral load monitoring, unnecessary regimen switches are common, resulting in increased treatment costs and loss of drug options. Additionally, late detection of treatment failure resulted in high frequencies of accumulated mutations conferring broad cross-resistance to NRTIs, which may impair the effectiveness of second-line regimens. Future studies should determine the optimal frequency of routine virological monitoring and examine the clinical benefits of early failure detection and timely switching. The development of more affordable point-of-care viral load assays is a public health priority for resource-limited settings.

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Chapter 6

Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in six sub-Saharan African countries: implications for second-line ART strategies

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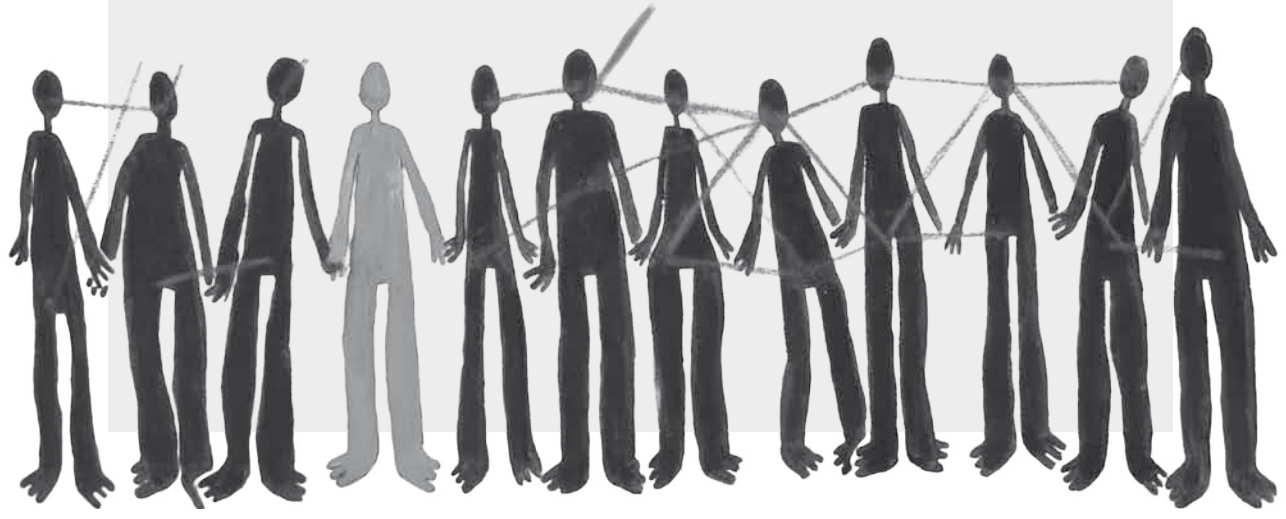

ABSTRACT

Background

HIV-1 drug resistance may limit the benefits of combination antiretroviral therapy (ART). This cohort study examined patterns of drug resistance mutations (DRMs) in individuals with virological failure on first-line ART at 13 clinical sites in six African countries, and predicted their impact on second-line drug susceptibility.

Methods

A total of 2588 HIV-1 infected antiretroviral-naïve individuals initiated ART consisting of different nucleoside reverse transcriptase inhibitor (NRTI) backbones (zidovudine, stavudine, tenofovir or abacavir, plus lamivudine or emtricitabine) with either efavirenz or nevirapine. Population sequencing after 12 months of ART was retrospectively performed if HIV-RNA >1000 copies/mL. The 2010 IAS-USA list was used to score major DRMs. Stanford algorithm was used to predict drug susceptibility.

Results

HIV-1 sequences were generated for 142 participants who virologically failed ART, of whom 70% carried ≥1 DRM and 49% had dual-class resistance, with an average of 2.4 DRMs per sequence (range 1–8). The most common DRMs were M184V (53.5%), K103N (28.9%), G190A (14.1%), Y181C (15.5%). Thymidine analogue mutations were present in 8.5%. K65R was frequently selected by stavudine (15.0%) or tenofovir (27.7%). Among participants with ≥1 DRM, the predicted HIV-1 susceptibility was reduced in 93% for efavirenz/nevirapine, in 81% for lamivudine/emtricitabine, in 59% for etravirine/rilpivirine, in 27% for tenofovir, in 18% for stavudine, and in 10% for zidovudine.

Conclusions

Early failure detection limited the accumulation of drug resistance. After stavudine failure in African populations, zidovudine rather than tenofovir may be preferred in second-line ART. Strategies to prevent HIV-1 resistance are a global priority.

INTRODUCTION

The rapid scale-up of access to combination antiretroviral therapy (ART) for HIV-1 infected persons in sub-Saharan Africa during the past decade, through a WHO-recommended public-health approach,[1] has dramatically reduced HIV-related mortality.[2] However, the widespread use of HIV clinical staging and, if available, CD4 cell counts to diagnose ART failure in resource-limited settings, rather than routine virological monitoring, is associated with the accumulation of HIV-1 drug resistance mutations,[3-5] which may limit subsequent drug options and constitutes a source for onward transmission. Studies from the region have reported high levels of drug resistance in individuals with prolonged first-line ART failure, including complex nucleoside reverse transcriptase inhibitor (NRTI) resistance profiles, such as K65R, Q151M and thymidine analogue mutations (TAMs), in addition to highly prevalent M184V and non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations.[4-8]

Most reports on HIV-1 drug resistance deal with subtype B infections in developed countries. There is limited knowledge of resistance pathways in the different HIV-1 non-B subtypes and their clinical relevance,[9] despite the fact that more than 90% of HIV-1 infections globally belong to non-B subtype variants.[10]

The PASER-M study is a prospective cohort of HIV-1 infected individuals from 13 clinical sites in 6 sub-Saharan African countries who initiated first-line ART including different NRTI backbones (zidovudine [37%], tenofovir [34%], stavudine [27%] or abacavir [3%]) and NNRTIs (efavirenz [60%] or nevirapine [40%]), in accordance with national guidelines.[11] The present study assessed HIV-1 DRMs in participants with virological failure of different first-line regimens, and predicted viral drug susceptibility to gain insight about the optimal strategies for second-line therapy.

METHODS

Study population

The PASER-M study includes clinical sites in Kenya (2), Nigeria (1), South Africa (3), Uganda (3), Zambia (3), and Zimbabwe (1).[11] We previously reported the participants' baseline resistance profiles,[12] and the effect of pre-treatment drug resistance on the immunological and virological patient outcomes after the first year of ART.[13]

For the present analysis, individuals were included if they were aged ≥18 years with HIV-1 infection and had initiated standard first-line NNRTI-based ART in accordance with national guidelines- that is, advanced immunodeficiency (CD4 cell count < 200 cells/μL) or advanced

HIV disease (WHO clinical stage 3 or 4) (13). Individuals who were previously exposed to any antiretroviral drugs for prevention and/or treatment were excluded. Other exclusion criteria were pregnancy at study screening, or –screened in Nigeria only– HIV-2 co-infection.

Participants provided written informed consent at enrollment. The study protocol was approved by the appropriate national and local research ethics committees at all collaborating sites and the Academic Medical Center of the University of Amsterdam in The Netherlands.

Data collection

Participants were followed-up in accordance with local standard-of-care guidelines. A single-drug substitution, due to toxicity or intolerance, was not considered a regimen switch. Plasma was collected at the baseline visit and after 12 months of ART (time window, 11-15 months), and stored for retrospective assessment of HIV RNA and genotypic drug resistance. Virologic failure was defined as a plasma HIV RNA value of ≥400 copies/mL. Participants who were switched to second-line ART earlier than month 12 (owing to locally diagnosed ART failure) were not included in the month 12 summary statistics.

Virological analysis

All virological testing was conducted at 1 of 2 reference laboratories in South Africa or Uganda, as previously described.[12] Briefly, HIV RNA was determined using NucliSens EasyQ real-time assay, version 2.0 (bioMérieux, Lyon, France) or COBAS Ampliprep/COBAS Taqman assay (Roche, Branchburg, New Jersey, USA). Population-based genotyping of HIV-1 protease and reverse transcriptase was undertaken in specimens with HIV RNA >1000 copies/mL, using in-house sequencing methods. HIV-1 subtypes were inferred from the *pol* sequences using the STAR algorithm[14] and, if required, the REGA tool (version 2.0).[15] Genotypic drug resistance was defined as the presence of ≥1 major amino acid substitution included in the International Antiviral Society-USA mutation list of December 2010.[16] Drug-classes considered were NRTIs, NNRTIs and protease inhibitors (PIs). HIV-1 drug susceptibility for each participant was predicted using the Stanford algorithm, version 6.1.0,[17] and was categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance. All sequences have been deposited in GenBank (accession numbers JQ480157-JQ480298).

Statistical analysis

Group comparisons for categorical data were done using chi-square or Fisher's exact test, and for continuous data using Kruskal-Wallis test, as appropriate. Mantel-Haenszel method was used to compute weighted odds ratios (OR) that adjusted for a single confounding factor. Reported p-values are two-sided and $p<0.05$ was considered statistically significant. All analyses were performed using Stata version 11 (StataCorp, Texas, USA).

RESULTS

Study population and clinical outcomes

Between March 2007 and September 2009, 2588 participants were enrolled. Table 1 shows the baseline characteristics of all participants (n=2588), and of those with a resistance test result by month 12 (n=142). Participants were initiated on ART regimens containing either zidovudine (964 [37.2%]), tenofovir (867 [33.5%]), stavudine (691 [26.7%]), or abacavir (66 [2.6%]), combined with either efavirenz (1543 [59.6%]) or nevirapine (1045 [40.4%]). Baseline resistance was detected in 140 (5.4%) participants; the proportions did not differ between the initial regimens.

A total of 2132 (82.4%) participants were retained in care up to 12 months of follow-up, of whom 2128 (82.2%) were still on first-line ART and 4 (0.2%) were switched to second-line therapy earlier than month 12 due to locally diagnosed ART failure. The remaining 456 (17.6%) participants were not retained, because they died (190 [7.3%]), were lost to follow-up (200 [7.7%]), transferred out (61 [2.4%]), or discontinued ART (5 [0.2%]) (figure 1).

HIV RNA by month 12 was assessed for 94.6% (2014 of 2128) of participants who were still on first-line ART. For all who initiated first-line ART, 70.3% (1820 of 2588; 95%CI 68.5-72.1) achieved viral suppression, and for those who had a 12-month HIV RNA result 90.4% (1820 of 2014; 95%CI 89.0-91.6) achieved viral suppression (figure 1).

Drug resistance mutations by drug regimen

Of the 166 participants with HIV RNA >1000 copies/mL by month 12, sequence results were available for 142 (85.5%); 14 specimens failed to amplify, and results were missing for 10 specimens. One hundred (70.4%, 95%CI 62.2-77.8) sequences harbored ≥1 DRM and 42 (29.6%) did not harbor any DRMs. The average number of DRMs (any class) per sequence was 2.4, with a range of 1–8. Detected DRMs were associated with NRTIs (82 [57.8%]), NNRTIs (86 [60.6%]) (figure 2). Dual-class resistance to NRTIs and NNRTIs was detected in 69 (48.6%) participants. Combinations of DRMs included M184V and NNRTI (64 [45.1%]), M184V and TAMs (10 [7.6%]), TAMs and NNRTI (8 [5.6%]), and M184V, TAMs, and NNRTI (7 [4.9%]) (figure 2). In 2 (1.4%) participants, DRMs known to be associated with PIs were observed (both tipranavir-associated Q58E); no triple-class resistance was detected. Exclusion of the 16 (9.6%) participants who had ≥1 single-drug substitutions during the first-year of ART did not significantly change the frequencies of the DRMs (not shown).

Of the NRTI-associated DRMs (figure 3), M184V was the most frequent (76 [53.5%]), followed by K65R (17 [12.0%]) and any TAM (12 [8.5%]). In 9 (6.3%) participants, ≥2 TAMs were detected and in 3 (2.1%) participants, ≥3 TAMs were detected. TAM-1 mutations

Table 1. Baseline characteristic of participants.

Variable	Baseline	Month 12			p-value a
	Overall (n=2588)	Overall (n=142)	No resistance (n=42)	Resistance (n=100)	
Sex					0.13
Women	1482 (57.3)	61 (43.0)	14 (33.3)	47 (47.0)	
Men	1106 (42.7)	81 (57.0)	28 (66.7)	53 (53.0)	
Age at initiation , mean years (SD)	38.0 (9.0)	36.0 (8.5)	36.7 (7.9)	35.7 (8.8)	0.43
Country					0.79
Zambia	555 (21.5)	28 (19.7)	9 (21.4)	19 (19.0)	
South Africa	593 (22.9)	26 (18.3)	9 (21.4)	17 (17.0)	
Uganda	602 (23.3)	38 (26.8)	8 (19.1)	30 (30.0)	
Kenya	425 (16.4)	22 (15.5)	7 (16.7)	15 (15.0)	
Zimbabwe	211 (8.2)	12 (8.5)	3 (7.1)	9 (9.0)	
Nigeria	17 (25.8)	16 (11.3)	6 (14.3)	10 (10.0)	
WHO clinical stage at initiation					0.51
1 or 2	1015 (39.2)	52 (36.6)	17 (40.5)	35 (35.0)	
3 or 4	1573 (60.8)	90 (63.4)	25 (59.5)	65 (65.0)	
Baseline HIV-1 drug resistance [b]	140 (5.4) [c]	15 (10.6)	1 (2.4)	14 (14.0)	0.10
Initial ART regimen					0.78
Efavirenz-based	1543 (59.6)	69 (48.6)	21 (50.0)	48 (48.0)	
Tenofovir-containing	720 (27.8)	33 (23.4)	10 (23.8)	23 (23.0)	
Stavudine-containing	430 (16.6)	11 (7.8)	5 (11.9)	6 (6.0)	
Zidovudine-containing	342 (13.2)	23 (16.2)	6 (14.3)	17 (17.0)	
Abacavir-containing	51 (2.0)	2 (1.4)	0 (0.0)	2 (2.0)	
Nevirapine-based	1045 (40.4)	73 (51.4)	21 (50.0)	52 (52.0)	
Zidovudine-containing	622 (24.0)	48 (33.8)	13 (31.0)	35 (35.0)	
Stavudine-containing	261 (10.1)	9 (6.3)	4 (9.5)	5 (5.0)	
Tenofovir-containing	147 (5.7)	14 (9.9)	3 (7.1)	11 (11.0)	
Abacavir-containing	15 (0.6)	2 (1.4)	1 (2.4)	1 (1.0)	
CD4 cell count, median cells/μL (IQR)	133 (62-204) [d]	120 (52-198	157 (136)	108 (127.5)	0.002
Plasma HIV RNA, median $\log_{10}$ c/mL (IQR)	5.00 (4.38-5.59) [e]	5.23 (4.53-5.67)	5.18 (1.31)	5.24 (.99)	0.19
HIV-1 subtypes [f]					0.14
A	611 (24.9)	37 (26.1)	14 (33.3)	23 (23.0)	
C	1329 (54.2)	69 (48.6)	22 (52.4)	47 (47.0)	
D	276 (11.3)	19 (13.4)	1 (2.4)	18 (18.0)	
CRF02_AG	116 (4.7)	6 (4.2)	2 (4.8)	4 (4.0)	
Other	118 (4.8)	11 (7.8)	3 (7.1)	8 (8.0)	

Baseline characteristics of all participants at baseline (n=2588) and of those who had a genotypic resistance test results by month 12 (n=142). Data are no. (%) unless stated otherwise. [a] P values for comparison between no resistance versus resistance outcome groups. [b] Defined as at least 1 drug resistance mutation of the 2010 International Antiviral Society–USA list. [c] Data available for n=2442. [d] Data available for n=2578. [e] Data available for n=2564. [f] Data available for n=2450. ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; SD, standard deviation; WHO, World Health Organization.

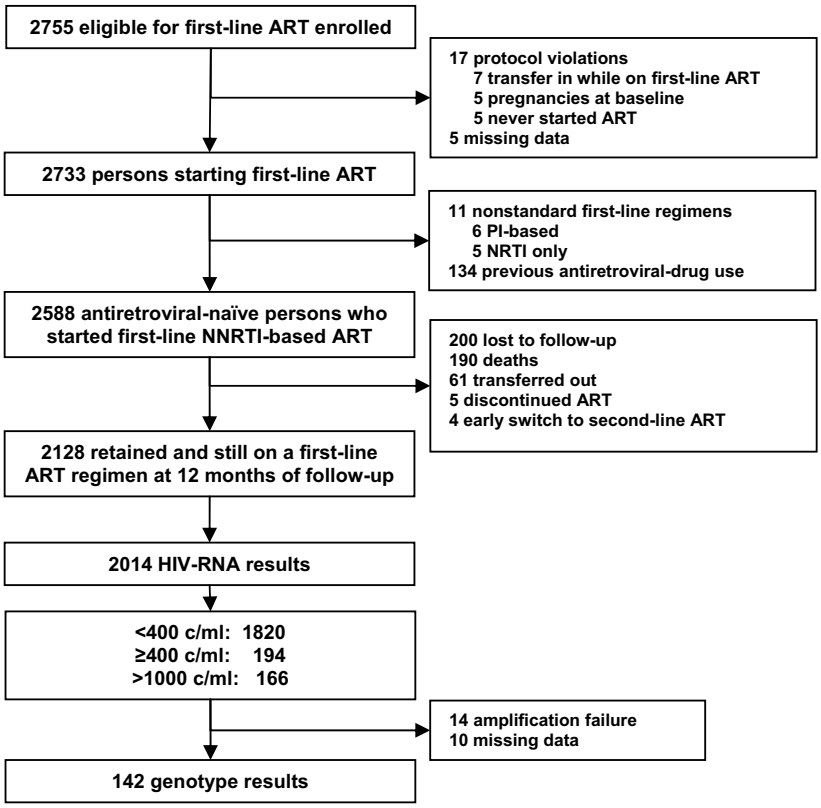

Figure 1. Study profile. ART, antiretroviral therapy; HIV, human immunodeficiency virus; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

included M41L and T215 F/Y (L210W not observed); TAM-2 mutations included D67N, K219E and K70R (K219Q not observed). In participants who failed regimens containing zidovudine, stavudine or tenofovir, ≥1 TAM was detected in 12.7% (9 of 71), 5.0% (1 of 20), and 4.3% (4 of 47), respectively; K65R in 0% (0 of 71), 15.0% (3 of 20) and 27.7% (13 of 47), respectively. Q151M was not detected.

Of the NNRTI-associated DRMs (figure 3), K103N (41 [28.9%]) was the most frequent, followed by Y181C (22 [15.5%]) and G190A (20 [14.1%]). In participants failing an efavirenz-based or nevirapine-based regimen, NNRTI-associated DRMs were detected in 58.0% (40 of 69) and 63.0% (46 of 73) , respectively (p=0.5); K103N in 33.3% (23 of 69) and 24.7% (18 of 73), respectively (p=0.3); Y181C in 7.3% (5 of 69) and 23.3% (17 of 73) participants, respectively (p=0.008); and V106M in 20.3% (14 of 69) and 1.4% (1 of 73), respectively (p<0.001). V106A only occurred after nevirapine exposure. Sixteen (11.3%) participants harbored only DRMs associated with NNRTIs, and 33 (22.5%) participants harbored

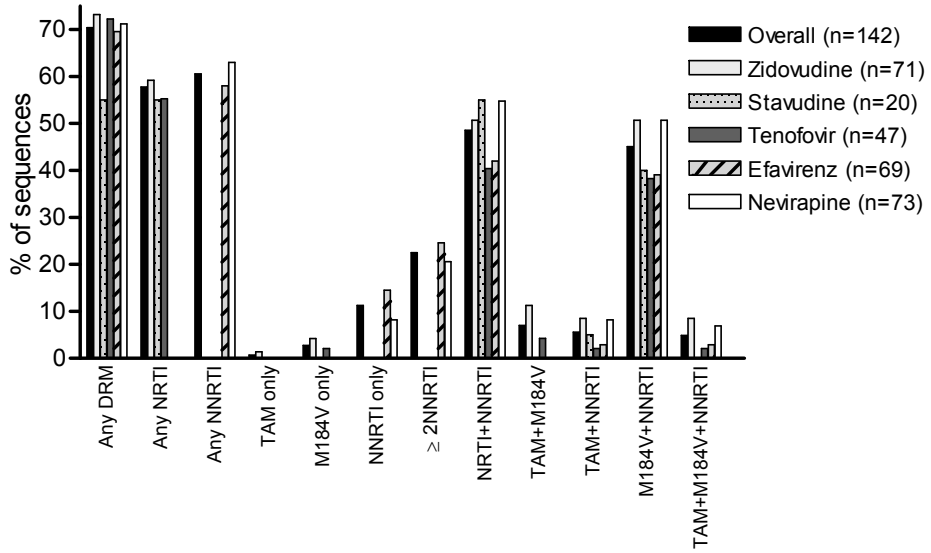

Figure 2. Frequencies of any drug resistance according to drug class and permutations by nucleoside reverse transcriptase inhibitor (NRTI) and by non-nucleoside reverse transcriptase inhibitor (NNRTI). Figure shows major International Antiviral Society–USA drug resistance mutations associated with NRTIs and NNRTIs in participants experiencing virological failure. The legend includes the number of patients exposed to each antiretroviral drug. DRM, drug resistance mutation; TAM, thymidine analogue mutation.

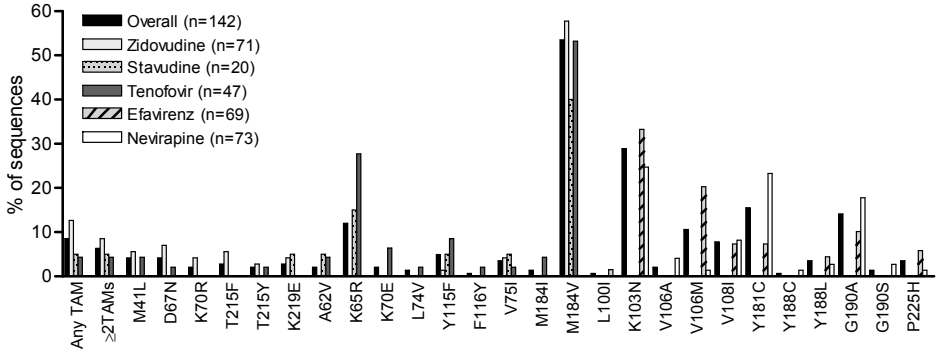

Figure 3. Frequencies of individual drug resistance mutations by nucleoside reverse transcriptase inhibitor (NRTI) and by non-nucleoside reverse transcriptase inhibitor (NNRTI). Figure shows major International Antiviral Society–USA drug resistance mutations associated with NRTIs and NNRTIs in participants experiencing virological failure. The legend includes the number of patients exposed to each antiretroviral drug. TAM, thymidine analogue mutation.

≥2 NNRTI-associated DRMs. K103N occurred as the only NNRTI mutation in 23 of 41 participants (56.1%), and occurred in combination with other NNRTI mutations in 18 participants (with Y181C [7], P225H [3], G190A [2], V106M [1], M230L [1], V106M+Y181C [1], Y181C+G190A [1], P225H+M230L [1], L100I+P225H [1]).

Compared with participants with virological failure by month 12 who did not harbor any DRM, those with ≥1 DRM had a marginally lower median HIV RNA (4.33 vs. 4.71 $\log_{10}$ copies/mL; p=0.04); this difference was least pronounced for NNRTI resistance (4.30 vs. 4.55 $\log_{10}$ copies/mL, p=0.05) and most pronounced for M184V (4.21 vs. 4.59 $\log_{10}$ copies/mL, p=0.02).

Of the 142 participants with a genotype result by month 12, 135 had a baseline genotype result (7 baseline specimens not collected); of these, 14 (10.4%) participants harbored ≥1 DRM before start of ART. Of the DRMs detected by month 12, 96% were newly acquired during the first year of ART.

HIV-1 subtype diversity

Among the 142 participants who had a resistance test result by month 12, HIV-1 subtype C was most commonly identified, followed by A, D, A/G recombinant, and other subtypes/recombinants (table 1). The subtype distribution did not differ significantly between those with or without DRMs (p=0.14). The DRMs according to subtype are shown in figure 4. K65R was more frequent in subtype C (12 [17.4%]) than non-C (5 [6.9%]) (p=0.05). After adjusting for differential tenofovir and stavudine exposure, the association with subtype was not significant: OR for C vs. non-C (by Mantel-Haenszel method) 1.27; 95%CI 0.41-3.93; p=0.68).

Compared with subtype C (13 [18.8%]), K103N occurred more frequently in D (10 [52.6%]; p=0.003) and A (13 [35.1%]; p=0.06), but did not differ between A and D (p=0.21). After adjusting for differential efavirenz and nevirapine exposure, K103N remained significantly associated with subtype: OR for C vs. non-C (by Mantel Haenszel method) 0.33; 95%CI 0.15-0.75; p=0.005, and OR for D vs. non-D 3.40; 95%CI 1.21-9.58; p=0.014. Frequencies of other DRMs, except for V106M, which was exclusively observed in subtype C, did not differ between HIV-1 subtypes (figure 4).

Predicted genotypic drug susceptibility

Of the viruses harboring ≥1 DRM (n=100), the predicted HIV-1 susceptibility to lamivudine and emtricitabine was reduced in the majority (81%), due to the high frequency of M184V (figure 5). For the other NRTIs, reduced HIV-1 susceptibility was predicted for abacavir in 42% of sequences, didanosine in 40%, tenofovir in 27%, stavudine in 18%, and zidovudine in 10%. For the NNRTIs, reduced HIV-1 susceptibility was frequently predicted for the first-generation drugs efavirenz and nevirapine (93%) and less frequently

for the second-generation drugs etravirine and rilpivirine (59%). High-level resistance was most frequently predicted for nevirapine (92%), followed by lamivudine/ emtricitabine (77%) and efavirenz (73%), but was uncommon for etravirine and rilpivirine (5%). Reduced HIV-1 susceptibility was not predicted for any of the ritonavir-boosted PIs.

DISCUSSION

This multi-country cohort study examined HIV-1 resistance mutation patterns in African patients with virological failure on different first-line ART regimens, and predicted their impact on second-line drug susceptibility. After the first year of ART, 70% of those with virological failure had a virus with ≥1 DRM, and dual-class resistance was observed in 49%. Nearly all (96%) DRMs detected by month 12 were newly acquired in the first year of ART. Previous studies from the region have reported higher frequencies (83-93% of those with virological failure) and complexity of DRM patterns in persons with prolonged ART failure, in the absence of viral load monitoring.[4-8] By contrast, in the present study, routine viral load testing after 12 months of ART enabled the relatively early detection of virological ART failure, which, to some extent, may have prevented the accumulation of resistance. Nonetheless, observed resistance patterns for this cohort were more extensive than for cohorts that received intensive virological monitoring in South Africa[18] and resource-rich countries.[19, 20] Therefore, our data underscore the importance of implementing routine viral load monitoring in ART programs in sub-Saharan Africa to prevent drug resistance accumulation.[3-5] The presence of DRMs was associated with lower HIV RNA values, which results from the reduced fitness of resistant variants.

In patients who failed a tenofovir-containing regimen, the K65R mutation, which is tenofovir's signature mutation, was commonly observed. Notably, K65R, rather than TAMs, was also frequently selected after stavudine failure. This finding concurs with previous clinical studies that reported high rates of K65R in subtype C–infected Africans who failed stavudine-containing regimens,[4, 5, 7] and contrasts with the low frequencies of K65R observed in subtype-B-infected individuals from developed countries.[21, 22] K65R and TAMs represent antagonistic pathways of NRTI resistance,[23] and K65R confers cross-resistance to all NRTIs except zidovudine.

Several mechanistic studies have demonstrated *in vitro* that the K65R mutation, which confers broad cross-resistance to the NRTI class, develops more readily in HIV-1 subtype C, the predominant subtype in sub-Saharan Africa, than in subtype B.[24-29] In our cohort, which included only non-B subtypes, K65R was detected more frequently in subtype C than in subtypes A or D. However, after adjusting for differential stavudine and tenofovir

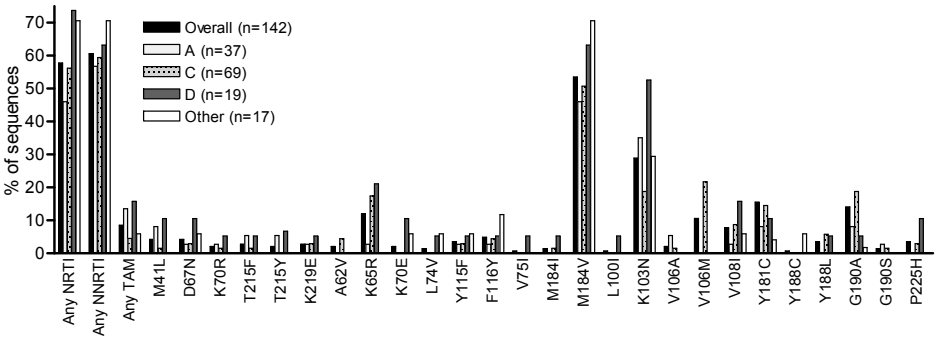

Figure 4. Frequencies of individual drug resistance mutations by human immunodeficiency virus type 1 (HIV-1) subtype. Figure shows major International Antiviral Society–USA drug resistance mutations associated with nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the participants experiencing virological failure. The legend includes the number of patients infected with each HIV-1 subtype. TAM, thymidine analogue mutation.

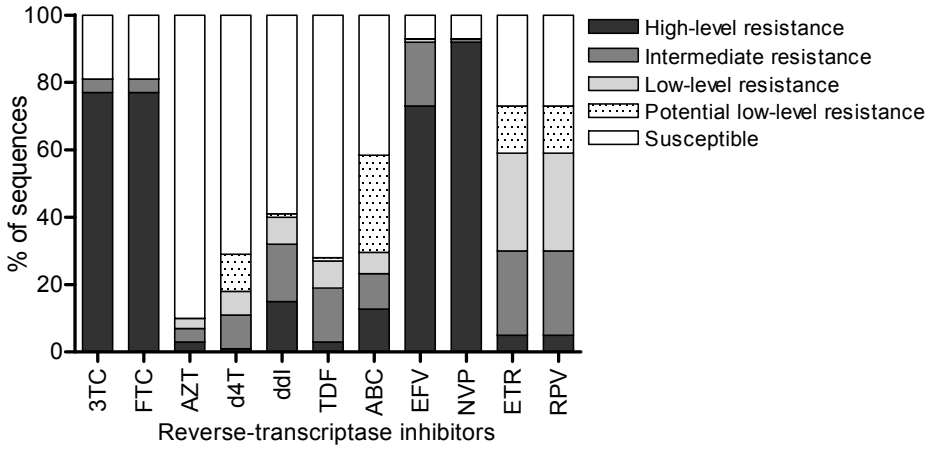

Figure 5. Predicted viral susceptibility to reverse transcriptase inhibitors. Figure includes the viruses that harbored ≥1 drug resistance mutation (n= 100). Genotypic drug susceptibility was predicted using the Stanford HIVdb algorithm (version 6.1.0). Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir.

use across the subtypes, the association was not statistically significant. This discrepancy with the published literature is most likely explained by the limited statistical power of the present study to assess the effects of subtype on the rates of individual DRMs. Nonetheless, overall, our data support the conclusion that K65R is frequently selected after stavudine or tenofovir use in African populations receiving first-line ART. Therefore, from a virological perspective, zidovudine rather than tenofovir (as currently recommended by the WHO guidelines[30]), may be preferred in second-line therapy for non-B subtype infected patients who fail a stavudine-containing first-line regimen in the absence of stringent viral load and resistance monitoring. This scenario would warrant enhanced toxicity monitoring, particularly for zidovudine-associated anemia.[31]

The WHO advises that stavudine be phased out of first-line regimens due to its serious adverse effects, to be replaced with either tenofovir or zidovudine.[30] Dose reduction (from 40 mg to 30 mg, and even 15-20 mg per day) has been suggested to limit the mitochondrial toxicities from stavudine.[32] However, suboptimal dosing of stavudine might in theory result in even more frequent emergence of K65R, particularly when used as part of NNRTI-based regimens. Emerging K65R will compromise future NRTI backbones, and clinical trials are ongoing to establish the effectiveness of ("simplified") PI-based second-line therapy without an effective NRTI-backbone.[33]

For second-line ART, WHO recommends a ritonavir-boosted PI (preferably lopinavir or atazanavir), with a dual backbone of 2 new or recycled NRTIs, that is, tenofovir plus lamivudine/emtricitabine (after use of stavudine or zidovudine in first-line), or zidovudine plus lamivudine (after use of tenofovir in first-line).[30] In our cohort, HIV-1 susceptibility to all PIs was preserved for all participants; zidovudine and tenofovir susceptibility was preserved in the majority of patients who failed first-line ART (90% and 73%, respectively). This means that the majority of patients who are switched empirically to second-line PI-based therapy will receive at least two active drugs. Preliminary studies in Africa have indeed suggested high chance of viral re-suppression with empirically prescribed second-line regimens.[34-38]

The second-generation NNRTIs rilpivirine or etravirine are being considered as second-line drugs in resource-limited countries in patients previously exposed to efavirenz or nevirapine, because of their different resistance profiles and suggested high genetic barrier. Susceptibility to the second-generation NNRTIs is preserved in viruses containing the K103N mutation, preferentially selected by efavirenz, but susceptibility is reduced in the presence of Y181C, preferentially selected by nevirapine.[39] In our cohort, viral susceptibility to the second-generation NNRTIs was already reduced in 59% of participants who had ≥1 DRM. Given that NRTI and NNRTI resistance mutations rapidly

accumulate in the absence of viral load monitoring,[4, 5] second-line regimens based on 2 NRTIs and a second-generation NNRTI are therefore unlikely to be effective. Instead, use of second-generation NNRTIs as part of first-line ART in resource-limited countries merits further evaluation.

In our cohort, K103N occurred more frequently in subtype D infections than in subtype C infections. By contrast, previous studies in women and infants after the use of single-dose nevirapine to prevent vertical HIV-1 transmission have suggested that K103N accumulates faster in subtype C than D, and faster in D than A.[40-42] Additional studies are needed to elucidate the virological mechanisms for different subtypes.

This study has some limitations. Population-based sequencing is not able to detect minority resistant viral strains, thus potentially underestimating resistance.[43] The lack of frequent serial blood sampling after ART initiation precluded a more detailed analysis of the evolution of DRMs after ART initiation.

The number of HIV-1 infected people accessing ART in resource-limited countries is projected to increase further in view of the goal of universal access and, particularly, the earlier start of ART and emerging treatment-for-prevention.[44,45] Before widely implementing such strategies, mathematical modeling as well as empirical data are urgently needed to investigate their acceptability, adherence as well as the possible consequences for the emergence and spread of drug resistance.

In conclusion, regular viral load monitoring aimed at early failure detection may limit the accumulation of HIV-1 drug resistance. The strengthening of national HIV treatment programs through robust supply chains, routine viral load monitoring and improved access to alternative drug regimens in sub-Saharan Africa to prevent drug resistance, is an urgent priority. Zidovudine rather than tenofovir may be the preferred second-line NRTI after first-line stavudine failure. The high frequencies of Y181C and accumulated NNRTI mutations limit the effectiveness of second-generation NNRTIs for use in second-line ART.

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Chapter 7

Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa

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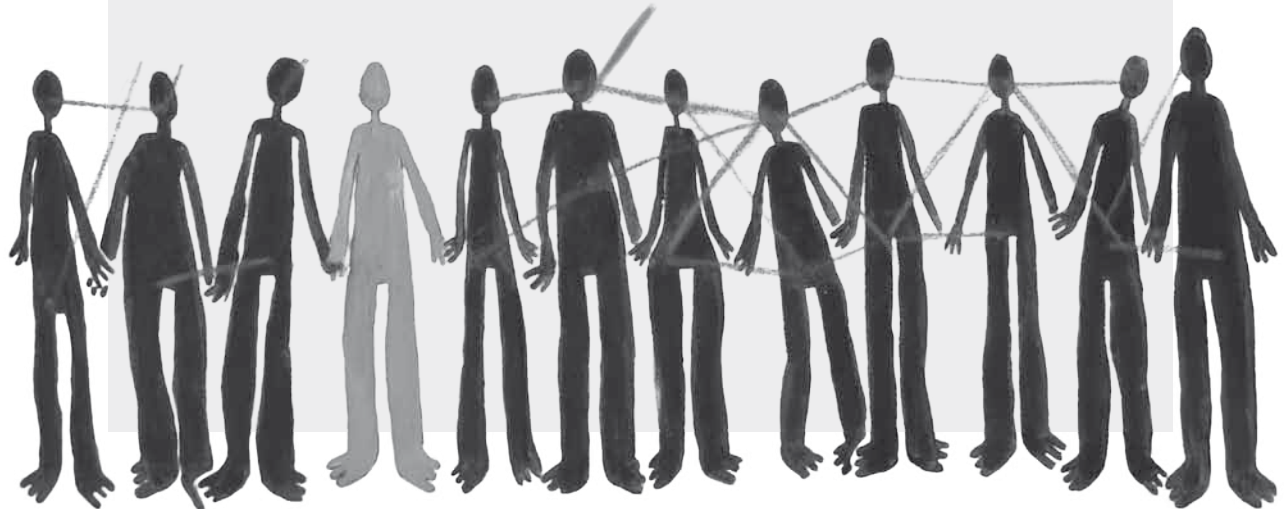

ABSTRACT

Patients failing antiretroviral treatment for extended periods of time are at risk of accumulating HIV drug resistance mutations (DRMs), which negatively influences second-line treatment. This retrospective study assessed the rate of DRM accumulation among South African patients with continued virological failure. Serial genotypic resistance testing was performed and DRMs were scored according to the 2009 IAS-USA list. Among 43 patients, 38 (88.4%) harbored ≥ 1 DRM. The median time between two sequential resistance tests was 5 months (IQR: 3–10). Thymidine analogue mutations accumulated at a rate of 0.07 mutation per month of drug exposure, which is faster than previously reported. Routine virological monitoring should be implemented in resource-limited settings to preserve susceptibility to second-line regimens.

INTRODUCTION

As the number of patients receiving antiretroviral treatment (ART) has expanded in South Africa, so too has the number experiencing first-line regimen failure.[1–3] HIV drug resistance data emerging from South Africa have generally revealed patterns similar to those seen in HIV-1 subtype B virus.[4–6] In contrast to other countries in sub-Saharan Africa, studies from South Africa have reported fewer thymidine analogue mutations (TAMs) at failure of first-line ART, likely to be related to the use of viral load (VL) monitoring in the public sector program.[5–7] A study from Malawi described complex resistance profiles in patients diagnosed with clinical and/or immunological treatment failure.[8] Similarly, a meta-analysis has also revealed a higher frequency of genotypic resistance in less frequently monitored patients in resource-limited settings.[9]

Patients receiving a virologically failing regimen containing nucleoside reverse transcriptase inhibitors (NRTIs) for extended periods of time are at risk of acquiring multiple TAMs, the K65R and/or Q151M mutations, which confer broad NRTI cross-resistance and may decrease the effectiveness of second-line treatment options. Limited data are available as to how fast TAMs and other NRTI mutations accumulate, especially in resource-limited settings. The current study aimed to assess resistance patterns and the rate of accumulation of mutations among South African patients with continued virological failure on first-line ART.

MATERIALS AND METHODS

Patients and study design

Patients receiving ART at the Charlotte Maxeke Academic Hospital in Johannesburg who had been diagnosed with virological treatment failure and referred for genotypic HIV resistance testing were eligible for this retrospective cohort study. ART was administered according to national treatment guidelines and virological failure was defined as a VL over 5000 RNA copies/ml confirmed on two separate occasions.[10] Genotyping conducted at the clinical visit at which ART was switched to second-line, is referred to as time point 0 (T0). For each patient, additional resistance testing was conducted retrospectively whenever specimens were available; the time point of first detection of viremia is denoted T - 1. Clinical outcome after switch to second-line treatment was also assessed and defined as T + 1.

Population genotype analysis

Population-based genotyping was performed using an in-house HIV drug resistance assay.[11] Briefly, viral RNA was extracted using the automated Roche MagNa Pure LC analyzer and the MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany). A 1.7-kb amplicon was generated by a reverse transcriptase (RT)-initiated polymerase chain reaction encompassing the entire protease (PR) and partial RT coding regions, using primers designed from the consensus HIV-1 subtype C sequence available on the Los Alamos Database (www.hiv.lanl.gov/). The amplicon was sequenced using five primers that ensure bidirectional coverage from codons 1–99 of PR and codons 1–230 of RT with an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, USA). Sequences were assembled, manually edited using Sequencher version 4.8 software (Genecodes, Ann Arbor, MI), and submitted to the ViroScore database (Advanced Biological Laboratories, France). Drug resistance mutations (DRMs) were scored according to the 2009 International AIDS Society-USA list.[12] The REGA HIV-1 subtyping tool[13] was used to determine HIV-1 subtype.

Statistical data analysis

Statistical analysis was performed using Stata version 10 (StataCorp LP, TX). The difference in frequency of DRMs occurring with and without TAMs was compared using Pearson's chi-square test or Fisher's exact test for small samples. A p-value of < 0.05 was considered significant. For patients with multiple resistance tests, the rate of mutation accumulation was calculated as the number of mutations detected at T0 that had not been detected at T-1, divided by the person-months of follow-up between the tests. Separate rates were calculated for all persons at risk at T-1 and for those with ≥ 1 TAM identified at T0. All patients with a resistance test at T-1 were considered as persons at risk for DRM accumulation.

RESULTS

Patient characteristics

Between January 2006 and August 2009, 43 patients were referred for resistance testing and switched to second-line ART. The median age was 33 years old [interquartile range (IQR): 28–40.3] and 69.8% of patients were female. The median VL at time of treatment switch was 26,000 HIV RNA copies/ml (IQR: 7500–106,000) and the median CD4 count was 169 cells/ml (IQR: 77–289). The median time spent on the first-line regimen was 22 months (IQR: 13–50 months). First-line ART consisted of NNRTI-based regimens, with the majority (n = 26) of patients receiving stavudine (d4T) in combination with lamivudine (3TC) as the NRTI component. Patients who experienced toxicity to d4T were changed to zidovudine (AZT, n = 10) or tenofovir (TDF, n = 2). All patients were infected with HIV-1

subtype C. For 37 patients with prior VL tests, the median time between first detection of viremia and treatment switch was 7 months (IQR: 4–13).

Mutational patterns at routine resistance testing (T0)

Table 1 shows resistance patterns at time of treatment switch (T0) for both patients with and without TAMs. In total, 38 (88.4%) patients harbored any DRM at time of failure and 34 (79.1%) had dual-class resistance. The most frequent NRTI mutation was the M184V (n = 32; 74.4%), followed by the TAMs (n = 23 with ≥ 1 TAM; 53.5%). Ten (23.3%) patients harbored ≥ 2 TAMs and the D67N TAM pathway was preferred. The frequencies of Q151M and K65R mutations were n = 1 (2.3%) and n = 3 (7.0%), respectively. The K103N (n = 21; 48.8%) and V106M (n = 12; 29.9%) were the most frequent NNRTI mutations observed. No significant difference was observed between the frequency ofM184V and K65R between the group with and without TAMs (p = 0.128 and p = 0.554, respectively). All patients were infected with HIV-1 subtype C.

Table 1. Resistance patterns for patients with thymidine analogue mutations versus those without thymidine analogue mutations

Resistance pattern			Overall (n=43)	No TAMs (n=23)	TAMs* (n=20)	*P*
Any resistance mutation			38 (88.4)	18 (78.3)	20 (100)	0.027
NRTI and NNRTI mutations			34 (79.1)	15 (65.2)	19 (95.0)	0.017
Any NRTI mutation			35 (81.4)	15 (65.2)	20 (100)	0.003
M184V		32 (74.4)	15 (65.2)	17 (85.0)	0.128	
M184V only		0	0	0	n/a	
≥ 2 TAMs		10 (23.3)	n/a	10 (50.0)	n/a	
≥ 3 TAMs		7 (16.3)	n/a	7 (35.0)	n/a	
K65R		3 (7.0)	2 (8.7)	1 (5.0)	0.554	
K70E		1 (2.3)	1 (4.4)	0	n/a	
Q151M		1 (2.3)	1 (4.4)	0	n/a	
Any NNRTI mutation			37 (86.1)	18 (78.3)	19 (95.0)	0.114
L100I	3 (7.0)	1 (4.4)	2 (10.0)	0.446		
K101E	6 (14.0)	2 (8.7)	4 (20.0)	0.266		
K103N	21 (48.8)	10 (43.5)	11 (55.5)	0.451		
V106M	12 (29.9)	5 (21.7)	7 (35.0)	0.265		
V108I	5 (11.6)	2 (8.7)	3 (15.0)	0.431		
Y188C/L	2 (4.7)	0	2 (10.0)	n/a		
G190A	9 (20.9)	4 (17.4)	5 (25.0)	0.405		
P225H			3 (7.0)	2 (8.7)	1 (5.0)	0.554

Data are n (%) of patients. * Frequencies of individual TAMs: M41L, 7 (16.3); D67N, 8 (18.6); K70R, 6 (14.0); L210W, 3 (7.0); T215Y/F, 14 (32.6); K219Q, 4 (9.3). TAM, thymidine analogue mutation; (N)NRTI, (non-) nucleoside reverse transcriptase inhibitor; n/a, not applicable.

Accumulation of mutations (T-1 to T0)

Figure 1 shows the increase of TAMs and any (N)NRTI mutations for 29 patients with paired resistance tests. The median time between T-1 and T0 for these patients was 5 months (IQR: 3–10). At T-1, the mean number of TAMs was 0.59 and at T0 this increased to 1.03. In absolute terms, 13 TAMs were acquired in a total of 190 person-months, resulting in a rate of accumulation of 0.07 TAM per month [confidence interval (CI): 0.04–0.11] for all persons at risk. Patients with ≥ 1 TAM at T0 (n = 13) accumulated mutations at a rate of 0.15 mutation per month (CI: 0.08–0.25). This group was further divided into patients with one to three TAMs and patients with four to five TAMs at T0, and the respective rates of TAM accumulation were 0.08 mutation per month (CI: 0.02–0.20) and 0.24mutation per month (CI: 0.12–0.41). The accumulation rate for any NRTI-associated mutations was 0.05 (CI: 0.03–0.09) per month, and for NNRT-associated mutations 0.03 (CI: 0.01–0.06) per month for all persons at risk at T-1.

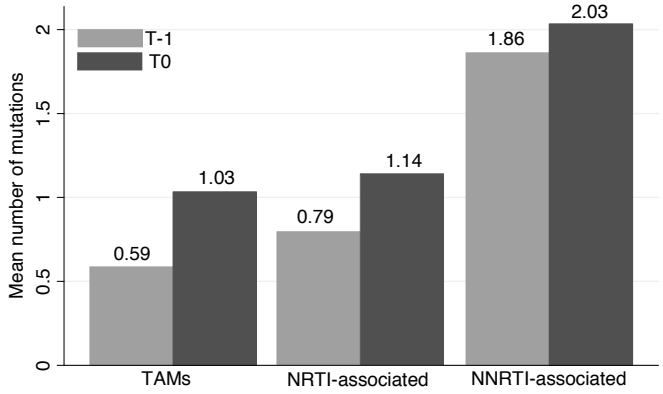

Figure 1. Increase of mutations between T-1 and T0 for 29 patients with multiple resistance tests. TAM, thymidine analogue mutation; (N)NRTI, (non-)nucleoside reverse transcriptase inhibitor. T0 is timepoint of the last resistance test, conducted at the visit at which ART was switched to second-line; T-1 is timepoint of first resistance test, conducted retrospectively at first detection of viremia.

Outcome on second-line treatment

Clinical outcomes on second-line treatment were assessed after a median of 22 months (IQR: 13.5–28). Twenty-five 58.1%) patients achieved full virological suppression, of which 14 (56%) had complex DRMs that included TAMs, K65R, or Q151M at T0. Seven (16.3%) patients failed second-line ART, of whom two never suppressed following treatment switch and five experienced virological rebound. Within this group, four (57.1%) subjects had TAMs, K65R, or Q151M at T0. The proportion with complex DRMs at T0 was not significantly different for patients who achieved virological suppression versus

those who failed second-line ART (p = 0.649). For the remaining 11 patients, no follow-up information was available.

DISCUSSION

This study provides important information on the accumulation of HIV drug resistance among patients with prolonged virological failure of first-line ART in South Africa. A considerable frequency of clinically significant mutations was observed, including multiple TAMs (23.3%), and K65R (7.0%) or Q151M (2.3%) mutations. TAMs accumulated at a high rate of 0.07 mutation per month of drug exposure after the first detection of viremia, resulting in one new TAM for every 14.6 months of continued drug exposure. While the South African 2010 national treatment guidelines recommend using TDF in first-line regimens,[14] the resistance data presented from failures of regimens containing d4T and AZT remain very relevant for resource-limited settings.

An unusual contribution of our study is the repeated resistance testing on patients with persistent viremia while receiving first-line ART, enabling us to assess the accumulation of mutations. This information is useful for informing clinical decisions, such as how rapidly to switch to second-line therapy after detecting viremia. A limitation of this study is the small sample size and erratic nature of sampling for resistance testing. When VL monitoring is available, deliberately leaving patients on failing regimens is no longer ethical and it is impossible to conduct prospective or randomized investigations. A second shortcoming of our study is the fact that mutations detected within the T-1 to T0 interval might reflect drug selective pressure before time point T-1. In the absence of baseline genotypic information, however, we were unable to assess the rate of mutation accumulation for the entire time of drug exposure. Nevertheless, our findings provide an estimate of mutation accumulation in patients left on failing regimens. This information is useful for clinicians and can guide them in their decision to switch regimens.

At first detection of viremia, NNRTI mutations were most commonly observed, followed by the M184V mutation. This is consistent with prior work from South Africa, which has documented first-line failure patterns with mainly NNRTI-associated mutations and multiple TAMs in only 12%.[6] Recent work using a cohort with a more stringent failure definition of 1000RNA copies/ml revealed less complex resistance profiles with a low frequency of TAMs.[15] In our study, TAMs accumulated after a longer duration of viremia. This mirrors the situation in other African countries in which monitoring is conducted based on clinical and/or immunological grounds, resulting in a high prevalence of TAMs and complex resistance mutations at failure.[8] The most common TAM detected was the

D67N, indicating that most patients had the more favorable TAM-2 pathway, as had been reported previously in South Africa.[6,16]

Although the number of patients with paired resistance tests in our study was limited, we estimated a rate of one additional TAM for every 14.6 months of drug exposure. This is considerably faster than the rate of one TAM in every 4.3 years described in a European study in which resistance testing was performed at HIV-RNA > 500 copies/ml.[17] To date, few studies in non-subtype B-infected patients in Africa have documented the rate of TAM accumulation. Prior work from South Africa reported that only 10% of patients had TAMs after 6 months of continued viremia on first-line ART.[18] The results found in our study population are therefore alarming, and imply that a prompt switch to second-line therapy is warranted after the first detection of viremia.

We separately evaluated the rate of accumulation in patients who had acquired ≥ 1 TAM at time of treatment switch (T0). These patients were found to develop 0.15 additional TAM per month, i.e., one TAM for every 6.5 months of continued drug exposure. Although evaluating a triple NRTI regimen, a study from Germany showed that TAM accumulation rates differed for patients acquiring one to three TAMs versus those acquiring four to five TAMs (0.04 and 0.23 mutation per month, respectively).[19] When we performed this analysis within our cohort, we found very similar rates of TAM accumulation (0.08 and 0.24 mutation per month, respectively), although this difference was not significant. Our sample was too small to evaluate specific risk factors for those patients with higher TAM accumulation rates. Also, in the absence of baseline genotypic information, we were unable to evaluate whether the TAM accumulation rate differed over time or over the course of the disease.

The accumulation of TAMs and complex mutations causes decreased activity of NRTIs,[20,21] bearing consequences for the effectiveness of the currently available second-line regimens in African countries. Once cross-resistance has developed, standard second-line regimens will primarily offer the benefit of the boosted PI, with limited or no additional effect of the NRTI backbone. Patients will thus receive functional monotherapy, which lowers the barrier for selection of PI resistance.[22] A study from Malawi has shown a high rate (85%) of virological suppression on second-line ART despite extensive NRTI resistance.[23] In our study, over half of the patients, including those with complex DRMs, achieved virological suppression on second-line ART. Despite these relatively favorable on-treatment outcomes, a study of PI monotherapy after failure of an NNRTI-based regimen revealed a high incidence of low-level viremia.[24] Therefore, patients with extensive NRTI resistance at time of switch to PI-based ART should receive intensive adherence counseling and frequent virological monitoring. A high rate of attrition, likely due to

poor tolerability and toxicity of second-line regimens, accounted for the low overall success rate on second-line ART in our study. Active patient tracing therefore remains of key importance after regimen switch.

In conclusion, the degree and pattern of HIV drug resistance detected after prolonged virological failure of first-line ART are likely to negatively impact future regimes containing NRTI backbones. These findings underscore the need to perform viral load testing to detect failure early and guide treatment switch. The standard of care in South Africa now includes yearly routine virological monitoring,[14] and this policy should be implemented in other African countries as well. Further research should focus on the optimal frequency of virological monitoring to reduce the risk of the accumulation of resistance mutations and protect the susceptibility to second-line and subsequent therapies.

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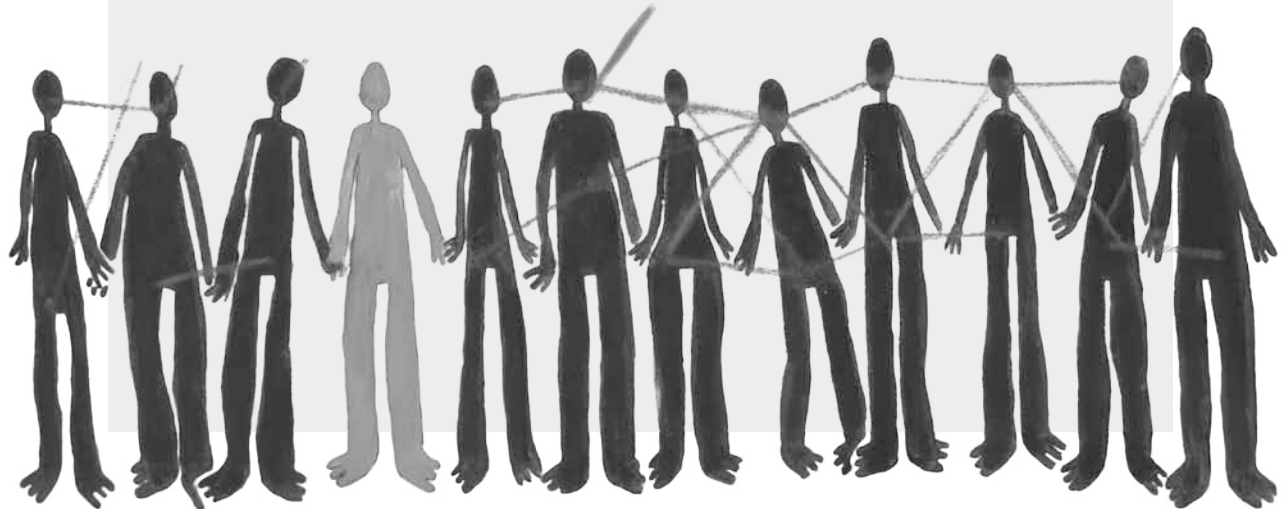
Part 3

Outcomes of first- and second-line antiretroviral therapy



Chapter 8

Early warning indicators for population based monitoring of HIV drug resistance in six African countries

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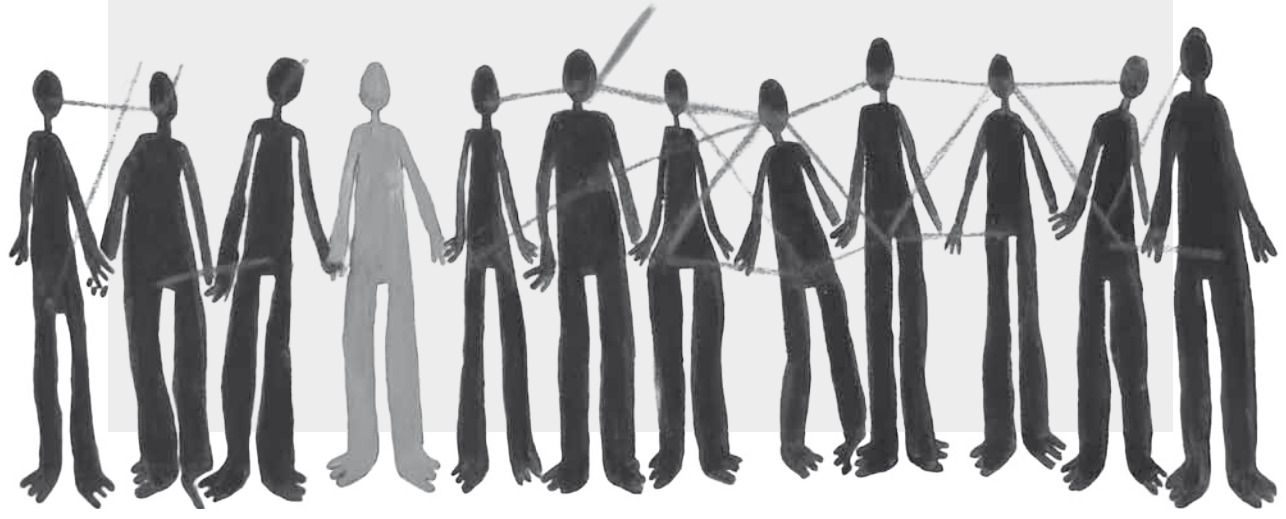

ABSTRACT

Human immunodeficiency virus (HIV) RNA testing and HIV drug resistance (HIVDR) testing are not routinely available for therapeutic monitoring of patients receiving antiretroviral therapy (ART) in resource-limited settings. World Health Organization HIVDR early warning indicators (EWIs) assess ART site factors known to favor the emergence of HIVDR. HIV drug resistance EWI monitoring was performed within the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) study, comprising 13 ART sites in 6 African countries. Early warning indicator assessment in the PASER network identified vulnerable aspects of ART programs and triggered interventions aimed at minimizing HIVDR emergence. Additionally, data suggest an advantage of medication possession ratio over on-time antiretroviral drug pickup in identifying patients at risk for HIVDR development.

INTRODUCTION

Major challenges to expanded access of antiretroviral therapy (ART) for human immunodeficiency virus (HIV)–infected individuals in sub-Saharan Africa arise from a deficient, under-resourced health infrastructure. Suboptimal delivery of HIV care and treatment may accelerate the emergence of drug resistant viruses, which could impair the sustained response to first-line ART and increase the subsequent spread to newly infected individuals.[1,2] Because HIV drug resistance (HIVDR) testing is not routinely available in resource-limited settings, the World Health Organization (WHO), as part of its global strategy for prevention and assessment of HIVDR, recommends that ART program factors, such as prescribing practices, patient retention, and drug supply and adherence, are monitored to optimize the quality of patient care.[3] World Health Organization HIVDR early warning indicators (EWIs) make use of data that are routinely collected in patients' medical and pharmacy records,[4] and feasibility of their implementation has been successfully demonstrated in sub-Saharan Africa.[5,6] However, a formal evaluation of the EWIs for their ability to identify possible HIVDR has not been performed.

Imperfect adherence to antiretroviral (ARV) medication is associated with poor virological response[7–10] and selection of drug resistant virus.[11,12] Patient adherence can be approximated using pharmacy pickup information, and studies have shown that drug pickup data may be considered an alternative to CD4 cell counts in predicting virological failure.[13,14] Another adherence measure, the medication possession ratio (MPR), defined as the amount of time an individual is in possession of ARVs divided by the time between ARV prescriptions,[15] has also been shown to be associated with treatment outcomes in resource-limited settings.[7,13,16–18]

We report the results of EWI assessment in a clinical network of 13 ART sites in 6 African countries. Additionally, we sought to evaluate the EWI on-time ARV drug pickup and the MPR as measures to determine possible HIVDR after 12 months of ART.

METHODS

Study Population and Design

Early warning indicators were collected as part of the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) study, a multicenter, prospective, observational cohort of HIV-infected adults who receive first- and second-line ART under routine circumstances at 13 clinical sites in 6 African countries (Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe). The sites collaborating on the PASER-M study comprise

6 public institutions, 3 non-governmental organizations, 2 private for-profit clinics, and 2 faith-based hospitals. Cohort and site characteristics have been previously described.[19] Exclusion criteria were pregnancy at study screening and re-initiation of first-line ART < 30 days after stopping previous ART. Any other prior ARV drug use was not an exclusion criterion. Participants were consecutively enrolled during a median site-specific enrollment period of 12 months. The appropriate institutional review boards approved the study, and all participants provided written informed consent.

EWI Data Abstraction

Definitions of the selected EWI followed WHO/HIVResNet guidance[4] and are summarized in Table 1. Data on PASER-M participants initiating first-line ART were used to abstract the following EWIs: ART prescribing practices, patients lost to follow-up (LTFU) at 12 months, patient retention of first-line ART, and viral load (VL) suppression at 12 months. The indicators "on-time ARV drug pickups" and "ARV drug supply continuity" were retrospectively collected from medical and/or pharmacy records. Indicator 4a (on-time ARV drug pickups) was modified from the WHO indicator and assessed for whether patients

Table 1. Definition of selected Early Warning Indicators

Indicator	Definition
1. ART prescribing practices	Percentage of patients initiating ART at the site who are prescribed, or pick up from the pharmacy, an appropriate[a] first-line ART regimen.
2. Patients LTFU at 12 months	Percentage of patients initiating ART at the site who are LTFU[b] 12 months later.
3. Patient retention on first-line ART	Percentage of patients initiating ART at the site who are taking an appropriate[a] first-line regimen 12 months later, excluding those who transferred out.
4a. On-time ARV drug pick-ups	Percentage of patients picking up all prescribed ARV drugs on-time[c] for two consecutive drug pick-ups after initiation of ART.
4b. On-time ARV drug pick-ups for 12 months	Percentage of patients picking up all prescribed ARV drugs on-time[c] during the first 12 months of ART or until classified as dead, transferred out or stopped ART.
6. ARV drug-supply continuity	Percentage of months in a designated year in which there were no ARV drug stock-outs.
8. VL suppression at 12 months	Percentage of patients initiating ART at the site with VL <1000 copies/ml after 12 months of first-line ART, excluding those who died or transferred out.

[a]An appropriate first-line ART regimen meets one or both of the following definitions: standard regimen listed in national ART guidelines and used according to those guidelines; regimen recommended in the WHO treatment guidelines. [b]Lost to follow-up is defined as a patient who has not returned to the clinic ≤90 days after the last missed appointment or drug pick-up during the first 12 months of ART, and who was not known to have transferred out, stopped therapy without restarting, or died. [c]On or before the date the previously dispensed drugs would have run out if taken according to schedule. Table adapted from WHO HIVDR EWI guidance document. EWI, early warning indicators; ART, antiretroviral therapy; ARV, antiretroviral; VL, viral load.

were on time for 2 consecutive drug pickups after initiation of ART; those who had been on ART for longer periods of time were not considered.

Medication Possession Ratio

The MPR was assessed using pharmacy pickup information during the first 12 months of ART for all patients with the exception of those who died or transferred out. The MPR was calculated by dividing the number of days a patient was in possession of ARVs by the 365 days in the first calendar year of ART.[15]

Laboratory Methods

HIV RNA testing for the survey was performed on EDTA anticoagulated plasma using the NucliSens EasyQ real-time assay version 2.0 (bioMérieux, Lyon, France) or the COBAS Ampliprep/COBAS TaqMan assay (Roche, Branchburg, New Jersey) at 2 reference laboratories in South Africa and Uganda.

Classification of Virological Outcomes

Viral load suppression, or HIVDR prevention, was defined as HIV RNA ≤1000 copies/mL after 12 months of first-line ART.[20] The outcome "possible HIVDR" applied to patients with HIV RNA > 1000 copies/mL after 12 months but also to patients who were LTFU, who were no longer on appropriate first-line regimens (due to stopping or switching ART), or from whom no follow-up specimen was available. Patients who transferred out or died in the first year of ART were censored from analysis because these outcomes are unlikely to be the result of HIVDR.[20]

Statistical Analysis

World Health Organization data abstraction tools[4] were used to capture and calculate on-time ARV drug pickups. The sensitivity, specificity, positive and negative predictive values of the EWI "on-time ARV drug pickup" and the MPR for the identification of virological outcomes (VL suppression or possible HIVDR) were calculated with 95% confidence intervals (CIs) using 2 x 2 tables. The positive and negative likelihood ratios were derived from the sensitivity and specificity. All analyses were performed using Stata software version 10 (StataCorp, College Station, Texas).

RESULTS

Study Sites and Population

During the recruitment period from March 2007 to September 2009, 2735 patients initiated first-line ART. Less than 5% of patients reported any previous ARV exposure (i.e.,

for prevention of mother to child transmission, mono/dual therapy and/or ART).[19] As shown in Table 2, it was feasible to monitor 5 of the selected indicators at all sites. The EWI "on-time ARV drug pickups" was monitored at 2 sites. All patients at 11 of 13 (84.6%) sites were prescribed an initial first-line regimen according to WHO guidelines.[21] At site 9, 2 patients received first-line protease inhibitor (PI)–based regimens, and at site 12, an

Table 2. Early Warning Indicators results by site

	ART prescribing practices	Patients LTFU at 12 months	Patient retention on first-line ART	On-time ARV drug pick-ups	Drug-supply continuity[a]	VL suppression at 12 months[b]
WHO target	100%	≤ 20%	≥ 70%	≥ 90%	100%	≥ 70%
Site 1	116/116 (100%)	13/116 (11.2%)	81/115 (70.4%)	-	12/12 (100%)	70/96 (72.9%)
Site 2	228/228 (100%)	10/228 (4.4%)	192/223 (86.1%)	178/228 (78.1%)	12/12 (100%)	173/206 (84.0%)
Site 3	239/239 (100%)	31/239 (13.0%)	165/227 (72.7%)	156/235[c] (66.4%)	12/12 (100%)	147/200 (73.5%)
Site 4	205/205 (100%)	20/205 (9.8%)	156/199 (78.4%)	-	NA[d]	139/185 (75.1%)
Site 5	204/204 (100%)	22/204 (10.8%)	162/193 (83.9%)	-	12/12 (100%)	132/185 (71.4%)
Site 6	223/223 (100%)	34/223 (15.3%)	177/220 (80.5%)	-	12/12 (100%)	154/214 (72.0%)
Site 7	203/203 (100%)	18/203 (8.9%)	174/202 (86.1%)	-	11/12 (91.7%)	140/193 (72.5%)
Site 8	215/215 (100%)	22/215 (10.2%)	176/215 (81.9%)	-	11/12 (91.7%)	156/200 (78.0%)
Site 9	221/223 (99.1%)	17/223 (7.6%)	173/222 (77.9%)	-	9/12 (75%)	159/192 (82.8%)
Site 10	220/220 (100%)	15/220 (6.8%)	184/213 (86.4%)	-	12/12 (100%)	152/199 (78.6%)
Site 11	223/223 (100%)	15/223 (6.7%)	200/217 (92.2%)	-	12/12 (100%)	169/215 (78.6%)
Site 12	229/230 (99.6%)	7/230 (3.0%)	211/230 (91.7%)	-	12/12 (100%)	186/223 (83.4%)
Site 13	206/206 (100%)	44/206 (21.4%)	148/205 (72.2%)	-	12/12 (100%)	127/199 (63.8%)
On target	11/13 (84.6%)	12/13 (92.3%)	13/13 (100%)	0/2 (0%)	9/12 (75%)	12/13 (92.3%)

ART, antiretroviral therapy; ARV, antiretroviral; VL, viral load; NA, not applicable; WHO, world health organization. [a]ARV drug supply continuity during the first year of the PASER-M survey. [b]Viral load as measured retrospectively for the PASER-M survey. [c]Patients who transferred out or died before the first pick-up were excluded from analysis. [d]No site dispensary.

inappropriate triple-nucleoside reverse transcriptase inhibitor (NRTI) combination was prescribed.

Twelve (92.3%) sites met the targets of ≤20% patients LTFU, and all sites retained ≥70% patients on first-line ART after 12 months. The overall proportion of patients LTFU within the first year of ART was 9.8% across sites (range, 3.0%–21.4%). Inappropriate switch to PI-based or inappropriate substitution to triple-NRTI regimens within the first 12 months took place in 19 patients at 7 sites. A total of 19 patients at 8 sites were switched to second-line ART after suspected treatment failure.

Neither of the sites at which ARV drug pickups were monitored met the target for this indicator: 78% of patients at site 2 and 66% of patients at site 3 were on time for 2 consecutive pickups. When assessing pickups for the entire first year of ART, 4% of patients at site 2 and 6% of patients at site 3 were always on time (data not shown). Calculation of the MPR revealed that 86% of patients at site 2 and 59% of patients at site 3 were in possession of ARVs 100% of the time in the first year of ART. Antiretroviral drug supply was uninterrupted at 75% of sites. Three sites reported stock-outs of fixed-dose combinations (FDCs), but not of individual ARV components, for a period of 1–3 months.

All but 1 site (92.3%) met the target of ≥70% of patients achieving VL suppression after 12 months of first-line ART. Overall, 76.0% of all patients in the cohort were classified as having VL suppression or HIVDR prevention. The remaining patients either had a VL >.1000 copies/mL (n = 166), had no available VL result (n = 148), were no longer on first-line ART (switch, n = 19; stop, n = 2), or were LTFU (n = 268) and were classified as having possible HIVDR.

Evaluation of Adherence Measures

As summarized in Table 3, the indicator "on-time ARV drug pickup" had a sensitivity of 46.6% (95% CI, 35.9%–57.5%) and a specificity of 79.4% (95% CI, 74.6%–83.7%) for determining possible HIVDR after 12 months of first-line ART. An MPR of 100% yielded 67.0% (95% CI, 56.2%–76.7%) sensitivity and 83.5% (95% CI, 79.0%–87.4%) specificity. The positive predictive value of on-time ARV drug pickup and MPR were 38.3% (95% CI, 29.1%–48.2%) and 52.7% (95% CI, 43.0–62.2%), respectively. The positive likelihood ratios for the 2 measures were 1.6 and 3.9, respectively.

Table 3. Performance of on-time ARV drug pick-up and the medication possession ratio to determine possible HIV drug resistance[a] at 12 months

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR (%)	NLR (%)
On-time ARV drug pick-ups	46.6 (35.9-57.5) [41/88]	79.4 (74.6-83.7) [255/321]	38.3 (29.1-48.2) [41/107]	84.4 (79.8-88.3) [255/302]	1.6	0.8
100% MPR	67.0 (56.2-76.7) [59/88]	83.5 (79.0-87.4) [268/321]	52.7 (43.0-62.2) [59/112]	90.2 (86.3-93.4) [268/297]	3.9	0.4

Numbers in parentheses refer to the 95% confidence interval. Numbers in square brackets refer to the numerator and denominator from which the relevant percentages are calculated. Medication possession ratio is defined as the amount of time an individual is in possession of ARVs divided by the time between ARV prescriptions. ARV, antiretroviral; MPR, medication possession ratio; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.
[a]Possible HIVDR defined as patients with viral load > 1000 copies/ml, patients lost to follow-up, patients who were no longer on appropriate first-line regimens (due to stopping or switching ART), or from whom no follow-up specimen was available.

DISCUSSION

The assessment of the WHO-recommended EWIs as part of the ongoing PASER-M study at 13 public and nongovernmental ART sites in 6 African countries has identified vulnerable aspects of ART program functioning that are potentially associated with the emergence of HIVDR. Feedback to the respective sites is expected to trigger interventions targeted at the most at-risk populations. The measurement of the MPR, as a proxy measure for drug adherence, appeared to have better performance to predict possible HIVDR after 12 months of ART than the indicator "on-time ARV drug pickups."

Monitoring the indicator "on-time ARV drug pickups" was not feasible at all sites because patient or pharmacy records were found to be incomplete or could not be easily accessed even though the required data may have been routinely collected. The complexity associated with extracting the data required for the drug pickup indicator has been reported previously.[5,6] The percentage of patients picking up ARVs on time for the entire first year of ART was unrealistically low, suggesting that this indicator may be too stringent or potentially signaling that pharmacy data may be unreliable. Notably, the indicator assessing 2 consecutive drug pickups after ART initiation achieved better performance in predicting virological outcomes. With a specificity of 79.4%, this cross-sectional indicator may be useful in correctly classifying persons in whom VL suppression will be achieved and could serve as a practical tool for clinics: adherence should be strongly reinforced in patients who fail to refill 2 drug pickups on time. Significantly, the MPR yielded higher sensitivity, specificity, and positive and negative predictive values than did ARV pickup information. As reflected by the positive likelihood ratio, patients with an MPR <100% were 3.9 times as likely to be classified as having possible HIVDR

after 12 months of ART compared with patients with perfect medication possession. The MPR was therefore more accurate in identifying patients at risk of HIVDR development than pickup information alone, which had a positive likelihood ratio of 1.6.

The assessment of ART prescribing practices is relevant as inappropriate drug combinations have been documented to lead to the development of HIVDR.[22] The rate of correct prescribing was high, but did not reach 100% at all sites. One patient received an inappropriate triple-NRTI regimen for unknown reasons. Protease inhibitor–based first-line regimens were prescribed for 2 patients diagnosed with Kaposi sarcoma at site 9, in spite of the recommendation to reserve these drugs for second-line therapy in the setting of limited formularies.[21]

Limiting attrition is essential because returning patients who were previously LTFU risk virological failure due to selected drug resistant virus.[23] The attrition rate was lower than has previously been reported in African ART programs[24] but varied substantially between sites. The site with the highest proportion of patients LTFU (21.4%) serves an urban, mobile population and performs limited patient tracing. Failure to correctly classify patients as deceased might have contributed to a high proportion presumed LTFU at both clinics that did not meet the indicator target. This underscores the importance of proper administration at ART sites for obtaining reliable EWI results. All sites met the target for patient retention on first-line ART at 12 months, which highlights the general effectiveness of first-line regimens and the success of managing toxicity with in-class substitutions. For 15 patients at 7 sites, inappropriate substitutions within first-line regimens to PI-based regimens occurred, limiting durable second-line options.

Because treatment interruptions may lead to HIVDR in resource-limited settings,[25] it is essential that ARV drug supply continuity be monitored strictly. The sites that did not meet the indicator target had stock-outs of FDCs that were replaced by the individual ARV components. Even though FDC stock-outs lasted up to 3 months (site 9), they did not result in stopping or changing of individual patients' regimens.

Lastly, the optional indicator "VL suppression at 12 months" identified 1 clinic in which <70% of patients achieved HIVDR prevention. This poor performance was largely due to the high rate of patients LTFU at this clinic. Patient retention and tracing are the most important programmatic factors to be targeted for improvement at this site, which failed to meet 2 of 5 EWI targets.

The current study has some limitations. Because the PASER-M study used eligibility criteria, the population may not be completely representative of the general clinic popula-

tion. However, additional selection bias is likely to be limited because all new patients who qualified for ART initiation were screened and enrolled in the study consecutively within a limited median time period of 12 months.[19]

In conclusion, this pilot assessment of EWIs in the PASER clinical network has identified deficiencies in ART site functioning that should be targeted to minimize the emergence of preventable HIVDR. Nine sites failed to meet at least 1 EWI target; this should raise concern and flag those aspects of the clinic or pharmacy that need to be improved. This report provides the first example of how the WHO site-based EWIs, part of the WHO global strategy for HIVDR prevention and assessment, have been successfully abstracted and evaluated within an ongoing population-based monitoring study. Early warning indicators approximating drug adherence appeared to be helpful in identifying patients at risk for the development of HIVDR who would benefit from targeted adherence support. Based on our findings, the assessment of on-time refilling for 2 consecutive drug pickups as opposed to the entire first year of ART was more feasible and informative. Our data suggest an additional advantage of the MPR over pickup information, because this measure was better able to predict possible HIVDR emergence. Further studies should focus on examining the MPR target or threshold necessary to prevent treatment failure and HIVDR. Based on its feasibility and performance, it is recommended to incorporate MPR in future EWI monitoring efforts to estimate patient adherence to ART. In addition to the ongoing laboratory-based studies to assess transmitted and acquired HIVDR within the PASER network, EWI monitoring will contribute to improved ART site functioning and quality of care for HIV-infected persons in sub-Saharan African countries.

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Chapter 9

Effect of pretreatment HIV-1 drug resistance on immunological, virological and drug resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study

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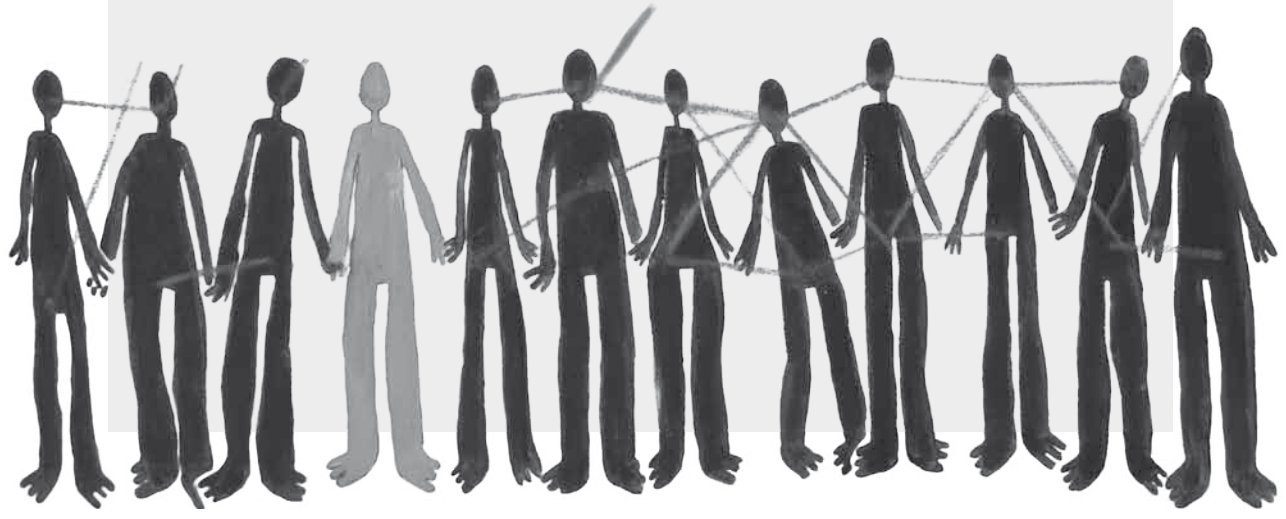


ABSTRACT

Background

The effect of pretreatment HIV-1 drug resistance on the response to first-line combination antiretroviral therapy (ART) in sub-Saharan Africa has not been assessed. We studied pretreatment drug resistance and virological, immunological, and drug resistance treatment outcomes in a large prospective cohort.

Methods

HIV-1 infected patients in the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort started non-nucleoside reverse transcriptase inhibitor-based ART at 13 clinical sites in six countries, from 2007 to 2009. We used the International Antiviral Society-USA drug resistance mutation list and the Stanford algorithm to classify participants into three pretreatment drug resistance categories: no pretreatment drug resistance, pretreatment drug resistance with fully active ART prescribed, or pretreatment drug resistance with reduced susceptibility to at least one prescribed drug. We assessed risk factors of virological failure (≥400 copies per mL) and acquired drug resistance after 12 months of ART by use of multilevel logistic regression with multiple imputations for missing data. CD4 cell count increase was estimated with linear mixed models.

Findings

Pretreatment drug resistance results were available for 2579 (94%) of 2733 participants; 2404 (93%) had no pretreatment drug resistance, 123 (5%) had pretreatment drug resistance to at least one prescribed drug, and 52 (2%) had pretreatment drug resistance and received fully active ART. Compared with participants without pretreatment drug resistance, the odds ratio (OR) for virological failure (OR 2.13, 95% CI 1.44–3.14; p<0.0001) and acquired drug resistance (2.30, 1.55–3.40; p<0.0001) was increased in participants with pretreatment drug resistance to at least one prescribed drug, but not in those with pretreatment drug resistance and fully active ART. CD4 count increased less in participants with pretreatment drug resistance than in those without (35 cells per μL difference after 12 months; 95% CI 13–58; p=0.002).

Interpretation

At least three fully active antiretroviral drugs are needed to ensure an optimum response to first-line regimens and to prevent acquisition of drug resistance. Improved access to alternative combinations of antiretroviral drugs in sub-Saharan Africa is warranted.

INTRODUCTION

A public-health approach based on standardized, affordable drug regimens and limited laboratory monitoring has been crucial for the scale-up of combination antiretroviral therapy (ART) in sub-Saharan African countries with high prevalences of HIV-1.[1] By 2010, more than 4 million people with HIV-1 in sub-Saharan Africa were receiving ART—about 40% of those in need.[2] Mutations in the HIV genome that confer drug resistance can diminish the virological response to ART.[3-6] Drug resistant variants, acquired because of selective pressure in people receiving ART, can be transmitted to people newly infected with HIV.[7, 8] In east, central, and southern Africa, reports suggest that transmitted drug resistance has increased, in parallel with the widespread distribution of ART.[9-12]

In developed countries, drug resistance testing to guide first-line therapy choices mitigates virological failure in people who have a transmitted drug resistant strain.[3-6] By contrast, in the absence of drug resistance testing in resource-limited countries, standard first-line combinations of antiretroviral drugs including two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) are prescribed empirically.[13, 14] The effect of emerging pretreatment drug resistance on the virological and immunological responses to the standard first-line ART regimens in sub-Saharan Africa has not been assessed.

This study investigates the effect of pretreatment drug resistance on the virological and immunological responses and the acquisition of drug resistance after the first year of ART and additional risk factors for virological failure and acquisition of drug resistance in the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort.[15]

METHODS

Study design and participants

PASER-M is a multicentre prospective cohort of people with HIV-1 receiving ART at 13 clinics in Kenya (two clinics), Nigeria (one), South Africa (three), Uganda (three), Zambia (three), and Zimbabwe (one). Cohort characteristics[15] and pretreatment drug resistance mutations in the antiretroviral-naive cohort[10] have been described previously.

Patients were included if they were aged 18 years or older, HIV-1 infected, and started first-line ART in accordance with national guidelines—i.e., when with advanced immunodeficiency (a CD4 cell count less than 200 cells per μL) or advanced HIV disease (WHO clinical stage 3 or 4).[13] To prevent recruitment of patients who were already receiving

ART, people who had received a first-line regimen in the previous 30 days were excluded. Use of ART (more than 30 days previously), or any previous monotherapy, dual therapy, or use of antiretroviral prophylaxis was allowed. Our analysis included participants who received a standard first-line regimen containing two NRTIs and an NNRTI, excluding those who had started receiving three NRTIs only or a regimen containing a protease inhibitor. Other exclusion criteria were pregnancy at study screening and—tested in Nigeria only—HIV-2 co-infection.

Participants provided written, informed consent at enrolment. The study protocol was approved by the appropriate national and local research ethics committees at the collaborating sites and by the Academic Medical Centre, University of Amsterdam, Netherlands.

Procedures

Participants were followed up in accordance with the local standard of care guidelines. Medical staff at each site extracted routine clinical and laboratory data recorded in medical records into standard case-report forms, which were then entered into a central online database. CD4 cell counts recorded closest to the ART start date were used as pretreatment counts. Throughout the study, a data monitoring team verified source data and reviewed study data.

Drug adherence was assessed at each follow-up clinic visit by two measures of self-reported adherence. For 3 day self-reports, we counted the number of follow-up visits at which the patients reported to have missed any pills during the previous 3 days. For the 30 day visual analogue scale, we averaged the number of pills taken at all follow-up visits and classified adherence accordingly as less than 80%, 80–94%, 95–99%, or 100%.[16, 17]

Patients were included in one of the following outcome categories: still on a first-line ART regimen after 12 months of follow-up, discontinued ART, transferred out, lost to follow-up, died, or switched to second-line ART because of treatment failure as judged by local criteria.[13] A single-drug substitution because of toxicity or intolerance was not classed as a regimen switch. Time was measured from the start of ART and ended at the earliest of either last follow-up visit or 12 months after starting ART (from 11 to 15 months); possible treatment interruptions were ignored.

Plasma was sampled at the baseline visit, after 12 months of therapy, and—if applicable—earlier, in case of treatment failure, and stored for later assessment of HIV RNA viral load and genotypic drug resistance. HIV viral load results after 12 months of follow-up

for participants who were still on a first-line regimen were classed as either suppression (<400 copies per mL) or failure (≥400 copies per mL).

All virological testing was done at either of two reference laboratories: one in South Africa or one in Uganda.[10] Viral loads were measured with NucliSens EasyQ real-time assay (version 2.0) or COBAS Ampliprep/COBAS Taqman assay. For baseline and follow-up specimens with a viral load greater than 1000 copies per mL, population-based genotyping of HIV protease and reverse transcriptase was done with in-house sequencing methods. HIV subtypes were inferred from the *pol* sequences with the STAR algorithm[18] and confirmed with REGA (version 2.0).[19] Genotypic drug resistance was defined as the presence of at least one major amino acid substitution included in the International Antiviral Society USA mutation list of December, 2010,[20] including revertant mutations at codon 215.[21] NRTIs, NNRTIs, and protease inhibitors were included.

Pretreatment drug resistance was defined in two steps. We used the International Antiviral Society USA mutation list to distinguish between participants with a virus with at least one drug resistance mutation and those with none.[20] For participants with a virus with at least one drug resistant mutation, we used the Stanford drug-susceptibility algorithm (version 6.0.9)[22] to classify participants into those receiving fully active ART (Stanford levels 1 [susceptible] or 2 [potential low-level resistance] for all prescribed drugs), or those receiving partly active ART (Stanford levels 3 [low-level resistance], 4 [intermediate resistance], or 5 [high-level resistance] for at least one of the prescribed drugs). All sequences have been deposited in GenBank (accession numbers HM119603–HM120150, HQ993572- HQ995497, JN630892-JN631033).

Statistical analysis

We estimated a required sample size of at least 190 people per site on the basis of anticipated virological failure and drop-out rates, and balanced against the cost of genotypic testing.[15] We did group comparisons for categorical data with a χ^2 test, and for continuous data with a one-way ANOVA or Kruskal-Wallis test,[23] as appropriate. We used multilevel analysis with random intercepts to assess the effect of baseline and prospective factors at the level of individuals and sites (while accounting for clustering of observations within sites) on two outcomes after 12 months of therapy—virological failure and acquired drug resistance (defined as the presence of at least one International Antiviral Society USA drug resistance mutation and viral load >1000 copies per mL). To account for missing data due to participant attrition, we did multiple imputations with the Markov chain Monte Carlo approach with five simulation datasets.

The main analysis was a model that incorporated the following baseline characteristics: pretreatment drug resistance, age, sex, clinical stage of disease, initial NRTI backbone (stavudine, zidovudine, tenofovir, or abacavir) and NNRTI drug (efavirenz or nevirapine), previous antiretroviral drug exposure (ART, single-dose nevirapine for prevention of mother-to-child transmission, combination prophylaxis for prevention of mother-to-child transmission, or other), pretreatment CD4 cell count (<50, 50–199, or ≥200 cells per μL), pretreatment viral load (log_{10}-transformed, fitted as a continuous variable), HIV subtype, anemia, body mass index (fitted as a continuous variable), cost to patient, year of ART initiation, government or non-government site, years of on-site ART experience, and patient to staff ratio. The prospective model also includes CD4 cell count increase 6 months after ART initiation (fitted as a categorical variable), single-drug substitution, 3 day and 30 day adherence (fitted as categorical variables), and routine viral-load test at 6 months after ART initiation (available at seven of 13 sites). All variables were assessed univariately and those associated ($p<0.10$) with the outcome were entered stepwise into the multivariate model. Biologically plausible interactions were examined in all multivariate models. We did several sensitivity analyses (webappendix table 3).

We estimated the mean changes in CD4 cell counts between the pretreatment drug resistance groups over intervals of 3 months by use of linear mixed models. All CD4 cell counts measured routinely before and after start of ART were used. The model was adjusted for age, sex, pretreatment CD4 cell count and viral load, HIV subtype, previous antiretroviral drug exposure, year of ART initiation, types of NRTI and NNRTI, and 30 day adherence. A sensitivity analysis included only participants who had viral suppression (HIV RNA <50 copies per mL) after 12 months of ART.

Reported p values are two-sided and a p value below 0·05 was judged significant. All analyses were done with Stata (version 11).

RESULTS

Between March, 2007, and September, 2009, 2733 participants were recruited (median 221 per site, range 116–239; figure 1). 2588 participants (95%) reported no previous exposure to antiretroviral drugs, 145 (5%) had previously had antiretroviral drugs, including ART (n=61), monotherapy or dual therapy (four), single-dose nevirapine for prevention of mother-to-child transmission (43), combination regimen for prevention of mother-to-child transmission (19), post-exposure prophylaxis (one), or unknown (23). Webappendix table 1 shows participant characteristics by site.

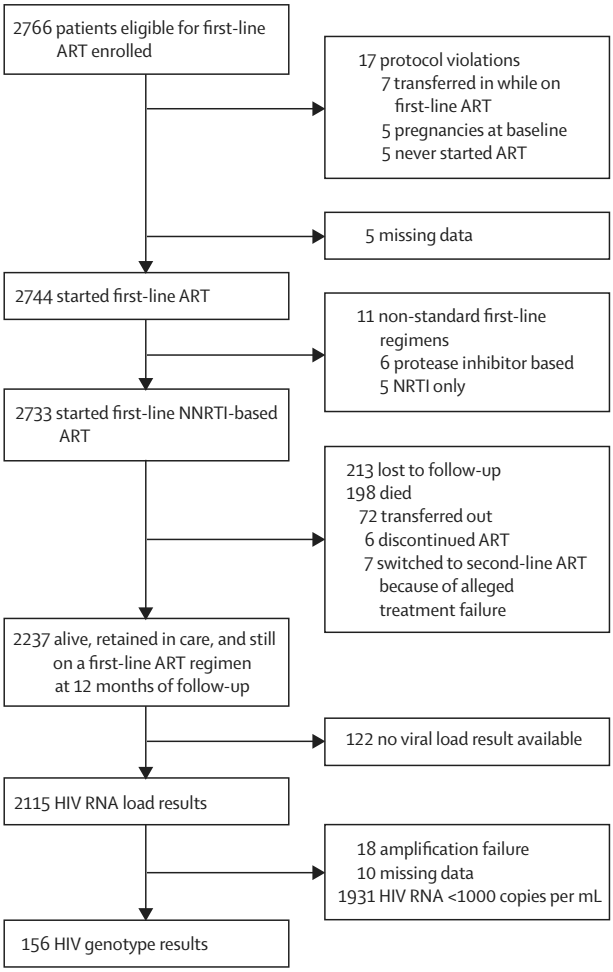


Figure 1. Study profile. ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

A pretreatment drug resistance test result was available for 2579 participants (94%; table 1). Pretreatment drug resistance was detected in 175 participants (7%), of whom 52 (30%) received fully-active ART and 123 (70%) had reduced susceptibility to at least one prescribed drug. Of these 123 participants, 99 (80%) had high-level resistance (Stanford 5), 11 (9%) had intermediate-level resistance (Stanford 4), and 13 (11%) had low-level resistance (Stanford 3) to at least one prescribed drug. The median number of active drugs for participants with pretreatment drug resistance to at least one prescribed drug was 2·0 (IQR 1.25–2.0). 1405 participants (54%) had HIV-1 subtype C, then 638 (25%) A, 296 (11%) D, 117 (5%) A/G recombinant, 68 (3%) G, 48 (2%) other recombinants, seven (<1%) other subtypes, and five (<1%) B. The distribution of pretreatment drug resistance and previous exposure to antiretroviral

Table 1. Patients' baseline characteristics

	All (n=2579)	No PDR (n=2404)	PDR (n=175)	P-value
Sex				0.494
Female	1493 (57.9)	1396 (58.1)	97 (55.4)	
Male	1086 (42.1)	1008 (41.9)	78 (44.6)	
Age – mean years (SD)	37.8 (9.0)	37.8 (9.0)	37.6 (9.3)	0.7361
18-29	506 (19.6)	470 (19.6)	36 (20.6)	0.947
30-39	1166 (45.2)	1088 (45.3)	78 (44.6)	
≥40	907 (35.2)	846 (35.2)	61 (34.9)	
Country				<0.0001
Zambia	551 (21.4)	522 (21.7)	29 (16.6)	
South Africa	601 (23.3)	566 (23.5)	35 (20.0)	
Uganda	606 (23.5)	530 (22.1)	76 (43.4)	
Kenya	424 (16.5)	405 (16.9)	19 (10.9)	
Zimbabwe	204 (7.9)	194 (8.1)	10 (5.7)	
Nigeria	193 (7.5)	187 (7.8)	6 (3.4)	
Calendar year of initiation				0.014
2007	618 (24.0)	585 (24.3)	33 (18.9)	
2008	1596 (61.9)	1470 (61.2)	126 (72.0)	
2009	366 (14.2)	349 (14.5)	16 (9.1)	
WHO clinical stage at initiation				0.936
1 or 2	1000 (38.8)	930 (38.7)	70 (40.0)	
3	1150 (44.6)	1073 (44.6)	77 (44.0)	
4	429 (16.6)	401 (16.7)	28 (16.0)	
Previous antiretroviral experience				<0.0001
No	2442 (94.7)	2302 (95.8)	140 (80.0)	
Yes	115 (4.5)	82 (3.4)	33 (18.9)	
Unknown	22 (0.9)	20 (0.8)	2 (1.1)	
Type of initial NRTI-backbone *				0.773
Zidovudine based	960 (37.2)	900 (37.4)	60 (34.3)	
Stavudine based	685 (26.6)	639 (26.6)	46 (26.3)	
Tenofovir based	870 (33.7)	805 (33.5)	65 (37.1)	
Abacavir based	64 (2.5)	60 (2.5)	4 (2.3)	
Type of initial NNRTI				0.459
Efavirenz	1542 (59.8)	1442 (60.0)	100 (57.1)	
Nevirapine	1037 (40.2)	962 (40.0)	75 (42.9)	
Pre-treatment body-mass index, median kg per m^2 (IQR) †	21.0 (18.8-24.0)	21.0 (18.3-23.7)	21.0 (18.4-23.6)	0.3553
Pre-treatment hemoglobin, median gram per dL (IQR) ‡	11.4 (9.9-12.9)	11.4 (9.9-12.9)	11.8 (10.5-13.2)	0.2300

Table 1 (continued)

	All (n=2579)	No PDR (n=2404)	PDR (n=175)	P-value
Pre-treatment CD4 cell count, median cells per µL (IQR) §	133 (62-204)	133 (62-204)	129 (53.5—204.5)	0.7148
<50	534 (20.8)	492 (20.5)	42 (24.0)	0.490
50-99	432 (16.8)	408 (17.0)	24 (13.7)	
100-199	910 (35.4)	845 (35.3)	65 (37.1)	
≥200	695 (27.0)	651 (27.2)	44 (25.1)	
Pre-treatment HIV-RNA, median log_{10} copies per mL (IQR) ¶	5.00 (4.36-5.59)	5.04 (4.46-5.62)	5.16 (4.58-5.74)	0.591 ||

Data are n (%), mean (SD), or median (IQR). Data are from participants who initiated an NNRTI-based first-line regimen and had a pretreatment genotypic resistance test result (n=154 excluded). p values refer to the comparison between PDR and no PDR groups. *Combined with lamivudine or emtricitabine. †Data available for 2562 patients. ‡Data available for 2526 patients. §Data available for 2568 patients. ¶Data available for 2561 patients. ||Adjusted for assay. PDR, pretreatment drug resistance; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor;

drugs did not differ between the initial ART regimens (data not shown). The prevalences of all pretreatment drug resistance mutations are listed in webappendix table 2.

Total follow-up was 2358 person-years, with a median of 11·9 months (IQR 11.2–12.2). Of 2733 participants, 2237 (82%) were retained in care and still receiving first-line ART after 12 months of follow-up. 489 (18%) were not retained, 198 (7%) because they had died, 213 (8%) lost to follow-up, 72 (3%) transferred out, and six (<1%) discontinued ART. Seven (<1%) patients switched to a second-line regimen in the first year of ART because of treatment failure (figure 1), one of whom had pretreatment drug resistance (webappendix figure 1). A slightly smaller proportion of patients with pretreatment drug resistance were retained on first-line ART (133 [76%]) than were those without (1979 [82%]; p=0·036); whereas all-cause mortality did not differ significantly between the groups (17 [10%] *vs.* 171 [7%]; p=0·201).

1942 participants (87%) were still taking their original regimen after 12 months, and 295 (13%) had a single-drug substitution because of toxicity, intolerance, or other reasons. 12 patients (1%) interrupted ART because of severe intolerance. 2218 participants (81%) had not missed any pills in the 3 days before any follow-up visit. 1517 patients (56%) had 100% 30 day adherence, 726 (27%) had 95–99% adherence, 201 (7%) had 80–94% adherence, and 37 (1%) had <80% adherence. Pretreatment drug resistance and adherence were not associated (data not shown).

Table 2. Risk factors of virological failure in the first year of first-line ART

	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Total	213									
Pre-treatment drug resistance										
No PDR	174	1			1			1		
PDR and fully-active ART	6	1.62	0.67, 3.92	0.282	1.01	0.55, 1.87	0.964	1.07	0.54, 2.11	0.853
PDR and partially-active ART	21	3.31	1.96, 5.59	<0.001	2.13	1.44, 3.14	<0.001	2.21	1.40, 3.50	0.001
Sex										
Women	108	1			1			1		
Men	105	1.53	1.15, 2.03	0.003	0.86	0.39, 1.86	0.695	0.52	0.21, 1.30	0.162
Age (yrs), mean (sd) *		0.76	0.64, 0.91	0.002						
For men					0.87	0.76, 1.01	0.06	0.87	0.61, 1.04	0.114
For women					0.74	0.64, 0.85	<0.001	0.67	0.74, 1.03	<0.001
Calendar year of ART initiation										
2007	64	1			1			1		
2008	116	0.55	0.36, 0.84	0.006	0.87	0.66, 1.13	0.291	0.72	0.53, 0.99	0.041
2009	33	0.60	0.33, 1.11	0.104	0.69	0.46, 1.04	0.075	0.67	0.42, 1.07	0.092
WHO clinical stage										
1 or 2	76	1								
3 or 4	137	1.34	1.00, 1.80	0.054						
Body Mass Index, kg/m^2 †		0.81	0.68, 0.97	0.019						
Pre-treatment viral load (log_{10} c/mL), median (IQR)		1.29	1.10, 1.52	0.002	1.20	1.08, 1.34	0.001	1.13	0.99, 1.28	0.071
Previous antiretroviral experience										
None	194	1			1			1		
ART	10	3.64	1.73, 7.69	0.001	2.62	1.50, 4.57	0.001	3.10	1.65, 8.81	<0.001
Single-dose nevirapine for PMTCT	5	1.95	0.73, 5.20	0.181	1.66	0.84, 3.30	0.148	2.04	0.94, 4.47	0.073
Other	3	2.01	0.57, 7.10	0.278	1.91	0.84, 4.36	0.124	1.86	0.75, 4.60	0.182
Type of initial NRTI-backbone ‡										
Zidovudine based	102	1								
Stavudine based	43	0.63	0.43, 0.91	0.015						
Tenofovir based	63	0.72	0.52, 1.01	0.057						
Abacavir based	5	1.04	0.40, 2.71	0.942						
Type of initial NNRTI §										
Efavirenz	104	1			1			1		
Nevirapine	109	1.50	1.13, 1.99	0.005	0.98 §	0.80, 1.20	0.864	1.12	0.88, 1.41	0.363

Table 2 (continued)

	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
CD4 gain six months after initiation, cells per µL ¶										
<0 (decrease)	18	1						1		
0-99	41	0.41	0.22, 0.76	0.004				0.36	0.22, 0.60	<0.001
100-199	28	0.30	0.16, 0.57	<0.001				0.27	0.16, 0.45	<0.001
≥200	17	0.27	0.13, 0.55	<0.001				0.31	0.18, 0.54	<0.001
3-day self-report adherence, no. ¶										
0	126	1						1		
1	52	2.39	1.76, 3.42	<0.001				1.31	0.94, 1.83	0.114
2	27	6.41	3.83, 10.73	<0.001				1.67	0.96, 2.88	0.068
3 or more	8	7.92	3.16, 19.83	<0.001				1.87	0.71, 4.94	0.208
30-day VAS adherence, %¶										
100	99	1						1		
95-99	57	1.10	0.76, 1.57	0.623				0.63	0.47, 0.85	0.003
94-80	38	3.69	2.39, 5.70	<0.001				1.75	1.14, 2.69	0.010
<80	10	9.44	3.97, 22.45	<0.001				3.70	1.71, 8.04	0.001

Table shows results of multilevel logistic regression, including the data from all participants who initiated a NNRTI-based first-line regimen. 3 day self-report is defined as the number of follow-up clinic visits at which the patient reported to have missed any pills in the 3 previous days. 30 day VAS is defined as the mean visual analogue score at all follow-up clinic visits. Total number of events=213. *Odds ratio for 10-year increase in age. †Odds ratio for 5 kg/m2 increase. ‡Combined with lamivudine or emtricitabine. §Inverse-probability weighting for NNRTI assignment: OR 0.91 (95% CI 0.69–1.19; p=0.475). ¶The baseline model did not include data for prospectively collected parameters. ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission; VAS, visual analogue score; OR, odds ratio; PDR, pretreatment drug resistance.

Viral load at 12 months' follow-up was assessed for 2115 (95%) of 2237 participants still taking first-line ART. 213 of 2733 patients (70%, 95% CI 68–71) who started first-line ART had viral suppression; 213 of 2115 (90%, 89–91) of those who were still on first-line ART had viral suppression (figure 1). Virological suppression at 12 months did not differ significantly between participants without pretreatment drug resistance (1697 [91%]) and those with pretreatment drug resistance and fully active ART (36 [86%]; p=0.274), but was significantly lower in participants with resistance to at least one prescribed drug (63 [75%]; p<0·0001).

Of the 184 participants with a viral load greater than 1000 copies per mL at 12 month follow-up, 156 (85%) specimens were successfully genotyped: 113 (72%) had one or

more major drug resistance mutation. Drug resistance mutations were associated with NRTIs (94, 60%), NNRTIs (98, 63%) and protease inhibitors (two, 1%). Dual-class resistance to NRTIs and NNRTIs was detected in 80 participants (51%), and no triple-class resistance was detected. Of the 113 participants who had one or more major drug resistance mutation after 12 months of ART, 20 (18%) had pretreatment drug resistance, 87 (77%) did not, and status was unknown in six (5%).

Compared with participants without pretreatment drug resistance, people who had resistance to at least one prescribed drug had an increased risk of virological failure (odds ratio [OR] 2.13, 95% CI 1.44–3.14; p<0·0001; table 2) and acquired drug resistance (2.30, 1.55–3.40; p<0.0001; table 3). By contrast, for those who had pretreatment drug resistance and were prescribed a fully active regimen, the ORs for virological failure (1.01, 0.55–1.87; p=0.964; table 2) or acquired drug resistance (0.97, 0.51–1.58; p=0.934; table 3) were not increased, compared with those without pretreatment drug resistance (webappendix table 3). Additionally, compared with participants with pretreatment drug resistance and reduced susceptibility to at least one prescribed drug, the risks of virological failure (0.48, 0.23–0.97, p=0.041) and acquired drug resistance (0.42, 0.20–0.88; p=0.022) were reduced in those with pretreatment drug resistance and fully active ART.

Independent of pretreatment drug resistance, participants who had virological failure or acquired drug resistance were more likely to have been previously exposed to ART or prevention of mother-to-child transmission, to have high pretreatment viral load, and to be younger (for women) at ART initiation, than were participants who did not (tables 2, 3). Participants who acquired drug resistance were also more likely to have a pretreatment CD4 count less than 50 cells per µL than were those who did not. Of the measures obtained prospectively after ART initiation, no CD4 cell count increase during the first 6 months of ART, and suboptimum (<95%) 30 day adherence were associated with virological failure (table 2) and acquired drug resistance (table 3) by month 12. In the univariate analysis the following covariables were not associated with virological failure: pretreatment CD4 cell count, treatment cost, severe anemia at initiation, HIV subtype, site administration, site ART experience, patient to staff ratio, single-drug substitution, and viral-load test at 6 months after initiation. In univariate analysis the following covariables were not associated with acquired drug resistance: free treatment, severe anemia at initiation, body-mass index, HIV-1 subtype, site administration, site ART experience, patient to staff ratio, single-drug substitution, viral-load test at 6 months after initiation. No significant effect modification across strata of pretreatment drug resistance and covariables occurred.

Table 3. Risk factors of acquired drug resistance in the first year of first-line ART

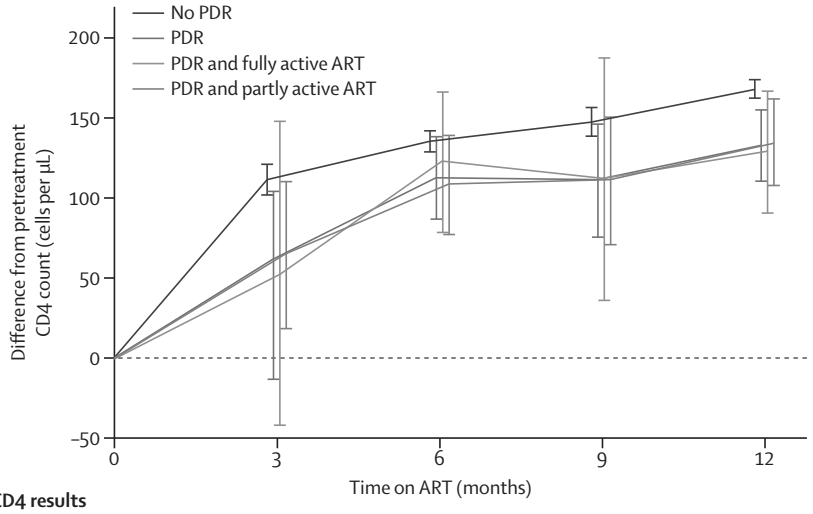
	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Total	113									
Pre-treatment drug resistance										
No PDR	87	1			1			1		
PDR and fully-active ART	4	2.11	0.73, 6.11	0.166	0.97	0.51, 1.58	0.934	1.05	0.52, 2.15	0.884
PDR and partially-active ART	16	5.03	2.77, 9.12	<0.001	2.30	1.55, 3.40	<0.001	2.42	1.53, 3.83	<0.001
Sex										
Women	56	1			1			1		
Men	57	1.54	1.05, 2.26	0.026	0.78	0.35, 1.76	0.555	0.45	0.17, 1.17	0.102
Age (yrs), mean (sd) *		0.75	0.59, 0.94	0.014						
For men					0.90	0.77, 1.04	0.151	0.91	0.76, 1.08	0.280
For women					0.75	0.65, 0.87	<0.001	0.67	0.56, 0.80	<0.001
Calendar year of ART initiation										
2007	33	1			1			1		
2008	62	0.56	0.32, 0.98	0.042	0.97	0.74, 1.27	0.819	0.78	0.57, 1.08	0.132
2009	18	0.63	0.29, 1.41	0.263	0.83	0.55, 1.27	0.394	0.76	0.47, 1.22	0.255
WHO clinical stage										
1 or 2	38	1								
3 or 4	75	1.46	0.97, 2.18	0.068						
Pre-treatment CD4 count, cells per µL										
<50	50	1			1			1		
50-199	43	0.62	0.40, 0.97	0.037	0.67	0.55, 0.83	<0.001	0.64	0.50, 0.81	<0.001
≥200	20	0.35	0.20, 0.63	<0.001	0.60	0.47, 0.76	<0.001	0.58	0.44, 0.77	<0.001
Pre-treatment viral load (log_{10} c/mL), median (IQR)		1.43	1.13, 1.80	0.002	1.12	1.00, 1.26	0.048	1.04	0.91, 1.19	0.594
Previous antiretroviral experience										
None	100	1			1			1		
ART	8	5.83	2.56, 13.31	<0.001	2.76	1.58, 4.85	<0.001	3.22	1.71, 6.05	<0.001
Single-dose nevirapine for PMTCT	3	2.16	0.63, 7.45	0.222	1.99	0.99, 3.99	0.052	2.34	1.06, 5.18	0.035
Other	1	2.21	0.16, 9.40	0.855	2.11	0.90, 4.93	0.084	1.98	0.78, 4.99	0.150
Type of initial NRTI-backbone †										
Zidovudine based	58	1			1			1		
Stavudine based	12	0.31	0.16, 0.58	<0.001	1.13	0.85, 1.50	0.398	1.05	0.76, 1.44	0.759
Tenofovir based	39	0.79	0.52, 1.20	0.272	1.08	0.84, 1.39	0.557	1.02	0.76, 1.38	0.883
Abacavir based	4	1.47	0.51, 4.28	0.478	1.99	1.13, 3.51	0.018	1.53	0.78, 3.00	0.221

Table 3 (continued)

	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Type of initial NNRTI										
Efavirenz	52	1			1			1		
Nevirapine	61	1.65	1.11, 2.46	0.014	0.96‡	0.77, 1.20	0.735	1.06	0.82, 1.38	0.636
CD4 gain six months after initiation, cells per µL §										
<0 (decrease)	11	1						1		
0-99	32	0.56	0.27, 1.16	0.117				0.34	0.20, 0.57	<0.001
100-199	13	0.24	0.10, 0.55	0.001				0.22	0.13, 0.38	<0.001
≥200	7	0.19	0.071, 0.51	0.001				0.27	0.15, 0.48	<0.001
3-day self-report adherence, no. §										
0	70	1						1		
1	25	1.87	1.16, 3.02	0.010				0.79	0.56, 1.13	0.205
2	12	3.90	2.00, 7.61	<0.001				0.64	0.35, 1.17	0.147
3 or more	6	8.44	3.15, 22.62	<0.001				1.03	0.37, 2.90	0.952
30-day VAS adherence, % §										
100	48	1						1		
95-99	35	1.50	0.93, 2.42	0.097				0.86	0.63, 1.16	0.325
94-80	24	4.46	2.60, 7.66	<0.001				2.46	1.57, 3.84	<0.001
<80	5	7.99	2.76, 23.11	<0.001				4.97	2.30, 10.74	<0.001

Table shows results of multilevel logistic regression, including the data from all participants who initiated a NNRTI-based first-line regimen. *OR for 10-year increase in age. †Combined with lamivudine or emtricitabine. ‡Inverse-probability weighting for NNRTI-assignment: OR 0.78 (95% CI 0.59–1.04; p=0.088). §The baseline model did not include data for prospectively collected parameters. PDR, pretreatment drug resistance; ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission; VAS, visual analogue score; OR, odds ratio.

The median gain in CD4 count between the start of ART and 6 months' follow-up was 110 (IQR 57–186) cells per µL and 149 (IQR 76–239) cells per µL at 12 months. Compared with participants without pretreatment drug resistance, CD4 count increased less in the first 3 months of ART (49 cells per µL; 95% CI 7–90; p=0.021) up to 12 months (35 cells per µL; 13–58; p=0.002) in all participants with pretreatment drug resistance; the difference was 40 cells per µL per 12 months (2–78; p=0.041) for those with pretreatment drug resistance and fully active ART, and 33 cells per µL (6–61; p=0.015) for those with resistance to at least one prescribed drug (figure 2).



Number of CD4 results					
No PDR	2396	442	1095	489	1527
PDR	175	24	80	38	113
PDR and fully active ART	52	4	26	10	39
PDR and partly active ART	123	20	54	28	72

Figure 2. Change in CD4 cell count, by pretreatment drug resistance. Linear mixed model (n=2439) adjusted for age, sex, pre-treatment CD4 count, pretreatment HIV RNA load, HIV-1 subtype, year of ART initiation, NRTI-backbone, NNRTI-drug, previous antiretroviral drug exposure, and adherence; not adjusted for HIV RNA loads at 12 months of therapy. Vertical bars=95% CI. PDR=pretreatment drug resistance. ART, antiretroviral therapy.

In a sensitivity analysis, which only included participants who had viral suppression (viral load <50 copies per mL) at 12 months' follow-up, the reduced CD4 increase for all participants with pretreatment drug resistance was of borderline statistical significance (28 cells per µL; 95% CI 1–55; p=0.039), compared with those without pretreatment drug resistance (webappendix figure 2).

DISCUSSION

Pretreatment drug resistance was strongly associated with virological failure and development of drug resistance after the first year of standard first-line NNRTI-based ART in patients in sub-Saharan Africa who received partly active regimens—i.e., that included at least one drug to which the virus had reduced susceptibility. By contrast, the response to ART was not reduced in patients who had a drug resistant virus when the prescribed regimen was predicted to be fully active. Our findings seem to be robust in sensitivity analyses that adjusted for possible effects of previous antiretroviral drug exposure and the M184V mutation (webappendix table 3). These findings are largely in agreement

with results from studies in developed countries (panel).[3-6] Notably, 70% of participants who had pretreatment drug resistance were empirically started on a suboptimum first-line regimen—nearly 5% of the total study population. In view of the limited potency and low genetic barrier of NNRTI-based regimens, our study emphasizes the need for at least three fully active antiretroviral drugs in first-line regimens in Africa to ensure an optimum virological response and to prevent the acquisition of NRTI and NNRTI drug resistance mutations.

Panel: Research in context

Systematic review

We searched PubMed for English-language studies, published between January, 2006, and September, 2011, with a sample size greater than 100, that assessed the effect of pretreatment HIV-1 drug resistance on response to first-line antiretroviral therapy. We identified seven cohort studies from Europe and none from resource-limited countries (appendix pp 9–10). Some studies showed no significant association between the presence of pretreatment drug resistance and virological [24-26] or immunological [24, 25] response to antiretroviral therapy. Other studies reported a decreased virological response in patients with pretreatment drug resistance compared with those without [3, 6]. A collaborative study including 10 056 patients from 25 European cohorts showed an increased risk of virological failure in patients with pretreatment drug resistance to at least one prescribed drug compared with those without pretreatment drug resistance, but no increased risk for those with pretreatment drug resistance who received fully active ART [5].

Interpretation

Our study confirms findings from studies done in high-income countries. The risk of virological failure and acquired drug resistance for patients in sub-Saharan Africa receiving non-nucleoside reverse transcriptase inhibitor-based regimens with pretreatment drug resistance to at least one prescribed drug was increased compared with those without pretreatment drug resistance and those with pretreatment drug resistance who received a fully active regimen. Improved access to alternative drugs with different modes of action and without cross-resistance to nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors is warranted in resource-limited countries to ensure continued effectiveness of HIV/AIDS treatment.

Overall, CD4 cell count recovered well during the first year of ART in patients with pretreatment drug resistance, although it was reduced compared with those without resistance. This difference is probably due to the higher proportion with virological failure among patients with pretreatment drug resistance, rather than by a direct effect of drug resistant virus on CD4 cell count. The small difference in CD4 gain after adjustment for differences in viral load at 12 months of ART could be due to the longer time needed to achieve viral suppression or by residual viral replication below the detection level of the HIV RNA assay in the pretreatment drug resistance group, compared with the group without. However, the clinical relevance of this difference might be small, because the overall immunological response in participants with pretreatment drug resistance was

favorable. By contrast with a European study (panel),[5] which showed a reduced CD4 cell count increase only in patients with transmitted resistance to at least one prescribed drug, but not in patients with transmitted resistance who received a fully active regimen, immunological outcomes did not differ between the ART susceptibility subgroups in our study, which might be because of a lack of statistical power.

Independent of pretreatment drug resistance, previous use of ART was strongly associated with virological failure and acquired drug resistance. Previous exposure to ART might result in minority-resistant viral strains not detected by population-based sequencing. Minority NNRTI resistance mutations might contribute to virological failure in patients starting an NNRTI-based regimen.[27-32] Furthermore, women who had had single-dose nevirapine to prevent perinatal transmission of HIV-1 had a slightly higher risk of virological failure than did antiretroviral-naive women. A randomized study in Botswana showed that previous exposure to peripartum single-dose nevirapine results in increased rates of virological failure if given less than 6 months before the start of subsequent NNRTI-based ART.[33] For this reason, the revised 2010 WHO HIV treatment guidelines[14] recommend initiating a regimen based on a protease inhibitor in women with previous exposure to antiretrovirals for prevention of mother-to-child transmission if they have received single-dose nevirapine alone or in combination with other drugs without an NRTI tail within 12 months of starting long-term ART, irrespective of the presence of NNRTI-associated pretreatment drug resistance. Because of the small numbers of women who had received ART for prevention of mother-to-child transmission in our study, we could not differentiate according to the time since prevention of mother-to-child transmission treatment exposure.

In the absence of routine virological monitoring, clinical proxy measures of treatment failure, obtained prospectively after ART initiation, might be helpful for clinical decision making as an early warning signal. In our study, we noted that failure to achieve an increase in CD4 cell count by month 6 as well as prolonged non-adherence below 95% was associated with virological failure and the acquisition of drug resistance by month 12. Sustained suboptimum drug concentrations leads to ongoing viral replication and selection for drug resistant variants. Suboptimum ART adherence predicts virological failure,[34] the development of drug resistance[35, 36] and death.[37] Our finding supports current recommendations for NNRTI-based regimens to strive for adherence levels above 95%. Average adherence was high in our cohort, which suggests that adequate rates of adherence can be achieved in African ART programmes; although self-reported adherence measures as used in our study can be affected by recall or social desirability bias.[38]

This study has some limitations. Attrition due to mortality and loss to follow-up in the first year of ART was high 489 (18% of patients enrolled), which is consistent with attrition estimates for ART programmes in sub-Saharan Africa.[2, 39] To account for the uncertainty introduced by these missing data, we used multiple imputations, which reduce bias and improve statistical efficiency. A sensitivity analysis that classed missing data as failures also suggests that our findings are robust. The overall virological failure after 12 months of therapy was 30% for all patients who started ART and 10% for those who were still on first-line ART, which compares favorably to a systematic review of 89 ART programmes in Africa.[40] Although the PASER network includes mostly free-access, routine ART programmes, non-government and urban sites were over-represented, and public and rural sites were under-represented. Therefore, caution is warranted when extrapolating the results to other settings where resource constraints might be even more substantial. Population-based sequencing cannot detect minority-resistant viral strains that are present below 25% of the viral population. Therefore, a proportion of participants with pretreatment drug resistance could have been misclassified as having none (false-negative), which might have led to underestimates of the effect on treatment outcome. Furthermore, the absence of serial blood sampling after ART initiation precluded a time-to-event analysis for virological failure and the development of pretreatment drug resistance.

Our study emphasizes the need for at least three fully active antiretroviral drugs to ensure an optimum response to first-line NNRTI-based ART regimens in sub-Saharan Africa and prevent the acquisition of HIV drug resistance. The findings have important implications for public health policy. Early mortality and attrition are major challenges to the success of ART programmes in Africa. Emerging drug resistance also jeopardizes the effectiveness of standard regimens. To maintain effective HIV/AIDS treatment in Africa, further studies and mathematical modeling are needed to establish optimum strategies for the prevention of drug resistance, ART monitoring, retention of patients, and use of protease-inhibitor-based regimens for specific high-risk groups, taking into account that pretreatment drug resistance frequencies in Africa will likely increase in the coming years.[9-12] Extended genotypic drug resistance testing as part of population-based surveillance is needed in regions where ART is scaled up. Improved access to alternative drugs with different modes of action and without cross-resistance to NRTIs and NNRTIs is warranted in resource-limited countries to ensure the continued effectiveness of HIV/AIDS treatment.

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WEBAPPENDIX

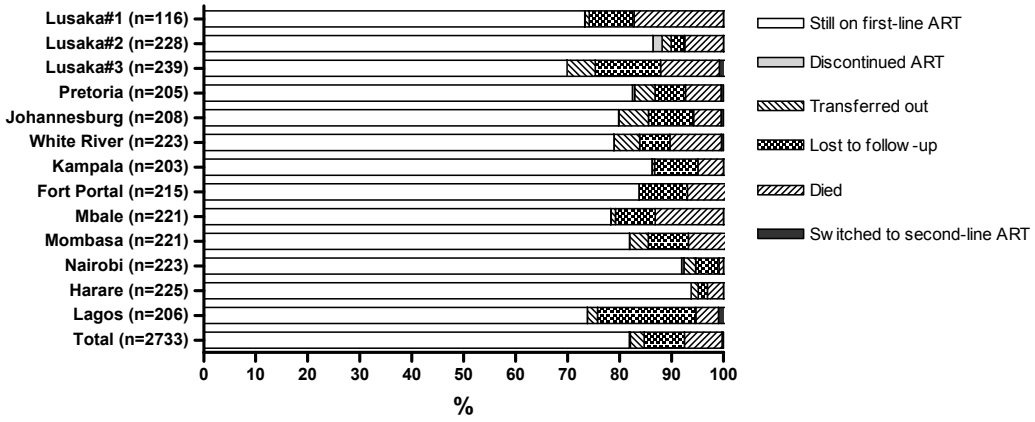

Figure 1. Bar chart of participants' clinical outcomes at 12 months of follow-up for each site. Bart chart shows data from all participants who initiated a NNRTI-based first-line regimen.

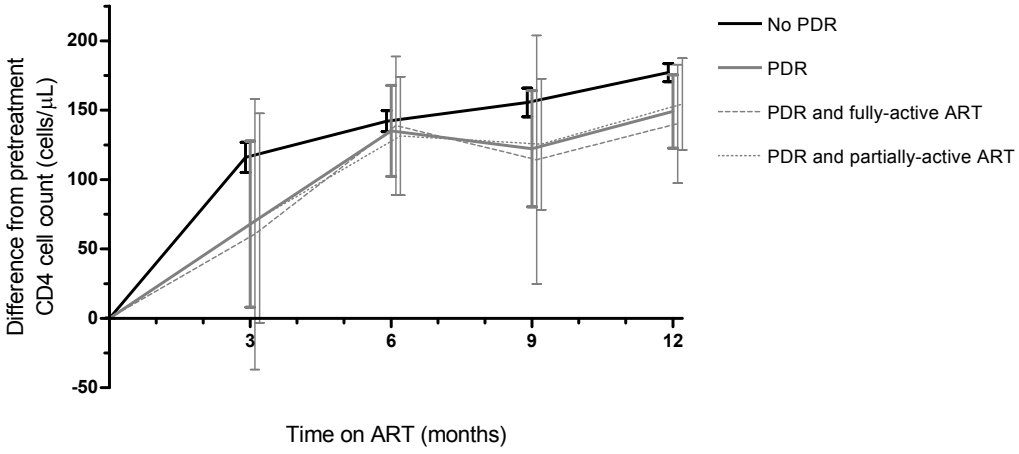

Figure 2. Change in CD4 cell count, by pre-treatment drug resistance. Sensitivity analysis: only includes participants with HIV-RNA<50c/mL after 12 months of therapy (n=1560). Linear mixed model adjusted for age, sex, pre-treatment CD4 count, pretreatment HIV-RNA load, HIV-1 subtype, calendar year of initiation, NRTI-backbone, NNRTI-drug, prior antiretroviral drug exposure, and adherence. Vertical bars = 95% confidence interval. PDR, pre-treatment drug resistance; ART, antiretroviral therapy.

Table 1. Baseline characteristics of all participants, by site.

Country			Zambia		South Africa			Uganda			Kenya		Zimbabwe	Nigeria
Sex														
Female	1592 (58.3)	53 (45.7)	133 (55.7)	133 (58.3)	113 (55.1)	155 (74.5)	132 (59.2)	113 (55.7)	120 (55.8)	117 (52.9)	131 (59.3)	121 (54.3)	147 (65.3)	124 (60.2)
Male	1141 (41.8)	63 (54.3)	95 (41.7)	106 (44.4)	92 (44.9)	53 (25.5)	91 (40.8)	90 (44.3)	95 (44.2)	104 (47.1)	90 (40.7)	102 (45.7)	78 (34.7)	82 (39.8)
Age – mean yrs (SD)	37.8 (9.0)	39.1 (8.9)	37.5 (8.7)	37.6 (9.4)	38.2 (8.3)	36.5 (7.4)	39.0 (10.0)	36.1 (8.8)	37.3 (10.3)	38.6 (9.6)	37.5 (8.6)	38.7 (8.7)	38.7 (8.7)	37.0 (8.2)
Calendar year of initiation														
2007	650 (23.8)	48 (41.4)	134 (58.8)	238 (99.6)	117 (57.1)	20 (9.6)	31 (13.9)	0 (0)	0 (0)	0 (0)	62 (28.1)	0 (0)	0 (0)	0 (0)
2008	1690 (61.8)	68 (58.6)	94 (41.2)	1 (0.4)	88 (42.9)	188 (90.4)	192 (86.1)	203 (100.0)	215 (100.0)	221 (100.0)	159 (72.0)	132 (59.2)	60 (26.7)	69 (33.5)
2009	393 (14.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	91 (40.8)	165 (73.3)	137 (66.5)
WHO clinical stage at initiation														
1 or 2	1078 (39.4)	74 (63.8)	92 (40.4)	97 (40.6)	84 (41.0)	136 (65.4)	59 (26.5)	76 (37.4)	77 (35.8)	46 (20.8)	81 (36.7)	66 (29.6)	102 (45.3)	88 (42.7)
3	1205 (44.1)	19 (16.4)	115 (50.4)	126 (52.7)	71 (34.6)	53 (25.5)	96 (43.1)	78 (38.4)	90 (41.9)	139 (62.9)	124 (56.1)	106 (47.5)	102 (45.3)	86 (41.8)
4	450 (16.5)	23 (19.8)	21 (9.2)	16 (6.7)	50 (24.4)	19 (9.1)	68 (30.5)	49 (24.1)	48 (22.3)	36 (16.3)	16 (7.2)	51 (22.9)	21 (9.3)	32 (15.5)
Type of initial NRTI-backbone [a]														
Zidovudine based	1017 (37.2)	13 (11.2)	60 (26.3)	10 (4.2)	20 (9.8)	10 (4.8)	18 (8.1)	122 (60.1)	66 (30.7)	130 (58.8)	171 (77.4)	106 (47.5)	159 (70.7)	132 (64.1)
Stavudine based	728 (26.6)	13 (11.2)	10 (4.4)	86 (36.0)	2 (1.0)	193 (92.8)	204 (91.5)	0	1 (0.5)	0	47 (21.3)	107 (48.0)	65 (28.9)	0 (0)
Tenofovir based	919 (33.6)	90 (77.6)	158 (69.3)	92 (38.5)	183 (89.3)	5 (2.4)	1 (0.5)	81 (39.9)	147 (68.4)	91 (41.2)	3 (1.4)	10 (4.5)	1 (0.4)	57 (27.7)
Abacavir based	69 (2.5)	0 (0)	0 (0)	51 (21.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	17 (8.3)
Type of initial NNRTI														
Efavirenz	1634 (59.8)	91 (78.5)	129 (56.6)	161 (67.4)	187 (91.2)	194 (93.3)	213 (95.5)	82 (40.4)	185 (86.1)	129 (58.4)	85 (38.5)	112 (50.2)	30 (13.3)	36 (17.5)
Nevirapine	1099 (40.2)	25 (21.6)	99 (43.4)	78 (32.6)	18 (8.8)	14 (6.7)	10 (4.5)	121 (59.6)	30 (14.0)	92 (41.6)	136 (61.5)	111 (49.8)	195 (86.7)	170 (82.5)
CD4 cell count at initiation -Median cells/μL (IQR) [b]	135 (63-206)	115.5 (47-184)	140.5 (83.5-197.5)	130 (58.5-201.5)	148 (60.5-235.5)	95.5 (28.3-163)	94.5 (34-155)	136 (53-219)	179 (99-259)	114 (50.5-177.5)	128 (61-195)	164 (104.5-223.5)	191 (114-268)	133 (66.5-199.5)

Table 1 (continued)

Country		Zambia		South Africa			Uganda			Kenya		Zimbabwe	Nigeria
HIV RNA at initiation - median log_{10} c/ml (IQR) [c]	5.00 (4.36-5.59)	4.88 (4.09-5.67)	4.99 (4.42-5.57)	5.20 (4.73-5.68)	4.56 (3.94-5.17)	4.71 (4.10-5.32)	4.99 (4.50-5.48)	5.40 (4.96-5.84)	5.23 (4.79-5.66)	5.59 (5.03-6.14)	4.62 (3.96-5.28)	4.66 (4.07-5.25)	4.68 (4.06-5.29)	5.10 (4.53-5.66)
Pre-treatment drug-resistance [d]														
No PDR	2404 (88.0)	103 (88.8)	207 (90.8)	212 (88.7)	189 (92.2)	181 (87.0)	196 (87.9)	170 (83.7)	175 (81.4)	185 (83.7)	203 (91.9)	202 (90.6)	194 (86.2)	187 (90.8)
PDR and fully-active ART	52 (1.9)	1 (0.9)	1 (0.44)	5 (2.1)	0 (0)	3 (1.4)	7 (3.1)	12 (5.9)	9 (4.2)	3 (1.4)	5 (2.3)	2 (0.9)	4 (1.8)	0 (0)
PDR and partially-active ART	123 (4.5)	5 (4.3)	9 (4.0)	8 (3.4)	8 (3.9)	6 (2.9)	11 (4.9)	12 (5.9)	18 (8.4)	22 (10.0)	6 (2.7)	6 (2.7)	6 (2.7)	6 (2.9)
PDR unknown	154 (5.6)	7 (6.0)	11 (4.8)	14 (5.9)	8 (3.9)	18 (8.7)	9 (4.0)	9 (4.4)	13 (6.1)	11 (5.0)	7 (3.2)	13 (5.8)	21 (9.3)	13 (6.3)
Previous antiretroviral experience	122 (4.5)													
No	2588 (94.7)	110 (94.8)	213 (93.4)	232 (97.1)	182 (88.8)	197 (94.7)	214 (96.0)	188 (92.6)	197 (91.6)	217 (98.2)	211 (95.5)	214 (96.0)	211 (93.8)	202 (98.1)
Yes	122 (4.5)	4 (3.5)	11 (4.8)	5 (2.1)	21 (10.2)	11 (5.3)	5 (2.2)	15 (7.4)	17 (7.9)	2 (0.9)	9 (4.1)	6 (2.7)	14 (6.2)	2 (1.0)
Unknown	23 (0.8)	2 (1.7)	4 (1.8)	2 (0.8)	2 (1.0)	0 (0)	4 (1.8)	0 (0)	1 (0.5)	2 (0.9)	1 (0.5)	3 (1.4)	0 (0)	2 (1.0)

Data are number (%) unless stated otherwise. [a] Combined with lamivudine or emtricitabine. [b] All n =2722; [c] All n =2706; [d] At least one drug-resistance mutation of the IAS-USA list, includes 52 participants who received a fully-active regimen (Stanford levels 1 or 2) and 123 who harbored drug-resistance to at least one drug in the prescribed regimen (Stanford levels 3, 4, or 5). PDR, pre-treatment drug-resistance; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; SD, standard deviation; IQR, interquartile range; WHO, World Health Organization.

Table 2. Pre-treatment prevalence of major IAS-USA drug-resistance mutations (n=2579).

NRTI mutations		NNRTI mutations		PI mutations	
Mutation	N (%)	Mutation	N (%)	Mutation	N (%)
M41L	19 (0.74)	L100I	4 (0.16)	D30N	1 (0.04)
A62V	3 (0.12)	K101P	0	V32I	0
K65R	4 (0.16)	K103N	57 (2.21)	M46I	5 (0.19)
D67N	3 (0.12)	V106A	0	M46L	6 (0.23)
T69INS	0	V106M	3 (0.12)	I47A	0
K70E	3 (0.12)	V108I	15 (0.58)	I47V	0
K70R	13 (0.50)	Y181C	25 (0.97)	G48V	0
L74V	1 (0.04)	Y181I	1 (0.04)	I50L	1 (0.04)
V75I	7 (0.27)	Y188L	1 (0.04)	I50V	1 (0.04)
F77L	1 (0.04)	Y188C	1 (0.04)	I54L	0
Y115F	1 (0.04)	Y188H	0	I54M	0
F116Y	0	G190A	22 (0.85)	Q58E	10 (0.39)
Q151M	0	G190S	1 (0.04)	T74P	0
M184I	0	P225H	2 (0.08)	L76V	2 (0.08)
M184V	32 (1.24)			V82A	0
L210W	7 (0.27)			V82F	0
T215C	0			V82L	0
T215D	2 (0.08)			V82S	0
T215E	0			V82T	0
T215F	6 (0.23)			N83D	1 (0.04)
T215G	0			I84V	0
T215H	0			N88S	0
T215I	4 (0.16)			L90M	7 (0.27)
T215L	0				
T215N	1 (0.04)				
T215S	1 (0.04)				
T215V	0				
T215Y	13 (0.50)				
K219E	4 (0.16)				
K219Q	6 (0.23)				

Pre-treatment genotypic drug-resistance was defined as the presence of at least one amino acid substitution included in the IAS-USA mutation list of December 2010, including the revertant mutations at codon 215. Drug-classes considered were NRTIs, NNRTIs and PIs. Drug-resistance mutations: the wild-type amino acid is given, followed by the codon of the reverse-transcriptase or protease gene, followed by the amino acid substitution conferring resistance. IAS-USA, International Antiviral Society of the USA; NRTI, nucleotide reverse transcriptase inhibitors; NNRTI, nonnucleotide reverse transcriptase inhibitors; PI, protease inhibitors. Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine, G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Table 3. Overview of sensitivity analyses on virologic endpoints in the PASER-M cohort.

Definition of virologic endpoint and/or patient sample	PDR groups	N	Virologic failure		Acquired drug-resistance	
			OR (95% CI)	p	OR (95% CI)	p
Virologic failure (≥400c/mL) (main analysis)		2733				
	No PDR		1		1	
	PDR and fully-active ART		1.01 (0.55, 1.87)	0.964	0.97 (0.51, 1.58)	0.934
	PDR and partially-active ART		2.13 (1.44, 3.14)	<0.001	2.30 (1.55, 3.40)	<0.001
Virologic failure (≥50c/mL)		2096				
	No PDR		1		n/a	
	PDR and fully-active ART		1.01 (0.45, 2.25)	0.981		
	PDR and partially-active ART		2.53 (1.53, 4.18)	<0.001		
Exclusion of patients with prior antiretroviral drug exposure		2588				
	No PDR		1		1	
	PDR and fully-active ART		0.97 (0.51, 1.85)	0.919	0.91 (0.46, 1.81)	0.797
	PDR and partially-active ART		2.11 (1.36, 3.25)	0.001	2.29 (1.48, 3.55)	<0.001
Exclusion of patients harbouring a virus with the M184V mutation before ART initiation		2701				
	No PDR		1		1	
	PDR and fully-active ART		1.02 (0.55, 1.88)	0.958	0.97 (0.51, 1.84)	0.929
	PDR and partially-active ART		2.61 (1.67, 4.06)	<0.001	2.75 (1.76, 4.31)	<0.001
Exclusion of patients with "possible drug-resistance"		2690				
	No PDR		1		1	
	PDR and fully-active ART		1.01 (0.54, 1.90)	0.965	0.99 (0.52, 1.88)	0.964
	PDR and partially-active ART		2.24 (1.51, 3.29)	<0.001	2.25 (1.52, 3.33)	<0.001
Missing equals failure *		2699				
	No PDR		1		1	
	PDR and fully-active ART		0.91 (0.47, 1.74)	0.768	0.89 (0.44, 1.78)	0.742
	PDR and partially-active ART		2.31 (1.44, 9.11)	<0.001	2.36 (1.58, 3.53)	<0.001

(table legend on next page)

Table 3 (legend)

Table shows results of multilevel logistic regression baseline models with multiple imputations for missing data. * Participants for whom no outcome HIV-RNA load result was available due to attrition (stopped ART, lost to follow-up, transferred out, died, switched to second-line ART) were classified as virologic failure (≥400 copies per mL). PDR=pre-treatment, drug-resistance; ART, antiretroviral therapy; CI, confidence interval; OR, odds ratio; n/a, not applicable

Summary of results

- Changing the definition of virological failure to ≥50 copies per mL showed the same results as the main analysis.
- Missing-equals-failure* analysis showed the same results as the main analysis.
- Exclusion of all participants with prior antiretroviral drug exposure from regression analysis showed the same results as the main analysis.
- Exclusion of all participants who harboured the M184V mutation (associated with recent prior use of ART) before start of treatment from regression analysis showed the same results as the main analysis.
- Participants with HIV-RNA load >1000 copies per mL but without major IAS-USA drug-resistance mutations after 12 months of ART have been classified as "No drug-resistance" in the main analysis. However, there might be a resistant virus below the sensitivity level of the population-based genotypic assay. Exclusion of this group of "possible drug-resistance" from regression analysis did not affect results.

Table 4. Overview of observational cohort studies investigating the effect of pretreatment drug resistance on treatment outcome.

Panel research in context: Systematic review

Reference	N	Cohort/ Study population	Year	Country	Definition of PDR	Main results
Wittkop *et al. (1)*	10,056	Eurocoord and CHAIN; Genotype was not used to guide treatment selection	≥1998	Europe	Stanford algorithm: at least low-level resistance to ≥1 drug	Patients with PDR to ≥1 prescribed drug had higher risk of VL≥500 c/mL; No risk difference for patients with PDR and fully-active ART.
Bansi *et al. (2)*	935	UK-CHIC; Genotype was not used to guide treatment selection	1999-2006	UK	Stanford algorithm: GSS calculated	Patients with GSS<3 had higher risk of VL≥50 c/mL (week 52)
Chaix *et al. (3)*	350	ANRS CO 06 PRIMO; Genotype was not used to guide treatment selection	1996-2005	France	ANRS: resistant to ≥1 drug	Proportion of patients with VL<400 c/mL lower in patients with PDR (week 24)
Bannister *et al. (4).*	277	EuroSIDA; Genotype was not used to guide treatment selection	1996-2004	Europe	Stanford: at least intermediate resistance to ≥1 drug	No difference for proportion of VL <500 c/mL (week 24)
Oette *et al. (5)*	269	RESINA; Genotype was used to guide treatment selection	2001-2003	Germany	Geno2pheno	No difference for proportion of VL <50 c/mL (week 48)
Pillay *et al. (6)*	201	Seroconverters CASCADE; Genotype was not used to guide treatment selection	1996-2003	Europe	Stanford: at least intermediate resistance to ≥1 drug	No difference in time to VL suppression <500 c/mL
Peuchant *et al. (7)*	172	ANRS CO3 Aquitaine Cohort	1996-2005	France	ANRS: resistant to ≥1 drug	Slower decrease in mean viral load in patients with PDR (one month)

Studies are sorted by descending sample size. ANRS, Agence Nationale de Recherches sur le Sida et les hépatites virales; GSS, Genotypic Sensitivity Score; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PDR, pretreatment drug-resistance; WHO, World Health Organization mutation list.

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Chapter 10

Second-line antiretroviral treatment successfully resuppresses drug resistant HIV-1 after first-line failure: prospective cohort in sub-Saharan Africa

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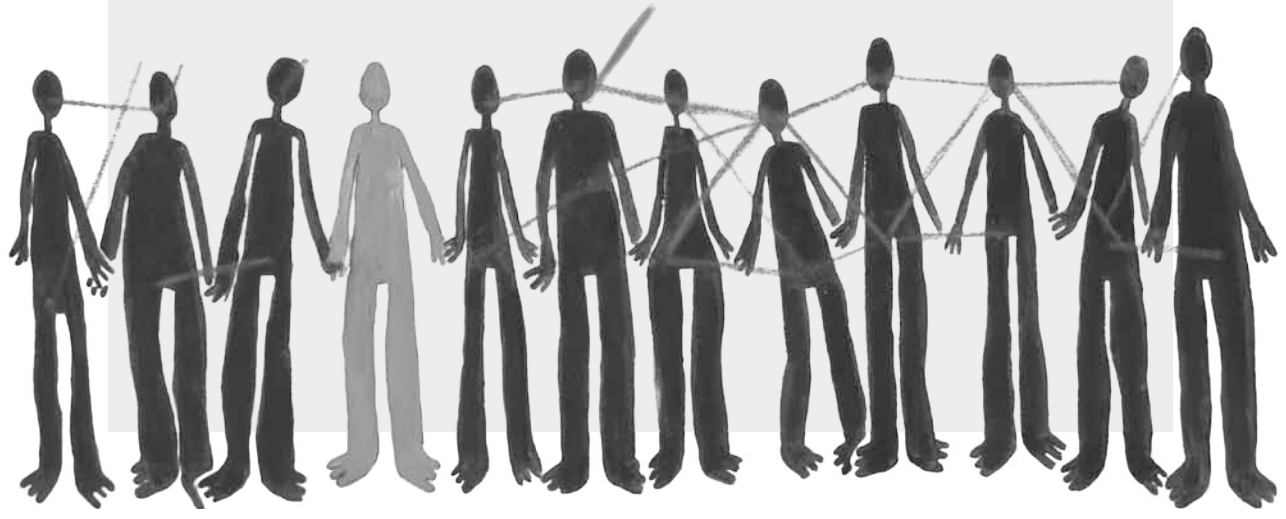

ABSTRACT

Little is known about the effect of human immunodeficiency virus type 1 (HIV-1) resistance mutations present at time of regimen switch on the response to second-line antiretroviral therapy in Africa. In adults who switched to boosted protease inhibitor-based regimens after first-line failure, HIV-RNA and genotypic resistance testing was performed at switch and after 12 months. Factors associated with treatment failure were assessed using logistic regression. Of 243 participants, 53% were predicted to receive partially active second-line regimens due to drug resistance. The risk of treatment failure was, however, not increased in these participants. In this African cohort, boosted protease inhibitors successfully resuppressed drug resistant HIV after first-line failure.

INTRODUCTION

With more human immunodeficiency virus type 1 (HIV-1) infected people receiving antiretroviral therapy (ART) in low-resource settings, treatment failure and the need to switch to second-line regimens is likely to increase. Reported regimen switching rates have been lower than expected,[1,2] due in part to actual rates of treatment success, but also because of restricted access to virological monitoring and second-line regimens. The absence of virological monitoring is associated with delayed switching and consequent accumulation of resistance mutations to nucleoside reverse transcriptase inhibitors (NRTIs).[3,4] Lack of access to genotypic resistance testing further complicates the selection of optimal second-line regimens. Few data exist on the impact of resistance mutations selected for by the first-line regimen on the response to empirically prescribed second-line ART in resource-poor settings.[5]

This study investigated the impact of acquired drug HIV-1 drug resistance mutations present at time of regimen switch on the response to second-line ART, within the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort in 6 sub-Saharan African countries.

METHODS

Study design and population

PASER-M is a prospective cohort of adults infected with HIV-1 who receive ART at 13 clinical sites in Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe. Cohort and site characteristics have been profiled elsewhere.[6] Participants were consecutively enrolled during a median site-specific enrolment period of 12 months between March 2007 and September 2009. The present analysis included participants who were switched to second-line ART after first-line failure had been diagnosed using clinical, immunological and/or virological failure criteria.[7] We excluded participants who had received protease inhibitors (PIs) prior to switch, or who were pregnant at study screening. Human immunodeficiency virus type 2 (HIV-2) co-infection was ruled out using an HIV-2 specific antibody test in endemic countries (i.e. Nigeria). The study protocol was approved by the appropriate national research ethics committees and the Academic Medical Center of the University of Amsterdam in The Netherlands. Participants provided written informed consent at enrolment.

Procedures

Participants were treated and followed-up as per local standard of care, generally in accordance with 2006 World Health Organization guidelines.[7] Medical staff at each site completed case-report forms at 3-month intervals, which were entered into an online

database. A data monitoring team reviewed study data. Drug adherence was assessed at each follow-up visit by 2 measures of self-reported adherence. For 3-day self-reports, the number of follow-up visits at which the patients reported to have missed any pills during the previous 3 days were counted. For the 30-day visual analogue scale, the number of pills taken at all follow-up visits was averaged and classified as <95% or >95%.

Blood collection was performed prior to regimen switch and after 12 months of follow-up (window, 11-15 months). Plasma specimens were batch-shipped to either of 2 reference laboratories in South Africa and Uganda for HIV-RNA determination, and genotypic resistance testing if HIV-RNA was >1000 copies/mL, as described elsewhere.[3] Drug resistance mutations (DRMs) were scored according to the 2010 International Antiviral Society-USA list.[8] HIV-1 subtypes were determined using the STAR algorithm and confirmed with the REGA subtyping algorithm (version 2.0). All sequences have been deposited in GenBank.

Drug resistance profiles at time of switch have been reported elsewhere.[3] Drug-susceptibility was scored using the Stanford algorithm (version 6.0.9)[9] in participants who harbored at least 1 DRM. Participants with Stanford levels 3 (low-level resistance), 4 (intermediate resistance), or 5 (high-level resistance) to at least one of their prescribed drugs were considered to have received partially-active ART. Participants with Stanford levels 1 (susceptible) or 2 (potential low-level resistance) to all prescribed drugs were considered to have received fully-active ART. Participants who had HIV-RNA <1000 copies/mL, or HIV-RNA >1000 copies/mL without DRMs were also considered to have received fully-active ART.

Among participants still in follow-up after 12 months, virological failure was defined as an HIV-RNA of ≥400 copies/mL. Immunological failure was defined according to WHO guidelines as a decrease in CD4 cell count to the value before regimen switch, a decline of at least 50% from highest measurement on treatment, or a persistent CD4 cell count <100 cells/mm^3.[7] Clinical failure was defined as the presence of a new WHO clinical stage 4 event or new diagnosis of pulmonary tuberculosis.

Statistical analysis

Group comparisons for categorical data were done using chi-square or Fisher exact test, and for continuous data using 1-way analysis of variance or Kruskal-Wallis test. Logistic regression with robust standard errors, accounting for clustering of observations within sites, was used to identify risk factors for the 2 outcomes virological failure and any type of failure. Attrition was additionally considered as virological failure. Any type of failure was defined as virological, immunological or clinical failure, or attrition. Explanatory variables at time of switch to second-line ART included the activity of the second-line regimen, age, sex, WHO clinical stage, CD4 cell count, and HIV-RNA load. Prospective parameters included any single-drug substitutions,

30-day and 3-day adherence. In addition to activity of the second-line regimen, age and sex, all variables univariately associated ($p<0.05$) with the outcome were stepwise entered into the multivariate model. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CI) and two-sided p-values, with $p<0.05$ regarded statistically significant. All statistical analyses were performed using Stata version 10 (StataCorp LP, TX, USA).

RESULTS

Study population

We enrolled 243 participants who switched to a second-line PI-based regimen after first-line ART failure (figure 1). HIV-RNA and genotypic test results were available for 232 participants (95.5%), for whom the predicted activity of the second-line regimen

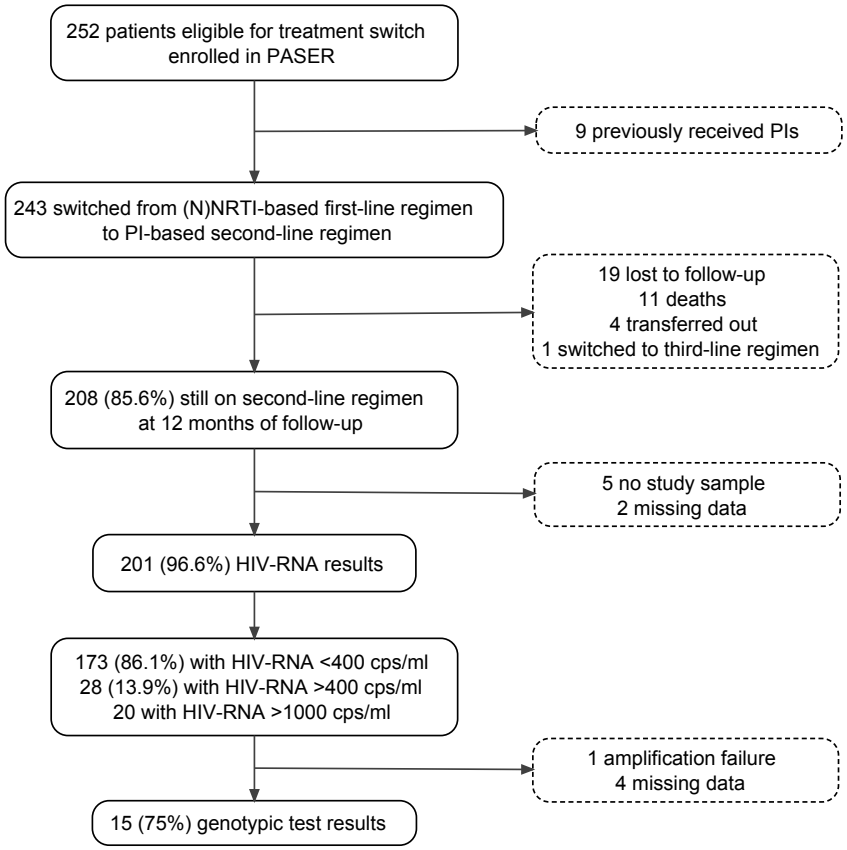

Figure 1. Study profile. PASER, PharmAccess African Studies to Evaluate Resistance; (N)NRTI, (non) nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

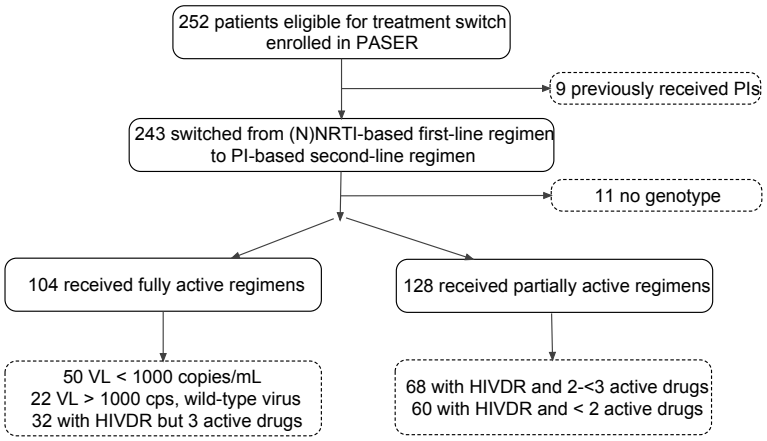

Figure 2. Predicted regimen activity at start of second-line ART. (N)NRTI, (non)nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

could be determined (figure 2). ART was predicted to be fully active for 104 participants (44.8%), comprising 50 participants (48.1%) with HIV-RNA <1000 copies/mL, 22 participants (21.2%) with HIV-RNA >1000 copies/mL and wild-type virus, and 32 participants (30.8%) with HIV-RNA >1000 copies/mL and drug resistant virus at Stanford levels 1 or 2. ART was predicted to be partially active for 128 participants (55.2%), harboring drug resistant virus with reduced predicted susceptibility to at least 1 prescribed drug. Of these, 60 (46.9%) received <2 active drugs (figure 2). Among participants with drug resistant virus, NRTI-associated DRMs were observed in 154 (96.3%), NNRTI-associated DRMs in 153 (95.6%) and dual-class resistance in 147 (91.9%).

At second-line start, WHO clinical stage 4 was diagnosed in 12 participants (11.5%) predicted to receive fully active regimens and in 25 participants (19.5%) with partially active regimens (p=0.014, Table 1). For the 2 groups, median CD4 cell count was 147 and 104 cells/mm^3 (p=0.001) and median HIV-RNA load was 3.2 and 4.7 $\log_{10}$ copies/ml (p<0.001), respectively. The median duration of first-line ART was 26.7 months; participants predicted to receive fully active regimens had shorter duration of previous ART than participants with partially active ART (22.8 versus 30.1 months, p=0.024). Subtype C was most commonly identified (n=77, 42.3%), followed by A (n=49, 26.9%), D (n=26, 14.3%), G (n=14, 7.7%), and circulating recombinant forms (CRFs) (n=16, 8.8%).

Response to second-line ART

After 12 months of follow-up, 208 participants were still on second-line ART and 201 had available HIV-RNA results (figure 1). Of these, 28 participants (13.9%) experienced virological failure. Eleven of 80 participants (13.8%) predicted to receive fully active

Table 1. Participant characteristics at second-line start, stratified by regimen activity

		Overall (n=232)	Fully active (n=104)	Partially active (n=128)	p^a
Women		116 (50)	56 (53.9)	60 (46.0)	0.291
Median age (years)		38.3 [34.1-45.1]	38.5 [34.4-44.5]	38.2 [33.8-45.9]	0.972
WHO clinical stage					0.014
Stage 1 and 2	119 (51.3)	57 (24.6)	62 (26.7)		
Stage 3	76 (32.8)	35 (33.7)	41 (32.0)		
Stage 4	37 (16.0)	12 (11.5)	25 (19.5)		
Median CD4 cell count (cells/mm^3)[b]		126 [66-205]	147 [92-230]	104 [51-185]	0.001
Median HIV-RNA (log_{10} copies/ml)		4.2 [3.3-5.0]	3.2 [1.6-4.2]	4.7 [4.1-5.2]	<0.001
Median duration of first-line regimen (months)[c]		26.7 [15.2-43.7]	22.8 [13.8-39.5]	30.1 [16.5-48.7]	0.024
ART regimen at failure[d]					0.001
ZDV, 3TC, NNRTI	102 (44.0)	39 (37.5)	63 (49.2)		
D4T, 3TC, NNRTI	95 (41.0)	59 (56.7)	36 (28.1)		
TDF, FTC, NNRTI	23 (9.9)	3 (2.9)	20 (15.6)		
NNRTI-based, other	5 (2.2)	2 (1.9)	3 (2.3)		
Triple NRTI	4 (1.7)	0	4 (3.1)		
Criteria used for switch[e]					0.006
Clinico-immunological only	60 (25.9)	36 (34.6)	24 (18.8)		
Targeted viral load testing	172 (74.1)	68 (65.4)	104 (81.3)		
Country of origin					0.002
Uganda	73 (31.5)	21 (20.2)	52 (40.6)		
South Africa	59 (25.4)	34 (32.7)	25 (19.5)		
Kenya	36 (15.5)	22 (21.2)	14 (10.9)		
Nigeria	30 (12.9)	16 (15.4)	14 (10.9)		
Zambia	24 (10.3)	9 (8.7)	15 (11.7)		
Zimbabwe		10 (4.3)	2 (1.9)	8 (6.3)	

Percentages are shown in parentheses and interquartile ranges in square brackets. ART, antiretroviral treatment; ZDV, zidovudine; 3TC, lamivudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; D4T, stavudine; TDF, tenofovir; FTC, emtricitabine.

[a]Categorical data were compared using the chi-square test; continuous were compared using the Wilcoxon rank-sum test. [b]230 observations. [c]227 observations. [d]229 observations.

[e]Clinico-immunological criteria for switch was defined as WHO clinical staging or CD4 counts only. Targeted viral load testing was defined as ART failure based on clinical and immunological information, confirmed with an HIV-RNA test.

regimens and 17 of 112 (15.2%) of those with partially active ART experienced virological failure (p=0.782). Of participants predicted to receive <2 active drugs, 7 of 51 (13.7%) experienced virological failure.

Of participants with an HIV-RNA load >1000 copies/mL after 12 months of follow-up, 15 of 20 (75%) had a valid genotype. Nine (60%) harbored ≥1 major DRMs. Detected mutations were associated with NRTIs (n=7, 46.7%), NNRTIs (n=8, 53.3%) and PIs (n=1, 6.7%). There were no significant differences in DRM patterns between participants predicted to receive a fully versus partially active second-line regimens.

CD4 cell counts were available for 173 of 208 participants still in follow-up. The median CD4 cell count gain was 146.5 cells/mm^3. Immunological failure was diagnosed in 21 participants (12.1%). Information on WHO clinical stage was available for all participants, and 13 (6.3%) experienced clinical failure. Clinical and immunological failure rates did not differ significantly for participants predicted to receive fully or partially active second-line regimens.

An HIV-related cause of death was recorded in 11 participants (4.5%). Other participants not retained in care were lost to follow-up (n=19, 7.8%) or transferred out (n=4, 1.6%). One person switched to a third-line regimen due to alleged virological failure before the 12 month visit. The attrition rates did not differ significantly for participants predicted to receive fully or partially active second-line regimens.

Risk factors for failure of second-line ART

In multivariate analyses, the predicted activity of the second-line regimen was not significantly associated with virological failure or any type of failure (Table 2). The risk of virological failure was increased for patients with 30-day adherence <95%, and reduced for increasing age. The risk of any type of failure was increased for patients with 30-day adherence <95% and for those in clinical stage 4 at second-line start.

DISCUSSION

In this assessment of routine ART programs in sub-Saharan Africa, the risk of second-line ART failure was not increased in participants who harbored virus with predicted reduced susceptibility to at least 1 prescribed second-line drug, compared to those who received ART that was predicted to be fully active. After 12 months of second-line ART, 14% of participants experienced virological failure, 5% died and 8% were lost to follow up. Our study demonstrates that empirically prescribed PI-based regimens can successfully

Table 2. Risk factors for second-line failure

	Virological failure[a]					Any type of failure[b]				
		Events (%)	Univariate	Multivariate	p	Events (%)	Univariate		Multivariate model	p
Activity of second-line regimen[c]										
Fully-active		31 (29.8)	1.0	1.0		44 (42.3)	1.0		1.0	
Partially-active		30 (23.4)	0.72 (0.38-1.36)	0.80 (0.33-1.91)	0.610	39 (30.5)	0.60 (0.37-0.99)		0.53 (0.24-1.17)	0.115
Sex										
Female	28 (23.0)	1.0	1.0			40 (32.8)	1.01.0	1.0		
Male	35 (28.9)	1.37 (0.74-2.51)	1.76 (0.65-4.72)	0.263		47 (38.8)	1.30 (0.73-2.32)	1.55 (0.57-4.27)	0.393	
Age (years)			0.98 (0.94-1.01)	0.94 (0.90-0.98)	0.006		0.99 (0.95-1.02)		0.96 (0.92-1.00)	0.083
Clinical stage at second-line start										
1-3		48 (23.5)	1.0	1.0		62 (30.4)	1.0		1.0	
4		15 (38.5)	2.03 (0.78-5.30)	2.44 (0.97-6.17)	0.059	25 (64.1)	4.09 (1.33-12.57)		5.25 (1.55-17.74)	0.008
CD4 count at second-line start										
≥100 cells/mm^3		35 (24.7)	1.0			53 (37.3)	1.0			
<100 cells/mm^3		27 (27.3)	1.15 (0.63-2.10)			33 (33.3)	0.84 (0.53-1.33)			
VL at second-line start (log_{10})			1.19 (0.97-1.45)				1.07 (0.87-1.31)			
Substitutions										
None		58 (13.2)	1.0	1.0		81 (35.4)	1.0			
Yes, ≥1		5 (33.3)	1.64 (0.78-3.46)	2.59 (0.95-7.08)	0.063	6 (42.9)	1.37 (0.64-2.95)			
3-day adherence[d]										
No pills missed	32 (16.6)	1.0				50 (25.9)	1.0			
Pills missed at ≥1 visit	12 (38.7)	3.18 (1.05-9.64)				18 (58.1)	3.96 (1.29-12.18)			
Average 30-day adherence (%)[e]										
≥95%		35 (17.2)	1.0	1.0		55 (27.0)	1.0		1.0	
<95%		7 (38.9)	3.07 (1.51-6.25)	2.90 (1.12-7.54)	0.029	11 (61.1)	4.26 (1.80-10.08)		4.08 (1.45-11.46)	0.008

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Table 2 (legend)
Multivariate logistic regression with robust standard errors. Data are given as odds ratio (95% confidence interval). VL, viral load. [a] Virological failure defined as HIV-RNA ≥ 400 copies/mL (n=28). Participants that died (n=11), were lost to follow-up (n=19), transferred out (n=4) or switched to third-line ART (n=1) were additionally considered as virological failure [b] Any type of failure defined as clinical, immunological or virological failure (n=63). Participants who were lost to follow-up, transferred out or switched to third-line ART were additionally considered as any type of failure. [c] Activity of second-line regimen for participants harboring DRMs based on the Stanford drug-susceptibility algorithm (version 6.0.9). [d] The number of follow-up visits at which the participant reported to have missed any pills during three days prior. [e] The average percentage of pills taken during 30 days prior to all follow-up visits.

resuppress HIV, even in the absence of a fully active NRTI backbone. This corroborates findings of a Malawian study among patients with extensive NRTI resistance at the time of switch.[5] Our study adds important insights to the limited available data on the effectiveness of second-line ART in resource-limited settings.[10,11]

The good immunological and virological response to partially active second-line regimens, i.e., in participants who harbored NRTI resistance, is likely explained by the high potency and genetic barrier to resistance of ritonavir-boosted PIs.[12] Our data suggest potential for the use of "simplified" PI-based regimens after failure of an NNRTI-based regimen. Preliminary results from the ACTG 5230 trial, evaluating PI monotherapy after first-line treatment failure in low-resource settings, showed promising results with 87% virological suppression after 6 months.[13] By contrast, a meta-analysis and recent trial have shown that boosted PI monotherapy is inferior to standard triple ART regimens and cannot be considered an alternative to standard treatment.[14,15] Therefore, long-term results from the ACTG 5230 and other ongoing studies, such as the EARNEST trial, are eagerly awaited.

Participants with predicted partially active second-line regimens had more advanced HIV disease, lower CD4 cell counts and higher viral loads, which is in line with the observation that prolonged ART failure leads to disease progression and drug resistance accumulation.[3,4] Starting second-line ART after the occurrence of an AIDS-defining event increased the risk of treatment failure, indicating that regimen switch should ideally occur before the onset of severe clinical symptoms. Early detection of first-line failure can be improved by implementing routine virological monitoring, which has also been shown to avert unnecessary switches in the absence of viral breakthrough.[3] Furthermore, access to affordable second-line drugs needs to be urgently improved to enable switching to second-line ART when appropriate.

Our finding that suboptimum adherence to second-line ART was associated with an increased risk of treatment failure expands on previous knowledge,[5,10] and underscores

the importance of meticulous long-term adherence. A limitation of our study is the fact that our findings apply to the first year of second-line ART. Longer term follow-up is required to determine if these early outcomes can be sustained over the following years of treatment. Secondly, there was an overrepresentation of urban sites in our cohort and therefore caution is warranted when extrapolating the results to other settings where resource constraints may be more significant.

In conclusion, our cohort study in 6 African countries showed that individuals on second-line ART achieve favorable virological outcomes after 12 months of follow-up, despite the fact that more than half were predicted to receive partially active regimens. Given that the provision of effective and safe ART requires life-long commitment, it is a global health priority to ensure access to viral load testing and to improve availability of second-line drugs.

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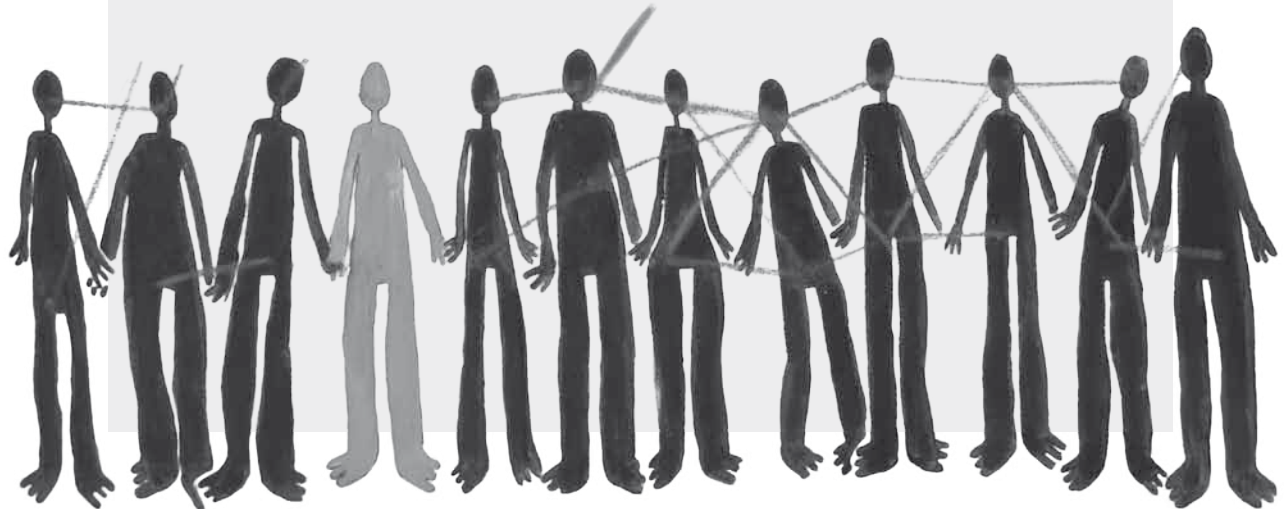
Part 4

Pediatric studies



Chapter 11

HIV-1-resistance-associated mutations after failure of first-line antiretroviral treatment among children in resource-poor regions: a systematic review

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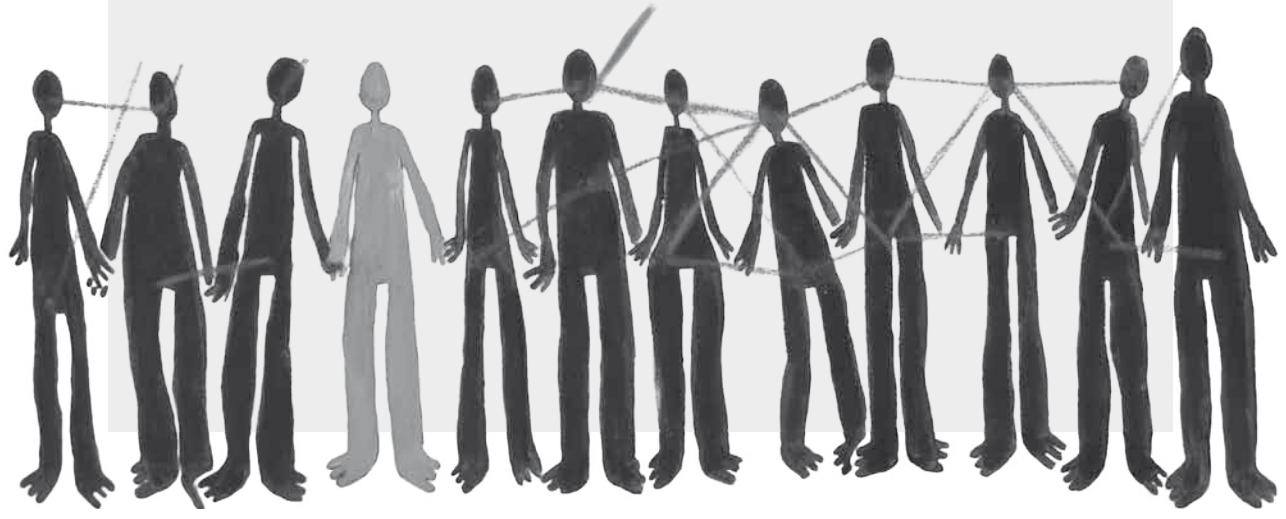

ABSTRACT

HIV-positive children are at high risk of drug resistance, which is of particular concern in settings where antiretroviral options are limited. In this Review we explore resistance rates and patterns among children in developing countries in whom antiretroviral treatment has failed. We did a systematic search of online databases and conference abstracts and included studies reporting HIV-1 drug resistance after failure of first-line pediatric regimens in children (<18 years) in resource-poor regions (Latin America, Africa, and Asia). We retrieved 1312 citations, of which 30 studies reporting outcomes in 3241 children were eligible. Viruses with resistance-associated mutations were isolated from 90% (95% CI 88–93%) of children. The prevalence of mutations associated with nucleoside reverse transcriptase inhibitors was 80%, with non-nucleoside reverse transcriptase inhibitors was 88%, and with protease inhibitors was 54%. Methods to prevent treatment failure, including adequate pediatric formulations and affordable salvage treatment options are urgently needed.

INTRODUCTION

Children are highly vulnerable to HIV infection, which can be transmitted from mother to child before, during, or after delivery.[1] Rates of disease progression and death in vertically infected infants are higher than those in older children or adults.[2,3] More than half of HIV-positive children who do not receive treatment are estimated to die before age 2 years.[4] Antiretroviral therapy (ART) has strikingly reduced morbidity and mortality in children.[5–7] In 2008, an estimated 38% of all children in need of treatment worldwide were receiving ART.[8] In view of treatment guidelines, which recommend that all HIV-positive children younger than 24 months should start treatment, irrespective of clinical or immunological status,[9] the uptake of ART is expected to increase.

The probability of virological suppression and long-term treatment success is lower in children than in adults.[10,11] Younger children typically have poorer virological responses than do older children,[12] which is associated with high viral loads before treatment[13] and the risk of sub-therapeutic drug concentrations (<90% of current recommended dose) owing to limited pediatric formulations, variable pharmacokinetics, and changes in bodyweight because of growth.[14] Furthermore, adherence is frequently hindered by multiple practical and social barriers throughout childhood and adolescence.[15] Suboptimum virological suppression might lead to the emergence of drug resistant virus, the consequences of which are particularly important for children who will need ART for longer periods of time than adults.

Most children with HIV infection live in resource-poor settings[16] and could, therefore, be most at risk of developing resistance to ART. Exposure to nevirapine for the prevention of vertical HIV transmission, especially for long periods of time, increases the risk of infection with drug resistant virus in infants and of treatment failure with standard first-line regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTIs).[17] Virological monitoring should be used to establish the optimum first-line therapy and time points for regimen switching in children. Identification of resistance mutations by genotype testing could further aid appropriate sequencing and rational use of antiretroviral medication. Financial and technical constraints, however, prevent routine use of viral load tests in developing countries, and information on resistance rates associated with pediatric antiretroviral treatment in these settings is scarce. At the same time, preservation of the first-line regimen is essential for patients living in resource-poor settings, as options are limited to two lines of ART.

We report a systematic review of the literature on acquired drug resistance among children in resource-poor settings after failure of ART. We explore the current knowledge of

resistance rates and patterns in relation to first-line pediatric regimens to gain insight into optimum switching strategies.

METHODS

Search strategy and selection criteria

We systematically searched PubMed, Embase, and Web of Science for articles available before March 1, 2010, with the following terms: "HIV", or "AIDS" or "human immunodeficiency virus" or "acquired immunodeficiency syndrome" AND "antiretroviral*" or "ARV" or "ART" or "HAART" AND "resistance" or "genotyp*" AND "paediatric" or "pediatric" or "infant*" or "child*" or "adolescent*". We manually searched the abstract databases of the following selected conferences held between Jan 1, 2008, and March 1, 2010: the International AIDS Society Conference (2008 and 2009); the Conference on Retroviruses and Opportunistic Infections (2008, 2009, and 2010); the International HIV Drug Resistance Workshop (2008 and 2009); and the International Workshop on HIV Pediatrics (2009). Retrieved reports were also manually screened for further potentially relevant full articles and abstracts.

We included original studies in which HIV-1-infected children were treated with first-line ART in Africa, Latin America, and Asia. Included studies had to report data on the frequency of HIV drug resistance in children who had been treated for at least 6 months. Clinical trials, observational cohort studies, and cross-sectional studies published in English were eligible.

We excluded studies in which patients were infected with HIV-2, were older than 18 years, lived in resource rich regions, were still receiving monotherapy or dual therapy at the time of study, or who had started second-line ART. Studies reporting HIV drug resistance in children before ART was started (i.e., transmitted or acquired resistance related to the use of antiretroviral drugs for the prevention of mother-to-child transmission) were not eligible. Previous use of drugs for prophylaxis of transmission or treatment was allowed.

After removal of duplicates, reports were initially assessed by title and abstract. For those not excluded, full-length versions were retrieved and assessed. If the description of the study was unclear in terms of eligibility criteria, we contacted the authors for further information. Eligible reports were independently reviewed by two authors (KCES and JCJC) and any disputes about inclusion were resolved by discussion between these authors.

Data extraction

We extracted data on locations and time frames of studies, type of study, method of analysis (intention to treat or on treatment), number of participants and age at entry, duration of follow-up, ART regimens, definitions of treatment failure, proportion of children with virological failure, virological cutoff for genotype tests, number of HIV genotype resistance tests, and proportion of children infected with HIV-1 and with at least one resistance-associated mutation.

Statistical analysis

We summarized drug resistance data. Mutations were analyzed according to the International AIDS Society 2009 update of drug resistance mutations.[18] Polymorphisms occurring in wild-type viruses and minor mutations were not included. We classified resistance mutations as being associated with nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs, or protease inhibitors (PIs). We calculated frequencies of specific mutations.

The proportions of children infected with HIV-1 that had at least one drug resistance mutation were pooled, irrespective of the type of first-line regimen. We used the Cochran Q test to assess heterogeneity between eligible studies.[19,20] In case of heterogeneity, random effects models were used to pool data. We used fixed effects models in homogeneous study sets. Virological outcomes were not pooled but were reported as the median (range) of failure rates at follow-up per study. Most studies presented on-treatment analyses and, therefore, the virological failure percentage for all studies was calculated with this approach.

RESULTS

1312 articles and abstracts were deemed potentially eligible after duplicate retrieved articles were removed. On the basis of the title and abstract, 1183 reports were excluded (figure 1) because they dealt with obviously distinct issues, such as comorbidity or adverse drug reactions, or because they did not report original data. Of the remaining 129 studies, 30 met the inclusion criteria, including 15 full-text papers and 15 conference abstracts (figure 1) and involved 3241 children. Among these, treatment was deemed to have failed in 1307 (40%).

15 studies reported virological failure and associated drug resistance levels in longitudinal cohorts of children receiving ART (n=2545). The other 15 studies described the cross-sectional analyses of HIV drug resistance rates and patterns of treatment failure

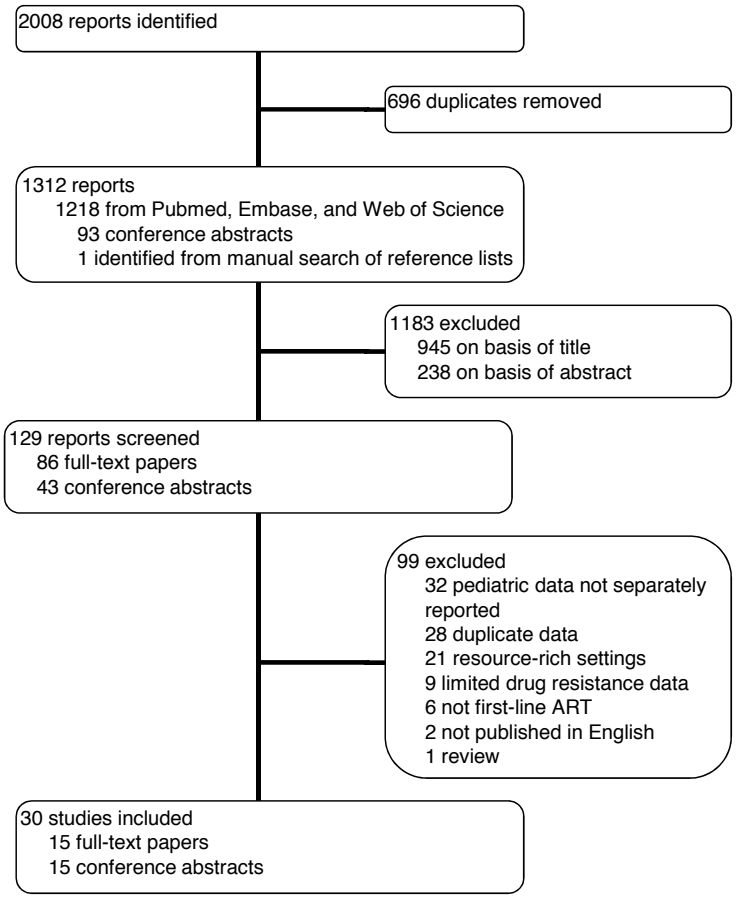

Figure 1. Search strategy and results. ART, antiretroviral therapy.

in children (n=696). None of the studies reported a clinical trial. Studies were done in 15 countries: nine in sub-Saharan Africa, three in Latin America, and three in Asia (table 1, table 2). Most of the included studies were published after 2007; six reports were published between 2003 and 2007 (table 1, table 2).

In the cohort studies the median duration of treatment ranged from 6 months to 50 months (table 1). Virological failure was defined as an HIV-RNA rebound of more than 50 copies per mL (one study), 400 copies per mL (seven studies), 500 copies per mL (one study), or 1000 copies per mL (six studies). Across the studies with the cutoff of 1000 copies per mL, treatment failure was seen in a median of 26% of patients (range 14–40%). Among the studies with more stringent definitions of virological failure, the median proportion of virological failure was 36% (range 13–71%). The highest proportion of failure (71%) was seen in an Argentinean cohort with the smallest sample size

Table 1. Summary of virological outcome and HIV-1 drug resistance data in cohort studies

	Number of patients	Median age at entry (years)	Regimen		Median follow-up (months)	Previous ART	Analysis	Viral detection cut-off (cps/ml)	Proportion with virological failure (%)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI							Any	NRTI	NNRTI	PI
Argentina 2008[21]	21	1	100%		6	NA	OT	>400	71%	15	100%	NA	NA	NA
Cambodia 2003-2005[63]	212	6	100%		16.6	Unspeci-fied 3%	OT/ITT	>400	19/26%*	36	94%	86%	89%	0
Thailand, 2002-2006[64]	202	7.1†	100%		50	sdNVP 2%	OT/ITT	>1000	21/26%*	39	97%	87%	95%	0
Central African Republic 2008[39]	52	7.9	81%	19%	6	NA	OT	>500	52%	26	77%	77%	54%	12%
Côte d'Ivoire 2000-2003[65]	115	6.35	30%	70%	10.2	Dual therapy 3%	OT	>1000	34%	38	71%	66%	24%	42%
Kenya 2001-2008[23]	90	7.9†	92%	8%	24	None	OT	>400	36%	32	67%	56%	38%	9%
Mali 2006-2007[66]	97	2.5	100%		6	NA	OT/ITT	>400	44/61%*	30	73%	70%	70%	0
Mozambique 2003-2007[67]	495	4	100%		18.5	sdNVP 0.2%, ZDV 0.6%	OT	>50	27%	84	92%	88%	92%	0
South Africa 2004-2008[68]	335	8.6†‡	74%	26%	>6	NA	OT	>1000	14%	46	87%	NA	NA	NA
South Africa 2009[53]	257	all < 2		100%	12	sdNVP 100%	OT	>400	13%	34	76%	74%	6%	35%
Uganda 2007[22]	104	0.5-1.8**	100%		12	sdNVP 34%	OT	>400	42%	15	100%	100%	100%	0
Uganda 2010[69]	126	NA	100%		24.8	NA	OT	>1000	14%	14	100%	100%	86%	0

Table 1 (continued)

	Number of patients	Median age at entry (years)	Regimen		Median follow-up (months)	Previous ART	Analysis	Viral detection cut-off (cps/ml)	Proportion with virological failure (%)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI							Any	NRTI	NNRTI	PI
Uganda 2004-2005[70]	250	9.2†	100%		12	NVP or ZDV 4%	OT/ITT	>400	26/34%*	3	100%	100%	100%	0
Uganda 2004-2006[71]	86	5.4-5.5**	100%		12 (n=59) 24 (n=27)	NA	OT	>1000	40%	33	88%	88%	85%	0
Zambia 2003-2005[72]	103	8	100%		24	NA	OT (n=74)	>1000	30%	26	92%	>77%	92%	0

ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NA, not available; sdNVP, single dose nevirapine; NVP, nevirapine; ZDV, zidovudine; OT, on treatment; ITT, intention to treat.* Virological failure in on-treatment or intention-to-treat analysis; ** range of medians; † mean; ‡ at time of treatment failure.

Table 2. Summary of HIV-1 drug resistance profiles in cross-sectional studies

	Number of patients	Median age (years)	Regimen			median follow-up (months)	Previous ART	Viral detection cut-off (cps/ml)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI	NNRTI + PI					Any	NRTI	NNRTI	PI
Argentina 2006-2008[73]	36	7.7	39%	61%		62.4	NA	NA	36	89%	89%	50%	53%
Brazil[74]	12	5.5	8%	75%	17%	36	NA	> 1000	10 RT, 12 PR regions	90%	80%	30%	75%
Brazil 2000-2001[30]	41	5.5	56%,	37%	7%	12	Mono/dual therapy 78%, ART 12 %	> 80	36 RT, 38 PR regions	94%	61%	56%	37%

Table 2 (continued)

	Number of patients	Median age (years)	Regimen			median follow-up (months)	Previous ART	Viral detection cut-off (cps/ml)	Genotype resistance test	Proportion with resistance-associated mutation (%)			
			NNRTI	PI	NNRTI + PI					Any	NRTI	NNRTI	PI
Brazil 2000-2006[75]	103	9†	22%	14%	63%	NA	NA	>1000	92	>90%	90%	70%	55%
Mexico[76]	25	10.9†		64%	36%	NA	NA	NA	25	72%	72%	24%	60%
Cambodia 2008[77]	53	10.9	100%			26.4	Unspecified 8%	NA	38	97%	>79%	97%	0
China 2005-2007[78]	41	11	100%			12	adult formulations 51%	> 1000	34	100%	>79%	100%	0
Thailand 2000-2007[79]	21	4.1	100%			6.3	NA	> 1000	21	57%	52%	43%	0
South Africa 2007-2008[38]	30	2.3		LPV/r 73%, RTV-sPI 27%		NA	RTV-sPI 30%	> 400	30	83%	83%	0	43%
South Africa[80]	25	7.3†	92%	8%		29.6	NA	NA	25	92%	>80%	>44%	4%
South Africa 2000-2003[81]	39	6	38%	62%		NA	Unspecified 28%	NA	39	92%	>37%	>20%	>20%
South Africa 2007[82]	86	NA	71%	29%		19.2	NA	> 1000	86	>79%	>79%	64%	3%
South Africa[83]	109	7.6†*	59%	41%		NA	NA	> 5000	109	92%	>71%	47%	14%
Senegal[84]	40	NA	100%			6	NA	> 1000	40	73%	68%	78%	13%
Uganda[85]	35	NA	100%			>13	NA	NA	35	100%	100%	100%	0

ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NA, not available; RT, reverse transcriptase; PR, protease; LPV/r, Lopinavir/ritonavir; RTV-sPI = ritonavir as single protease inhibitor. † mean; * n=41.

(n=21) and a short follow-up time of 6 months.[21] For children receiving regimens that included NNRTIs or PIs the median failure rates were 29% (range 14–71%) and 54% (range 13–90%), respectively.

Virological failure in the cross-sectional studies was defined as an HIV-RNA rebound of more than 80 copies per mL (one study), 400 copies per mL (one study), 1000 copies per mL (six studies), or 5000 copies per mL (one study); six studies did not define the cutoff values (table 2). The duration of ART before treatment failure was available in ten studies and ranged from 6 months to 62 months (table 2).

Across all studies, antiretroviral regimens were specified for 2952 (91%) children. Most children (n=2303, 78%) were treated with an NNRTI-based regimen that contained either nevirapine or efavirenz. PI-based regimens were reported in 569 (19%) patients. In southern Africa, ritonavir-boosted lopinavir and full-dose ritonavir were the most commonly used PI agents. Children in Latin America received nelfinavir and, occasionally, ritonavir or indinavir. In this region, regimens combining a PI and an NNRTI plus one or two NRTIs were used in 79 (3%) children. In west Africa, nelfinavir and indinavir were used. In all regions, the NRTI backbones were stavudine, zidovudine, and lamivudine, and occasionally didanosine. One child received a triple NRTI regimen. Previous use of antiretroviral drugs was reported in 12 studies and included single-dose nevirapine or long-term nevirapine or zidovudine for the prevention of perinatal transmission (seven studies) and use of these drugs in monotherapy or dual therapy regimens (two studies). Maternal exposure to antiretroviral drugs was only reported in two studies and involved single-dose nevirapine.

HIV-1 subtypes were determined for 598 children in 13 (43%) studies (table 3). The most prevalent was subtype C, which was noted in 252 (42%) patients and seen mainly in southern Africa. Subtype B was detected 108 times (18%) in Latin America and Asia. The recombinant form CRF02_AG was found in 91 patients (15%) and occurred almost uniquely in central and west Africa. Subtypes A and D were found in 23 (4%) and 14 (2%) patients, respectively, mostly in east Africa. In Latin America subtypes F and BF mosaics were found in ten (2%) and 33 (6%) patients, respectively.

The reverse transcriptase and protease regions were sequenced, respectively, in 1127 (86%) and 1131 (87%) of 1307 children. Genotype tests were done with commercial assays in 29 (97%) studies; in one study an in-house assay was used.[31] The proportion of patients in which the virus had at least one resistance-associated mutation ranged from 57% to 100%. The pooled proportion of children with virus with any resistance-associated mutation was 90% (95% CI 88–93). In a subgroup analysis done according

Table 3. Number of children receiving antiretroviral treatment, by HIV-1 subtype and region

HIV-1 subtypes	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
A	23 (4%)			5 (4%)	16 (26%)	2 (1%)
B	108 (18%)	74 (62%)	34 (100%)			
C	252 (42%)	2 (2%)			2 (3%)	248 (98%)
D	14 (2%)			3 (2%)	8 (13%)	3 (1%)
A/D/C*	35 (6%)				35 (56%)	
F	10 (2%)	10 (8%)				
BF mosaics	33 (6%)	33 (28%)				
CRF02_AG	91 (15%)			90 (70%)	1 (2%)	
CRF11_cpx	11 (2%)			11 (8%)		
CRF06_cpx	5 (<1%)			5 (4%)		
CRF01_AE	4 (<1%)			4 (3%)		
Other CRF	12 (2%)			12 (9%)		
Total	598	119	34	130	62	253

* Subtypes not specified further; CRF, circulating recombinant form.

to geographical regions, the pooled proportions of drug resistant HIV among children in whom treatment had failed varied from 75% (67–82) in central and west Africa to 96% (91–100) in Asia (figure 2). A second subgroup analysis to test the frequency of resistance-associated mutations in relation to duration of ART showed at least one mutation at the time of treatment failure in 76% (69–83) of children who had received ART for less than 1 year, and that this proportion notably increased with longer duration of treatment (97%, 94–99; figure 3)

In 27 (90%) studies the rates of resistance associated with specific drug classes were reported for 1027 children. All these children had been exposed to NRTIs and 80% (95% CI 75–86; range 52–100) had at least one related resistance mutation associated. NRTI resistance varied by geographical region and ranged from 70% (95% CI 62–78) in central and west Africa to 95% (95% CI 89–100) in east Africa (figure 2). For children exposed to NNRTIs (n=774), HIV-1 with mutations conferring resistance was seen in 88% (95% CI 84–92; range 43–100). Within the different regions the respective prevalence ranged from 79% (95% CI 71–87) in central and west Africa to 91% (95% CI 83–99) in Asia (figure 2). Among children who had used PIs (n=320) 54% (95% CI 39–69; range 12–86) were infected with HIV-1 that had at least one major protease mutation. These mutations were detected in 76% (95% CI 39–69) of children in Latin America, 51% (95% CI 35–67) in central and west Africa, and 31% (95% CI 18–43) in southern Africa. No children in Asia received PIs. In one study in east Africa,[23] three (60%) of five children exposed to PIs had major associated mutations (figure 2).

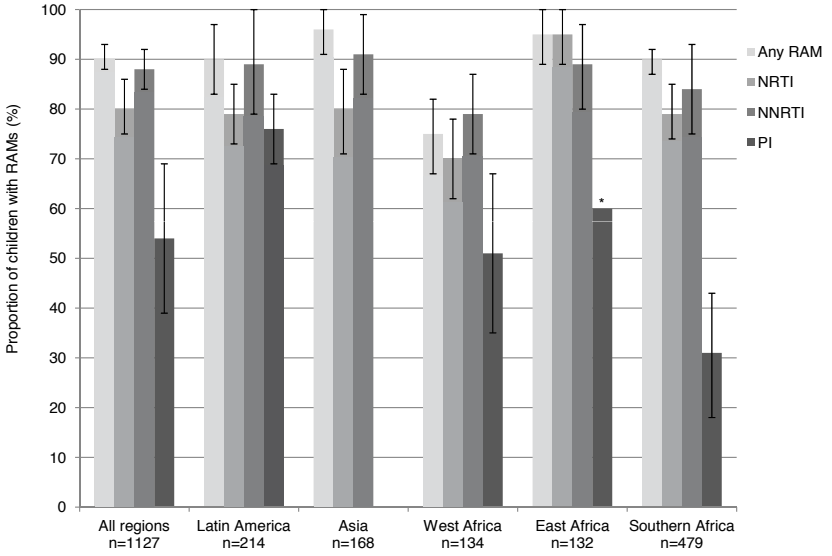

Figure 2. Frequency of at least one resistance-associated mutation, by region and drug class. Error bars indicate 95% CI. RAM, resistance-associated mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. * Proportion reported in one study.

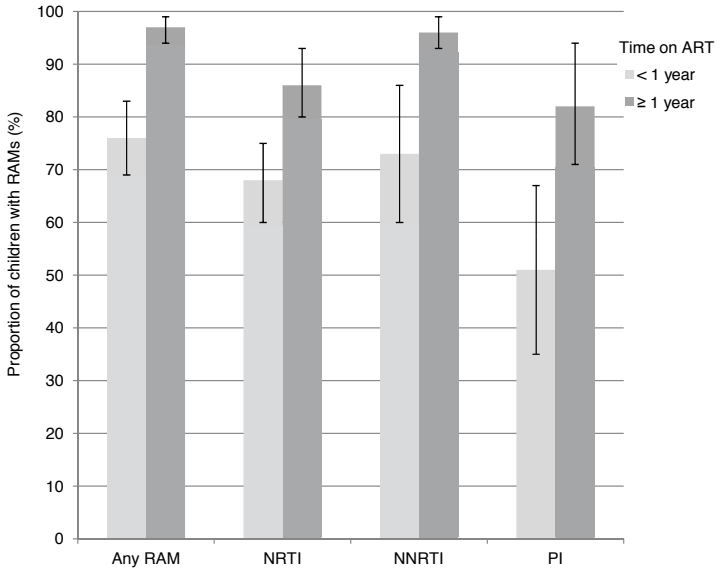

Figure 3. Frequency of resistance-associated mutations in relation to duration of ART. Error bars indicate 95% CI. ART, antiretroviral therapy; RAM, resistance-associated mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

The frequencies of specific resistance-associated mutations were provided in 24 (80%) studies, for 934 children, all of whom had been exposed to NRTIs. 695 (74%) patients had also been treated with NNRTIs and 297 (32%) had received a PI. Major mutations occurred at 36 different codons (table 4). All 16 NRTI-associated mutations listed in the 2009 update of the International AIDS Society drug resistance mutations were reported.[18] The most prevalent mutation was the M184V mutation, which was recorded in 587 (63%) patients. Thymidine analogue mutations were seen in 522 (56%) patients. The mutations K65R and Q151M were reported in 25 (3%) and 21 (2%) patients, respectively. Mutations at nine different codons associated with NNRTI resistance were reported. The K103N mutation was the most frequently detected (260 [37%] occurrences). Other frequently reported mutations were Y181C/I (112 [16%]), G190S/A and G190A (each 90 [13%]), and V106A/M (67 [10%]). 11 different major PI-associated protease mutations were noted. The most frequent were V82A (51 [17%] occurrences), L90M (44 [15%]), and I54V (36 [12%]).

By region, the most frequent NRTI-associated mutation was M184V (40% in Latin America, range 60–76% across regions), and of the thymidine analogue mutations it was T215F/Y (57% in Latin America, 16% in Asia, and 5% in Africa). In the NNRTI class, the most frequent overall was K103N (60% in Latin America, range 23–42% across regions), although V106A/M was predominant in southern Africa (21%), and Y181C/I was predominant in Asia (31%). Protease mutations were seen predominantly in Latin America and southern Africa; the D30N, M46I/L, I84V, and L90M mutations occurred most frequently in Latin America.

DISCUSSION

We identified 15 peer-reviewed papers and 15 abstracts that provided information on the emergence of drug resistance to ART among children in resource-poor regions. Most studies originated from sub-Saharan Africa. At least one resistance-associated mutation was found in 90% of children in whom first-line ART had failed. The drug-class-specific resistance rates were 88% for NNRTIs, 80% for NRTIs, and 54% for PIs. Despite geographical and virological differences, the drug resistance rates are similar to those reported for children living in Europe.[51–53] The risk of drug resistance increased with the duration of ART.

When we compared the frequencies and patterns of mutations between the regions included in this systematic review we noted several important differences. The frequencies of reverse transcriptase and protease mutations in Latin America contrasted greatly with those in other regions: the M184V mutation, which is rapidly selected by lamivu-

Table 4. Number of children with reported major drug-associated mutations in children who experienced treatment failure, by drug class and region

Associated with NRTIs	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
M41L	92 (10%)	66 (48%)	10 (8%)	3 (2%)		13 (3%)
A62V	6 (<1%)			2 (1%)	1 (1%)	3 (<1%)
K65R	25 (3%)		8 (6%)	3 (1%)	3 (3%)	11 (3%)
D67N	113 (12%)	55 (40%)	19 (15%)	4 (3%)	4 (4%)	31 (7%)
T69ins	5 (<1%)		4 (3%)	1 (<1%)		
T69S/A/D/N	27 (3%)	17 (12%)		2 (1%)	3 (3%)	5 (1%)
K70R	79 (8%)	32 (23%)	9 (7%)	8 (6%)	5 (5%)	25 (6%)
L74V	14 (1%)	2 (1%)	2 (2%)	2 (1%)	1 (1%)	7 (2%)
V75I	12 (1%)		4 (3%)		2 (2%)	6 (1%)
V75A/L/M	3 (<1%)	3 (2%)				
F77L	3 (<1%)		3 (2%)			
Y115F	4 (<1%)		3 (2%)		1 (1%)	
F116Y	3 (<1%)	1 (<1%)				2 (<1%)
Q151M	21 (<1%)		9 (7%)			12 (3%)
M184V	587 (63%)	55 (40%)	92 (71%)	80 (60%)	75 (76%)	285 (66%)
M184I	25 (3%)					25 (6%)
L210W	61 (7%)	40 (29%)	10 (8%)	4 (3%)	1 (1%)	6 (1%)
T215F/Y	134 (14%)	79 (57%)	21 (16%)	7 (5%)	5 (5%)	22 (5%)
T215I/D/N	4 (<1%)			2 (1%)	1 (1%)	1 (<1%)
K219Q/E	43 (5%)	1 (<1%)	12 (9%)	2 (1%)	4 (4%)	24 (6%)
K219N/K	1 (<1%)			1 (<1%)		
Total*	934	138	130	134	99	433
Associated with NNRTIs	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
L100I	18 (3%)	15 (15%)	2 (2%)	1 (1%)		
K101E/H	47 (7%)	13 (13%)		6 (6%)	5 (5%)	23 (8%)
K103N	260 (37%)	61 (60%)	38 (29%)	22 (23%)	25 (27%)	114 (42%)
V106A/M	67 (10%)	5 (5%)		3 (3%)	1 (1%)	58 (21%)
V106V/I	2 (<1%)			2 (2%)		
V108I	20 (3%)	2 (2%)	9 (7%)	1 (1%)	4 (4%)	4 (1%)
Y181C/I	112 (16%)	14 (14%)	40 (31%)	16 (16%)	11 (12%)	31 (11%)
Y188L	22 (3%)	2 (2%)	3 (2%)	3 (3%)	2 (2%)	12 (4%)
Y188H	1 (<1%)			1 (1%)		
G190A	38 (5%)	2 (2%)	10 (8%)	9 (9%)	10 (11%)	7 (3%)
G190A/S	52 (7%)	15 (15%)	7 (5%)			30 (11%)
G190S/E	1 (<1%)			1 (1%)		
P225H	17 (2%)		2 (2%)	1 (1%)		14 (5%)
Total*	695	101	130	97	94	273

Table 4 (continued)

Associated with PIs	All regions	Latin-America	Asia	Central and West Africa	East Africa	Southern Africa
D30N	13 (4%)	12 (13%)				1 (<1%)
V32I	3 (1%)	2 (2%)				1 (<1%)
M46I	20 (7%)	3 (3%)		3 (8%)	2 (40%)	12 (8%)
M46I/L	27 (9%)	26 (27%)		1 (3%)		
I47V	5 (2%)	5 (5%)				
I50V	1 (<1%)					1 (<1%)
I54V	36 (12%)	11 (12%)		1 (35)		24 (15%)
I54V/L	1 (<1%)			1 (3%)		
L76V	1 (<1%)					1 (<1%)
V82A	51 (17%)	12 (13%)				39 (24%)
V82F	1 (<1%)			1 (3%)		
V82I	5 (2%)			5 (14%)		
V82A/F/T/S	31 (10%)	24 (25%)				7 (4%)
I84V	11 (4%)	11 (12%)				
N88S	11 (4%)			8 (22%)	2 (40%)	1 (<1%)
N88D	5 (2%)	4 (4%)				1 (<1%)
L90M	44 (15%)	28 (29%)		8 (22%)	1 (20%)	7 (4%)
L90A	3 (1%)					3 (2%)
Total*	297	95		37	5	160

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. Thymidine analogue mutations are indicated in **bold**; * Number of children with exposure to specific drug class.

dine or emtricitabine, was seen less frequently in Latin American studies, whereas more thymidine analogue mutations were reported. These differences might be explained by the use of regimens in Latin America that combine NNRTIs, PIs, and one or more NRTIs but do not always contain lamivudine or emtricitabine.[54] In other regions, where lamivudine or emtricitabine are generally included in triple ART regimens, the M184V mutation was more likely to be the first mutation to appear and the emergence of thymidine analogue mutations was delayed.[55] Furthermore, previous exposure to ART drugs in monotherapy or dual therapy regimens or for the prevention of vertical transmission, might have occurred more often in Latin America. In Brazil, guidelines formerly recommended that children with non-advanced HIV disease receive two NRTIs,[56] and this practice was reported in a Brazilian study.[38] Long-term administration of zidovudine to infants is recommended in Brazil[57] and Argentina,[58] and in Mexico zidovudine and lamivudine are recommended[59] to prevent vertical transmission of HIV. Previous research

suggests, however, that the development of resistance after zidovudine prophylaxis during pregnancy is limited.[60,61]

The overall rate of protease mutations was significantly higher in Latin America than in other regions, which is likely to be related to children in this region having high exposure to this drug class. PIs have been used for longer in Latin America than in other regions and, therefore, the degree of transmitted PI resistance might be higher. For instance, PIs became available in Brazil in 1996.[62] The type of PI used probably also affects the development of resistance. Ritonavir-boosted lopinavir only became available in around 2003, and, therefore, nelfinavir (mostly without boosting) is the most widely studied PI in Latin American studies. Adequate plasma concentrations of this drug are seldom reached in children,[63] which leads to acquired resistance. In a small study from Argentina sub-therapeutic plasma concentrations of nelfinavir were measured in most children.[21] Studies from Latin America frequently reported the D30N and N88D mutations, which are specifically selected by nelfinavir.[18]

In southern Africa, ritonavir-boosted lopinavir and full-dose ritonavir are widely used in pediatric regimens. The most frequently reported mutations from this region were V82A, I54V, and M46I, which are selected by lopinavir and ritonavir.[18] One study compared rates of major PI mutations between children exposed to ritonavir-boosted lopinavir and ritonavir alone, and found that the latter is significantly associated with an increased risk of resistance.[44] In west Africa, a small number of children received indinavir, among a high proportion of whom treatment failure and drug resistance were reported.[39] The bioavailability of indinavir is variable in children, which contributes to sub-therapeutic plasma concentrations.[64]

In Asia, where NRTI resistance after treatment failure is high among children, frequencies of the T69ins and Q151M mutations were raised. These mutations, which confer resistance to multiple NRTIs, might indicate accumulated resistance if use of a failing treatment regimen is continued. Although virological criteria were used to define treatment failure in most studies we identified from Asia, monitoring of viral load is unlikely to have been used routinely to detect early treatment failure. Another contributing factor to the high rate of resistance could be the undisclosed use of previous ART exposure through monotherapy, dual therapy, or both, which could in turn be related to the early introduction of antiretroviral treatment in the region. In Thailand, for instance, zidovudine monotherapy started being used in 1992, dual therapy of zidovudine plus didanosine or zalcitabine in 1995, and ART was introduced in 2001.[65] Lastly, the Y181C/I mutation in the NNRTI drug class also occurred more frequently in Asia than in other regions.

This difference could be due to unreported use of nevirapine to prevent mother-to-child transmission of HIV.

Some of the differences in findings between regions might be attributable to diversity in HIV-1 subtype. The V106A/M mutation was found in 21% of cases in southern Africa, compared with less than 5% in other regions. The NNRTI mutation V106M seems to be found most frequently in subtype C HIV-1, whereas V106A is more frequently seen in subtype B.[66] V106A in subtype B is mainly associated with resistance to nevirapine but not efavirenz. By contrast, V106M confers broad cross-resistance. We noted no differences in the prevalence of the K65R mutation between HIV subtypes, although this mutation develops more rapidly in populations infected with subtype C.[67,68]

Rates of drug resistance at the time of virological failure seem to be similar for children and adults in resource-poor regions.[66,69,70] A previous systematic review that compared outcomes in frequently and infrequently monitored adult patients found a prevalence of 88% resistance at time of virological failure in the latter group.[71] As the rates of virological failure are, however, higher in children,[10,11] having a similar resistance rate to that in adults translates into an overall higher rate of increased emergence of drug resistance among children.

The pattern of mutations in the reverse transcriptase region of children was largely similar to that seen in adult patients in sub-Saharan Africa.[72] The frequency of the K103N mutation in HIV-1, however, was much lower among children with NNRTI exposure than in adults (37% vs. 52%). This finding corroborates that of the HIVNET 012 study,[73] which identified that the K103N mutation was selected more frequently in women than in their infants after single-dose nevirapine.[73] Mutations in the protease region also differ between adults and children. The M46I variant of the M46I/L mutation was detected in 16% of all children with PI exposure, compared with in 8% of adults. The various codon substitutions at the V82 location were more common in children than in adults (30% vs. 16%). One possible explanation for the occurrence of mutations at these two locations in children might be the use of full-strength ritonavir as the sole PI, which is associated with the emergence of major protease mutations.[44]

We could not assess whether prophylactic antiretroviral medication during the perinatal period contributed to the frequencies of resistance mutations at the time of treatment failure. Most studies did not report perinatal prophylaxis or did not separately report results for exposed children. Only one study[31] compared outcomes in children who were exposed to perinatal single-dose nevirapine with those in children who were not. The rate of virological failure and subsequent resistance development were increased in the

exposed children. The diversity in NNRTI mutations in the exposed children was greater than that in unexposed children, and included the V106A, V108C/V, and Y181C mutations. These findings are consistent with those from other investigations, which have shown that exposure to single-dose nevirapine affects treatment outcomes.[74] Various studies are in progress to assess this effect further.[75,76]

A limitation of our study is that the reported rates of drug resistance associated with particular drug classes might not be due only to the ART regimen. Transmitted resistance, which is of particular concern in children who have become infected despite maternal antiretroviral medication or acquired resistance because of the previous prophylactic or therapeutic use, might have increased drug resistance. We decided to include studies in which children had previously received antiretroviral drugs for two reasons. First, we could not confirm that studies with no information on previous antiretroviral medication had excluded children with any previous exposure. Second, exposure before ART reflects the reality of pediatric treatment programmes.

Another shortcoming of this systematic review is that we could not analyze patient adherence as a predictor of HIV drug resistance. Four included studies assessed adherence in relation to virological outcomes and one study assessed the association with resistance. Heterogeneous adherence measures were used, however, and direct comparison was not possible. Good adherence seemed generally to be related to virological suppression. In one study drug resistance was reported to be less likely in children in whom treatment had failed than in those with good adherence,[30] which is similar to previous findings in adults.[77]

Lastly, we could not relate the selection of specific resistance mutations to the antiretroviral regimens used. The reviewed studies provided only summary data on resistance-associated mutations rather than individual patients' data. Therefore, the frequencies of mutations associated with resistance to single or multiple drugs could not be assessed.

The highest rates of resistance were identified for nevirapine and efavirenz and reflect the low genetic barriers of these drugs. The reported mutations preclude further use of first-generation and possibly even second-generation NNRTIs. Etravirine has shown promising results in children in developed countries,[78] but is not yet licensed for pediatric use. It might, however, be less effective in resource-poor settings, where widespread use of nevirapine is associated with increased risk of acquiring the Y181C and G190S/A mutations and, therefore, possibly reduced susceptibility to etravirine. In the studies we assessed these mutations were detected, respectively, in 16% and 13% of children exposed to NNRTIs.

The reported NRTI mutations selected for stavudine, zidovudine, lamivudine, and, infrequently, didanosine. Extensive NRTI resistance could jeopardize the recycling of NRTIs in second-line ART. This issue is of particular concern in young children, for whom the number of licensed NRTIs is limited. The frequencies of K65R and Q151M mutations, which confer broad NRTI cross-resistance, were low, but the various thymidine analogue mutations were reported in 56% of patients. The number of patients with multiple thymidine analogue mutations, which would be indicative of accumulated resistance, was not, however, possible to calculate. The NRTI tenofovir would be suitable to include in the NRTI backbone of second-line ART, as it retains potency despite multiple thymidine analogue mutations. This drug has been associated with promising clinical outcomes in studies,[79,80] but is not yet formulated or licensed for young children. Furthermore, the risk of toxic effects to the kidneys and bone will restrict its use in resource-poor settings, where monitoring is limited.

For children with resistance to NRTIs and NNRTIs, salvage therapy with a dual-boosted PI regimen that uses low-dose ritonavir in combination with lopinavir and saquinavir has been investigated.[81] A mean viral load lower than 400 copies per mL was seen in 39 (78%) of 50 children in Thailand after 12 weeks of therapy, but significant increases in lipid concentrations in serum were observed. Further safety and efficacy data are needed to establish the usefulness of double-boosted PI regimens for children in whom the NRTI backbone is no longer expected to be active.

The number of children with PI exposure and subsequent resistance was limited in the reports we assessed. Our findings suggest, however, that nelfinavir and single-dose ritonavir are associated with a raised risk of drug resistance, which highlights the need for effective PIs in formulations suitable for children. The mutations selected for by ritonavir and lopinavir confer cross-resistance, but HIV that is resistant to older PIs may still be susceptible to newer PIs, such as darunavir and tipranavir. The lopinavir-associated V82A mutation even has a positive impact on virological response to darunavir.[82] Darunavir boosted with ritonavir and tipranavir are among the new regimens licensed for use in children and pediatric formulations are available.[83,84] Darunavir has a low pill burden and is not associated with serious adverse events. Furthermore, the first studies of the integrase inhibitor raltegravir in children show promising results, although pediatric formulations are required.[85]

This Review reveals a high proportion of children with drug resistant HIV-1 infections after first-line ART failure. One of the underlying causes could be the shortage of appropriate formulations for children and difficulties in calculating correct doses. The occurrence of triple-class resistance is rare in resource-poor regions, especially when

WHO treatment guidelines are followed. Our subgroup analysis points to high resistance rates in regions with extensive antiretroviral exposure (i.e., Asia and Latin America). In the context of increased access to medication in all resource-poor settings, commonly without testing of viral load to detect early failures, the rate of resistance to at least two drug classes is likely to increase. The policy to start treatment immediately after diagnosis in infants will lead to greater demand for feasible sequencing of regimens early in childhood. Ideally, active second-line agents should be selected on the basis of resistance testing, but this option is seldom available outside research settings because of the high cost and complex logistics. Access to second-line options remains limited in developing countries and, therefore, children are likely to continue taking failing regimens for longer periods of time, which promotes mutation accumulation and hinders the chance of virological control with new drugs. The future of an increasing number of children will depend on the availability of new-generation antiretroviral medications, and on pharmaceutical companies, donor agencies, and policy makers prioritizing the development of second-line and salvage pediatric formulations. Moreover, to make these regimens accessible on a global scale, low-cost generic drugs or major price reductions of patented versions are necessary.

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Chapter 12

High rates of thymidine analogue mutations and dual-class resistance among HIV-infected Ugandan children failing first-line antiretroviral therapy

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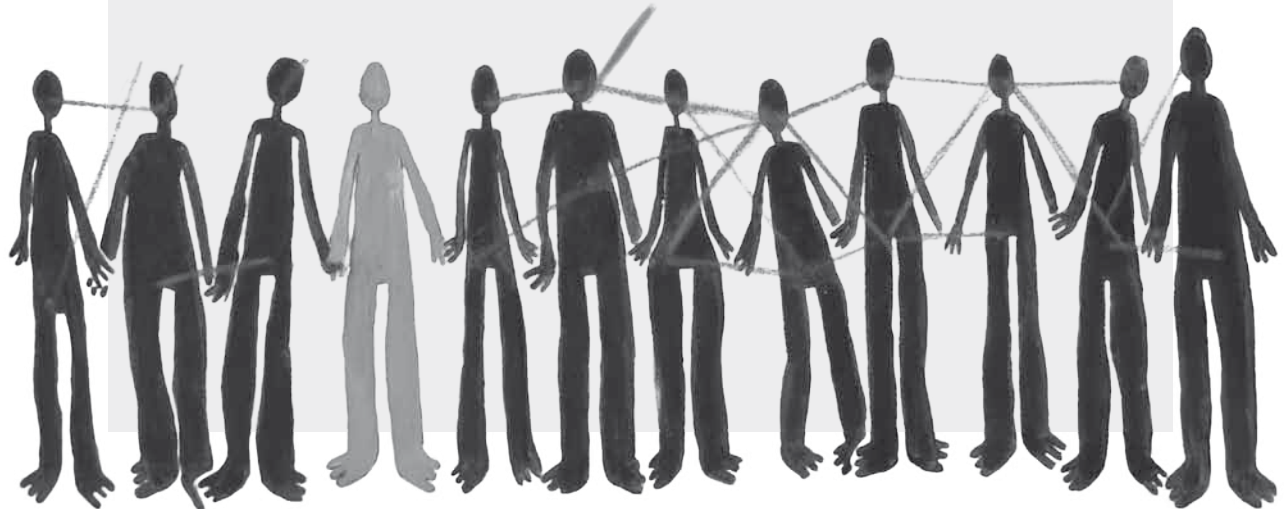
Submitted



ABSTRACT

Background

HIV-infected children are at high risk of acquiring drug resistant viruses, which is of particular concern in settings where antiretroviral drug options are limited. We aimed to assess resistance patterns and predict viral drug susceptibility among children with first-line antiretroviral therapy (ART) failure in Uganda. A cross-sectional analysis of children switching ART regimens due to first-line failure was performed at three clinical sites in Uganda.

Methods

HIV-RNA determination and genotypic resistance testing on all specimens with HIV-RNA >1000 copies/ml was performed. Major drug resistance mutations were scored using the 2011 International Antiviral Society–USA list. The Stanford algorithm was used to predict drug susceptibility.

Results

At the time of switch, 44 genotypic resistance tests were available for 50 children. Viruses with dual-class resistance in the nucleoside (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) drug-class were isolated in 98% (95% confidence interval [CI] 88-100%). Forty-six percent (95% CI 30-61%) of children harbored multiple thymidine analogue mutations. M184V was identified as the only NRTI mutation in 27% (95% CI 15-43%). HIV susceptibility to NRTIs, with the exception of tenofovir, was reduced in ≥60% of children.

Conclusion

Ugandan children experiencing first-line ART failure in our study harbored high rates of dual-class and accumulated HIV drug resistance. Methods to prevent treatment failure, including adequate pediatric formulations and alternative second-line treatment options are urgently needed.

INTRODUCTION

An estimated 3.4 million children are currently infected with HIV-1 worldwide, of whom 90% reside in sub-Saharan Africa.[1] Antiretroviral therapy (ART) has strikingly reduced morbidity and mortality in children.[2] However, younger children typically have poorer virological responses than adults,[3-5] which is attributable to high viral loads before treatment,[6] and the risk of sub-therapeutic drug concentrations owing to limited pediatric formulations, variable pharmacokinetics and changes in bodyweight.[7] Suboptimum virological suppression fosters the emergence of drug resistant viruses, the consequences of which are particularly important for children as they require ART for longer periods of time than adults.[8]

In resource-limited settings, exposure to nevirapine for the prevention of mother-to-child transmission (PMTCT) increases the risk of HIV drug resistance development and subsequent treatment failure of standard first-line regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTIs).[9, 10] Due to financial and technical constraints, virological monitoring to enable early failure detection is often not available. Furthermore, the identification of resistance mutations using genotypic testing, which could aid appropriate sequencing of antiretroviral medication, is seldom performed. As a consequence, children in resource-limited settings are empirically switched to a second-line regimen, typically containing ritonavir-boosted lopinavir and two new and/or recycled nucleoside reverse transcriptase inhibitors (NRTIs).

In Uganda, an estimated 200,000 children are HIV-infected and the Joint Clinical Research Centre (JCRC) is an important provider of HIV care and treatment in Uganda. In this cross-sectional study, we describe the resistance patterns among children failing first-line ART at three JCRC clinical sites, and predict viral drug susceptibility in order to gain insight into the optimal strategies for second-line therapy.

MATERIALS AND METHODS

Study design and population

The Monitoring Antiretroviral Resistance in Children (MARCH) study is a multicenter prospective observational cohort of HIV-1 infected children who receive ART at 3 clinical sites in Uganda. The national JCRC network – consisting of 7 clinical sites – has over 20 years of experience with ART, from conducting clinical trials to nationwide roll-out programs since 2003. Site and cohort characteristics have been described previously.[11]

Potential participants were informed of the study and screened for eligiblity by the study staff. Children aged 12 years or below, that initiated a first- or second-line ART regimen were enrolled. For the current analysis, only children requiring a treatment switch from first- to second-line ART due to treatment failure were included. Local definitions of clinical, immunogical and virological treatment failure were in accordance with WHO guidelines, i.e. virological failure was defined as a persistent viral load >5000 copies/ml after at least 6 months of treatment.[12] The ethical committees of JCRC in Uganda and the Academic Medical Center of the University of Amsterdam in The Netherlands 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. Socio-demographic and clinical data were collected using electronic case report forms, which were aggregated in a web-based database.

Procedures

Routine laboratory results including CD4 cell count and serum hemoglobin concentration were obtained from local laboratory records. Immunodeficiency for age was classified according the 2010 WHO guidelines.[12] HIV-RNA and genotypic resistance testing on all specimens with HIV-RNA >1000 copies/ml was performed at a central reference laboratory. For HIV-RNA determination, the COBAS Ampliprep/COBAS Taqman HIV-1 test (Roche, Branchburg, NJ, USA) was used. For HIV-1 genotyping, an in-house sequencing method encompassing the whole of PR and codons 1-300 of RT with a Beckman Coulter CEQ 8000 analyzer (Beckman Coulter Inc., Fullerton, CA, USA) was used.[13] Sequences were assembled and manually edited using BioEdit software version 7.1.3.0. Subtypes were determined using MEGA 5.10 software. Drug resistance mutations were scored according to the 2011 International AIDS Society-USA list.[14] HIV drug susceptibility for each participant was predicted using the Stanford algorithm, version 6.1.0[15] and was categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance. The proportions of virus with drug resistance mutations were calculated with 95% confidence intervals (CI). Analyses were performed using Stata version 10 (StataCorp LP, TX, USA).

RESULTS

Between January 2010 and July 2011, 50 children who switched to second-line after first-line ART failure were enrolled. The patients originated from Kampala (n=29), Mbale (n=18) and Fort Portal (n=3). The median age was 5.5 years old (interquartile range [IQR] 4.0-10.1) and 50% (25/50) of the participants were girls (Table 1). The mother was the primary caregiver for most children (n=30, 60%); 9 (18%) children were orphans. At the

Table 1. Characteristics of children with first-line antiretroviral therapy failure

	Total (n=50)
Age (years)	5.5 (4.0-10.1)
Sex, female	25 (50.0)
Living situation	
With 2 parents	13 (26.0)
With 1 parent	24 (48.0)
Other	13 (26.0)
Mother current ART	
First-line ART	23 (46.0)
Second-line ART	3 (6.0)
Not on ART	5 (10.0)
Mother deceased	14 (28.0)
Unknown	5 (10.0)
WHO clinical stage	
1 or 2	27 (54.0)
3 or 4	23 (46.0)
Weight-for-age z-score[a]	
Underweight (< -2 SD)	9 (24.3)
Height-for-age z-score	
Stunting (< -2 SD)	25 (50.0)
CD4 % (in <5 year)	23.2 (14.0-30.0)
CD4 cell count (in ≥5 year)	460.5 (86.0-645.5)
Total lymphocyte count (x 10^9/L)[b]	3.0 (1.8-3.9)
Hemoglobin (g/dl)[c]	11.3 (10.3-12.7)
Viral load (log_{10})	4.8 (4.0-5.2)
PMTCT exposure	
Yes	13 (26.0)
No	37 (74.0)
Previous ART regimen	
AZT/3TC/NVP or EFV	35 (70.0)
D4T/3TC/NVP	10 (20.0)
ABC/3TC/NVP or EFV	4 (8.0)
AZT/3TC/ABC	1 (2.0)
Duration of first-line ART (months)[d]	29.8 (25.1-48.4)

Data presented as n (%) or median (interquartile range). ART = antiretroviral therapy, SD = standard deviation, PMTCT = prevention of mother-to-child transmission, AZT = zidovudine, 3TC = lamivudine, NVP = nevirapine, EFV = efavirenz, D4T = stavudine, ABC = abacavir. [a]Weight-for-age z-score calculated for ages 0 to 10 year only, n=37; [b]Total lymphocyte count, n=48; [c]Hemoglobin, n=48; [d]Duration of first-line ART, n=49.

Table 2. Mutational patterns among children with first-line antiretroviral therapy failure and available genotypic test results

Resistance pattern	Overall (n=44)
Any mutation	44 (100) [92-100]
Any NRTI	43 (97.7) [88-100]
Any TAM	28 (63.6) [28-78]
≥ 2 TAMs	20 (45.5) [30-61]
≥ 3 TAMs	16 (36.4) [22-52]
TAM+M184V	28 (63.6) [28-78]
M184V only	12 (27.3) [15-43]
Any NNRTI	44 (100) [92-100]
≥ 2 NNRTIs	24 (54.5) [39-70]
NRTI+NNRTI	43 (97.7) [88-100]
TAM+NNRTI	28 (63.6) [28-78]
M184V+NNRTI	41 (93.2) [81-99
TAM+M184V+NNRTI	28 (63.6) [28-78]

Data are presented as n (%) [95% confidence interval]. NRTI = nucleoside reverse transcriptase inhibitor, TAM = thymidine analogue mutation, NNRTI = non-nucleoside reverse transcriptase inhibitor.

time of the child's enrolment, 26 (52%) mothers used ART. The decision to switch to second-line ART was based on virological failure in 48 (96%) and immunological failure in 2 (4%). Overall, 23 (46%) children had a WHO stage 3 or 4 event, 9 (24%) children were underweight and 25 (50%) were stunted. Of the children with virological failure, 24 (50%) were also classified as immune-deficient based on their CD4 percentage or CD4 cell count for age.

First-line ART had been received for a median of 30 months (IQR 25.1-48.4). The most common previous regimen consisted of zidovudine/lamivudine with either nevirapine (n=23, 46%) or efavirenz (n=12, 24%), followed by stavudine/lamivudine/nevirapine (n=10, 20%) and abacavir/lamivudine with nevirapine (n=3, 6%) or efavirenz (n=1, 2%). Single-drug substitutions within NRTI drug class were common (n= 23, 46%). Thirteen (26%) children had been exposed to nevirapine for PMTCT (Table 1).

Genotypic tests results were obtained for 44 (88%) children: 4 specimens had a plasma viral load below the sequencing threshold (1000 copies/ml) and 2 specimens failed amplification. Subtype D was most commonly identified (n=19, 43%), followed by A (n=16, 36%), C (n=1, 2%) and various circulating recombinant forms (n=8, 18%). Drug resistance mutations were detected in all children with a genotypic test result (Table 2). Within the NRTI drug class, M184V was present in 41 (93%) and multiple thymidine analogue mutation (≥ 2 TAMs) in 20 (46%). In 64% of children M184V occurred in combination with

≥ 1 TAM and in 27% the M184V was the only NRTI mutation identified. The most frequent NNRTI mutations were K103N (n=17, 39%) and G190A (n=16, 36%). Dual-class NRTI and NNRTI resistance was present in 43 (98%) children (Table 2). Figure 1 depicts frequencies of individuals mutations by the type of NRTI or NNRTI used in the (most recent) first-line regimen. TAMs appeared more frequently when zidovudine was used, whereas K65R was less common (Figure 1A). Multiple NNRTI mutations were observed more frequently when efavirenz was used as opposed to nevirapine (Figure 1B).

Predicted HIV susceptibility to lamivudine and emtricitabine was reduced in nearly all (98%) children due to the high frequency of M184V (Figure 2). For the other NRTIs, reduced susceptibility was predicted for abacavir in 71% of sequences, zidovudine in 59%, stavudine in 68%, didanosine in 61%, and tenofovir in 39%. For the NNRTIs, reduced HIV susceptibility was frequently predicted for efavirenz and nevirapine (98%) and less frequently for the second-generation drugs etravirine and rilpivirine (52%). Reduced susceptibility was not predicted for any of the ritonavir-boosted protease inhibitors (PIs).

DISCUSSION

This cross-sectional study among Ugandan HIV-infected children examined mutational resistance patterns after first-line ART failure and predicted the impact on second-line drug susceptibility. Nearly all children harbored dual-class resistance and multiple TAMs were detected in 46%. HIV susceptibility to NRTIs, with the exception of tenofovir, was severely reduced, jeopardizing the recycling of NRTIs in second-line ART. This issue is of particular concern in young children, for whom the number of licensed NRTIs is limited.

The most frequently observed mutation in our study, the M184V, associated with lamivudine and emtricitabine, delays resistance to zidovudine and stavudine.[16] Despite this increased susceptibility, 64% of patients harbored TAMs in combination with the M184V mutation. The rate of TAMs detected in our study is very high compared to previous reports among children.[8] This may be attributed to the fact that the majority of children were referred from outside clinics and had a longer duration of treatment failure. It also likely reflects clinicians' reluctance to switch to second-line ART, due to regimen cost and subsequent loss of drug options.

After failure of an NNRTI-based regimen in children, the WHO recommends a ritonavir-boosted PI, with a dual backbone of 2 new or recycled NRTIs, i.e. abacavir or zidovudine plus lamivudine.[12] Didanosine is proposed as a possible alternative to lamivudine. In our cohort, susceptibility to abacavir, zidovudine and didanosine was preserved in 29%,

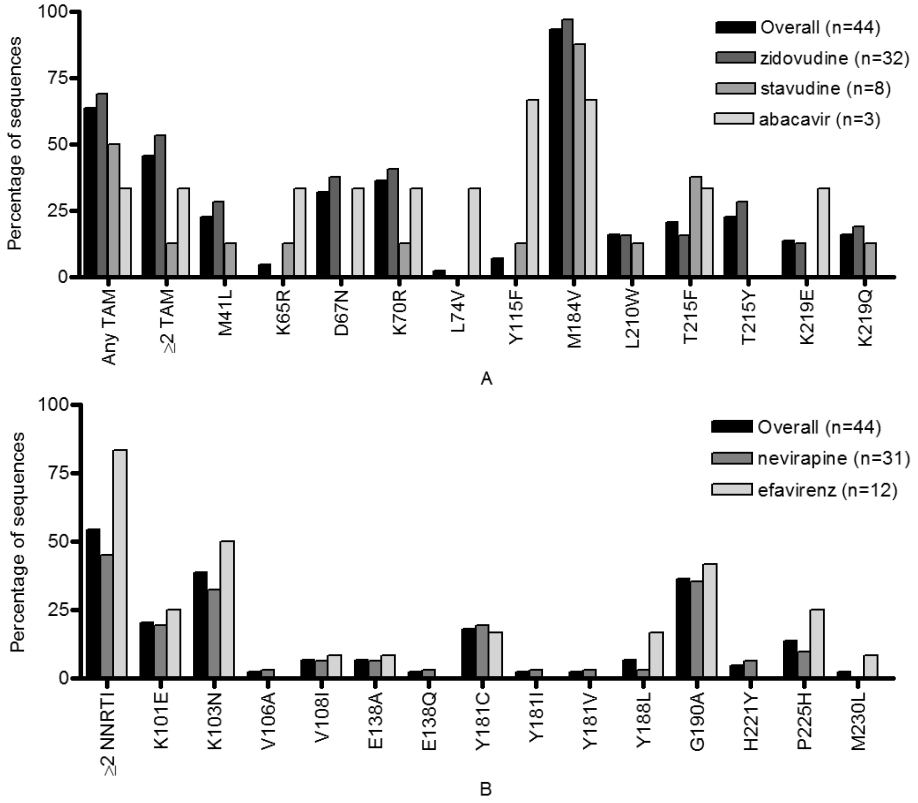


Figure 1. Frequencies of individual drug resistance mutations by nucleoside (A) and non-nucleoside reverse transcriptase inhibitors (B). The legend includes the number of patients exposed to each antiretroviral drug. TAM = thymidine analogue mutation.

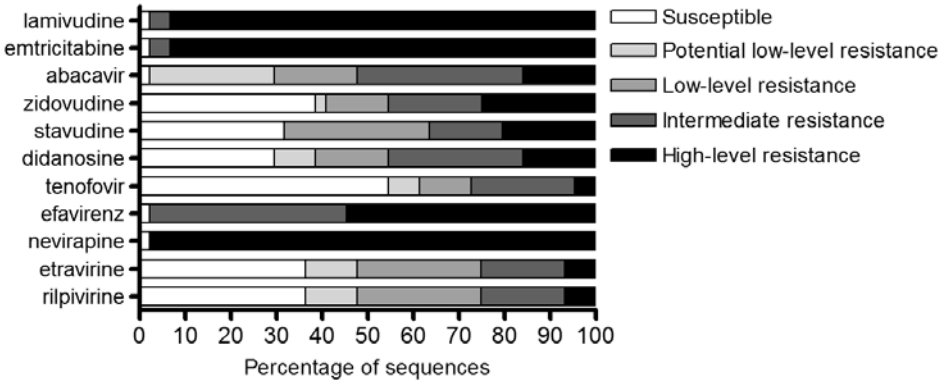


Figure 2. Predicted HIV susceptibility to nucleoside and non-nucleoside reverse transcriptase inhibitors in children with first-line antiretroviral therapy failure (n=44). Genotypic drug susceptibility was predicted using the Stanford HIVdb algorithm (version 6.1.0).

41% and 39% of children, respectively. Therefore, the recommended NRTI backbone in second-line ART would be expected to have very limited effect, while at the same time exposing children to considerable toxicity.[17] The most suitable NRTI to include in second-line ART would be tenofovir, which retains potency despite multiple TAMs. This drug has been associated with promising clinical outcomes in studies,[18, 19] and its recent approval by the US Food and drug Administration (FDA) for children ≥ 2 years of age is particularly encouraging.[20] However, the risk of toxic effects to the kidneys and bone[18] will restrict its use in resource-poor settings, where monitoring is often limited. Therefore, constructing an active NRTI backbone for the children in this study becomes a major challenge.

An alternative strategy for second-line would be the use of boosted-PI monotherapy, which has shown promising preliminary results in adults.[21] In children, this strategy would have several advantages such as fewer pills, fewer drug interactions and less NRTI-associated toxicity. Future research should investigate long-term outcomes of PI monotherapy in the pediatric population. In our study, we profiled the HIV susceptibility to second generation NNRTIs. Susceptibility to etravirine and rilpivirine is reduced in the presence of G190A and Y181C[22] and these mutations were present in 36% and 18% of children, respectively. Etravirine has shown promising results in children,[23, 24] but is not yet licensed for use. For rilpivirine, no data in the pediatric population are yet available.[25] Clearly, the use of newer agents, such as integrase inhibitors, should be explored.

A limitation of our study is the small sample size. However, as resistance data on children is scarce, we believe that the results of this small cohort are important in informing clinicians in resource-limited settings when they are faced with the challenge of changing ART regimens without the aid of genotypic resistance testing. Second, a limitation related to the cross-sectional design is that the prevalent cases identified in this study may not be representative of all incident cases with virological failure.

This study reveals a high proportion of children with multiple TAMs and dual-class resistance after first-line ART failure. As a result, second-line regimens will primarily offer the benefit of the boosted-PI, and limited or no additional effect of the NRTI backbone. Deferred treatment switch is common in resource-limited settings[26, 27] and results in increased mortality.[28, 29] Therefore, the obstacles to second-line drug access and usage must be addressed by reducing costs and increasing the availability of new-generation antiretroviral medications with non-overlapping resistance profiles and tolerable formulations for children.

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Chapter 13

Barriers to initiation of pediatric HIV treatment in Uganda: a mixed methods study

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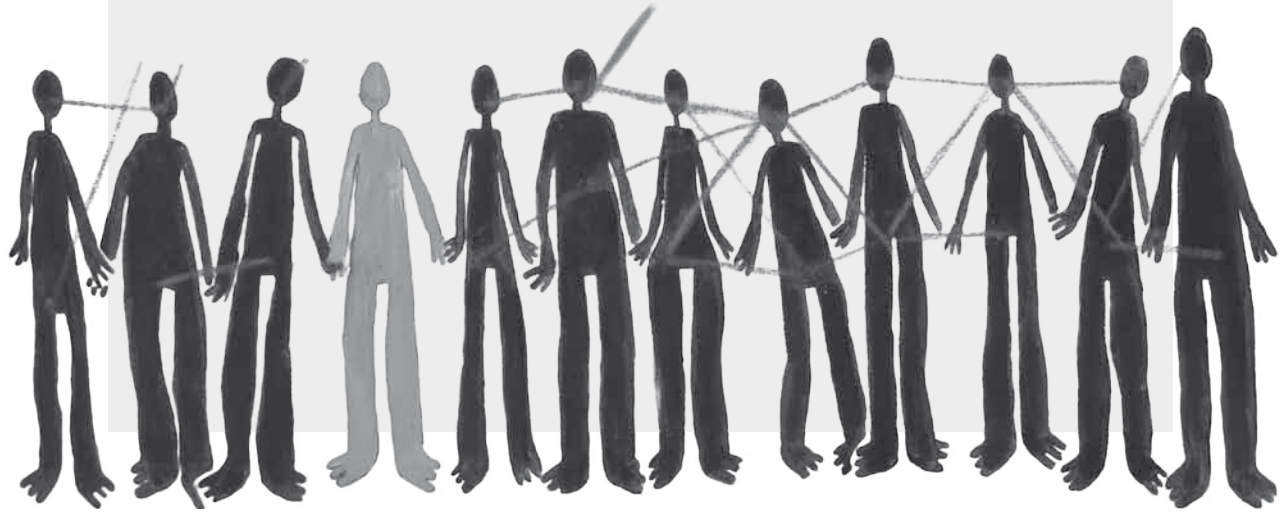

ABSTRACT

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-Sahara 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.

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).

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 eligiblity 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

Table 1. Characteristics of the Joint Clinical Research Centre (JCRC) sites

	Kampala	Fort Portal	Mbale
Location	National capital	District capital	District capital
Population [a]	1,659,600	47,100	91,800
Catchment area	Urban	Urban and rural	Urban and rural
HIV prevalence [b]	5-9.9%	5-9.9%	5-9.9%
Number of adults in care at JCRC (% of total) [c]	15306 (85.7)	5880 (87.3)	3027 (85.7)
Number of children in care at JCRC (% total) [c]	2553 (14.3)	858 (12.7)	506 (14.3)
Number of adults receiving ART at JCRC (% of adults in care) [c]	6096 (39.8)	2575 (43.8)	2505 (82.8)
Number of children receiving ART at JCRC (% of children in care) [c]	888 (34.8)	340 (39.6)	349 (69.0)

[a] Source: Uganda Bureau of Statistics (UBOS) 2011.
[b] Source: UNAIDS Epidemiological Factsheet Uganda 2009.
[c] Source: Monitoring and Evaluation records at JCRC, 2011.

use of ARVs for PMTCT was allowed. The ethical committees of 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.

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

Table 2. Clinical and demographic characteristics of the 'Monitoring of Antiretroviral Resistance in Children' cohort participants

		Overall	Study Site			P-value
		n = 306 (100)	Kampala n = 91 (29.7)	Fort Portal n = 112 (36.6)	Mbale n = 103 (33.7)	
Sex	*Male*	152 (49.8)	43 (47.3)	55 (49.1)	54 (52.9)	0.719
Age, median years (IQR)		4.8 (2.2-8.6)	4.0 (1.5-8.5)	4.2 (1.9-8.5)	5.8 (3.0-8.7)	0.127
Age groups	*<2 years old*	76 (24.8)	32 (35.2)	29 (25.9)	15 (14.6)	0.005
	2-5 years old	84 (27.5)	18 (19.8)	37 (33.0)	29 (28.2)	
	5-12 years old	146 (47.7)	41 (45.1)	46 (41.1)	59 (57.3)	
Age at (first) confirmed HIV+ test	*Median (IQR)*	3.7 (1.6-6.8)	3.0 (1.3-7.2)	3.3 (1.1-6.8)	4.5 (2.7-6.8)	0.264
WHO clinical stage	*Stage 3 and 4*	221 (72.2)	36 (39.6)	92 (82.1)	93 (90.3)	<0.001
HIV-TB co-infection	*Pulmonary tuberculosis*	31 (10.1)	18 (19.8)	6 (5.4)	7 (6.8)	0.001
Severe immunodeficiency [a]	*CD4count-for-age*	67 (31.0)	36 (40.0)	9 (21.4)	22 (26.2)	0.047
	CD4%-for-age	102 (51.3)	57 (63.3)	18 (42.9)	27 (40.3)	0.008
Viral load, median $\log_{10}$ cps/mL (IQR) [c]		5.0 (4.4-5.5)	5.2 (4.7-5.6)	5.1 (4.2-5.5)	4.7 (4.1-5.3)	0.001
Main reason for ART initiation [d]	*HIV diagnosis <24 months*	30 (9.8)	20 (22.0)	9 (8.0)	1 (1.0)	<0.001
	Immunological status	102 (33.3)	55 (61.4)	25 (22.3)	22 (21.4)	
	WHO clinical stage	174 (56.9)	16 (17.6)	78 (69.6)	80 (77.7)	
Time between HIV test and ART initiation, median days (IQR)	*<2 years old*	43 (19-85)	40 (18-66)	48 (23-103)	43 (26-86)	0.299
	2-5 years old	97 (26-400)	117 (34-309)	197 (29-546)	64 (15-180)	0.302
	5-12 years old	258 (29-802)	242 (28-634)	296 (35-757)	216 (21-848)	0.474
PMTCT exposed	*Yes*	14 (4.6)	11 (12.1)	3 (2.7)	-	<0.001
Drugs for PMTCT	*Single dose NVP*	9 (2.9)	6 (6.6)	3 (2.7)	-	0.025
	NVP	4 (1.3)	4 (4.4)	-	-	0.008
	AZT	2 (0.7)	2 (2.2)	-	-	0.093
	Unknown	1 (0.3)	1 (1.1)	-	-	0.306
Breastfeeding	*Yes*	24 (7.9)	10 (11.1)	12 (10.7)	2 (1.9)	0.023
"Is there enough food in the household?"	*Yes*	300 (98.0)	87 (95.6)	110 (98.2)	103 (100.0)	0.087

Data are presented as n (%) unless otherwise indicated. [a] Severe immunodeficiency, defined as CD4 percentage <25% or CD4 count <1500 cps/mm^3 below 12 months old, CD4 percentage <20% or CD4 count < 750 cps/mm^3 between 12-35 months old and CD4 percentage <15% or CD4 count <350 cps/mm^3 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.

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.

Risk factors for late disease stage at presentation

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

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

		n = 306
Underweight (WAZ < -2 SD)	*< 5 years old*	42.7 (34.6-50.7)
	5-12 years old	24.5 (15.2-33.7)
Severe underweight (WAZ < -3 SD)	*< 5 years old*	26.1 (18.9-33.3)
	5-12 years old	12.8 (5.5-20.0)
Stunting (HAZ < -2 SD)	*< 5 years old*	62.0 (53.9-70.1)
	5-12 years old	39.0 (30.4-47.5)
Severe stunting (HAZ < -3 SD)	*< 5 years old*	40.0 (31.8-48.2)
	5-12 years old	18.4 (11.5-25.3)
Wasting (WHZ < -2 SD)	*< 5 years old*	21.7 (14.9-28.4)
	5-12 years old	NA
Severe wasting (WHZ < -3 SD)	*< 5 years old*	7.0 (2.7-11.3)
	5-12 years old	NA
BMI-for-age z-score (< -2 SD)	*< 5 years old*	16.3 (10.2-22.3)
	5-12 years old	8.6 (3.6-13.7)
Mid-upper arm circumference n (%) [a]	*≤ 13.5 cm*	55 (37.9)

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).
[a] 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/m^2).

time at the clinic. The sensitivity analysis, excluding 22 children in whom MTCT was not confirmed, yielded similar associations (data not shown).

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

OR		Univariate analysis			Multivariate analysis		
		95% CI	P-value	OR	95% CI	P-value	
Sex	*Female*	1					
	Male	0.99	0.56-1.74	0.963			
Age group	*5-12 years old*	1			1		
	2-5 years old	1.20	0.60-2.41	0.612	1.90	0.85-4.22	0.117
	0-2 years old	1.45	0.72-2.92	0.303	2.83	1.23-6.50	0.014
Current living situation	*With both parents*	1			1		
	With one parent	1.39	0.71-2.73	0.340	2.05	0.96-4.35	0.062
	With siblings/relatives/other caregiver/in institution	2.46	1.14-5.31	0.021	3.93	1.65-9.38	0.002
Primary caregiver	*Mother*	1					
	Father	0.80	0.22-2.94	0.738			
	Grandparent	3.07	1.06-8.92	0.039			
	Other relative / friend	0.92	0.45-1.91	0.826			
	Institution	3.09	0.54-17.64	0.203			
Mother's health status	*Healthy*	1					
	Sick	1.66	0.49-5.60	0.416			
	Deceased	1.59	0.71-3.58	0.260			
	Unknown	1.10	0.28-4.24	0.894			
Father's health status	*Healthy*	1					
	Sick	1.27	0.43-3.74	0.660			
	Deceased	1.78	0.82-3.85	0.142			
	Unknown	2.17	0.81-5.81	0.122			
Highest level of education of the caregiver	*Illiterate*	1					
	Literate / primary school	0.77	0.32-1.83	0.550			
	Secondary school	0.55	0.21-1.44	0.223			
	Post-secondary school	0.49	0.17-1.45	0.197			
Occupation of the caregiver	*Wage-employed*	1			1		
	Self-employed	1.82	0.78-4.26	0.166	2.50	1.00-6.28	0.051
	None/at home/student	3.24	1.43-7.34	0.005	4.26	1.75-10.35	0.001
PMTCT experienced	*Yes*	1			1		
	No	5.07	1.24-20.79	0.024	5.66	1.21-26.51	0.028
Time between HIV+ diagnosis and ART initiation	*0-31 days*	1					
	> 30 days	0.93	0.50-1.73	0.818			

Table 4 (continued)

OR		Univariate analysis			Multivariate analysis		
		95% CI	P-value	OR	95% CI	P-value	
Transportation time	*< 1 hour*	1					
	1 hour or more	0.85	0.45-1.58	0.606			
Transportation costs	*1st quartile (UGX[a] 500-1500)*	1			1		
	2nd quartile (UGX 1500-3000)	1.07	0.51-2.23	0.857	0.96	0.43-2.14	0.915
	3rd quartile (UGX 3000-4000)	0.89	0.34-2.33	0.818	0.85	0.30-2.38	0.756
	4th quartile (UGX 4000-15000)	2.09	0.85-5.15	0.108	2.51	0.92-6.85	0.072
Waiting time at clinic	*< 2 hours*	1					
	2 hours or more	0.83	0.35-1.98	0.676			

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.

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.

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.

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

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

- **i.** Improve antenatal care attendance and linkage to ART clinics
- **ii.** Involve men during antenatal care
- **iii.** Increase awareness of care and treatment services
- **iv.** Improve community outreach for HIV testing
- **v.** Facilitate transport to clinics

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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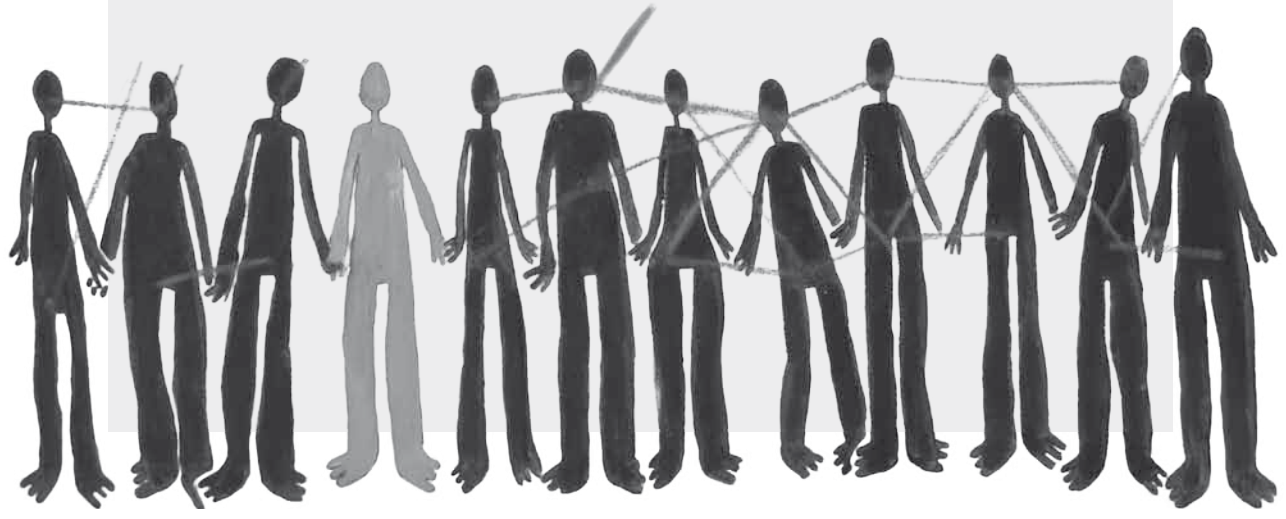
Chapter 14

General discussion



Since its introduction 15 years ago, combination antiretroviral therapy (ART) has saved millions of lives worldwide. In Africa, over five million HIV-infected people are currently estimated to receive ART, impacting nearly 50% of those in need.[1] Treatment programs in resource-limited countries have efficacy rates similar to those reported for developed countries.[2] However, from the moment ART was introduced and scaled-up in resource-limited countries, little attention has been paid to the emergence of HIV drug resistance and its potential consequences. Drug resistant HIV may severely restrict therapeutic options, and treatment costs will greatly increase when second and third-line ART regimens are needed. Therefore, a major challenge that will confront national HIV treatment programs is how to prevent and manage mounting drug resistance.

To address this challenge, the 'PharmAccess African Studies to Evaluate Resistance' or PASER program was established with the aim of developing regional capacity for the population-level assessment of acquired and transmitted HIV drug resistance. The PASER network consists of clinical sites, laboratories and research groups in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe. Within the PASER program, the PASER-Monitoring protocol assessed baseline and acquired resistance in adults receiving first- or second-line ART, and the PASER-Surveillance protocol assessed transmitted drug resistance in recently infected individuals. A spin-off project, the 'Monitoring Antiretroviral Resistance in Children' or MARCH pediatric cohort, was initiated in Uganda with similar objectives as the adult study. The studies included in this thesis were conducted as part of the PASER and MARCH programs.

PART 1: EPIDEMIOLOGY OF TRANSMITTED HIV DRUG RESISTANCE

Initial evidence of studies in resource-limited settings, using the World Health Organization (WHO) threshold survey methodology, suggested that in the first years of ART scale-up, the prevalence of transmitted drug resistance was limited, i.e. below 5%.[3] Studies performed in 2009-2010 as part of the PASER-Surveillance program have reported a rise in transmitted drug resistance in East Africa. Our study carried out among 70 recently diagnosed individuals in Kampala, Uganda, revealed drug resistance mutations in 8.6% (**chapter 2**). This study suggested increasing resistance levels compared with a previous survey in the same geographic region in which no drug resistance was found.[4]of this information or reproduction of this materialis strictly forbidden unless prior written permission is obtainedfrom Adobe Systems Incorporated.File: docxtoSangamML.xsltAuthor : Sachin Soni

In Mombasa, Kenya, we conducted a similar study using the WHO threshold survey methodology (**chapter 3**). Among 68 participants, the prevalence of transmitted drug

resistance was found to be as high as 13.2%. Although no prior surveys have been conducted in Mombasa, this level exceeds what has been previously reported in Kenya.

Reported transmitted drug resistance levels in these two urban African settings were highest for non-nucleoside reverse transcriptase inhibitors (NNRTIs), which is consistent with the widespread use of this drug class for first-line ART, the low genetic barrier of NNRTIs for resistance, and the relative fitness of NNRTI-resistant strains.[5, 6] The findings in Kampala and Mombasa corroborate other recent studies reporting an increase of transmitted drug resistance in parallel to the widespread distribution of ART in other African countries.[7-9] A Virology Laboratory CREMER/IMPM/IRD, Yaounde, Cameroon bCentral Hospital, Yaounde, Cameroon cUMI 233 TransVIHMI, Institut de Recherche pour le Developpement (IRD Indentifying recent infections is complicated and instead, HIV drug resistance can be measured among people at the time of ART initiation, although mutations may disappear due to reversion.[10] The PASER-Monitoring study assessed baseline (or pretreatment) resistance among chronically infected, antiretroviral-naïve persons and detected a drug resistance mutation in 5.6% before ART initiation.[11] The prevalence of resistance mutations appeared higher in Uganda (12%), where antiretroviral drugs were available ahead of other African countries. In this study, pretreatment resistance was estimated to increase with time since the start of the ART roll-out by 38% per year. In agreement, a recent meta-analysis also demonstrated an increasing prevalence of drug resistance since ART scale-up, in particular in East Africa.[12] Importantly, this study also found that the prevalence of mutations was not different between recently or chronically infected populations.

In 2010, the WHO recommended that the threshold for ART initiation be changed from 200 to 350 CD4 cells/mm^3 – substantially increasing the number of people eligible for ART.[13] In addition to the individual clinical benefits, earlier initiation of ART may reduce HIV incidence rates and the concept of treatment as prevention has attracted attention as a means to reduce the global HIV epidemic.[14, 15] We investigated the effect of the subsequent increased ART coverage on future transmitted drug resistance trends in East Africa using a mathematical model (**chapter 4**). This model was based on the rates of acquired and transmitted resistance rates as detected in the PASER-M and PASER-S studies, respectively, in Kampala and Mombasa. We estimated that in 10 years time, the prevalence of transmitted drug resistance will increase to 12% and 13% if ART is started at CD4 <350 cells/mm3 and to 18% and 19% if ART started at CD4 <500 cells/mm3 in Kampala and Mombasa, respectively. Our model confirmed that resistance will consist predominantly of NNRTI-associated mutations. This, however, can be prevented by providing wider access to virological monitoring and boosted PIs for second-line therapy. Importantly, we predict that the number of infections that will be averted by earlier ART initiation will far exceed the number of infections with a drug resistant virus.

PART 2: ACQUIRED HIV DRUG RESISTANCE IN PERSONS FAILING FIRST-LINE ANTIRETROVIRAL THERAPY

Increasing transmitted drug resistance is ultimately caused by increasing rates of virological failure and acquired resistance in persons receiving ART. A challenge for healthcare workers in resource-limited countries is how to identify patients who are experiencing therapy failure and to make timely decisions on switches to second-line therapy. The WHO public-health approach to ART administration recommends using clinical and/or immunological definitions of ART failure if viral load testing is not available.[13] The studies in part two of this thesis underscore the poor ability of these clinical and immunological criteria to diagnose ART failure. We demonstrated that among 250 patients who started second-line ART, first-line treatment failure had been diagnosed in 64 patients based on clinical and immunological criteria alone, in absence of viral load testing (**chapter 5**). In this group, an unnecessary switch occurred in 47%, as later determination of viral load revealed <1000 copies/ml. The use of targeted viral load testing by the treating clinician reduced the rate of unnecessary switches to 12%. In the same cross-sectional study, a high frequency (88%) of clinically significant mutations was detected among patients with clinical and/or immunological first-line failure and a viral load >1000 copies/ml. Extensive cross-resistance within the nucleoside reverse transcriptase inhibitor (NRTI) drug class was present in 48%, which is indicative of prolonged virological failure. In these patients, a switch to second-line ART would be expected to offer the benefit of the boosted protease inhibitor (PI), but limited or no additional effect of the NRTI backbone.

Drug resistance mutations associated with first-line regimens were investigated in more detail in our prospective study of 2588 patients with no prior exposure to antiretroviral medication (**chapter 6**). During a routine measurement after 12 months of first-line ART, 142 patients were evaluated for drug resistance after a viral load >1000 copies/ml was detected. Of these, 70% had at least one mutation and 49% had dual-class resistance. Compared to the study in chapter 5, in which most patients had prolonged treatment failure, extensive NRTI resistance was less common in this cohort where a routine viral load test was carried out after 12 months. For example, multiple thymidine analogue mutations, conferring broad NRTI cross-resistance, were found in 38% of patients in chapter 5 but only in 6% of patients in the prospective cohort in chapter 6. Hence, routine viral load measurement prevented prolonged virological failure and the accumulation of mutations. In chapter 6, the K65R mutation was frequently (12%) selected for after failure of stavudine or tenofovir containing regimens. This finding suggested that, after failure of a stavudine containing first-line regimen, zidovudine rather than tenofovir (as recommended by the current WHO guidelines)[13], may be the preferred second-line NRTI. The data reported in chapters 5 and 6 also indicated that the second-generation NNRTIs

etravirine or rilpivirine are unlikely to be effective as part of second-line ART, given the high frequencies of Y181C and G190A mutations.

We estimated the rate at which thymidine analogue mutations accumulate during continued ART failure in **chapter 7**. Among a small cohort comprising 43 South African patients, repeated resistance testing was performed in patients with persistent viremia while receiving first-line ART. Overall, thymidine analogue mutations were found to accumulate at a rate of 0.07 mutations per month, resulting in one new mutation for every 14.6 months of continued drug exposure. This was considerably faster than the rate of one thymidine analogue mutation in every 4.3 years described in a European study.[16] In patients who acquired at least 1 thymidine analogue mutation, the rate of accumulation was found to be 0.15 mutations per month, i.e. one mutation in 6.5 months. Again, we concluded that the degree and pattern of drug resistance detected after prolonged virological failure in this cohort are likely to negatively impact future regimens containing NRTI backbones.

The poor sensitivity and specificity of WHO clinical and immunological criteria to diagnose ART failure have been also pointed out by other studies in Africa.[17, 18] False-positive cases (clinical or immunological failure but suppressed viral load) cause unnecessary switching to second-line ART. In line with our observations, a Kenyan study estimated that up to 58% of participants would be misclassified as having ART failure and prematurely switched to second-line if only immunological criteria would be used.[19] We conclude that unnecessary regimens switches will exhaust drug options and augment costs. The use of targeted viral load testing to confirm suspected treatment failure and guide regimen switching is therefore likely to be cost-saving.[20] False-negative cases are more common as viral load elevation failure usually precedes clinical or immunological deterioration. This results in prolonged virological failure and subsequent accumulation of mutations, as was also clearly demonstrated by a study from Malawi confirming our work.[21] Overall, deferred treatment switch is common in resource-limited settings,[22, 23] and causes increased mortality.[24, 25] We conclude that prolonged failure could be prevented by routine viral load testing, for instance at 6-monthly or yearly intervals. When NRTI resistance, and especially the accumulation of thymidine analogue mutations, is less extensive, it might still be possible to construct an active NRTI backbone in second-line ART.

PART 3: OUTCOMES OF FIRST- AND SECOND-LINE ANTIRETROVIRAL THERAPY

In response to the rapid scale-up of ART in resource-limited settings, the WHO has formulated Early Warning Indicators, which constitute a key element of the organization's Global Strategy for HIV Drug Resistance Prevention and Assessment.[26] The indicators are ART site factors, such as prescribing practices, attrition rates and drug supply continuity, which may be associated with the emergence of HIV drug resistance and can be subsequently acted on to prevent or minimize resistance.[27] Within the PASER network, we assessed the Early Warning Indicators and identified vulnerable aspects of care (**chapter 8**). Of concern, nine out of thirteen sites failed to meet the target for at least one indicator. We studied the indicator "on-time antiretroviral drug pick-up" in detail among patients from two sites in Lusaka, Zambia. We found that this indicator was difficult to measure and had a low sensitivity (47%) for determining possible drug resistance after 12 months of first-line ART. Instead, the medication possession ratio (defined as the number of days that a person is in possession of drugs divided by the number of days between prescriptions) was better able to identify patients at risk for HIV drug resistance at 12 months, with a sensitivity of 67%. We therefore recommend including the medication possession ratio in the Early Warning Indicators.

Besides assessing the ability of ART programs to prevent HIV drug resistance emergence, part three of this thesis also evaluates the response to ART in patients with preexisting resistance. In **chapter 9**, the presence of pretreatment resistance was investigated among 2579 patients starting standard NNRTI-based first-line ART. We found that 123 (5%) patients harbored resistance to at least one prescribed drug and consequently received a suboptimal first-line regimen. In these patients, the risk of virological failure and further acquisition of resistance after the first year was more than double compared to patients without pretreatment resistance. The rate of virological failure was 10% of all patients who were still on treatment after 12 months. These findings were largely in agreement with results from a large collaborative analysis in Europe.[28] Our study emphasized the need for at least three fully-active antiretroviral drugs in first-line regimens to ensure a good virological response and to prevent the further acquisition of resistance. Independently of pretreatment resistance, we found that previous use of antiretroviral drugs and prolonged non-adherence below 95% were associated with virological failure and the further acquisition of resistance.

With more people receiving ART in resource-limited settings, treatment failure and the need to switch to second-line regimens is likely to increase. At present, less than 3% of patients are receiving second-line treatment, even though the proportion with virological failure on first-line ART is substantially higher.[1, 29] In **chapter 10**, we studied

the impact of resistance mutations selected for by the first-line regimen on the response to empirically prescribed second-line ART. Among 243 patients who started boosted PI-based regimens, 55% were predicted to receive partially active regimens because they harbored drug resistant virus with reduced susceptibility to at least one prescribed second-line drug. After 12 months, the virological failure rate among those still on treatment was 14% and was not increased in those patients receiving partially active regimens. The development of major PI mutations was very infrequent. Similar to our findings in the cohort on first-line ART, adherence levels below 95% increased the risk of second-line failure. We concluded that empirically prescribed PI-based second-line regimens can successfully re-suppress drug resistant HIV, even in absence of fully-active NRTI backbone. This corroborates the results of a Malawian study among patients with extensive NRTI resistance at the time of switch.[30] These findings bring up the question of whether boosted PI monotherapy could be used as second-line ART. This would have several potential advantages such as lower costs, fewer pills, fewer drug interactions and less NRTI-associated toxicity. Ongoing studies, such as the ACTG 5230 and EARNEST trials, will hopefully be able to answer this question in the coming years.[31]

PART 4: PEDIATRIC STUDIES

Pediatric HIV research and clinical care has always lagged behind progress for adults and has therefore been referred to as a neglected disease.[32, 33] Due to financial and technical constraints, monitoring HIV drug resistance in resource-limited settings is infrequently performed in adults and even less common in children. Most children with HIV infection live in resource-limited settings and unfortunately are at high risk of developing resistance to ART. In a systematic review, we searched the available literature reporting acquired drug resistance after failure of first-line pediatric regimens (**chapter 11**). We retrieved 30 eligible studies reporting outcomes of 3241 children. Viruses with resistance-associated mutations were isolated from 90% of children and most mutations were associated with the NNRTI (88%) and NRTI (80%) drug-classes. PI resistance was less common (54%) and found mostly in South-America and South-Africa where these drugs are used more frequently. A subgroup analysis pointed to higher resistance rates in regions with extensive antiretroviral exposure (i.e., Asia and Latin America).

As part of the MARCH pediatric study, we studied resistance patterns among children failing first-line ART at three clinical sites of the Joint Clinical Research Centre in Uganda (**chapter 12**). A total of 369 children were enrolled in the MARCH study, of which 319 initiated first-line ART regimens and 50 were switched to second-line after first-line ART virological failure. In the latter group, genotypic tests results were obtained for 44

children and 100% of these harbored drug resistant HIV. The observed rate of thymidine analogue mutations (any mutation in 64%, multiple mutations in 46%) was higher than reported by studies included in the systematic review in chapter 11. HIV susceptibility to NRTIs, with the exception of tenofovir, was reduced in ≥60% of children. Tenofovir, however, is only licensed for use in children above 12 years of age and therefore the selection of an active NRTI for second-line ART in young children is practically impossible. The extensive resistance patterns observed in this cohort could be due to clinicians' reluctance to switch to second-line ART, due to regimen costs and complexity. Both the systematic review and the MARCH study in Uganda underscore the fact that second-line drug access must be improved by increasing the availability of new-generation drugs with non-overlapping resistance profiles and tolerable formulations for children. The MARCH study is currently still ongoing in Uganda and will yield important prospective data regarding the efficacy of first- and second-line pediatric regimens.

In **chapter 13** we studied the barriers to initiation of pediatric HIV treatment in Uganda, drawing on both quantitative and qualitative data sources. The median age of children initiating first-line ART in the MARCH cohort was high (4.8 years) and 72% of children were diagnosed with a WHO clinical stage 3 or 4 event. This was surprising in light of the current guidelines which recommend initiation of ART in all HIV-infected children under the age of two, regardless of clinical symptoms or immunodeficiency.[34] We found that the main risk factors for late presentation were loss to follow-up between perinatal care and the pediatric HIV clinic, high transportation costs, caregivers' unawareness of HIV symptoms, fear and stigma. The problem of late presentation therefore requires a multifactorial approach, addressing both health system and individual-level factors. Over half of HIV-infected children without treatment will die before the age of two and therefore improving access to care is lifesaving in this vulnerable young age group.[35] Although HIV drug resistance was not studied in this chapter, those factors influencing health-seeking behavior might also influence retention in care and treatment adherence once ART is initiated. The challenge of optimizing service delivery so that it integrates HIV prevention, diagnosis, retention in care and lifelong treatment adherence is one of the key elements of the WHO Treatment 2.0 initiative.[1, 36] In the context of pediatric HIV care in Uganda, we demonstrated that these factors, along with the prevention of HIV drug resistance, are essential to achieve sustained and universal access.

FUTURE PERSPECTIVES

The aim of this thesis was to assess HIV drug resistance among adults and children in Africa. Data from PASER, MARCH as well as other studies now provide evidence that transmitted drug resistance in Africa is gradually increasing. Although HIV-infected persons thereby risk a suboptimum response to first-line regimens, this should not be an impediment to further scale-up of ART in the region. Universal access is yet to be attained, and the United Nations have expressed their commitment to do so by working toward the provision of ART to 15 million people worldwide by 2015.[37] HIV incidence rates are declining, but the yearly number of persons newly infected with HIV remains higher than the number of persons who start ART.[1] Although outside the scope of this thesis, it is a global priority to improve the prevention of HIV infection and to eliminate mother-to-child transmission. In addition, in order to curb the HIV epidemic, it is imperative that those who are infected with HIV at present have access to life-saving ART. Besides providing benefit to the individual patient, mounting scientific evidence suggests that increased access to ART is also contributing to a decline in the number of people acquiring HIV infection.[38]

HIV drug resistance poses a potential threat to the success of global HIV control and increased access to treatment should be accompanied by measures to protect the sustained effectiveness of ART regimens. First, African health systems must be prepared to provide care to more people, at an earlier stage of HIV infection and for a longer period of time. Procurement and supply management systems must be improved to prevent stock-outs and treatment interruptions, which are linked to the emergence of HIV drug resistance.[39, 40] Besides infrastructure, increased investments in human resources for health, including clinicians, counselors and laboratory staff, are critical. The WHO-defined Early Warning Indicators aim to identify specific health system deficiencies that are associated with HIV drug resistance development. Early Warning Indicator assessment is comparatively inexpensive and should become integrated into routine ART program monitoring and evaluation systems. To facilitate more widespread implementation, it is essential that the process of data abstraction, analysis, and reporting is as simple and straightforward as possible.

A second measure to prevent HIV drug resistance is the introduction of low-cost viral load measurement technologies. There is now compelling evidence from PASER and other studies that viral load testing is an important tool to preserve first-line regimens, guide regimen switching and prevent the accumulation of mutations. A recent model of HIV transmission predicted that resistance transmission in resource-limited settings will be reduced if routine viral load monitoring is introduced.[41] The scaling-up of viral-load

testing for individual patient management in Africa is possible since recent technological advances enable lower test cost, simplified sample storage and shipment using dried blood spots, as well as simpler real-time PCR machines that require less technical expertise.[42, 43] Dried blood spots are particularly suitable for use in children, as small blood volumes are required, which can be obtained from a finger- or heel prick. The Affordable Resistance Test for Africa (ART-A) project, coordinated by PharmAccess Foundation and the Amsterdam Institute for Global Health and Development, has developed a low-cost testing protocols for both viral load and genotypic resistance testing. Future research should focus on the implementation of the point-of-care qualitative viral load test under different field conditions. Furthermore, additional clinical studies should determine the optimal frequency of routine virological monitoring and examine the long-term benefits of early failure detection and timely switching.[44]

Third, in order to assess and respond to emerging HIV drug resistance, population-based resistance surveillance should be routinely integrated into national HIV treatment programs. Surveillance efforts should be directed at adults and children, the latter being an especially vulnerable population. Since observational data, meta-analyses and mathematical models have pointed to increasing levels of transmitted drug resistance, surveying resistance in populations prior to ART initiation should be prioritized. Given resource constraints, it seems most practical to sample chronically infected persons at the time of ART initiation, which will then be able to directly inform current ART guidelines. Although resistance among newly diagnosed persons most likely reflects true transmission of resistant virus, the challenges in identifying recent HIV infections result in labor-intensive and more expensive surveys. Standardized surveillance data from different ART programs and countries should be assembled in collaborative studies in order to increase the representativeness of research findings and have a stronger impact on policy makers. The Global HIV Drug Resistance Network (HIVResNet) coordinated by the WHO, has a leadership role in this respect and has published the first HIV Drug Resistance Global Report in 2012.[45]

As a fourth measure to respond to HIV drug resistance, ART guidelines should be adapted according to the findings of local or regional surveillance efforts. PASER results have demonstrated that pretreatment resistance will lead to higher rates of treatment failure and further resistance acquisition, and first-line ART regimens may have to be altered in specific populations in order to prevent this. Differentiation of therapy is already recommended by the pediatric guidelines: children below the age of two who were previously exposed to nevirapine should receive a boosted PI instead of NNRTI-based first-line regimens.[34] Similarly, PI-based first-line regimens should also be considered in adults who report previous use of (NNRTI-based) ART or PMTCT. Randomized clinical

studies, such as those that have been conducted in children,[46] are necessary to prove the benefits of first-line PI-based therapy in adults with previous antiretroviral exposure. For second-line ART, guideline recommendations may also have to be reconsidered. Studies in this thesis have shown that after failure of NNRTI-based regimens, a switch to second-line might offer the benefit of the boosted PI but only the toxicity of the NRTIs. Boosted PI monotherapy is an attractive option, but long-term efficacy still needs to be assessed.

As a final recommendation, it is essential to expand the available ART armamentarium for Africa. Guideline changes as mentioned above can only be implemented if PIs are made available as generic medicines or pressure is exerted on the pharmaceutical industry to reduce prices. Also, the introduction of viral load monitoring will only be of value if the cost of second-line (and hopefully, eventually, third line) regimens is sufficiently low to allow programs to adopt virologically determined switching. Especially for children, additional drugs that are licensed for use, well-tolerated, and have non-overlapping resistance profiles are urgently needed. Additional research is needed to examine the possible role of new drug classes (e.g. integrase inhibitors) in second and third-line therapy.

In conclusion, lifelong care is required for the effective and safe administration of ART to the millions of HIV-infected Africans who will eventually need it. At the beginning of the 20th century, high-level political commitment, international funding and pressure on pharmaceutical companies resulted in the large-scale introduction of ART in sub-Saharan Africa. At present, sustained investments are required to allow the further scale-up of treatment and optimize service delivery as emphasized by the Treatment 2.0 initiative.[1] The goal of making HIV treatment available to more people is currently under pressure due to declining resources and donors loosing political will. The findings in this thesis raise concern about a suboptimal response to the currently used antiretroviral drugs and call for the expansion of resistance surveillance efforts in Africa. Comprehensive HIV treatment must include strategies to monitor and prevent HIV drug resistance and provide options for the growing number of adults and children for whom standard first-line regimens will no longer be effective.

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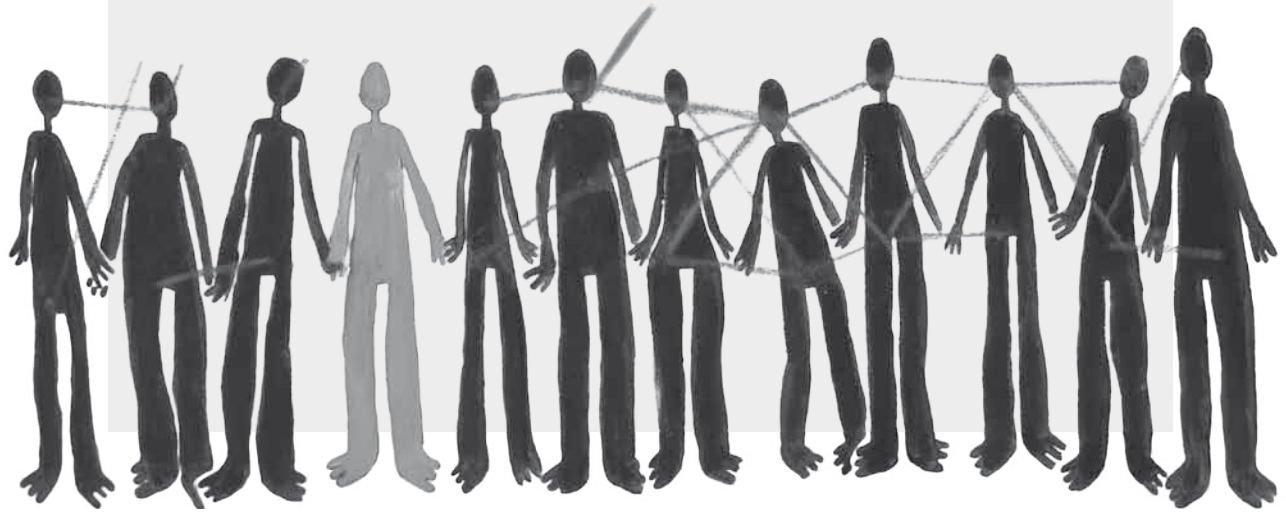
Addendum



SUMMARY

One of the biggest medical advances of the beginning of this century has been the expanded access to treatment for HIV-infected people living in sub-Saharan Africa. This region has been disproportionally hard-hit by the HIV epidemic: 68% of all HIV-infected people (23 million out of the global 34 million) live in Africa. Pressure by activist groups, political commitment and price-negotiations with the pharmaceutical industry have led to the implementation of large-scale treatment programs from 2002 onward, financially supported by international funds such as the 'Global Fund to Fight AIDS, Tuberculosis and Malaria' and the American 'President's Emergency Plan for AIDS Relief'. This has resulted in access to life-saving treatment for approximately 5 million Africans, which is an estimated 50% of those in need of treatment. Keys to this success have been the administration of standard first-line treatment and limited laboratory monitoring. This is called the 'public health approach' and is outlined in guidelines by the World Health Organization.

HIV treatment

HIV, the Human Immunodeficiency Virus, causes AIDS, the Acquired Immune Deficiency Syndrome. HIV infects and destroys specific human blood cells, called CD4 cells, which are crucial to fighting off infections. Without intervention, people with HIV will progress to severe immunodeficiency, AIDS and eventually death, typically within about 10 years. The goal of HIV treatment, also called antiretroviral therapy (ART), is to stop HIV from replicating, allow for recovery of the immune system, and prevent the occurrence of AIDS-related infections. HIV drugs are classified according to where they halt the virus replication cycle. Nucleoside reverse transcription inhibitors (NRTIs) was the first drug class approved for treatment in 1987. However, when treating HIV with just one drug, it soon became apparent that HIV has the ability to change its genetic code by acquiring mutations, thereby enabling the virus to 'escape' from the drug prescribed. This phenomenon is called "HIV drug resistance". In 1996, the drug classes non-nucleoside reverse transcription inhibitors (NNRTIs) and protease inhibitors (PIs) were introduced, resulting in major advances when patients could be treated with potent combinations of three or more drugs. The chance of HIV drug resistance developing in the presence of triple therapy is decreased, but near-perfect patient adherence to medication is required to maintain adequate drug levels in the blood and achieve long-term suppression of the virus. As HIV has a very high replication and mutation rate, there is always a chance of HIV drug resistance development in the long-run.

Antiretroviral resistance in Africa

A potential downside of the large-scale introduction of ART on the African continent is the emergence of HIV drug resistance. This risk is increased by the weak health systems in many African countries, characterized by poor infrastructure, intermittent drug supply, and shortage of skilled staff. As a result, there may not be enough support for patients receiving treatment, and laboratory monitoring is rarely performed. In developed countries, antiretroviral treatment is accompanied by regular measurements of the HIV viral load – which is expected to be suppressed in patients receiving adequate treatment. Before the start of treatment or in the event of suspected treatment failure, an HIV drug resistance test is performed to evaluate the susceptibility of the virus to the drug prescribed. Neither the viral load test nor the HIV drug resistance test is routinely available in Africa, owing to the high costs and technical requirements involved. Furthermore, if therapy fails, alternative treatment (also called second-line treatment) is generally not available or affordable. Therefore, if a patient in Africa does not respond well to the standard first-line therapy or experiences treatment failure, there are very limited means to diagnose the problem or to provide second-line therapy.

Resistance research

The development of resistance in a patient receiving treatment is called "acquired resistance". If the HIV viral load is not suppressed despite treatment (a phenomenon termed "virological failure") the virus can accumulate mutations which work to its benefit. The length of the duration of virological failure is directly related to the development of more complex resistance patterns. The patient is at risk of developing AIDS-related illness again, but may also be able to spread the drug resistant virus to a newly-infected person. The latter is call "transmitted resistance". In order to evaluate the extent of HIV drug resistance in Africa, PharmAccess African Studies to Evaluate Resistance (PASER) was established as a multi-national network in 2006. PASER is coordinated by the PharmAccess Foundation and the Amsterdam Institute for Global Health and Development (AIGHD). It is part of a larger Dutch initiative, called 'Linking African and Asian societies for an enhanced response to HIV/AIDS', the LAASER program. The aim of LAASER is to strengthen the capacity to measure HIV drug resistance within a network of clinical and laboratory sites in Africa and Asia. The collaborating clinical sites conduct observational patient studies and the laboratories have developed quality-assured genotypic HIV drug resistance testing. The health professionals, researchers and laboratory staff involved have received specialized training in HIV treatment and in the implications of HIV drug resistance.

In six African countries - Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe - thirteen clinics participated in a prospective study including approximately 3,000 adults receiving ART who were followed up for at least two years. In this group, the extent of

HIV drug resistance before and after the start of treatment was measured. Additionally, two studies have been conducted among newly-diagnosed HIV-infected persons who had never been exposed to antiretroviral drugs, to evaluate transmitted drug resistance. A spin-off project, the 'Monitoring Antiretroviral Resistance in Children' (MARCH) study was initiated in Uganda and focuses on HIV drug resistance among children, a group for which access to treatment has lagged behind and for which data on drug resistance development is scarce. The studies included in this thesis were conducted as part of the PASER and MARCH programs.

Part 1: Epidemiology of transmitted HIV drug resistance

After the introduction (**chapter 1**), part 1 describes the epidemiology of transmitted HIV drug resistance among newly-diagnosed persons in Kampala, Uganda and Mombasa, Kenya. In Kampala, a study carried out among 70 recently-infected individuals revealed a drug resistance mutation in 9% (**chapter 2**). An identical study in Mombasa found a prevalence of 13% (**chapter 3**). As previous research had shown that transmitted drug resistance was below 5% in African populations, our studies suggest a rise in resistance among the urban populations of Kampala and Mombasa. It is likely that the extent of resistance is related to the length of time since the large-scale introduction of HIV treatment. In **chapter 4**, we used a mathematical model to predict trends in transmitted resistance over the next decade, during which access to ART is expected to be further scaled up. Depending on the number of people for whom ART will be available, transmitted resistance is expected to rise to a level between 12-19% in East Africa. As ART was available in East Africa ahead of other African countries, the findings of increasing HIV drug resistance in this region provide an important warning to the rest of the continent.

Part 2: Acquired HIV drug resistance in persons failing first-line antiretroviral therapy

Part 2 is an examination of patients who fail first-line therapy and become eligible for second-line therapy. In settings in which doctors do not have access to an HIV viral load test, they rely on clinical and/or immunological criteria to diagnose treatment failure. If AIDS-related clinical symptoms are present although the HIV viral load is suppressed, a doctor may inappropriately decide to switch to second-line therapy. In **chapter 5**, we point out that this is a frequent problem, occurring in almost 50% of patients who did not have a viral load test prior to switching to second-line therapy. This unnecessary switch reduces therapeutic options for the patient and increases overall costs. It is, therefore, likely that performing a viral load test in this situation is cost-effective. In the same study, we analyzed resistance mutations among 183 patients in whom the viral load was confirmed to be high. Due to the presence of extensive resistance, the virus had often lost susceptibility to two medication classes, the NRTIs and NNRTIs. This

means that second-line therapy will consist of the third class, the protease inhibitor, as the only active agent. **Chapter 6** is an investigation in more detail of HIV mutations among 142 patients in whom a routine viral load test was conducted after the first 12 months of therapy. The specific mutational patterns were less complex and consequently, susceptibility to the NRTI drug class was better preserved compared to patients in chapter 5. This difference can be explained by the fact that for patients in chapter 6 virological failure was detected "early" during a routine measurement whereas patients in chapter 5 all had AIDS-related clinical symptoms, indicative of prolonged virological failure. The findings from these two studies underscore the fact that routine viral load testing among patients receiving ART can prevent the accumulation of mutations. The speed at which this accumulation can occur was studied in **chapter 7.** Among a small cohort comprising 43 South African patients, repeated resistance testing was performed in patients with a persistently elevated viral load while during treatment. Viruses were found to accumulate mutation to NRTI drugs at an average rate 1 mutation in six-and-a-half months. Future studies should determine the optimal frequency of routine viral load monitoring to prevent HIV drug resistance and ensure the patients remain free of AIDS-related diseases.

Part 3: Outcomes of first- and second-line antiretroviral therapy

In part 3 we investigate the effectiveness of ART in preventing HIV drug resistance or suppressing HIV once resistance has emerged. In response to the rapid scaling up of ART in resource-limited settings, the World Health Organization has formulated Early Warning Indicators, which assess ART site characteristics, such as prescribing practices, patient attrition rates and drug supply continuity, which may be associated with the emergence of HIV drug resistance. Within the PASER network, we evaluated the Early Warning Indicators and identified vulnerable aspects of care (**chapter 8**). Of great concern is the fact that nine out of thirteen sites failed to meet the targeted goal for at least one indicator. In **chapter 9** we examined the response to ART in patients with preexisting HIV drug resistance. In the PASER study, nearly 2600 people started first-line ART, of whom 5% harbored drug resistance to at least one prescribed drug. In these patients, the risk of virological therapy failure and further acquisition of HIV drug resistance after the first year was more than double compared to patients without resistance. This study confirmed the importance of suppressing HIV with three active agents (two NRTIs and one NNRTI) in the first-line. The situation is very different when starting second-line ART after first-line failure. Guidelines recommend that for second-line ART, a protease inhibitor is combined with two NRTIs. In **chapter 10**, we studied the response to second-line ART in 243 patients who harbored substantial NRTI resistance. We found that the protease inhibitor drug class was successful in re-suppressing drug resistant HIV, even though NRTI activity was diminished. These findings bring up the question of whether

the protease inhibitor could be used as a mono-therapy, with the advantage of lower costs, fewer pills, and less NRTI-associated toxicity. Ongoing studies will hopefully be able to answer this question in the coming years.

Part 4: Pediatric studies

Part 4 focuses on pediatric studies. A review of the literature (**chapter 11**) leads to the conclusion that in children with HIV treatment failure complex resistance patterns are present. In Africa, this mainly concerns resistance to the NRTI and NNRTI medication classes. As part of the MARCH pediatric study conducted in Uganda, HIV drug resistance was studied in 50 children failing first-line ART (**chapter 12**). All children harbored drug resistant viruses, and the effectiveness of NRTIs was severely reduced. This is of particular concern in children, for whom the number of licensed HIV drugs is limited. Both the systematic review and the MARCH study in Uganda underscore the fact that second-line drug access must be improved by reducing costs and increasing the availability of new drugs for children. In **chapter 13**, we concentrate on the question of why children enter into care at relatively advanced age and with advanced disease symptoms. Mortality among HIV-infected infants is very high and therefore early diagnosis and immediate treatment are essential. The main risk factors for late entry into care were inconsistent referral from prenatal to pediatric care, high transportation costs to the clinic, caregivers' unawareness of HIV symptoms, fear and stigma. Although HIV drug resistance was not studied in this chapter, those factors influencing the motivation of people to seek health care might also influence the patient's retention in care and treatment adherence once ART is initiated.

Chapter 14 consists of a general discussion and underscores the notion that the prevention of HIV drug resistance requires long-term commitment. In the fight against the global HIV epidemic, prevention of new infections among adults and the elimination of mother-to-child transmission should be the foremost priorities. It is imperative, in addition, that those who are presently infected with HIV have access to life-saving ART. Studies included in this thesis have provided evidence for a gradual rise in HIV drug resistance in sub-Saharan Africa. As drug resistance jeopardizes the effectiveness of ART, we strongly advocate five measures to protect the standard first-line regimens for adults and children. First, African health systems must be strengthened to meet increasing demands. For example, drug supply systems must be improved to prevent treatment interruptions for patients. Second, the introduction of low-cost viral load testing should be implemented for routine monitoring for patients on treatment. This is possible, since recent technological advances have enabled lower test cost and have simplified the storage and shipment of samples. Third, surveillance of resistance should be integrated into national HIV treatment programs. As individual resistance testing is not feasible, studies should focus on representative populations prior to ART initiation. Results from

such surveillance among adults and children will be able to directly inform current ART guidelines. Fourth, ART guidelines should be adapted to the findings of local or regional surveillance efforts. In certain populations or in regions with high drug resistance, prescribing the standard first-line regimen may no longer be useful; instead, the protease inhibitor, usually reserved for second-line treatment, may have to be used immediately. Fifth, these guideline changes are only possible if protease inhibitors, as well as other second-line medicines, are available at cheaper prices. The local production of generic medicines should be strengthened and great pressure should be exerted on the pharmaceutical industry in order to secure price reductions.

In conclusion, lifelong care and effective treatment is required for the millions of HIV-infected Africans who will eventually need it. The goal of making HIV treatment available to more people is currently under pressure due to the decline of donors and resources, and to the loss of political will. Sustained investments are required to allow the scaling up of comprehensive HIV treatment, including strategies to monitor, prevent and treat drug resistant HIV.

NEDERLANDSE SAMENVATTING

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Eén van de grootste medische succesverhalen van het begin van deze eeuw is het massaal beschikbaar maken van antiretrovirale medicijnen voor de bestrijding van HIV in sub-Sahara Afrika. Afrika ten zuiden van de Sahara is de regio die het hardst wordt getroffen door de wereldwijde HIV epidemie; 68% van alle HIV-geïnfecteerden in de wereld (23 van in totaal 34 miljoen mensen) leeft in dat gebied. Onder grote druk van verschillende actiegroepen en na onderhandelingen met de farmaceutische industrie werden er vanaf 2002 in Afrika grootschalige behandelprogramma's gestart met financiering van internationale fondsen, zoals het 'Global Fund to Fight AIDS, Tuberculosis and Malaria' en het 'President's Emergency Plan for AIDS Relief'. Naar schatting hebben 5 miljoen Afrikanen nu toegang tot levensreddende HIV medicatie. Dit is circa de helft van alle mensen die de behandeling nodig zouden hebben. Eén van de sleutels tot dit succes is het feit dat behandelprogramma's - op advies van de Wereldgezondheidsorganisatie (WHO) - goedkope, standaard eerstelijns medicatie voorschrijven en minimale laboratorium tests gebruiken.

HIV behandeling

HIV (het Humaan Immunodeficiëntie Virus) veroorzaakt AIDS (het 'Acquired Immune Deficiency Syndrome'). Virus deeltjes infecteren een belangrijke groep bloedcellen - de CD4 cellen - die de menselijke immuniteit regelen. Hierdoor wordt een HIV-positief persoon vatbaar voor allerlei infecties en aandoeningen die op den duur - gemiddeld binnen 10 jaar na infectie - tot AIDS zullen leiden. Het doel van de HIV behandeling is het onderdrukken van het virus (de HIV 'viral load') waardoor het immuunsysteem de kans krijgt om te herstellen en er geen AIDS-gerelateerde aandoeningen meer kunnen optreden. HIV medicatie, ofwel antiretrovirale medicatie, remt op verschillende niveaus de vermenigvuldiging van het virus en wordt op basis hiervan ingedeeld in klassen. De eerste klasse, de nucleoside reverse transcription inhibitors (NRTIs), is al beschikbaar sinds 1987. Echter, bij de bestrijding van HIV met slechts één middel werd al snel duidelijk dat er mutaties in het virus ontstonden die de gevoeligheid voor het middel verminderden. Dit fenomeen wordt HIV resistentie of antiretrovirale resistentie genoemd. In 1996 werden de klassen van non-nucleoside reverse transcription inhibitors (NNRTIs) en protease remmers aan het arsenaal van middelen toegevoegd. Dit resulteerde in een grote doorbraak: patiënten konden worden behandeld met een sterke combinatie van minstens drie middelen waarmee het virus langdurig onderdrukt kon worden. De kans op het ontstaan van HIV resistentie is bij trouw gebruik van triple-therapie sterk

verminderd, maar niet volledig uitgebannen. HIV is een snel delend en snel muterend virus en op de lange termijn blijft het risico op resistentie ontwikkeling bestaan.

Antiretrovirale resistentie in Afrika

Een potentiële keerzijde van het grote succes van HIV behandeling in Sub-Sahara Afrika is de opmars van antiretrovirale resistentie. Het gebrekkige gezondheidszorgsysteem in veel Afrikaanse landen verhoogt dat risico, onder andere vanwege onbetrouwbare medicijnvoorraden en het gebrek aan medisch personeel en ondersteunende diensten, zoals laboratoria. Door deze beperkte laboratorium infrastructuur is het vaak niet mogelijk is om de HIV viral load in het bloed van de patiënt te bepalen. Dit is in tegenstelling tot de situatie in Europa, waar regelmatig gecontroleerd wordt of de viral load onderdrukt blijft. In Westerse landen wordt ook gebruik gemaakt van een HIV resistentie test voor aanvang van behandeling of bij verdenking van therapiefalen, om de gevoeligheid van het virus voor verschillende medicijnen te bepalen. Door de hoge kosten en technische vereisten van deze laboratoriumtests, worden de viral load en HIV resistentie testen niet standaard toegepast in Afrika. Bovendien zijn er, in geval van toxiciteit of therapiefalen, weinig alternatieve medicatieopties (ofwel tweedelijns therapie) beschikbaar.

Resistentieonderzoek

In het geval van virologisch therapiefalen, waarbij het virus blijft delen terwijl de patiënt de medicijnen (al dan niet volledig) heeft ingenomen, kunnen HIV mutaties accumuleren en zal het virus steeds resistenter worden. Dit wordt verworven resistentie genoemd. Behalve het feit dat een patiënt met HIV resistentie minder goed reageert op (eerstelijns) therapie en zo opnieuw AIDS kan krijgen, kan het resistente virus ook overgedragen worden op nieuw-geïnfecteerden. Dit laatste wordt overgedragen resistentie genoemd. Om zicht te krijgen op de mate van HIV resistentie in Afrika, werd in 2006 een grootschalig resistentieonderzoek gestart met de naam PASER, afkorting voor 'PharmAccess African Studies to Evaluate Resistance'. PASER wordt gecoördineerd door de stichting PharmAccess en het Amsterdam Institute for Global Health and Development (AIGHD). Het maakt deel uit van een Nederlands initiatief, het 'Linking African and Asian Societies for an Enhanced Response to HIV/AIDS' ofwel het LAASER programma. De doelstelling van LAASER is om door de opbouw van een netwerk van klinieken en laboratoria bij te dragen aan de ontwikkeling van een kennis- en capaciteitsinfrastructuur op het gebied van HIV resistentie in Afrika en Azië. In de aangesloten klinische centra wordt observationeel onderzoek verricht en de laboratoria richten zich op kwaliteitsgecontroleerde resistentiediagnostiek. Betrokken medici, verpleegkundigen en laboranten krijgen onderwijs over HIV behandeling, resistentiepreventie en de klinische implicaties van resistentie.

Als onderdeel van PASER werden in 13 klinieken in 6 Afrikaanse landen - Zambia, Zuid-Afrika, Oeganda, Kenia, Zimbabwe en Nigeria - circa 3,000 patiënten voor 2 jaar gevolgd na het starten van antiretrovirale behandeling. In deze studie is resistentie voor aanvang en tijdens HIV behandeling gemeten. Tevens werden er 2 studies verricht onder nieuw-geïnfecteerden die nog nooit in aanraking met HIV medicatie waren gekomen om de mate van overgedragen resistentie te bepalen. Naast volwassenen, richtte het onderzoek zich ook op kinderen, een groep patiënten voor wie de HIV behandeling in Afrika veel langzamer op gang is gekomen dan voor volwassenen. De MARCH studie (Monitoring Antiretroviral Resistance in Children) vond plaats in Uganda. De onderzoeken die beschreven worden in dit proefschrift zijn uitgevoerd als deel van de PASER en MARCH studies.

Deel 1: Epidemiologie van overgedragen HIV resistentie

Na de introductie (**hoofdstuk 1**) wordt in deel 1 de epidemiologie van overgedragen HIV resistentie in Kampala, Oeganda en Mombasa, Kenia beschreven. Als onderdeel van PASER zijn in 2009-2010 twee studies verricht onder circa 70 nieuw-geïnfecteerden in deze twee steden (**hoofdstuk 2 en 3**). Terwijl uit eerdere soortgelijke studies gebleken was dat overgedragen resistentie in Afrika minder dan 5% was, laten deze twee onderzoeken zien dat dit percentage is gestegen tot respectievelijk 9% en 13% onder de stedelijke bevolking van Oeganda en Kenia. Deze stijging heeft plaatsgevonden sinds de uitbreiding van de nationale behandelprogramma's in 2000-2003. In **hoofdstuk 4**, hebben we met behulp van een wiskundig model een voorspelling gedaan over hoe de overgedragen resistentie in deze twee steden zich in de toekomst zou kunnen ontwikkelen. Afhankelijk van de hoeveelheid mensen die toegang zullen hebben tot behandeling, zal de prevalentie van overgedragen resistentie naar schatting stijgen tot 12-19% in Oost Afrika. Aangezien de introductie van HIV medicatie in Oost Afrika relatief vroeg heeft plaats gevonden, is de situatie in deze regio een boodschap en waarschuwing voor de toekomst van andere Afrikaanse landen.

Deel 2: Verworven resistentie onder patiënten met therapiefalen

Deel 2 beschrijft de aandachtspunten bij het vervangen van eerstelijns therapie door tweedelijns therapie. In **hoofdstuk 5** blijkt dat artsen, wanneer ze geen HIV viral load kunnen meten, vaker patiënten onterecht tweedelijns therapie voorschrijven dan wanneer ze wel over deze test beschikken. In onze studie had 50% van de patiënten klinische symptomen passend bij therapiefalen, terwijl er geen verhoogde viral load aanwezig was. In deze groep was het mogelijk geweest om patiënten met eerstelijns medicatie te blijven behandelen. Het onnodig wisselen van medicatie brengt extra kosten met zich mee en waarschijnlijk is het dus kostenbesparend om wel een viral load test te doen alvorens tweedelijns therapie te starten. In hoofdstuk 5 zijn ook resistentiepatronen

bestudeerd onder 183 patiënten met virologisch falen en terechte medicatie wissel. De twee eerstelijns medicatie klassen (de NRTIs en NNRTIs) waren grotendeels of geheel uitgeschakeld. Dit betekent dat van de tweedelijns therapie alleen nog de effectiviteit van de derde klasse (de protease remmer) te verwachten is. In **hoofdstuk 6** wordt nader ingegaan op de HIV mutatie patronen van 142 patiënten die na 12 maanden eerstelijns therapie een standaard viral load test kregen. Het blijkt dat als HIV resistentie wordt ontdekt tijdens een jaarlijkse routine controle, er minder complexe mutaties optreden dan wanneer er wordt gewacht tot de patiënt klinische symptomen van therapiefalen vertoont zoals in hoofdstuk 5. Doordat bij de onderzochte groep in hoofdstuk 6 therapiefalen "vroeg" gedetecteerd werd, vond er minder accumulatie van HIV mutaties plaats en bleef de gevoeligheid voor tweedelijns medicatie beter bewaard. De snelheid waarmee resistentie accumulatie plaatsvindt, is onderzocht in **hoofdstuk 7**. In een relatief kleine studie onder 43 patiënten met virologisch falen zijn herhaaldelijke resistentie tests gedaan. Patiënten die bij de laatste test één of meer HIV mutaties hadden voor de NRTI medicijn klasse, hadden deze mutaties in 6.5 maanden verworven. Toekomstige studies zullen moeten uitwijzen met welke frequentie de viral load het beste gemeten kan worden om HIV resistentie accumulatie te voorkomen en klinische uitkomsten te verbeteren.

Deel 3: Uitkomsten van eerste- en tweedelijns therapie

In deel 3 is onderzocht hoe effectief antiretrovirale behandeling is, enerzijds om HIV resistentie te voorkomen en anderzijds om al bestaande HIV resistentie te behandelen. De zogeheten 'Early Warning Indicators', opgesteld door de WHO, zijn bedoeld om HIV resistentie ontwikkeling te voorkomen dan wel vroegtijdig op te sporen **(hoofdstuk 8).** Deze indicatoren worden gebruikt om eigenschappen van HIV klinieken in kaart te brengen, zoals de medicijnvoorraad en de regelmaat waarmee patiënten op afspraken komen. Binnen het PASER netwerk zijn de Early Warning Indicators gemeten en daarmee zijn de kwetsbare onderdelen van de zorg aangetoond. Negen van de dertien klinieken voldeed niet aan de doelstelling voor tenminste één van de indicatoren. In **hoofdstuk 9** is de response op eerstelijns HIV behandeling onderzocht in patiënten met al bestaande of wel pre-existente HIV resistentie. Binnen de PASER studie zijn circa 2600 personsen met eerstelijns medicatie gestart, waaronder 5% met pre-existente resistentie. In deze groep was het risico op therapiefalen en vervolgens verdere resistentieontwikkeling tweemaal zo hoog in vergelijking tot patiënten zonder pre-existente resistentie. Deze bevindingen bevestigen dat het van essentieel belang is om HIV met drie actieve middelen (2 NRTIs en 1 NNRTI) te onderdrukken. **Hoofdstuk 10** laat zien dat de situatie anders is bij het wisselen naar tweedelijns therapie nadat de eerstelijns therapie gefaald heeft. In dit hoofdstuk hebben we aangetoond dat bij 243 patiënten met uitgebreide NRTI resistentie, de protease remmer in het tweedelijns regime in staat is om het virus

opnieuw te onderdrukken). Het is de vraag of de protease remmer ook als monotherapie ingezet zou kunnen worden bij dergelijke patiënten, met als voordeel minder pillen en bijwerkingen en lagere kosten. In de komende jaren zal het antwoord op deze vraag hopelijk gegeven kunnen worden door klinische studies die op dit moment uitgevoerd worden.

Deel 4: Kinderstudies

In deel 4 worden de resultaten van studies onder kinderen gerapporteerd. HIV behandeling bij (jonge) kinderen is complex en een onderbelicht onderzoeksgebied. Een literatuur studie laat zien dat bij kinderen met therapiefalen, uitgebreide resistentie patronen aanwezig zijn (**hoofdstuk 11**). In Afrika betreft dit voornamelijk resistentie voor de NRTI en NNRTI medicatieklassen. In het kader van de MARCH studie in Oeganda, zijn de resistentie patronen in 50 kinderen met eerstelijns therapiefalen beschreven (**hoofdstuk 12**). Bij alle kinderen was er sprake van HIV resistentie, waardoor de gevoeligheid voor de NRTI medicijn klasse ernstig verminderd was. Aangezien er maar een beperkt aantal middelen is geregistreerd voor kinderen, is het heel lastig om actieve medicijnen te vinden voor tweedelijns therapie. De resultaten van zowel het literatuur onderzoek en de MARCH studie maken duidelijk dat nieuwe medicijnen, zonder kruisresistentie, voor kinderen dringend nodig zijn. In **hoofdstuk 13** hebben we onderzocht waarom kinderen in een laat stadium, dat wil zeggen op latere leeftijd en met vergevorderde ziekteverschijnselen, naar de kliniek kwamen. De sterfte onder HIV-geïnfecteerden kinderen is hoog en het is dan ook essentieel dat diagnose en behandeling op jonge leeftijd plaatsvinden. De belangrijkste factoren die voor vertraging zorgen zijn een slechte doorverwijzing van prenatale zorg naar gespecialiseerde kinderklinieken, hoge transportkosten en angst en onwetendheid van de verzorger van het kind. In hoofdstuk 13 is geen HIV resistentie gemeten, maar we kunnen wel aannemen dat de factoren die belemmerend werken bij de toegang tot zorg ook invloed hebben op therapietrouw na het starten van behandeling.

Hoofdstuk 14, de discussie, onderstreept het feit dat aandacht voor HIV resistentie een lange termijn visie vergt. In de wereldwijde strijd tegen HIV hebben de preventie van nieuwe infecties onder volwassenen en het elimineren van de transmissie van moeder naar kind de grootste prioriteit. Daarnaast is levensreddende behandeling nodig voor degenen die al HIV-geïnfecteerd zijn. Studies in dit proefschrift tonen aan dat er toename is van HIV resistentie in sub-Sahara Afrika. Gezien het feit dat deze toename een negatieve invloed heeft op de standaard eerstelijns medicatie, doen we een vijftal aanbevelingen om de effectiviteit van HIV behandeling te waarborgen voor volwassenen en kinderen. Ten eerste moeten Afrikaanse gezondheidszorg systemen verbeterd worden om aan een toenemend aantal mensen zorg te kunnen leveren. De 'Early Warning

Indicators' kunnen gebruikt worden om er voor te zorgen dat de voorzieningen van een HIV-kliniek, zoals de medicijnvoorraad en patiënten administratie, aan minimum eisen voldoen. Ten tweede zou een goedkope viral load test geïntroduceerd kunnen worden voor het regelmatig controleren van patiënten tijdens de behandeling. Gezien recente technologische ontwikkelingen is het mogelijk geworden om een bloedmonster op kamertemperatuur te bewaren en te verzenden. Op deze manier kunnen de testkosten verminderd worden. Ten derde zou HIV resistentie onderzoek geïntegreerd moeten worden in de nationale HIV zorg. Het bepalen van HIV resistentie voor individuele patiënten is op dit moment nog te kostbaar en daarom zouden deze onderzoeken gedaan moeten worden onder representatieve populaties voor aanvang van de HIV behandeling. Een vierde manier om te reageren op HIV resistentie is het aanpassen van de behandelrichtlijnen op basis van de uitkomst van lokale of regionale HIV resistentie studies. Zo kan het nodig zijn om in bepaalde populaties of regio's met hoge HIV resistentie, de eerstelijns medicatie te veranderen. Tenslotte is het van groot belang dat de beschikbaarheid van protease remmers en andere tweedelijns medicatie toeneemt. De lokale productie van generieke medicijnen zal moeten worden versterkt en er zal opnieuw met de farmaceutische industrie moeten worden onderhandeld om forse prijs reducties te realiseren.

HIV medicatie is nog steeds niet voor iedereen beschikbaar in Afrika. Duurzame investeringen zullen nodig zijn om een toenemend aantal mensen te blijven behandelen in de komende jaren. Om de effectiviteit op de lange duur te waarborgen is het nodig om de kwaliteit van behandelprogramma's in Afrika te verbeteren en strategieën te ontwikkelen om HIV resistentie te meten, te voorkomen en te behandelen.

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CURRICULUM VITAE

Kim Sigaloff completed secondary school in 1997 at the Barlaeus Gymnasium in Amsterdam, graduating cum laude. After spending a year at the College of Wooster in the United States, she studied medicine at the University of Amsterdam and obtained her medical degree in 2005. During her studies she was actively involved in the International Federation of Medical Students' Associations (IFMSA) and as an international board member coordinated training workshops and student exchanges. For a research internship, she spent four months in Thailand with the Oxford Tropical Medicine Research Programme. In 2003, she did a clinical internship at the University Hospital in Maputo, Mozambique. At the time, antiretroviral treatment was not yet publicly available in Mozambique, and witnessing the hopeless situation of HIV-infected patients admitted to the Internal Medicine department made a deep impression.

After graduation, Kim worked in the Onze Lieve Vrouwe Gasthuis (OLVG) hospital in Amsterdam at the department of Internal Medicine. In 2007 she spent four months as a research assistant at the Department of Global Health and Social Medicine at Harvard Medical School in Boston. Thereafter she started her residency in internal medicine at the Westfries Gasthuis (WFG) hospital in Hoorn. She joined PharmAccess in 2009 as a project coordinator for the PharmAccess African Studies to Evaluate Resistance (PASER). During her three years at PharmAccess and the Amsterdam Institute for Global Health and Development (AIGHD) she supervised the follow-up of participants in the PASER cohort, developed sub-studies and completed her PhD work. Currently, Kim has resumed her residency at the Academic Medical Center (AMC) in Amsterdam and will continue with a clinical fellowship program in infectious diseases at the Leiden University Medical Center (LUMC).

Kim lives together with Diederik van Wijk and their 2-year old daughter Lisa Alex in Amsterdam.