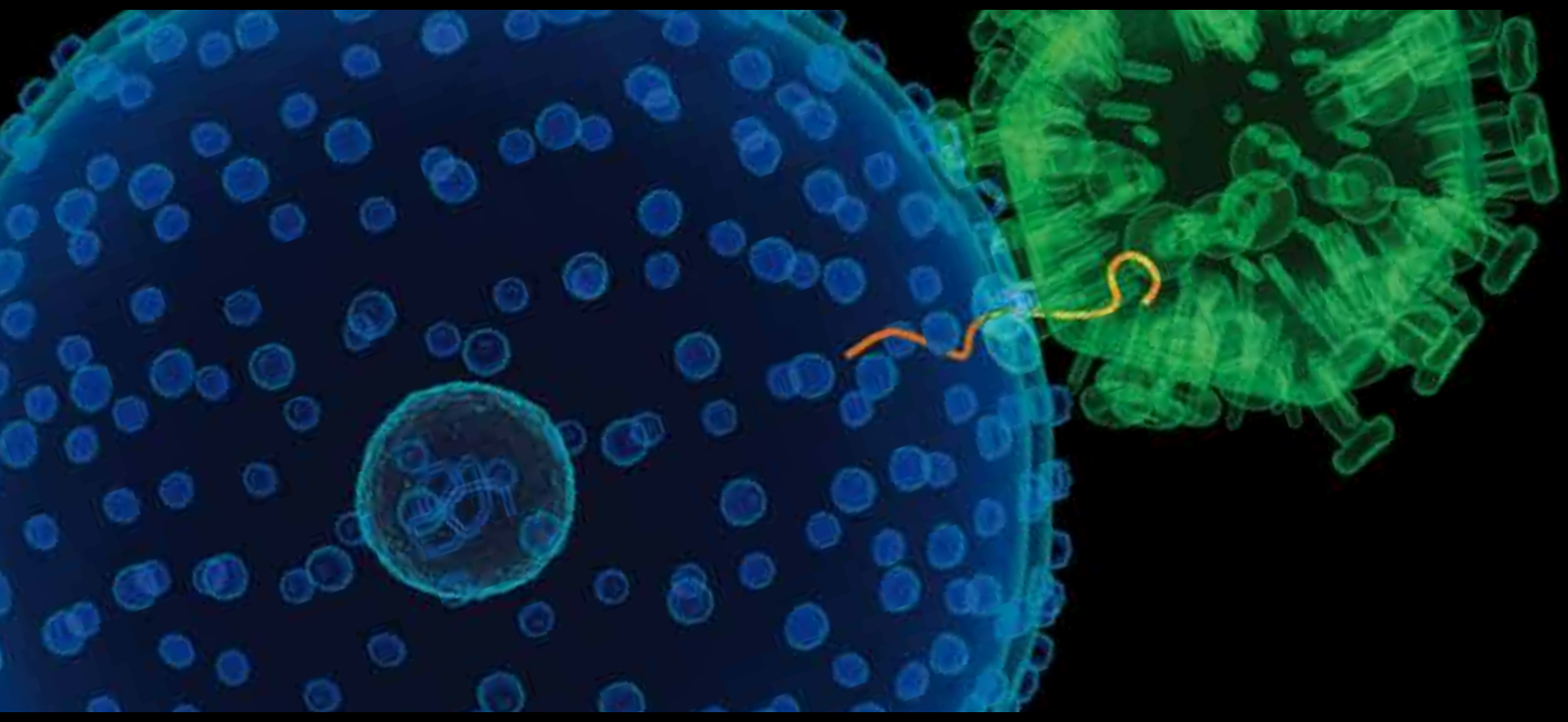
AIDS Research and Treatment

Antiretroviral Treatment in Resource-Limited Settings

Guest Editors: Ann Duerr, Esper Kallas, Joep Lange, and Robin Wood



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Editorial Board

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Research Article

Virological Breakthrough: A Risk Factor for Loss to Followup in a Large Community-Based Cohort on Antiretroviral Therapy

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Background. We have previously shown that 75% of individuals on antiretroviral therapy (ART) in a resource-limited setting who experienced virological breakthrough to >1000 copies/mL were resuppressed after an intensive adherence intervention. This study examines the long-term outcomes of this group in order to understand the impact of the adherence intervention over time. *Methods*. ART-naïve adults commencing ART between September 2002 and December 2009 were reviewed. Those who achieved suppression (<50 copies/mL) were categorised by subsequent viral load: any >1000 copies/mL (virological breakthrough) or not. Those with breakthrough were sub-categorised by following viral load into failed (VL > 1000 copies/mL) or resuppressed (VL < 1000 copies/mL). Their outcome (lost-to follow-up, death, in care on first-line therapy or in care on second-line therapy) was determined as of the 13th April 2010. *Findings*. 4047 ART-naïve adults commenced ART. 3086 had >2 viral loads and were included in the analysis. 2959 achieved virological suppression (96%). Thereafter 2109 (71%) remained suppressed and 850 (29%) experienced breakthrough ($n = 283$ (33%) failed and $n = 567$ (67%) resuppressed). Individuals with breakthrough were younger ($P < .001$), had lower CD4 counts ($P < .001$), and higher viral loads ($P < .001$) than those who remained suppressed. By 7 years the risk of breakthrough was 42% and of failure 15%. Fewer adults with breakthrough remain in care over time ($P < .001$). Loss to care is similar whether the individuals failed or resuppressed. *Interpretation*. While 67% of those who experience initial virological breakthrough resuppress after an adherence intervention, these individuals are significantly less likely be retained in care than those who remain virologically suppressed throughout.

1. Introduction

Adherence to antiretroviral therapy is the key to successful treatment outcomes at both individual and programmatic levels. It has been shown that individuals taking 95% or more of their medication will maintain virological suppression and that this is readily achieved in resource-limited settings [1–3]. More recent data from larger nonnucleoside-based ART regimens show that these regimens allow for lower levels of adherence after initial viral suppression, perhaps >80%, before virological breakthrough occurs [4, 5].

Poor ART adherence can be assessed by the use of a selection of objective or subjective adherence measures. These include 3-day recall, a visual analogue scale [6], and a count of tablet returns or pharmacy refills [7]. Many ART programmes in resource-limited settings have had the opportunity to implement adherence-focused systems from the outset and have shown exceptionally high proportions of individuals with virological suppression at any visit [8–10].

If poor adherence is persistent or is not detected, virological breakthrough may occur. At this point, intensive adherence support is required to initiate a change in tablet-taking behaviour which can result in resuppression of the HIV before resistance develops. In 2007, this group showed that 75% of individuals with virological breakthrough to >1000 copies/mL resuppressed to <50 copies/mL at the subsequent viral load after an intensive adherence

intervention [10]. The impact of this increase in viral load on the longer term course of ART is unknown. There is a possibility that the period of poor adherence with subsequent inadequate ART drug levels may have allowed the HIV to generate resistance mutations resulting in earlier failure than observed in those with no episodes of virological breakthrough.

In the previously published study, numbers of naive adults on treatment were limited ($n = 929$) and outcomes could only be determined to 32 months.

The objective of this study was to examine the long term programmatic outcomes of individuals who had previously experienced virological breakthrough to >1000 copies/mL and then resuppressed. We aimed to determine whether the impact of the intervention was longstanding or whether it resulted in differing outcomes including increased rates of virological failure and loss to followup/retention in care, compared to those with continued virological suppression.

2. Methods

2.1. Site Description. Antiretroviral delivery at the Hannan Crusaid Treatment Centre (HCTC) has been described previously [10]. The HCTC is one of the Department of Health ART sites in the Nyanga district, a township near Cape Town, South Africa. An estimated 300,000 people live in this district, which had an antenatal HIV-1 seroprevalence rate of 27.9% in 2008 [11]. The clinic opened in September 2002 and by December 2009 cared for 2991 adults and 270 children needing ART. Antiretroviral therapy and associated monitoring costs are provided free of charge in South Africa. HIV RNA load and CD4 cell count assessments were completed pretreatment and every 16 weeks while taking ART. Viral load assays were done using the branch DNA hybridisation technique (Bayer HIV-1 RNA 3.0 assay (branch DNA)). Additional safety tests were performed according to the 2004 edition of the national ART protocol and varied according to the treatment regimen [12].

2.2. Drug Regimens. Until the 2010 edition of the South African National Antiretroviral guidelines, first-line ART included stavudine (d4T), lamivudine (3TC), and either efavirenz or nevirapine. Zidovudine (AZT) or tenofovir could be substituted for d4T-related toxicity. ART for second-line therapy included AZT, Didanosine (ddI), and lopinavir/ritonavir [12, 13].

2.3. Adherence Support. The HCTC employed HIV-positive lay counselors to educate each individual prior to initiating ART and to provide on-treatment adherence support. Treatment preparedness included three small group education sessions and a home visit by a counselor assigned to the area where the individual lived. Further home visits occurred monthly after starting treatment and continued until viral suppression was achieved. Thereafter, home visits were only carried out if the individual was flagged as nonadherent, experienced virological breakthrough, or was identified as a defaulter through missed clinic visits.

2.4. Adherence Interventions. Patients on ART with a viral load >1000 copies/mL (virological breakthrough) at any followup visit received a targeted adherence intervention. These individuals were required to attend extracounselling sessions, which focussed on adherence issues, and weekly home visits were recommenced. The individual was issued with a pill box and a dosing diary. The viral load was repeated six to eight weeks after this intervention. Based on the second test, patients were either regarded as having resuppressed or having failed treatment. Patients with a second viral load >1000 copies/mL were prepared for second-line therapy while resuppression to a viral load <1000 copies/mL allowed for continuation on first-line therapy, with high-frequency home visits continuing. If the viral load had fallen to <50 copies/mL routine clinic care was recommenced.

2.5. Study Design and Analysis. Data were accessed from clinical (age, gender, WHO stage) and laboratory records (CD4 counts and viral load) that were maintained on all patients included in the HCTC ART programme. These records were transferred on a weekly basis to an off-site database. Outcomes which included death, loss to followup, or transfer out were noted in the database and the date of the outcome was captured.

2.6. Definitions. "Virological breakthrough" refers to any individual whose viral load reached >1000 copies/mL after previous suppression to <50 copies/mL. "Resuppressed" refers to those individuals whose viral load subsequent to the one at virological breakthrough was <1000 copies/mL. "Virological failure" refers to individuals with two consecutive viral loads >1000 copes/mL. "Retention in care" refers to individuals still receiving care either on-site or at another site (transfer out). "Losses to care" included deaths and individuals lost to followup.

2.7. Treatment Cohort. Patients commencing ART at the clinic between 2 September 2002 and 31 December 2009 were reviewed retrospectively. Those <15 years of age and those who were nonnaïve at entry to the programme were excluded. Also excluded were those patients who had 1 or no viral load after commencement of treatment as well as those who never achieved virological suppression on ART. All individuals who achieved suppression after ART commencement and later experienced virological breakthrough were analysed and divided into those who went on to virological failure and those who resuppressed and continued on first line. Long-term outcomes including death, transfer out to care in another service, or loss to followup were determined for all individuals in the cohort.

2.8. Statistical Analysis. Demographic and baseline data were described using medians and proportions as appropriate. Baseline characteristics were compared using nonparametric statistics for data not normally distributed.

Patients were right censored on 13 April 2010 if they remained in care. Patients who were more than 12 weeks late for a scheduled visit were considered lost to followup,

and their last visit to the clinic was used as last date in care. Patients who were transferred out were considered to still be in care at the time of censoring. Kaplan-Meier survival analysis was used to assess risks of failure and loss to care.

2.9. Ethical Review. The University of Cape Town Research Ethics Committee approved data capture from the ART site at the HCTC. All individuals who enrolled onto the programme provided written informed consent.

3. Results

As of the 31st December 2009, 4967 individuals had commenced ART at the HCTC. After excluding 375 children under the age of 15 years and 545 adult individuals who were transferred in on ART from another site, 4047 ART-naïve adults were available for review (Figure 1). A further 961 people were excluded from the analysis since they had had fewer than two viral loads on treatment, either as they died early into treatment ($n = 256$, 27%), were lost to followup ($n = 258$, 27%), transferred to care elsewhere ($n = 121$, 13%), or were within the first eight months of treatment ($n = 326$, 33%). A small proportion of the cohort never suppressed on treatment and experienced early failure ($n = 127$, 4.1%). These individuals were also excluded (Figure 1). The demographics of the remaining 2959 individuals used for this analysis are noted in Table 1.

The majority of individuals had at least one point where they suppressed their viral load to <50 copies/mL ($n = 2959$, 96%). Of those that initially suppressed on treatment, the majority did not have a subsequent viral load >1000 copies/mL ($n = 2109$, 71%). The remaining 850 individuals (29%) experienced virological breakthrough in that they had at least one viral load >1000 copies/mL while on treatment (Figure 1). Those with virological breakthrough were significantly younger, had a lower baseline CD4 count ($P = .0003$), and a higher viral load ($P = .002$) than those who remained suppressed throughout (Table 1).

Of the 850 individuals who experienced virological breakthrough, 567 resuppressed and 283 failed virologically (Figure 1). There was no significant difference in the age or stage between the above groups at baseline; however, individuals who failed ART had significantly lower CD4 counts and higher viral loads at baseline than those who resuppressed.

The median time to breakthrough was 1.39 years (IQR 0.75–2.46 years), and to failure, 1.51 years (IQR 0.99–2.64 years). Figure 2 is a Kaplan-Meier survival curve showing the risk of virological breakthrough and subsequent failure for the whole cohort ($n = 2959$). At seven years into treatment, up to 42% of the population on ART have a risk of experiencing at least one viral load >1000 copies after prior suppression. Only 15% have a risk of going on to fail suggesting that 67% of those who experienced breakthrough might be expected to resuppress at the next viral load.

Table 2 describes the long-term outcome of those who resuppressed. Of those who never experienced virological breakthrough 77% remained in care at the time of this study.

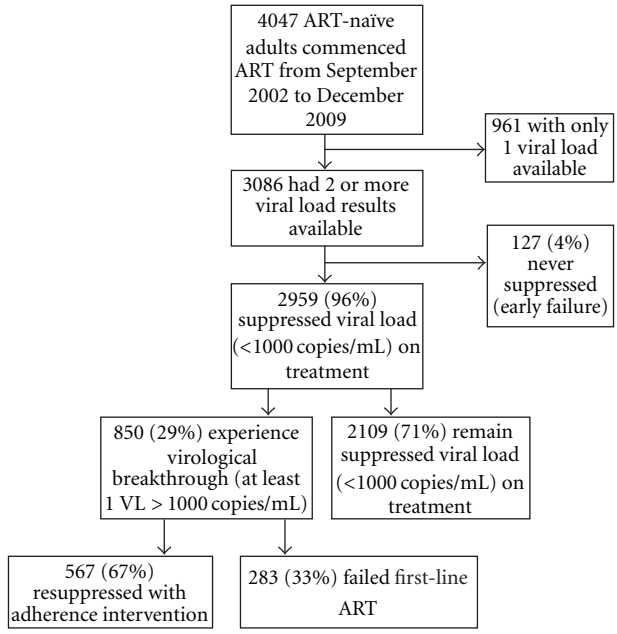

Figure 1: A flow diagram describing the cohort with virological breakthrough (>1000 copies/mL) as a subset of ART-naïve adults commencing treatment at the HCTC and subsequent outcomes.

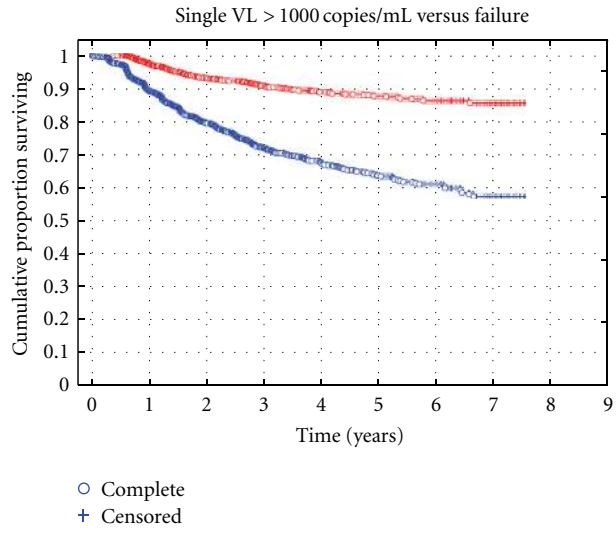

Figure 2: A Kaplan-Meier survival curve depicting risk of an initial virological breakthrough (first viral load >1000 copies/mL after initial suppression—lower curve) and subsequent risk of virological failure (second consecutive viral load >1000 copies/mL—upper curve). Of those with virological breakthrough an expected 66% will resuppress after an adherence intervention.

There was some loss due to transfer out to another clinical setting ($n = 186$, 8.9%) and to death ($n = 64$, 3.1%). Eleven percent ($n = 235$) were lost to followup. The outcomes for those who had a single viral load >1000 copies/mL and who then resuppressed differ significantly, with fewer remaining in care ($n = 348$, 61%; $P = .0000$), largely as a function of increased loss to followup ($n = 123$, 23%; $P = .0000$).

Table 1

	All naïve adults with initial viral suppression: $n = 2959$			Naïve adults with initial virological breakthrough: $n = 850$		
	Remain suppressed	Initial viral breakthrough		Resuppressed	Failed	
Total number	2109	850	—	567 (67%)	283 (33%)	—
Female gender: n (%)	1415 (67)	585 (68)	$P = .7733$*	386 (68)	200 (71)	$P = .7419$*
Age at treatment start: mean years (±SD)	35 (±8.7)	33 (±8.0)	$P = .0000$**	34 (±8.3)	32 (±7.5)	$P = .7591$**
WHO stage 1 or 2: n (%)	611 (29)	228 (27)	$P = .5099$*	159 (28)	67 (24)	$P = .2981$*
WHO stage 3 or 4: n (%)	1495 (71)	622 (73)	$P = .7068$*	406 (72)	216 (76)	$P = .5663$*
WHO stage unknown	3	0	—	0	0	—
Baseline CD4: median cells/mm^3 (IQR)	117 (62−168)	110 (50−163)	$P = .0003$#	110 (57−171)	83 (34−138)	$P = .0000$#
Baseline viral load: median copies/mL (IQR)	4.82 (4.39−5.26)	4.94 (4.51−5.30)	$P = .0002$#	4.86 (4.48−5.24)	5.07 (4.70−5.48)	$P = .0000$#

* Chi-squared.
** T-test.
# Mann-Whitney U test.

Table 2: Programmatic outcome of those who resuppressed compared to those who never experienced virological breakthrough.

	Never had breakthrough ($n = 2109$)	Breakthrough and resuppressed ($n = 567$)	P value
Lost to followup: n (%)	235 (11)	132 (23)	$P = .0000$*
Died on treatment: n (%)	64 (3.1)	25 (4.4)	$P = .1184$*
Transfer out: n (%)	186 (8.9)	62 (11)	$P = .1623$*
Continue in care: n (%)			
(i) On first line	1624 (77)	302 (53)	$P = .0000$*
(ii) Failed first line	0 (0)	46 (8)	$P = .0000$*

* Chi-squared (df = 1).

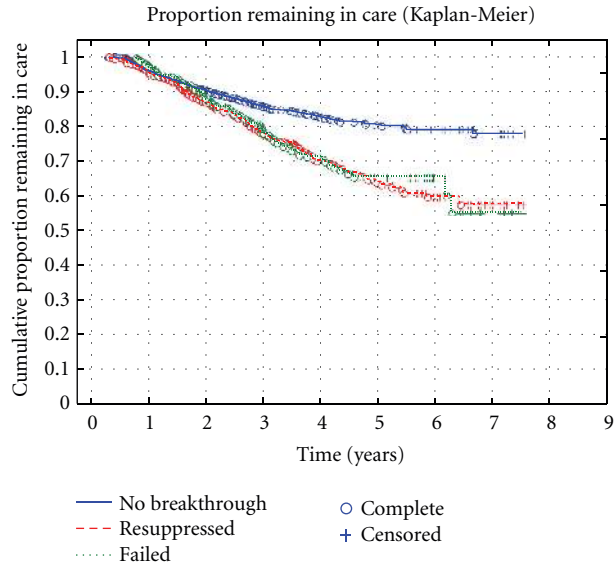

Figure 3: A Kaplan-Meier survival curve depicting risk of loss to care overtime. Losses include deaths and those lost to followup. Those who never experience virological breakthrough are more likely to remain in care overtime. Losses to care are greater in those who experience breakthrough and do not differ by future virological outcomes (failed or resuppressed).

There was no significant difference in proportion transferred to care elsewhere ($n = 62$, 11%; $P = .1623$) or in deaths ($n = 25$, 4.4%; $P = .1184$).

While all individuals who never experienced virological breakthrough remain on first-line therapy, 46 individuals (8%) of those who resuppressed after breakthrough went on to fail at a time subsequent to their next consecutive viral load and switched to second-line therapy.

Figure 3 is a Kaplan-Meier survival curve showing the risk of loss to care described above overtime. At seven years into care, it can be expected that 78% of those who remain suppressed throughout their ART remain in care. Losses are due to either death or loss to followup. The curves for those who experienced virological breakthrough do not differ as a function of resuppression or failure, with only 55–58% of these groups remaining in care by 7 years.

4. Discussion

The objective of this study was to determine whether those individuals on ART who experienced a single episode of

virological breakthrough after prior successful suppression had similar long-term virological outcomes to individuals who remain suppressed on ART throughout.

Of note, the majority of this treatment cohort achieved virological suppression (96%), a much higher proportion than noted in a recent systematic review of sub-Saharan African ART programmes where only 78% of treated patients achieved virological suppression at six months [14]. Seventy-one percent of this group continued to be suppressed at the censor date for this analysis. Of the 29% who experienced virological breakthrough, two-thirds resuppressed after the intensive adherence intervention: a similar proportion to that seen in 2005 [10]. However, the risk of experiencing virological breakthrough continued to increase overtime, so that by seven years in care there was a relatively high risk (42%) that an individual would have experienced breakthrough and a 15% risk of failure of first-line therapy.

Preliminary data from 2005 showed that the risk of failure remained relatively low in individuals who had virological breakthrough [10]. This study, of an expanded cohort, suggests that these individuals have different outcomes from those who remained successfully suppressed. While 77% of those who remained suppressed throughout were retained in care seven years into the programme, this only applied to 58% of patients who experienced virological breakthrough. This is largely a function of increasing loss to followup overtime. These individuals were younger and more ill with lower CD4 counts and higher viral loads at the time of starting ART. It is of note that the risk of loss to followup for patients who have virological breakthrough and then resuppress is the same as for patients who fail treatment.

A systematic review of patient retention in ART programmes estimated rates across 33 cohorts to range from 24 to 77% at only two years into the programme. Death and loss to followup accounted for the majority of these losses, as in our programme [15].

Although the overall outcomes of our programme may seem reasonable compared to those of other current programmes, as the expansion of antiretroviral services continue, so must the effort to improve adherence to care. While our adherence intervention appears successful in that two-thirds of those who experience virological breakthrough did resuppress it is important to note that this intervention does not impact on loss to followup.

Previous studies have identified subgroups of patients who are at risk of LTFU. These include pregnant women and younger people on ART [16]. This study identified patients with virological breakthrough as another of these subgroups. It seems that those who struggle to adhere to therapy, initially noted by an increase in viral load, signaling less than ideal adherence to taking ART tablets, may also not adhere to the program. It should also be considered that the intensity of the adherence intervention itself, while successful for some individuals, may deter others from remaining in care. It is a weakness of this study that this is left to speculation. Qualitative information from individuals lost to followup from this programme would be required to answer this in detail.

Intensive adherence programmes may result in retained first-line therapy for those individuals who have an initial viral breakthrough but they do not of themselves retain people in care. Remaining on first-line therapy is an important goal not only in terms of cost saving but also in terms of preserving treatment options in resource-constrained settings. However, increasing attention is being drawn to retention to care in these settings in order to secure public health impact. Adherence interventions should be targeted to those who have an increased risk of loss to followup and need to be broadened to address issues related to loss to care as well as tablet-taking behavior.

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Research Article

Evaluation of WHO Criteria for Viral Failure in Patients on Antiretroviral Treatment in Resource-Limited Settings

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Our objective was to evaluate outcomes in patients with sustained viral suppression compared to those with episodes of viremia. *Methods*. In a prospective cohort of patients started on ART in Uganda and followed for 48 months, patients were categorized according to viral load (VL): (1) sustained-suppression: (VL ≤1,000 copies/mL) (2) VL 1,001–10,000, or (3) VL >10,000. *Results*. Fifty-Three (11.2%) and 84 (17.8%) patients had a first episode of intermediate and high viremia, respectively. Patients with sustained suppression had better CD4+ T cell count increases over time compared to viremic patients ($P < .001$). The majority of patients with viremia achieved viral suppression when the measurement was repeated. Only 39.6% of patients with intermediate and 19.1% with high viremia eventually needed to be switched to second line ($P = .008$). *Conclusions*. The use of at least one repeat measurement rather than a single VL measurement could avert from 60% to 80% of unnecessary switches.

1. Introduction

Data from developed countries suggest that episodes of low (400–1000 copies/mL) and transient viremia while on antiretroviral treatment (ART) have limited consequences on patients' clinical and immunological outcomes and have a low risk of developing drug resistance [1–4], while episodes of viremia >1,000 copies/mL have been associated with new clinical events [5]. The optimal threshold at which patients should be switched to second line is still debated. It has been reported that patients may not have detrimental effects on CD4 T-cell counts [6] and clinical progression of disease [7] despite having detectable viral load (VL) above 10,000 copies/mL. The 2006 World Health Organization (WHO) guidelines recommended switching patients to second-line ART if the VL was >10,000 copies/mL. Therefore, patients with VL between 1000 and 10,000 copies/mL were usually maintained on first-line treatment and no specific guidance was available to clinicians [8]. Recently the WHO published a document [9] recommending that patients with a VL >5,000 copies/mL should have a VL repeated and, if the VL remains >5,000 copies/mL, should prompt therapy change.

The objective of our study was to evaluate clinical, immunological, virological, and therapeutic outcomes in patients with sustained viral suppression compared to patients with first episodes of viremia (categorized by magnitude) after at least 6 months of ART.

2. Methods

We analyzed data from a prospective cohort of patients started on first-line ART between April 2004 and April 2005 and followed for 4 years at the Infectious Diseases Institute, Kampala, Uganda. Patients were started on stavudine, lamivudine, and nevirapine (provided by Global Fund), or on zidovudine plus lamivudine plus efavirenz (provided by the US President's Emergency Plan for AIDS Relief).

A detailed description of the study has been published elsewhere [10]. Briefly, the study subjects are assessed

clinically every 3 months and laboratory testing including CD4+ T-cell count by FACS Count (Becton Dickinson, Mountain View, California, USA), and HIV-1 VL (Amplicor HIV-1 Monitor PCR Test, version 1.5, Roche Diagnostic, GmbH Molecular Systems, Pleasanton, California, USA), with a detection limit of 400 copies/mL is performed every 6 months. The study was approved by the Institutional Review Board of Makerere University Faculty of Medicine and the Uganda National Council for Science and Technology (no: MV 853).

Patients were included in the analysis if they had reached 6 months of followup on ART. They were categorized according to VL measurements: (1) subjects with sustained suppression: (VL ≤1,000 copies/mL at each measurement), (2) subjects with a first episode of intermediate viremia (VL between 1,001–10,000 copies/mL), or (3) subjects with a first episode of high viremia (VL >10,000 copies/mL). We also recategorized patients with a first episode of viremia between 1,000–5,000 copies/mL and those with a first episode of viremia >5,000 copies/mL for a second analysis according to the new WHO recommendation.

Clinical outcome was defined as the occurrence of new opportunistic infections or death after reaching a first episode of viremia. Immunological outcome was defined as the median increase in CD4+ T-cell count at the end of the study and the cumulative probability of reaching a CD4+ T-cell count of 200 cells/μL in patients that had not reached 200 cell/μL at month 6. The virological outcome was defined as the proportions of patients with a consecutive subsequent VL ≤1,000, 1,000–10,000 and >10,000 copies/mL. Finally, the therapeutic outcome was defined as the proportion of patients switched to second line. The standard operating procedure in our clinic for patients with episodes of viremia is to switch patient with 2 consecutive VL >1,000 copies/mL. However, in clinical practice patients are often not switched after 2 consecutive VLs >1,000 copies/mL. This is due to a variety of reasons: clinician reluctance to switch patients due to the lack of subsequent treatment options, subsequent low measures (<10,000) of detectable VLs, and finally irregular supply of the second-line drugs.

2.1. Statistical Analysis. We compared the baseline characteristics of patients in different VL groups. We compared baseline characteristics using the Kruskal Wallis test for continuous variables (age, body mass index, hemoglobin, CD4+ count) and chi-square for categorical variables (gender, WHO staging, ART regimen).

We used the Kruskal Wallis test to compare median CD4+ T-cell count increase at followup, chi-square tests to compare proportion of outcomes and proportion of patients that did not achieve a CD4+ T-cell count number >200 cell/μL at month 6, and Kaplan Maier curves to describe the probability of reaching a CD4+ T cell count >200 cell/μL in patients with a CD4+ T cell count ≤200 cell/μL after 6 months of followup.

3. Results

Of the 559 patients enrolled in the study, 474 reached at least 6 months on ART; of these patients, one patient had no VL measurement available after 6 months on ART, and therefore, 473 (84.6%) were included the analysis. Sixty-seven died [11], 13 were lost to followup, 4 were transferred other facilities, and 1 withdrew consent before reaching 6 months on ART. The patients were followed up for a median time of 48 months (range 6–48).

3.1. Baseline Characteristics and Patients Classification. The majority of the patients (n = 336, 71%) had sustained suppression throughout the study, 53 (11.2%) patients had a first episode of intermediate viremia (1,000–10,000 copies/mL) after a median time of 40 weeks (IQR 26–74) and 84 (17.8%) patients had a first episode of high viremia (>10,000 copies/mL) after a median time of 48 weeks (IQR 28–74) (P = .624).

The baseline characteristics were similar in the three groups except that a higher proportion (90.6%) of patients with a first episode of intermediate viremia were started on an nevirapine-based regimen as compared to patients with sustained suppression (71.7%) and patients with a first episode of high viremia (72.6%) (P = .009) (Table 1).

3.2. Opportunistic Infections and Death. We did not observe differences in the proportion of deaths in patients with sustained suppression (6.9%) compared to patients with a first episode of intermediate (5.7%) and high viremia (11.9%) (P = .25).

The proportion of patients experiencing at least one WHO grade 3 or 4 clinical event after 6 months of ART was similar in the three groups (21.1% (sustained), 28.2% (intermediate viremia), and 24.5% (high viremia) P = .4). A similar proportion of patients with a first episode of intermediate and high viremia experienced an opportunistic infection before (22.6% versus 27.4%, P = .53) and after the episode of viremia (7.5% versus 8.3%, P = .866).

3.3. Immunologic Response. The median CD4+ T-cell count increase at different study intervals was higher in patients with sustained suppression compared to those patients with intermediate and high viremia (P = .001) with a total increase at year 4 (or at the last available observation) of 186 cells/μL, 167 cells/μL, and 107 cells/μL, resp. (Figure 1(a)).

Overall, 220 (46.4%) patients did not achieve a CD4+ T-cell count >200 cells/μL at month 6. Only 42.9% (144/336) of those patients who achieved sustained suppression did not achieve a CD4+ T-cell count >200 cells/μL, compared to 58.5% (31/53) and 53.6% (39/84) of those with a first episode of intermediate and high viremia, respectively (P = .033). In the patients that had not achieved this threshold by month 6, the probability of achieving >200 cells/μL by year 4 was higher in the patients with sustained suppression (86.8%) and the patients with a first episode of intermediate viremia (96.8%) compared to the patients with a first episode of high viremia (55.6%) (P = .017) (Figure 1(b)).

Table 1: Comparison of the baseline characteristics of 473 patients classified in three groups according to their level of viremia.

Patients characteristics	Sustained suppression* 336 (71.0%)	Intermediate viremia* 53 (11.2)	High viremia* 84 (17.8)	*P* value
Female, number (%)	240 (71.4)	30 (56.6)	56 (66.7)	.085
Age (years), median (IQR)	35 (30–42)	34 (30–42)	34 (28–38.5)	.168
CD4+ count median cell/μL (IQR)	107 (35–174)	85 (25–154)	89.5 (26–165)	.246
BMI (Kg/m^2), median IQR	20.1 (18.3–22.5)	20.6 (18.5–20.5)	20.0 (18–22.5)	.816
WHO Stage 3 and 4, number (%)	291 (86.6)	51 (96.2)	76 (90.5)	.102
Hemoglobin median g/dL (IQR)	11.7 (10.4–13)	11.9 (10.8–13.2)	11.6 (10.5–13)	.792
ART, number (%) Nevirapine	241 (71.7)	48 (90.6)	61 (72.6)	.009
Efavirenz	95 (28.3)	5 (9.4)	23 (27.4)	

* Patients were categorized according to viral load measurements after 6 months on treatment. (1) Sustained suppression. Viral load ≤1,000 copies/mL at each measurement. (2) Subjects with a first episode of intermediate viremia. Viral load between 1,001–10,000 copies/mL. (3) Subjects with a first episode of high viremia. Viral load >10,000 copies/mL.
BMI: body mass index; ART: antiretroviral treatment; IQR: interquartile range.

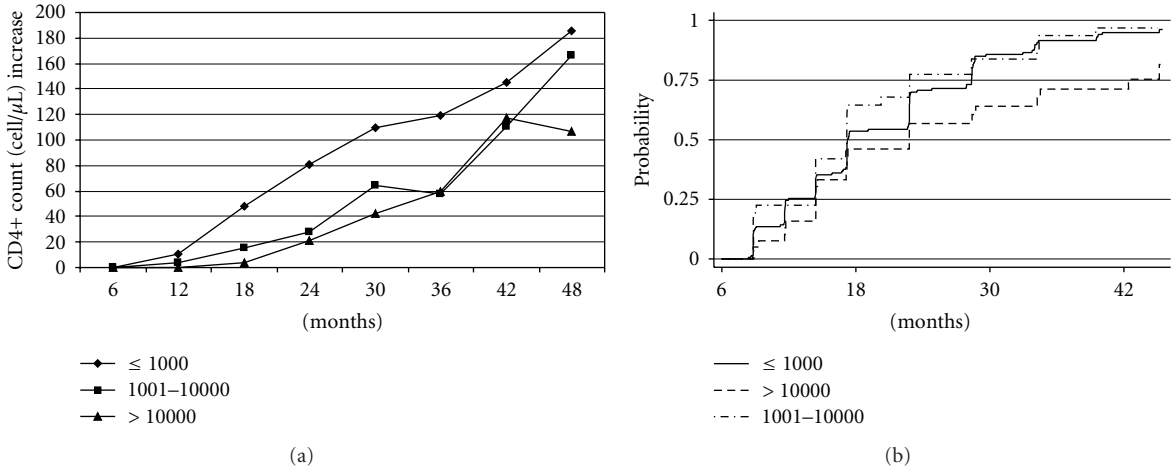

Figure 1: (a) Median increase in CD4+ T-cell count in patient with sustained suppression (viral load VL ≤1,000 copies/mL at each measurement), patients with a first episode of intermediate viremia (viral load between 1,001–10,000 copies/mL), and high viremia (viral load >10,000 copies/mL) over time. (b) Probability of achieving CD4+ T-cell count >200 cells/μL in patient with sustained suppression (viral load VL ≤1,000 copies/mL at each measurement), patients with a first episode of intermediate viremia (viral load between 1,001–10,000 copies/mL, and high viremia (viral load >10,000 copies/mL) over time.

3.4. Confirmed Viral Failure after the First Episode of Viremia. Of the 137 patients with at least one viremic episode, 15 (10.9%) were excluded from this analysis because their first viremic episode occurred on their last available visit. As shown in Table 2(a), we did not find differences in the proportion of patients with a consecutive subsequent VL ≤1,000 ($n = 37$, 72.5% versus $n = 41$, 57.8%), 1,000–10,000 ($n = 3$, 5.9% versus $n = 5$, 7.0%), and >10,000 ($n = 11$, 21.6% versus $n = 25$, 35.2%) (P value = .166) in patients with a first episode of intermediate and high viremia.

3.5. Switch to Second Line. Reassuringly, none of the patients with sustained suppression were switched to second line. Interestingly, a higher proportion of patients with a first episode of intermediate viremia ($n = 21$, 39.6%) as compared to patients with a first episode of high viremia ($n = 16$, 19.1%) were switched to second line treatment ($P = .008$).

In a subanalysis, we also evaluated the outcomes of 51 (9.1%) patients that had a first episode of viremia between 400 and 1000 copies/mL; clinical and immunological outcomes were similar to patients with sustained viral suppression below 400 copies/mL. only 5 had a subsequent confirmed viral failure.

3.6. Recategorizing Patients Using 5,000 Copies/mL as a Cutoff. Thirty-seven of the 473 (7.8%) total patients had a first viremic episode between 1,000 and 5,000 copies/mL after a

Table 2: Comparison of the subsequent viral load measurement in patients with a first episode of viremia of 1,001–10,000 copies/mL and >10,000 copies/mL. Comparison of the subsequent viral load measurement in patients with a first episode of viremia of 1,001–5,000 copies/mL and >5,000 copies/mL.

(a)

First episode of viremia (copies/mL)	Number of patients*	Subsequent viral load (copies/mL)			P value
		≤1,000	1,001–10000	>10000	
1,001–1,0000	51	37 (72.5)	3 (5.9)	11 (21.6)	.166
>10000	71	41 (57.8)	5 (7.0)	25 (35.2)	

* Viral load was available for 122/137 (89.1%) patients with a first episode of viremia.

(b)

First episode of viremia (copies/mL)	Number of patients*	Subsequent viral load (copies/mL)			P value
		≤1,000	1,000–5,000	>5,000	
1,001–5,000	37	30 (81.1)	1 (2.7)	6 (16.2)	.017
>5,000	85	47 (55.3)	4 (4.7)	34 (40.0)	

* Viral load was available for 122/137 (89.1%) patients with a first episode of viremia.

median time of 60 weeks (IQR: 26–96) and 85 (18%) patients had a first viremic episode >5,000 copies/mL after a median time of 39 weeks (IQR: 25–74) ($P = .331$).

As expected, we found no differences in the proportion of patients who died or developed opportunistic infections across the 3 groups, and between patients with first viremic episode between 1,000–5,000 copies/mL and patients with a first viremic episode between 5,000–10,000 copies/mL.

The median CD4+ T-cell count increase at year 4 (or at the last available observation) was higher in patients with sustained suppression (186 cells/μL) compared to patients with a first viremic episode between 1,001–5,000 copies/mL (167 cells/μL), and patients with a first viremic episode >5,000 copies/mL (100 cells/μL) ($P < .001$). Interestingly, patients with a first episode of viremia between 1,000–5,000 copies/mL had a higher median increase in CD4+ T-cell count as compared to patients with a first episode of viremia between 5,000 and 10,000 copies/mL (167 versus 52 cells/μL) ($P < .001$). In addition, we found that patients with a first viremic episode >5,000 copies/mL had a lower probability of reaching a CD4+ T-cell count of 200 cells/μL as compared to either patients with sustained suppression or patients with a first viremic episode between 1,001–5,000 copies/mL ($P = .017$). However, the probability of reaching a CD4+ T-cell count of 200 cell/μL by year 4 was similar in patients with a first viremic episode between 1,000 and 5,000 and in patients with viremia between 5,000 and 10,000 (P value = .35).

As shown in Table 2(b) a higher proportion of patients with a first viremic episode between 1,001–5,000 copies/mL had a consecutive subsequent VL ≤1,000 copies/mL as compared with patients with viremia >5,000 copies/mL (81.1% versus 55.3%) (P value = .017).

While the proportion of patients that needed to be switched to second line did not differ statistically ($P = .293$) between the patients with a first episode of viremia between 1,001 and 5,000 copies/mL (33.3%) and those with a first episode of viremia >5,000 copies/mL (24.5%) ($P = .293$).

4. Discussion

In our cohort, the majority of the patients (71%) achieved sustained suppression defined as a VL ≤1,000 copies/mL at each visit throughout the study period. Overall, patients with a first episode of intermediate and high viremia do not experience in the medium term (4-year followup) more OIs or deaths as compared to patients that achieve sustained suppression.

Despite the WHO recommendation to switch patients to second line ART if the viral load exceeds 10,000 copies/mL, patients with viremic episodes between 1,001–10,000 copies/mL need to be closely followed because they have an impaired CD4+ T-cell count rise as compared to patients with sustained suppression. Moreover, the long-term consequences of keeping patients on first line with these levels of viremia on morbidity and mortality are not known.

When we analyzed the VL obtained after a first episode of viremia, we found that the majority of the patients with high (72.5%) and intermediate (57.8%) viremia subsequently achieved viral suppression. In our clinic, patients with viral failure receive counseling to re-enforce adherence on their following monthly routine visit, so that VL rebound can be reversed in patients with virus susceptible to the same antiretroviral treatment.

Conversely, 21.5% of the patients with viremia between 1,001–10,000 copies/mL, who should be kept on first-line therapy according to the 2006 WHO criteria, experienced a subsequent confirmed viral failure.

In our study, a higher proportion of patients with a first episode of intermediate viremia as compared to ones with a first episode of high viremia were later judged by the clinicians to be in need of second line treatment. An analysis from the UK Collaborative HIV Cohort Study [12] showed that mutations are more frequent in resistance tests performed at VLs between 300 to 10,000 copies/mL and decrease at VLs above 10,000. It is likely that first episodes of very high levels of viremia in our cohort occurred in patients that had discontinued medications without

clinicians' knowledge and that, in the long run, manage to achieve suppression after adherence counseling. On the other hand, a first presentation with intermediate viremia could be a sign of emerging drug resistance and, therefore, these patients are likely to experience a subsequent detectable viral load and increasing VL over time.

Because of the new WHO recommendations [9], we also analyzed the treatment outcomes using a cutoff of 5,000 copies/mL. Patients with a first episode of viremia between 1,001–5,000 copies/mL do not seem to have an impaired immune reconstitution; moreover, more than 80% of the patients in this group have a subsequent VL ≤1,000 copies.

Despite the newer guidelines provided by WHO, monitoring ART in resource limited settings is still very challenging. Previous research has shown that the currently proposed criteria for assessing treatment failure using immunologic response in the absence of a VL performs poorly in cohorts from resource limited settings, and researchers are advocating for accessible and cheap VL testing [13–17]. However, the ideal timing for VL testing is unclear. Some suggested strategies are to perform VLs on patients suspected to be failing before switching to second line [18], or as an adherence assessing strategy [19].

Our study suggests that one VL is not enough to make a clinical decision on whether to switch treatment to second line. In this cohort in Uganda, almost 60% of the patients with high viremia (>10,000 copies/mL) will subsequently achieve sustained suppression and only 19% were ultimately judged by the clinicians to be in need of second line. This study shows that the use of subsequent VLs measurement to identify patients failing ART could have averted 60% to 80% of unnecessary switches to second-line treatment. While the new cutoff suggested by the WHO seems to correctly identify patients that can be still kept on first-line, only a minority of patients with a first episode of any level of viremia had a persistently detectable VL that prompted our physicians to switch the patients to second line therapy.

Our study has some limitations. First, the follow-up time after patients had a first viremic episode may not have been long enough to show the effects on morbidity and mortality of remaining on a first line regimen with detectable viremia. The other limitation of the study is that that we do not perform routine genotype resistance testing on detectable samples; therefore, we do not know the effect on the accumulation of resistance mutations in these patients.

In conclusion, although the long-term consequences of keeping patients with detectable viremia on first-line therapy on clinical events are not known, in resource-limited settings clinicians must weigh the risk of incurring viral resistance in patients with a detectable VL against the high cost of second line ART [20] and the relatively high proportion of patients in our study who subsequently suppressed with adherence counseling. Strong consideration should be given to adherence counseling after the first detectable viral load and a repeated measurement of VL before switching these patients to a second-line regimen in resource limited settings.

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Research Article

Utilization Patterns and Projected Demand of Antiretroviral Drugs in Low- and Middle-Income Countries

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Background. The rapid scale-up of antiretroviral therapy in resource-limited settings has greatly increased demand for antiretroviral medicines and raised the importance of good forward planning, especially in the context of the new 2010 WHO treatment guidelines. *Methods*. Forecasting of the number of people receiving antiretroviral therapy from 2010 to 2012 was produced using three approaches: linear projection, country-set targets, and a restricted scenario. Two additional scenarios were then used to project the demand for various antiretroviral medicines under a fast and slower phase-out of stavudine. *Results*. We projected that between 7.1 million and 8.4 million people would be receiving ART by the end of 2012. Of these, 6.6% will be on second-line therapy. High variation in forecast includes reductions in the demand for d4T and d4T increases in the demand for tenofovir, emtricitabine followed by efavirenz, ritonavir, zidovudine and lopinavir; lamivudine, atazanavir, and nevirapine. *Conclusion*. Despite the global economic crisis and in response to the revised treatment guidelines, our model forecasts an increasing and shifting demand for antiretrovirals in resource-limited settings not only to provide treatment to new patients, but also to those switching to less toxic regimens.

1. Introduction

In the past five years, low- and middle-income countries have aggressively scaled up HIV treatment. By the end of 2009, more than 5.2 million people were receiving antiretroviral therapy [1]. This represented an increase of more than 1.2 million people from the end of 2008 and a 13-fold expansion over the previous six years. This rapid scale-up has greatly increased the demand on antiretroviral medicines and raised the importance of good forward planning.

The new forecasts presented here update previous estimates [2–4] by adding updated information on the number of people receiving antiretroviral therapy and data from the annual WHO AIDS Medicines and Diagnostics Service (AMDS) survey on the use of antiretrovirals [5–7]. Two major elements taken into account for developing updated forecasts were the financial crisis and the progressive implementation of the new WHO antiretroviral therapy (ART) recommendations published in 2010 [8].

Under the revised WHO guidelines, an estimated 14.6 million (13.4–15.4 million) people in low- and middle-income countries will be eligible for ART, a 45% increase from the 10.1 million (9.0–11.1 million) people in need of ART under the previous guidelines. While the predicted expansion of HIV treatment is well below 14.6 million, the figures in this paper are not a statement about what should be or will be accomplished in 2012. Rather, these estimates indicate what is likely to be accomplished in the absence of meaningful changes in the conditions driving treatment scale-up.

2. Methods

Estimates of the number of people receiving antiretroviral therapy were forecast from 2010 to 2012 using three approaches: linear projection, country-set targets and restricted scenario. To forecast the demand for antiretroviral medicines, two additional scenarios were included: a fast and slower phase-out of stavudine, with a baseline consumption taken from the 2009 WHO survey on antiretroviral use. Once the number of patients on treatment was estimated, these numbers were translated into volumes of active pharmaceutical ingredients.

2.1. Multicountry Survey on ARV Use. In March 2009, the WHO Department of HIV and AIDS conducted the third annual survey to assess antiretroviral use in low- and middle-income countries. A standard questionnaire was sent to the 43 countries with the highest number of people receiving antiretroviral therapy as of December 2008. National AIDS programmes of 39 countries responded and completed the questionnaires; these were collected by the WHO countries and regional offices and forwarded to the WHO AMDS team in Geneva.

2.2. Forecasting the Number of Patients Receiving Treatment

2.2.1. Choice of Forecasting Approach. Three forecast approaches were considered: linear projection, country-set targets, and restricted scenario. The linear projection is based on projections of historical trends of the number of people receiving antiretroviral therapy by country, from the data reported between December 2006 and December 2008 for each country. This approach is a linear extrapolation using a regression line through the last three data points to determine the average annual increase. This increase is added to the estimated number of people receiving antiretroviral therapy as of December 2008. This approach has the advantage of being grounded in actual country-observed data and is easy to implement and understand. Since it could result in a projection greater than total estimated need, all projections were capped at the level established for the country-set target scenario. Recent growth has been approximately linear, so this approach represents the best projection, assuming that past trends continue.

The second scenario was adjusted using targets set by countries for the number of people that they expect to reach with antiretroviral therapy by 2012. These targets take into account the realities in each country and their goals for increasing coverage. During a consultation at the WHO in Geneva in October 2009, five countries reported their targets: India, Kenya, South Africa, Thailand, and Zambia. Together, these countries account for approximately 40% of people receiving antiretroviral therapy. In the country-set target projection, it is assumed that the total number of people receiving antiretroviral therapy will grow at the same rate as the projected number for the five countries.

The two scenarios using linear projection and country targets may be over-optimistic if the economic crisis results in slower growth in international and national financing of antiretroviral therapy programmes [9–11]. A restricted scenario was therefore created to consider this possibility. Under the restricted scenario, the number of people receiving antiretroviral therapy grows at only 75% of the annual rate of the target scenario.

The three methods described above were applied to each of the 154 countries in the analysis and the results were aggregated to regional and interregional totals.

This exercise defined the number of people receiving antiretroviral therapy as the number of people receiving antiretroviral therapy at the end of a given year. This includes people who started antiretroviral therapy in that year, as well as those who started in previous years and remained on treatment.

2.2.2. Retention Assumptions. The proportion of people receiving antiretroviral therapy in one year who continue on antiretroviral therapy through the same programme in a subsequent year is called the retention rate. A number of countries estimate retention rates from their programme data. Published data from the *Towards Universal Access 2009* report have been consolidated into first year and subsequent years, resulting in a 79.5% overall retention rate for the first year and 95.8% for subsequent years [5].

2.2.3. First- and Second-Line Antiretroviral Therapy. The current proportion of people receiving second-line therapy was extracted from the 2009 WHO survey on antiretroviral use. The number of people receiving first-line antiretroviral therapy in any year is projected as the number of people initiating antiretroviral therapy in that year plus those people receiving first-line therapy in the previous year who survive to this year and do not switch to second-line therapy. For countries without information on the number of people receiving second-line therapy, a regional average from the survey was applied: 2.8% for sub-Saharan Africa; 8.2% for Central America and the Caribbean; 9.1% for Eastern and Central Europe; 30% for Latin America; 0.2% for South-East Asia; 2.0% for Western Pacific.

2.2.4. Rate of Switching from First- to Second-Line Antiretroviral Therapy. Data from a systematic review of monitoring strategies, treatment failure, and attrition rates, conducted by WHO in collaboration with the Australian National Centre in HIV Epidemiology and Clinical Research, were used to estimate the regional failure rates [12]. The review showed that when viral load monitoring is used, a failure rate of 6% is detected and that when the CD4 count is used there is a 1.9% detected failure rate. Data from the review were used to estimate failure rates for Africa (2.6%), Latin America (2.6%), and Asia (1.1%). For countries in all other regions, the average of 1.9% was used. For Latin American countries where viral load is used routinely, including Argentina, Brazil, Mexico, and Venezuela, the 6% rate from the meta-analysis was used.

2.3. Forecasting Demand for Antiretroviral Medicines. One of the expected impacts of the new WHO recommendations on

the use of antiretroviral drugs in low- and middle-income countries will be a progressive phasing out of stavudine and an increase in first-line regimens based on zidovudine and tenofovir [8]. The antiretroviral demand forecasts presented are based on two scenarios, which consider either a fast or slow phase-out of stavudine. The two scenarios were developed for both first- and second-line regimens, and the population was divided into two categories within each scenario: people already receiving antiretroviral therapy and people initiating antiretroviral therapy, for whom the new recommendations apply differently. The baseline is taken from the 2009 WHO AMDS survey on antiretroviral use.

3. Results

3.1. Use of Antiretroviral Therapy in Low- and Middle-Income Countries as of December 2008. Thirty-nine national AIDS programmes responded to the survey, representing a total of 3.4 million people receiving antiretroviral therapy, or about 85% of the estimated 4.0 million people receiving antiretroviral therapy in resource-limited countries as of December 2008. In these 39 countries, 93% of the people receiving antiretroviral therapy were adults (3.2 million adults) and 7% were children (252 000 children).

An exploratory data analysis confirmed that the pattern of use of first- and second-line regimens was similar among the 37 countries that scaled up treatment programmes after the publication of WHO's public health approach to antiretroviral therapy in 2002 [13]. However, in Brazil and Mexico, where the expansion of treatment programmes started earlier, the pattern of antiretroviral use was significantly different, particularly for the level of use of second-line regimens. For this reason, data from Brazil and Mexico were analysed separately from data from other programmes that have scaled up more recently and are presented separately.

Figure 1 shows the composition and distribution of the first- and second-line regimens most commonly used in the 37 low- and middle-income countries. In antiretroviral treatment programmes, the vast majority of adults (98%) were receiving first-line regimens and 2% of patients were receiving second-line regimens. A vast majority (99%) of people on first-line ART, and 87% of those on second-line ART, were receiving regimens recommended by the WHO [14]. More than half of the patients on first-line regimens (62%) were receiving stavudine and 56% were receiving nevirapine as the nonnucleoside component. Among the 2% of patients receiving second-line regimens, a majority (55%) were on a regimen containing zidovudine and 43% were on one containing tenofovir. Lopinavir boosted with low-dose ritonavir (LPV/r) was the predominant protease inhibitor, received by 95% of adults. A vast majority of children (97%) were receiving first-line regimens (237 000 children), with an almost equal distribution between regimens containing stavudine and zidovudine (49% and 47%, resp.). Brazil and Mexico reported frequent use of second-line regimens in adults (20% and 13%, resp.) and very low use of stavudine and nevirapine in first-line regimens. A high proportion of children in Brazil and Mexico were receiving second-line therapy (40% and 13%, resp.).

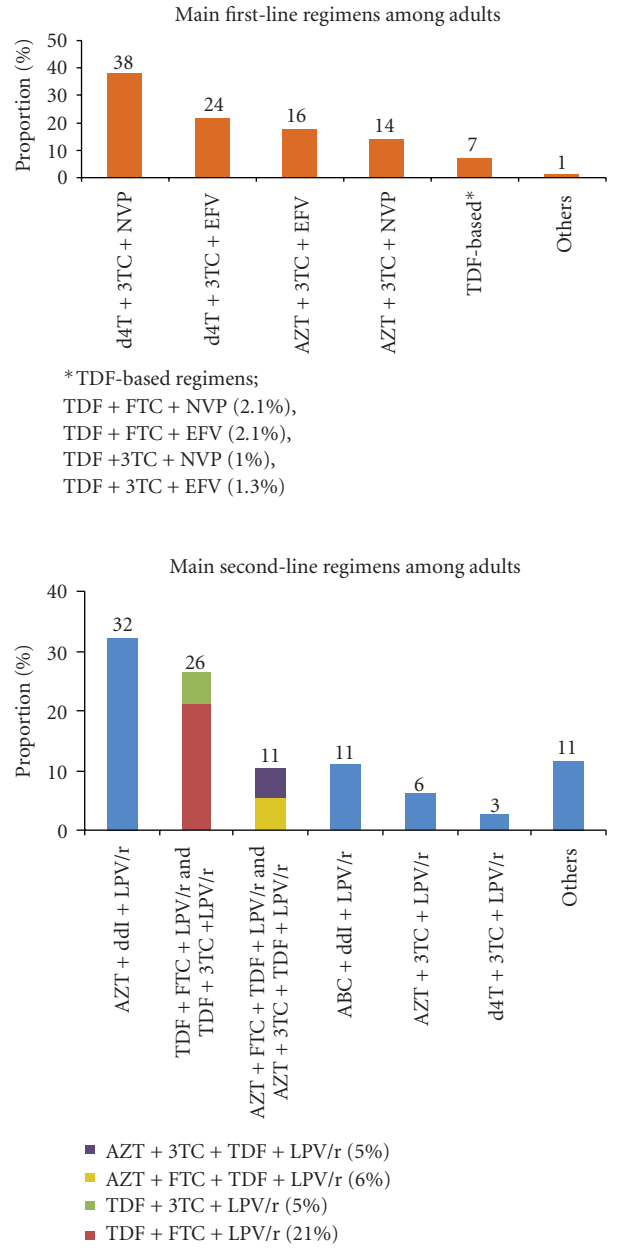

Figure 1: First- and second-line antiretroviral regimens received by adults in 37 low- and middle-income countries ($n = 2\,870\,000$ and $n = 67,500$), December 2008.

3.2. Antiretroviral Market Trends in 17 Countries from 2006 to 2008. An analysis of 17 countries (Burkina Faso, Burundi, Cambodia, Cameroon, Côte d'Ivoire, Ethiopia, India, Kenya, Lesotho, Namibia, Nigeria, Rwanda, Swaziland, the United Republic of Tanzania, Uganda, Zambia, and Zimbabwe.) that responded to all three rounds of the WHO survey on ARV use (2006–2008) showed the antiretroviral market trends over the three-year period [5]. The analysis confirmed that the proportion of adults receiving second-line regimens in antiretroviral therapy programmes remains low with 2.3% in 2008 (3.5% in 2007 and 2% in 2006). There was a net increase in tenofovir use in 2008, with 9% and 56% of adults

receiving tenofovir-based regimens on first and second-line respectively. (1.5% for first line and 32% for second line in 2007, and <1% and 11% in 2006). The analysis also showed a slow decrease in stavudine use, with 57% of adults on first-line regimens receiving stavudine in the 17 countries in 2008 (68% in 2007 and 67% in 2006) and an increase in zidovudine use, with 35% of adults on first-line regimens receiving zidovudine in 2008 (26% in 2007 and 29% in 2006).

3.3. Evolution in National Antiretroviral Therapy Guidelines. The survey showed that WHO 2006 treatment recommendations have largely been adopted and implemented by national HIV programmes, despite a few people still receiving treatment regimens that are not in line with WHO guidelines (such as second-line regimens without a protease inhibitor backbone) [14]. There is a constant evolution in national treatment guidelines; half the countries were in the process of revising adult (16 countries) and paediatric (19 countries) protocols at the time of the survey. The most frequently planned changes in adult guidelines were the introduction of tenofovir (nine countries), a change from stavudine as the preferred first-line option (seven countries), an increase in the CD4 threshold for treatment initiation to below 250 cells/mm^3 (two countries) or below 350 cells/mm^3 (four countries), and the introduction of viral load testing to monitor treatment (three countries).

3.4. Forecast Numbers of People Receiving Antiretroviral Therapy in Low- and Middle-Income Countries by 2012. Figure 2 shows the forecast demand for ART, based on linear projections, country-set targets, and the restricted scenario, which projects between 7.1 and 8.4 million people receiving ART in low- and middle-income countries by the end of 2012. The baseline number of people receiving ART was 4.0 million by the end of 2008 and is projected to increase to 5.0 million by the end of 2009, 6.0 million by the end of 2010, 6.9 million by the end of 2011, and 7.9 million by the end of 2012, according to linear projection. Annual growth is slightly slower than the growth observed between 2007 and 2008. It is expected that countries with the greatest number of people receiving antiretroviral therapy would experience slower enrolment of new patients, after achieving high coverage levels. When using upper and lower estimates of the number of people receiving antiretroviral therapy between 2006 and 2008 in each country, the estimated annual increase ranged from 0.98–1.0 million people per year. The country-set target scenario forecasts lower numbers than the linear projection in 2009 and 2010, but greater numbers by 2012, reaching a total of 8.4 million (6.3% greater than the linear projection). The restricted scenario forecasts the lowest numbers receiving antiretroviral therapy, reaching only 7.1 million by 2012 (10% less than the linear projection).

Table 1 shows detailed results for the linear projection approach, where the number of people receiving second-line therapy is estimated to increase from around 190 000 in 2008 to 520 000 in 2012, equivalent to an increase from 4.8% to 6.6%. Under the country-set target scenario, the number of people receiving second-line therapy is estimated to increase from about 190 000 in 2008 to 530 000 in 2012, a relative increase from 4.8% to 6.4%. Under the restricted scenario, the number of people receiving second-line therapy is estimated to increase from about 190 000 in 2008 to 500 000 in 2012, or from 4.8% to 7.1%.

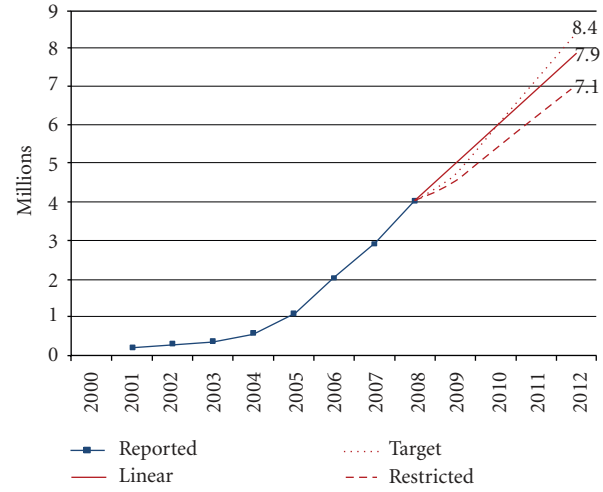

Figure 2: Number of people receiving antiretroviral therapy in low- and middle-income countries, reported on December 2008 and projected for December 2009–December 2012, linear projection and two scenarios (millions).

Table 1: People receiving antiretroviral therapy in low- and middle-income countries, by type of regimen, reported for 2008 and forecast for 2009–2012, linear projection (millions).

	2007	2008	2009	2010	2011	2012
Number on first line (millions)	2.84	3.84	4.74	5.62	6.49	7.35
Percent	95.1%	95.2%	94.8%	94.4%	93.9%	93.4%
Number on second line (millions)	0.15	0.19	0.26	0.34	0.42	0.52
Percent	4.9%	4.8%	5.2%	5.6%	6.1%	6.6%
Total (millions)	2.99	4.03	5.00	5.96	6.92	7.88

3.5. Forecasting Demand for Antiretroviral Medicines. Table 2 presents the resulting forecast quantities of antiretroviral products required globally, using the linear projection approach. The greatest variation in forecast demand is for stavudine, which ranges from a decrease in demand of 39% (from 2008 to 2012) with fast phase-out, to an increase of 27% with slower phase-out. For other antiretroviral molecules, the greatest annual increases are forecast for tenofovir and emtricitabine; followed by efavirenz, ritonavir, zidovudine, and lopinavir; lamivudine, atazanavir, and nevirapine.

Table 2: Demand for antiretroviral medicines for the linear projection with fast and slower phase-out of stavudine, 2008–2012 (person-years of antiretroviral therapy) (millions).

	Year												
	2008	2009			2010			2011			2012		
Molecule	Reported[a]	Fast d4T phase-out[a]	Slower d4T phase-out[a]	Difference between scenarios	Fast d4T phase-out[a]	Slower d4T phase-out[a]	Difference between scenarios	Fast d4T phase-out[a]	Slower d4T phase-out[a]	Difference between scenarios	Fast d4T phase-out[a]	Slower d4T phase-out[a]	Difference between scenarios
d4T	1.92	2.12	2.23	5%	2.00	2.41	20%	1.68	2.48	47%	1.18	2.43	105%
ZDV	1.26	1.67	1.60	−4%	2.15	1.92	−11%	2.68	2.23	−17%	3.26	2.56	−22%
3TC	3.23	4.03	4.00	−1%	4.73	4.70	−1%	5.40	5.36	−1%	6.06	5.96	−2%
NVP	2.00	2.34	2.30	−2%	2.70	2.61	−3%	3.00	2.85	−5%	3.26	3.00	−8%
EFV	1.15	1.72	1.72	0%	2.14	2.16	1%	2.59	2.64	2%	3.08	3.14	2%
ABC	0.05	0.06	0.07	12%	0.06	0.09	46%	0.05	0.11	115%	0.04	0.14	297%
ddI	0.08	0.09	0.10	9%	0.09	0.12	35%	0.07	0.14	95%	0.03	0.15	360%
IDV	0.01	0.01	0.01	36%	0.01	0.02	142%	0.01	0.03	345%	0.01	0.05	750%
LPV	0.18	0.22	0.22	0%	0.28	0.28	1%	0.34	0.35	3%	0.41	0.43	4%
TDF	0.21	0.54	0.50	−8%	1.02	0.89	−13%	1.63	1.38	−15%	2.37	1.99	−16%
FTC	0.10	0.25	0.24	−2%	0.38	0.36	−4%	0.55	0.51	−6%	0.74	0.69	−7%
NFV	0.01	0.01	0.01	−6%	0.01	0.01	−20%	0.01	0.00	−45%	0.00	0.00	−100%
RTV	0.16	0.22	0.22	2%	0.28	0.29	6%	0.34	0.38	10%	0.41	0.48	15%
SQV	0.00	0.00	0.00	69%	0.00	0.01	228%	0.00	0.01	448%	0.00	0.02	730%
ATV	0.03	0.03	0.03	8%	0.03	0.04	28%	0.04	0.06	54%	0.04	0.08	88%

[a] Units are millions of person-years of antiretroviral therapy.

4. Discussion

We forecast that an estimated 7.9 million people will be receiving antiretroviral therapy in low- and middle-income countries by 2012, based on the linear projection model. Taking into account the strengths and weaknesses of each method, the results of the linear projection are being used as the main basis for analysis and forecasting. Recent growth has been approximately linear and may be the best approach to extrapolate past trends over short periods of time. The other two scenarios are included to show the range of uncertainty around the projections (7.1–8.4 million).

An important strength of these projections is the inclusion of two unique sources of data: the latest WHO annual survey on antiretroviral use and a country target-setting consultation on future antiretroviral therapy coverage. Although conservative, these are still optimistic projections, since many countries would require formidable implementation efforts and additional funding to scale up treatment services at the pace observed in the recent past and to continue enrolling an estimated 83,000 new people on ART per month. However, treatment demand and the supply of funding to purchase treatment were found difficult to separate. Demand for ART in low-resource countries where donors provide the majority of ART funding may be more subject to the availability of funding from donor agencies than domestic constraints.

The projected estimates under the linear model suggest that coverage would grow by 2.9 million people in three years, representing a 60% increase over the number of people receiving ART by the end of 2012. However, this forecast must be viewed within the context of various factors likely to influence future demand: the global economic downturn, the delivery capacity of poor countries with the greatest HIV burden, and the implementation of the new WHO guidelines.

The global financial crisis is threatening countries' ability to sustain the progress made so far with respect to antiretroviral coverage, as well as efforts to attain universal access goals. It is therefore necessary to better understand the effect of the financial crisis on antiretroviral demand, its possible consequences, and what can be done to avoid negative impact. After years of significant increases for international AIDS assistance provided by the G8, European Commission, and other donor governments, funding remained essentially flat over the 2008-2009 period. Disbursements were $7.6 billion in 2009, compared to $7.7 billion in 2008. The observed decrease in disbursements between 2008 and 2009 is difficult to interpret, due to currency fluctuations and reporting cycle [9–11]. The global economic crisis is not the only obstacle and other donor priorities, such as global health, could likely impact the supply of aid for ART.

Some of the countries with the fastest growth in the number of people receiving ART are reaching high levels of coverage and may be nearing a saturation point of what they can deliver with their existing health care infrastructure. In some other cases, a saturation point may also be reached while high coverage remains to be attained. Growth in ART coverage in these countries can therefore be expected to slow down in the near future. The changes in the new WHO recommendations to initiate ART at a threshold of CD4 count of 350 cells/mm^3 will lower coverage levels in countries, especially those with very high burden of disease. The capacity of the health system to enroll new patients and to sustain patients on ART in the long term will also prove to be a central challenge.

The other factor influencing demand is the standardization of treatment and the application of clinical guidelines. The demand scenarios for antiretroviral medicines are based on the assumption that most low- and middle-income countries will progressively implement the new WHO treatment recommendations. Since the introduction of WHO ART guidelines in 2002 (and their subsequent updates in 2003 and 2006), successive AMDS surveys have shown an increasing rate of country compliance with WHO guidelines. Adoption of the new guidelines involves two key components: the duration of the transition period for countries to fully implement the new recommendations and the level of continued use of stavudine.

Treating the maximum possible number of new patients has been the priority for many governments and donors, resulting in increased demand for less expensive formulations (first-line stavudine-containing regimens procurement price dropped to between $64 and $122) [15]. One of the major recommendations of the 2010 WHO ART guidelines is to provide more durable, efficacious, and tolerable antiretroviral first-line regimens by substituting other options for stavudine-containing regimens. Many countries have already started to change their national guidelines or have entered a transition period to phase out stavudine in favour of zidovudine or tenofovir for people initiating first-line antiretroviral therapy.

A trend comparison across results from the 2006–2008 surveys for 17 countries confirms a decrease in the use of stavudine and an increase in the use of zidovudine and tenofovir. Countries may choose a longer transition period to phase out stavudine in order to reduce the cost burden of scaling up the number of people initiating antiretroviral therapy. Despite substantial reduction over the years, first-line regimens that include tenofovir or zidovudine cost up to three times more than stavudine-based regimens, and prices of second-line regimens are still high. The annual price per patient for first-line regimens ranges from $136 to $243 in low-income countries, and from $116 to $667 in lower-middle-income countries. For second-line regimens, the annual price per patient ranges from $572 to $803 in low-income countries and from $818 to $1545 in lower-middle-income countries. In upper-middle-income countries, annual prices of first-line regimens range from $161 to $1033, while second-line regimens range from $3393 to $3647 [15]. The transition period would most likely depend on the availability of resources over the next three years. Certain countries with large treatment volumes, such as Kenya, Nigeria, and South Africa, will continue to be instrumental in driving demand.

Opportunities for a faster phase-out include new prequalified formulations of fixed-dose combinations, possible

further price reductions for medicines, and available funding for antiretroviral treatment programmes. In addition, some countries are already seeking efficiency savings through better procurement of medicines and laboratory tests, to sustain and expand treatment programmes [16].

Given these factors, while we forecast an increasing-linear demand for antiretroviral medicines, its trajectory would more likely occur within the boundaries of the country-set targets and the restricted scenario. While the country-set target scenario uses data provided by the countries themselves, it may be over-optimistic if the economic crisis results in slower growth in international and national financing of antiretroviral therapy programmes. The resulting slower growth is difficult to estimate. If funding restrictions reduce the rate of growth by 25%, as per the restricted scenario, then 800,000 fewer people would be receiving antiretroviral therapy by 2012.

As in any other model, our projections have some limitations. First, the projections are based on a small number of past observation years. Second, while the WHO surveys are broadly representative of ART programmes operating across low- and middle-income countries, their general applicability requires careful consideration. This is especially true when extrapolating information from large country programmes that account for a high number of patients to countries with small-sized programmes operating in a wide variety of conditions. Programme size and other features of ART services that affect scale-up rates require further investigation. Future projections should address the barriers preventing countries from rapid scale-up and the implementation of standard guidelines.

Our results support the notion that the demand of ART in resource-limited settings will keep increasing and that not only will new patients be starting ART, but those in long-term care will also be shifted to less toxic antiretroviral drugs. Although the price of antiretroviral medicines has been the most important factor affecting their demand, government and donors are shifting to less toxic ARVs and bulk purchasing will eventually push prices down. It is expected that the use of more patient-friendly ARVs will be also associated with better retention rates and better overall outcomes [17, 18].

In conclusion, our demand estimates are still below the accelerated coverage required to reach universal access in resource-limited countries. Using classical methods of demand forecasting, we estimated the quantities of both antiretroviral medicines and the number of clients receiving treatment, based on historical demand. We believe this information to be relevant for informed demand planning. These forecasts should not downplay the fact that many people living with HIV would benefit from an accelerated scale-up of ART. This information should be supplemented with projections using different approaches and based on strategic targets. These reports should be made available to donors and governments as part of the continuing effort to provide information about the resources needed to reach universal access to HIV treatment and care.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgment

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Research Article

Outcomes of Universal Access to Antiretroviral Therapy (ART) in Georgia

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Since 2004, Georgia achieved universal access to free antiretroviral therapy (ART). A retrospective cohort study was conducted to evaluate the outcomes of Georgia's ART program. The study included adult patients enrolled in the ART program from 2004 through 2009. Of 752 patients, 76% were men, 60% were injection drug users (IDU), 59% had a history of an AIDS-defining illness, and 53% were coinfected with hepatitis C. The median baseline CD4 cell count was 141 cells/mm^3. During followup, 152 (20%) patients died, with the majority of deaths occurring within 12 months of ART initiation. Mortality was associated with advanced immunodeficiency or the presence of incurable disease at baseline. Among patients remaining on treatment, the median CD4 gain was 216 cell/mm^3 and 86% of patients had viral load <400 copies/ml at the last clinical visit. The Georgia ART program has been successful in treating injection drug users infected with HIV.

1. Introduction

The advent of highly active antiretroviral therapy in the mid 1990s fundamentally altered the natural history of HIV infection in industrialized countries, resulting in dramatic reduction in AIDS-related morbidity and mortality [1–3]. However, for many years access to HAART in low- and middle-income countries was limited, primarily due to the price of antiretroviral drugs that made them beyond the reach of most patients. The momentum generated by the World Health Organization's "3 by 5" strategy resulted in considerable progress in expanding access to lifesaving treatment in resource-limited countries [4]. Analyses of HIV treatment programs in low- and middle-income countries have already shown positive outcomes in terms of response to therapy and declining mortality [5–11].

Georgia, Armenia, and Azerbaijan make up a group of former Soviet republics known as the South Caucasus Republics. With the fall of the Soviet Union and the independence of all these republics in 1991, much of the social structure supporting health care became increasingly dysfunctional and the system of national healthcare that held a high standard for all Soviet citizens fell into disarray as economies crashed and conflicts within and between the countries disrupted services and infrastructure. Each one of these countries has its unique set of problems and issues, but, in general, their declining economic situations, coupled with rising drug use and commercial sex work, and their

geographic proximity to Russia and Ukraine, countries with emerging epidemics, make the South Caucasus a region ripe for the spread of HIV.

Georgia is located at the juncture of Eastern Europe and Western Asia and is bordered by Russia, Azerbaijan, Armenia, and Turkey. In 2009, the population of Georgia was estimated to be 4,615,807 in a territory of 26,900 sq miles. The GDP per capita in 2009 was approximately $4,300 US ranking 103 in the world, and the GINI coefficient was 40.8 ranking 59th in the world. Georgia regained independence in August of 1991, followed by a two-year civil war that led to large population migration with internal displacement, severe poverty, a dysfunctional economy, and disruption of many national services including healthcare. The country was stabilized in 1995, but the dire economic position of the Georgian government necessitated conversion of the state-funded health care system to a market-driven system. Nearly half of all Georgians are now forced to forego professional medical care when sick, opting instead for advice from friends and relatives, traditional medicine, or no care [12].

The first case of HIV in Georgia was reported in 1989. As of December 31, 2009 a cumulative 2,236 HIV cases had been reported. Among them 1,151 persons developed AIDS and 479 died. Males constitute the majority (74%), of the reported HIV/AIDS cases with injection drug use (IDU) responsible for HIV acquisition among nearly 60% of all reported cases. The estimated HIV prevalence in Georgia is less than 0.1%. Antiretroviral drugs have been available in the country since 1990s, but the access was limited only to those able to afford them. Since 2004, through support from the Global Fund, Georgia became the first country among the newly independent states (NIS) of Eastern Europe to achieve universal access to antiretroviral therapy (ART). This major achievement has been acknowledged in the joint WHI/UNAIDS/UNICEF report on universal access [4]. ART coverage estimation is based on standard WHO methodology using SPECTRUM projection software. According to this report, Georgia is among few low- and middle-income countries with highest attainable coverage. The objective of this study is to evaluate outcomes of Georgia's free ART program.

2. Methods

2.1. Study Design. A retrospective cohort study was conducted at the Infectious Diseases, AIDS and Clinical Immunology Research Center (IDACIRC), in Tbilisi, Georgia, which is the country's referral institution for HIV/AIDS diagnosis, treatment, and care. The study population included all adult (age $\geq$18 year) patients enrolled in the ART program from 2004 through September 30 2009, who started therapy for at least six month prior to April 1, 2010.

2.2. Description of the ART Program in Georgia. The National ART Program is coordinated by the IDACIRC, and antiretroviral drugs are dispensed in the IDACIRC clinic in the capital city of Tbilisi as well as at three affiliated regional centers in the cities of Kutaisi, Batumi, and Zugdidi. HIV infected persons are identified through state and donor funded HIV testing and counseling (HTC) services and screening programs. All persons with positive screening test results are referred to IDACIRC for confirmatory testing and if confirmed are initially assessed at IDACIRC. Patients have the option to continue clinical care either at central or regional levels.

Provision of therapy is governed by the National HIV/AIDS Treatment and Care guidelines developed based on the protocols of WHO as well as the guidelines of major Western countries [13–16]. The first guidelines were developed in 2004 and have been regularly updated thereafter. At the time that the patients included in this analysis were started on ART, treatment was recommended when the CD4 cell count was $\leq 200/mm^3$ or if the patient had an AIDS defining illness. ART was also recommended at CD4 cell count of $\leq 350/mm^3$, based on the CD4 cell decline rate, a high HIV-1 viral load, and coinfection with viral hepatitis. Currently, steps are being taken towards implementation of the recommendation to initiate treatment in all patients with CD4 cell count of $\leq 350/mm^3$.

Identification of patients in need of treatment is based on following those who don't qualify for therapy every 3-4 months and monitoring the CD4 cell count and HIV viral load. This allows for the timely identification of those in need of treatment.

The recommended initial regimen consists of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). A ritonavir (r) boosted protease inhibitor (PI) is recommended in cases when an NNRTI cannot be prescribed. Currently tenofovir (TDF) + emtricitabine (FTC), zidvudine (AZT), or abacavir (ABC) + lamivudine (3TC) are used for the NRTI component of initial regimen. Stavidine (d4T) is no longer recommended after the 2007 revision of National Guidelines; however the drug is reserved for short-term use for situations when AZT, ABC, or TDF cannot be used because of severe toxicity. Since 2008, patients are tested for HLA B $*$ 5701 before starting on an ABC-containing regimen. Efavirenz (EFV) is the preferred NNRTI with nevirapine (NVP) being recommended as an alternative to EFV.

Selection of subsequent regimens in treatment-experienced patients is based on the drug resistance profile with the goal of providing patients with at least two, and preferably three, fully active drugs. In addition to boosted PIs (ATV/r, DRV/r, FPV/r, LPV/r), new classes of drugs are also now available for highly treatment-experienced patients, such as the integrase strand transfer inhibitor raltegravir (RAL), the fusion inhibitor enfuvirtide (ENF), and the CCR5 antagonist maraviroc (MVC). Recently, the new NNRTI etravirine (ETV) has also become available.

As per the Georgian National guidelines, the standard of ART monitoring relies upon laboratory monitoring of CD4 count, HIV-1 viral load, and development of resistance based on a resistance-genotype detection when indicated. Virological failure is defined as confirmed plasma HIV-1RNA >400 copies/ml 6 months after starting therapy or plasma HIV-1 RNA >50 copies/ml 12 months after starting therapy in a patient who is on potent ART.

Georgia was the first NIS country to introduce genotypic resistance testing in 2005 into routine clinical practice. HIV drug resistance testing is used to guide treatment decisions during virologic failure and to make decisions as to the most effective subsequent regimen.

Special attention is paid to adherence to therapy as an important determinant of treatment success. A program to promote and maintain antiretroviral adherence has been developed that includes maintenance of an adherence diary, pill identification by shape and color, patient self-report about the medication intake in the preceding 3- to 7-day period and medication refill using pharmacy records. In addition, to improve adherence, mobile units to deliver home-based adherence support operate countrywide.

2.3. Laboratory Assays. Plasma HIV-1 RNA levels were initially measured using Amplicor HIV-1 Monitor test, version 1.5 (Roche Molecular Diagnostics, Germany), with lower limit of detection of 400 copies/ml. Since 2006, the real-time PCR assay COBAS TaqMan HIV-1 test (Roche Molecular Diagnostics, Germany) has been in use with a lower limit of detection of 40 copies/ml.

Determination of CD4+ cell count is based on the single-platform immunophenotyping technique using the FACSCalibur flow cytometer (Becton-Dickinson, USA) with four-color direct immunofluorescence reagent MultiTEST CD3/CD8/CD45/CD4.

For genotypic resistance testing, the TruGene HIV-1 Genotyping Kit was employed according to the manufacturer's instructions using OpenGene DNA Sequencing System (Siemens Medical Solutions Diagnostics, Germany). The Guidelines Rules version 14.0 and Stanford University algorithm (http://hivdb.stanford.edu/) were used for resistance interpretation. Mutations listed by the International AIDS Society-USA Panel were considered [17].

2.4. Statistical Analysis. Data were obtained from the National HIV/AIDS electronic database, operated by IDACIRC. The database contains information on all reported HIV cases, including demographic, epidemiological, clinical, and laboratory data. Information on all patients initi-ating HAART from 2004 through 2009 was extracted. Observations were censored as of April 1, 2010. Descriptive statistics were performed to assess distribution of covariates. Normality of continuous variables was evaluated using Q-Q plots. Kaplan-Meier product-limit estimator method was used to assess probability of survival and probability of virological failure. Predictors of mortality were evaluated in multivariate Cox proportional hazards model. The proportional hazards assumption was tested and was met for the final model. All tests were two-sided at a significance level of 0.05. Statistical analyses were performed using SAS v 9.2 (SAS Institute, Cary, NC, USA).

2.5. Ethical Approval. Study was approved by Institutional Review Board (IRB) of the Infectious Diseases, AIDS and Clinical Immunology Research Center. The study was based on information routinely collected as part of the standard of clinical care of HIV infected individuals.

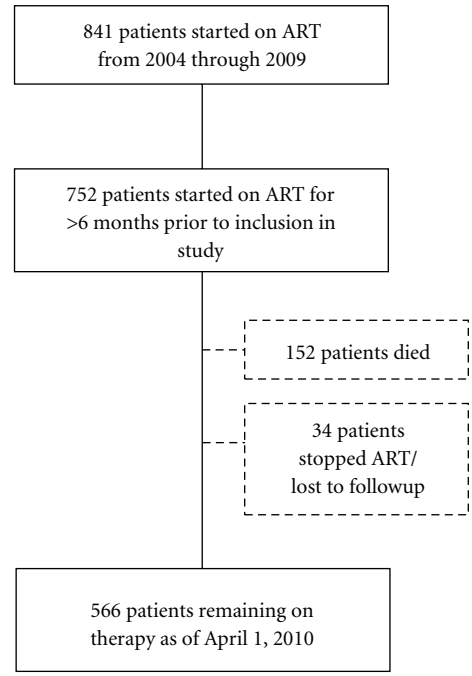

Figure 1: Cohort profile.

3. Results

Since 2004, over 1,800 patients have been seen for HIV clinical care at the IDACIRC and of these, 841 adults met the treatment initiation criteria and were enrolled in the HAART program (through December 2009). This analysis includes 752 adult patients who had started therapy at least six months prior to inclusion in the study (Figure 1).

Table 1 summarizes the baseline characteristics of these 752 patients. Their median age was 37 years (Interquartile range [IQR] 33–43), and 75% were men. The most common mode of HIV transmission was injection drug use (IDU)-60%, followed by heterosexual contact (35%). The median CD4 cell count at treatment initiation was 141 cells/mm^3 (IQR 76–208), with approximately one third of patients having a CD4 cell count less than 100 cells/mm^3. Median viral load was 5.4 $\log_{10}$ copies per ml (IQR 4.8–5.8). Fifty-nine percent of patients had a history of an AIDS defining illness (ADI), with 32.4% having a history of active tuberculosis. More than half of the patients had antibodies against Hepatitis C virus (HCV), 8% of patients had evidence of chronic Hepatitis B infection, and 6% had dual infection with HCV and HBV. Fourteen percent of patients had cirrhosis. Almost 3% of patients had a malignancy.

All but 5 patients were started on an NNRTI-based regimen, most frequently with EFV (82.9%). AZT + 3TC was the most common NRTI component of the first ART regimen (53.0%), followed by ABC + 3TC (27.2%) and d4T + 3TC (18.3%). After the 2007 revision of the national HIV/AIDS Treatment and Care guidelines, all patients on d4T were switched to AZT, ABC, or TDF.

The median duration of followup was 24 months (IQR 10–45 months). During followup, 152 (20.2%) patients died

Table 1: Baseline characteristics.

Characteristic	$n = 752$
Age, median years (IQR)	37 (33–43)
Gender, *n* (%)	
Male	570 (75.8)
Female	182 (24.2)
Mode of transmission, *n* (%)	
Injection drug use	454 (60.4)
Heterosexual contact	265 (35.2)
Male-to-male sex	17 (2.3)
Blood recipient	6 (0.8)
Other/not identified	10 (1.3)
CD4 cell count, median cells/mm^3 (IQR)	141 (76–208)
HIV RNA load, median $\log_{10}$ copies/ml (IQR)	5.4 (4.8–5.8)
AIDS defining illness, *n* (%)	446 (59.3)
Malignancy, *n* (%)	22 (2.9)
Liver related diseases, *n* (%)	
Anti-HCV+	397 (52.8)
HbsAg+	63 (8.4)
Anti-HCV+/HbsAg+	47 (6.3)
Cirrhosis	104 (13.8)
Initial HAART regimen, *n* (%)	
AZT + 3TC + EFV	310 (41.2)
AZT + 3TC + NVP	86 (11.4)
AZT + 3TC + LPV/r	3 (0.4)
ABC + 3TC + EFV	187 (24.9)
ABC + 3TC + NVP	15 (2.0)
ABC + 3TC + LPV/r	2 (0.3)
TDF + FTC + EFV	9 (1.2)
TDF + FTC + NVP	2 (0.3)
d4T + 3TC + EFV	126 (16.7)
d4T + 3TC + NVP	12 (1.6)

Table 2: Cox proportional hazards model analysis of factors associated with death.

	Univariate analysis, HR (95% CI)	Multivariate analysis, HR (95% CI)
Age		
<35 years	1	
>35 years	1.52 (1.07–2.16)	NS
Gender		
Female	1	1
Male	2.14 (1.38–2.32)	1.96 (1.19–3.24)
Mode		
Non-IDU	1	
IDU	1.99 (1.39–2.86)	NS
CD4		
>100 cells/mm^3	1	1
<100 cells/mm^3	2.50 (1.82–3.45)	2.06 (1.48–2.87)
Viral load		
<100,000 copies/ml	1	
>100,000 copies/ml	1.67 (1.15–2.45)	NS
AIDS defining illness		
No	1	1
Yes	2.69 (1.90–3.81)	2.01 (1.36–2.96)
Anti-HCV		
Negative	1	
Positive	1.40 (1.01–1.93)	NS
HbsAg		
Negative	1	
Positive	1.77 (1.10–2.83)	NS
Cirrhosis		
No	1	1
Yes	2.05 (1.24–3.40)	1.95 (1.36–2.81)

HR = Hazard ratio, CI = Confidence interval, NS = Not significant.

and 34 (4.5%) patients self-discontinued ART/were lost to follow-up, with overall retention rate of 84%.

Kaplan-Meier estimates of survival probability were 0.84 (95% CI: 0.82–0.87), 0.80 (95% CI: 0.77–0.83), 0.78 (95% CI: 0.75–0.81), and 0.77 (95% CI: 0.74–0.80) at 12, 24, 36, and 48 months, respectively (Figure 2). Of 152 patients, 115 (75%) died within 12 months of HAART initiation. Median time to death was 3 months (IQR 1–10). Most common causes of death were tuberculosis (34 cases, 22%) and end stage liver disease (29 cases, 19%). Eleven percent of patients died due to incurable malignancies at baseline, including Kaposi's sarcoma, HIV-related lymphomas, invasive cervical cancer, lung cancer, and breast cancer. Other causes of death included cryptococcal meningitis, cardiovascular diseases, wasting syndrome, and infectious diseases of unknown origin.

Factors associated with mortality were assessed in a multivariate Cox proportional hazards model. The following baseline factors were associated with death: male gender (Hazard ratio [HR] 1.96, 95% CI 1.19–3.24), CD4 cell count <100 cells/mm^3 (HR 2.06, 95% CI 1.48–2.87), history of an AIDS-defining illness (HR 2.01, 95% CI 1.36–2.96), cirrhosis (HR 1.95, 95% CI 1.36–2.81). Other covariates did not show statistical significance in multivariate analysis (Table 2). Presence of active TB was fitted into the multivariate model independently from other ADIs; it showed only active TB to be marginally significant with an HR of 1.40 (95% CI 1.01–1.97).

Among 566 patients still on ART, the median gain of CD4 cells was 216 cells/mm^3 (IQR 112–348) and 487 (86%) patients had an HIV-1 viral load measurement of <400 copies/ml at their last clinical visit. Data on medication refill adherence were available starting from 2007; the refill adherence rates were 85% in 2007, 83% in 2008, and 92% in 2009.

Eighty-two patients (10.9%) experienced virological failure. All of them have been tested for the presence of drug resistance virus while on a failing regimen. Median time to virologic failure was 16 months (IQR: 10–28 months). Kaplan-Meier probability of failure at 12, 24, 36, and

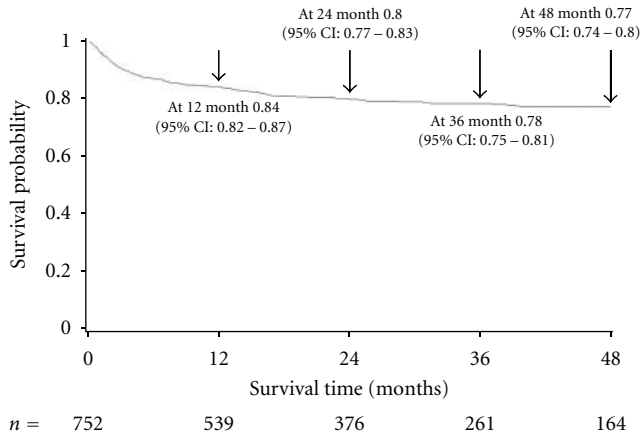

Figure 2: Kaplan-Meier curve of survival probability.

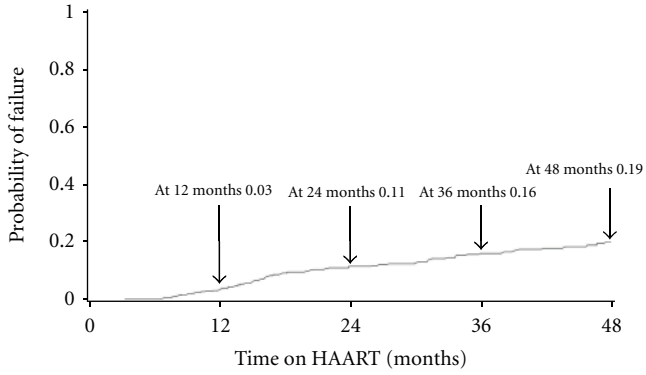

Figure 3: Kaplan-Meier curve of virological failure probability.

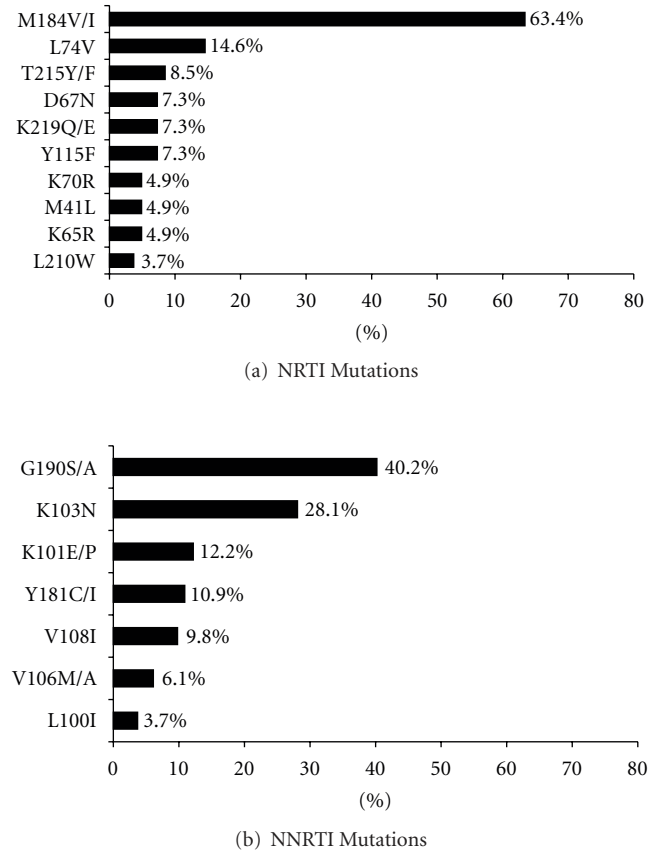

Figure 4: Frequency of resistant mutations in reverse transcriptase gene.

48 months were 0.04, 0.11, 0.16, and 0.19, respectively (Figure 3). Of 82 patients, 65 (79.3%) had mutations consistent with antiretroviral resistance. Resistance to a single drug class (NRTI or NNRTI) was found in 4 (4.9%) patients; dual-class drug resistance to NRTI and NNRTI occurred in 61 (74.4%) patients.

The frequency of drug resistance mutations in the reverse-transcriptase (RT) gene is shown in Figure 4. The most commonly detected NRTI mutation was M184V/I (63.4%). The frequency of thymidine analogue mutation (TAM) (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) was relatively low, with only 14 (17.1%) patients having virus with any TAM. Only five (6.1%) patients had viruses with ≥3 TAMs. G190S/A was the most frequent NNRTI mutation (40.2%), followed by K103N (28.1%). No major PI mutations were detected.

All patients with drug resistant viruses were switched to second line regimens with boosted PIs. At present, of 56 patients remaining on second-line therapy, 61%, 21%, 11%, and 7% receive LPV/r, ATV/r, DRV/r, and FPV/r based regimens respectively. Three patients are on salvage regimens, consisting of ENF plus optimized background therapy.

4. Discussion

We report the outcomes of the universal ART access program in Georgia. The program builds upon the successful training of young clinicians and scientists who started in their training in the care of HIV/AIDS patients in the 1990s with the goal of providing high quality clinical care in an environment that promotes research. The capacity to provide universal access to ART has been further strengthened through the support of the Global Fund. Under this framework, the approach to ART provision taken by Georgia has been designed to maximize effectiveness of the intervention.

The IDACIRC mandate—as the lead agency for the provision of all HIV care activities in the country—ensures universal access and high retention on therapy, with less than 5% drop-out rate. In addition, a coordinated approach to ART monitoring, including availability of laboratory monitoring (CD4 cell counts, HIV-1 viral load and HIV genotypic resistance testing), and adherence counseling contributes to the achievement of optimal outcomes. Establishment of mobile units for providing home-based adherence support and monitoring resulted in improvement of medication refill adherence from 83% in 2008 to 92% in 2009. Good immunological and virologic responses seen in our cohort also serve as an evidence of good overall adherence.

The probability of virological failure in our study was similar to that reported from developed countries [18, 19]. In contrast to most other resource-limited countries, where treatment monitoring is limited to clinical findings and changes in CD4 cell counts [20], viral load and drug resistance testing are performed in Georgia as part of the standard of care. Earlier, we have shown feasibility and effectiveness of routine use of these laboratory tools in early identification of patient failing on ART and in improving clinical outcomes in patients with drug resistant viruses [21]. Our current analysis corroborates previous findings: overall the small number of mutations and low frequency of TAMs is suggestive of shorter exposures to failing regimens among our patients, thus preventing the opportunity for mutations to accumulate.

Interestingly, the most common NNRTI mutation detected in our study was G190S/A but not K103N. It is possible that G190S/A is the favoured NNRTI mutation in HIV subtype A1—which is the most common circulating strain in Georgia [22]. Further investigation of clade-specific resistance pathways is needed to provide more detailed picture, which may have important implications for the clinical management of infection with HIV subtype A1.

An important challenge that remains to be addressed and that has been identified through this study is the early mortality of patients who start ART in Georgia. As shown in our analysis, the highest mortality rate was observed within the first year of ART initiation, with 75% of all deaths occurring in the first 12 months. Our results are consistent with findings from other resource-constrained countries reporting increased risk of mortality early after starting ART [23–26]. In our study, the majority of deaths were due to either advanced HIV disease, as evidenced by severe immunodeficiency due to the history of an ADI or the presence of incurable non-AIDS defining conditions. At present a large proportion of persons in Georgia continue to enter health care very late in the course of their chronic HIV infection, often resulting from missed opportunities to test for HIV in healthcare settings. This is particularly evident among male IDUs, whose HIV disease is further complicated by other comorbidities, such as hepatitis C. This also explains the twofold increased risk of dying in men compared to women. Expanding HTC services, especially among most-at-risk populations, is the key for ensuring earlier HIV diagnosis and treatment initiation.

The most common causes of death in our cohort were TB and end stage liver disease, together accounting for more than 40% of all deaths. Multivariate analysis showed a strong association between cirrhosis and death, emphasizing the problem of coinfection with viral hepatitis, especially with hepatitis C. Dually infected patients virtually have no access to anti-HCV therapy because of prohibitively high costs. Due to this fact, HIV/HCV coinfected patients die from hepatic disease despite the successful antiretroviral therapy. The high prevalence of coinfection with HCV among patients with HIV in Georgia may prevent the full realization of the benefits of ART, and may compromise the cost-effectiveness of ART. Recent studies have shown that the availability of ART does not decrease the risk of mortality among patients with a dual infection with HIV and HCV [27]. Moreover, HIV/HCV coinfected IDUs have been shown to be at 7-fold increased risk of dying from end stage liver disease compared to HCV monoinfected IDUs [28].

Another important challenge is HIV/TB coinfection. The high prevalence of this coinfection in our cohort was not a surprise given the overall high burden of TB in the country [29]. However, the high mortality from TB is of particular concern. Collaboration between the HIV/AIDS and TB services in Georgia is excellent, and all patients have free access to both TB treatment and ART. However, it is clear that the outcomes are not as good as we would have predicted. One possible challenge is limited information on TB drug susceptibility as these data were only available for very few patients. Taking into account the high prevalence of multidrug resistant TB in Georgia (7% in newly diagnosed and 27% in previously treated patients [30]), TB drug resistance may have contributed to excess death from TB similar to that experienced in other countries [31].

As with all studies, limitations should be mentioned. First, the study was based on data available in national HIV/AIDS database. Adverse events of ART are carefully monitored, the data are not entered into electronic database, and therefore we were not able to determine contribution of adverse events to outcomes of interest. A further limitation is that the only baseline information on drug abuse was available and this precluded us from assessing the impact of current drug use on outcomes.

In summary, the HIV epidemic in Georgia has entered a new phase. ART has been successfully introduced in Georgia, including in IDU population. The next stage is to maximize and sustain the benefits of ART. Mortality can be substantially reduced by improving earlier HIV diagnosis and initiation of ART. The comprehensive program for the identification and treatment of patients with HIV/AIDS in Georgia, a relatively low income country, has been quite successful in limiting premature mortality and morbidity from this epidemic disease. Increased efforts and new strategies are needed to effectively address the intersecting HIV/TB and HIV/HCV epidemics and their resulting mortality.

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Review Article

Antiretroviral Therapy for HIV-2 Infection: Recommendations for Management in Low-Resource Settings

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HIV-2 contributes approximately a third to the prevalence of HIV in West Africa and is present in significant amounts in several low-income countries outside of West Africa with historical ties to Portugal. It complicates HIV diagnosis, requiring more expensive and technically demanding testing algorithms. Natural polymorphisms and patterns in the development of resistance to antiretrovirals are reviewed, along with their implications for antiretroviral therapy. Nonnucleoside reverse transcriptase inhibitors, crucial in standard first-line regimens for HIV-1 in many low-income settings, have no effect on HIV-2. Nucleoside analogues alone are not sufficiently potent enough to achieve durable virologic control. Some protease inhibitors, in particular those without ritonavir boosting, are not sufficiently effective against HIV-2. Following review of the available evidence and taking the structure and challenges of antiretroviral care in West Africa into consideration, the authors make recommendations and highlight the needs of special populations.

1. Introduction

HIV-2 represents a distinct lineage of HIV, stemming from SIVsm instead of the SIVcpz responsible for HIV-1. Like HIV-1 it appears to have made the transition to humans more than once, giving rise to eight distinct groups, of which groups A and B account for nearly all of the cases identified thus far [1]. HIV-2 differs from HIV-1 most strikingly in its lower rate of progression and infectivity, with the majority of those infected likely to be long-term nonprogressors [2–4]. Those with progressive disease experience the same likelihood of morbidity and mortality as are seen with HIV-1 [5, 6]. People with advanced HIV-2 infection require treatment with antiretroviral therapy (ART), but most individual antiretroviral drugs and regimens have been designed and optimized for HIV-1 and cannot be assumed to provide optimal viral suppression for HIV-2 infection. In some instances, antiretroviral susceptibility differs significantly between HIV-1 and HIV-2, such that HIV-2 is intrinsically resistant to two of the major classes of antiretroviral drugs: the fusion inhibitors and the nonnucleoside reverse transcriptase inhibitor- (NNRTI-) based regimens that are the standard therapy for HIV-1 in West Africa [7, 8].

The challenge of treating HIV-2 infection falls mainly upon West Africa [6], with current prevalence estimates ranging up to 1% where reported, compared with HIV-1 prevalence rates of up to 3.4%, therefore comprising a substantial portion of all HIV infections in the subregion [9]. The exception to this is Guinea-Bissau, where the prevalence amongst adults was estimated to be 8%–10% two decades ago [10]. This has now changed to a current prevalence of around 4%, compared to an HIV-1 prevalence of 2.9% in rural areas and 4.2% in urban areas [11–13]. European countries with colonial links to West Africa such as Portugal, France, and the United Kingdom, as well as other countries with prior Portuguese ties, such as Angola, Brazil, India, and Mozambique, also have sizeable cohorts of HIV-2 infected individuals [14–18]. Although the absolute numbers of patients infected with HIV-2 in European cohorts are small, the earlier availability of ART in these countries has provided

some data to guide treatment recommendations in resource-poor settings.

Given the prevalence of HIV-2 in West Africa, it is imperative that up-to-date recommendations be available for the antiretroviral management of HIV-2 in these clinical settings, characterized by the use of standardized first-, and in some cases second-line regimens based on limited formularies, with treatment decisions driven by protocol, that are also highly sensitive to cost. At the time of writing, therapeutic drug monitoring, viral load measurement, and genotypic resistance testing are not routinely available in West Africa, nor are coreceptor tropism assays or HLA typing (to guide the safe use of CCR5 receptor blockers or abacavir, resp.). The monitoring and care of HIV in sub-Saharan Africa has, however, been a litany of barriers brought down, and the "impossible" becomes the standard, so these recommendations seek to strike a balance between optimal and current management trends.

Clinical trials of ART in HIV-2 are few compared with HIV-1, primarily because of HIV-2's lower prevalence and virulence, not to mention its concentration among some of the world's poorest people. Until there is better evidence from randomized controlled trials, judgment of what constitutes good care in HIV-2 management must therefore rely on both *in vitro* as well as *in vivo* data from small cohort studies and case series, theoretical assertions, and parallels with HIV-1 therapeutics.

As will be apparent to experienced clinicians and program officers, numerous potential factors have been left out of this work that might influence program-level decisions about ART for HIV-2 in West Africa. This is especially true where such factors affect both HIV-1 and HIV-2 infections in the same way. The current work is not intended as an exhaustive review of all aspects of a public health approach to the use of ART, nor is it intended to function as an ART primer. However, in the absence of universally accepted treatment guidelines for HIV-2, the authors seek to provide their own recommendations, based on the available literature, HIV-2 treatment meetings, discussions with colleagues from major HIV-2 treatment centers in Europe and Africa, and from personal experiences between 2003–2010 at the Genito-Urinary Medicine clinic at the MRC Laboratories in The Gambia, where ART was provided to HIV-2 infected people.

2. Selecting First- and Second-Line ART Regimes in HIV-2

2.1. Natural Polymorphisms and Patterns of Genotypic Resistance in HIV-2. The most crucial difference between HIV-1 and HIV-2 when considering suitable ART regimes is the lack of susceptibility of the latter to what would now be called first-generation NNRTIs, nevirapine, and efavirenz [19, 20]. The natural resistance of HIV-2 to these drugs is due to differences in the amino acid residues that make contact with the NNRTI in the binding pocket of HIV-1 and HIV-2, particularly the Y181I and Y188L natural polymorphisms seen in HIV-2, which significantly reduce NNRTI binding [7]. It is worth noting that HIV-1 mutations at these positions result in complete resistance to NNRTIs [20, 21]. Although etravirine is reported to have more activity against HIV-2 than previous NNRTIs, the presence of L181 and other structural differences in the HIV-2 NNRTI-pocket makes HIV-2 naturally resistant to etravirine as well (reviewed in [22, 23]).

Nucleos(t)ide reverse transcriptase inhibitors (NRTIs) have a similar potency in both HIV-1 and HIV-2. Earlier *in vitro* work demonstrating lower potency for zidovudine (AZT) [24] appeared to be an artifact of the assay used, and more recent work demonstrates similar potency in both HIV-2 and HIV-1 [25]. The development of NRTI resistance in HIV-2 shares many parallels with that in HIV-1, although key differences are worth highlighting that are of clinical relevance. The M184V mutation occurs rapidly, both *in vitro* [26] and *in vivo* [27] in approximately 83% of patients failing a lamivudine (3TC)-containing regimen [28, 29]. As in HIV-1, it is associated with high-level phenotypic resistance to 3TC and emtricitabine (FTC) in HIV-2-infected individuals [30]. Although HIV-1 and HIV-2 share some classic NRTI resistance patterns, the preference for alternative resistance pathways has been noted, in addition to unique resistance patterns in HIV-2. AZT resistance in HIV-1 occurs via two well-documented pathways. The most common and preferred pathway is marked by the accumulation of the six thymidine-associated mutations (TAMs): M41L, D67N, K70R, L210W, T215Y, and K219Q/E [31]. The less common pathway is via the Q151M mutation, which also tends to develop later. The TAM mutations are conspicuously absent in the AZT resistance profiles of HIV-2 patients [27, 28, 32–35]. In place of TAMs, AZT resistance in HIV-2 often involves the Q151M mutation, which occurs faster and with a much higher frequency and potency than in HIV-1 [30, 36]. Considering that this mutation causes multi-NRTI resistance in HIV-2, its high frequency raises real concerns [29, 37]. Unlike HIV-1 where K65R leads to TDF, ABC, ddI, and d4T resistance [38], K65R in HIV-2 does not cause phenotypic resistance to TDF, but causes high-level resistance to 3TC and FTC, and low-level resistance to ddI [30]. Current evidence suggests that this mutation rarely occurs in HIV-2 except during suboptimal mono- or dual-therapy with NRTIs that mostly do not include TDF [27, 39, 40], where it is often associated with the Q151M mutation. There is, however, limited experience with widespread use of TDF containing regimes in first-line HIV-2 ART, and firm conclusions on a reduced frequency of K65R in HIV-2 infection cannot be drawn with confidence. Of note, in a recent Senegalese study, two (of 23) patients exhibited K65R mutations at follow-up while on an AZT/3TC/indinavir (IDV) regimen, although in one case the mutation was present at baseline prior to commencing ART [35]. These data together with the phenotypic data by Smith et al. [30] indicate that K65R arises primarily due to 3TC/FTC pressure in HIV-2. The potential fragility of currently available NRTI backbones for use in HIV-2 therapy is highlighted by the finding that Q151M combined with K65R or M184V results in high-level AZT and 3TC resistance, whereas the presence of all three mutations in combination confers class-wide NRTI resistance, although it should be noted that these mutations

result only in low level resistance (4-5 fold) to d4T and TDF [30]. Lastly, the L74V mutation is rarely documented in HIV-2 [41], with one report of L74I in an HIV-2-infected patient on dual therapy (which included ddI) [40].

HIV-1 and HIV-2 proteases have an amino acid sequence similarity of about 50%, substantially less than that observed in their reverse transcriptase enzymes. These sequence differences are reflected in very distinct natural polymorphisms in the HIV-1 and HIV-2 proteases, most of which occur outside the functionally relevant areas [42]. Several HIV-2 natural polymorphisms correspond to drug resistance mutations in HIV-1. These include the major drug resistance mutation M46I, conferring resistance to indinavir (IDV), and several minor mutations, L10V, V32I, M36I, I47V, A71V, and G73A, that may decrease the activity of nelfinavir (NFV) and amprenavir (APV) [32, 43–46]. Several *in vitro* cultural and cell-free assays using individual PIs have suggested that while IDV, saquinavir (SQV), lopinavir (LPV), darunavir (DRV), and tipranavir (TPV) may exert full activity against wild-type HIV-2 [47–52], NFV and APV show a significant reduction in activity [46, 53]. A more in-depth study (kinetic inhibition assays) has shown that LPV, SQV, TPV, and DRV exhibit the highest potency in this order and that atazanavir (ATV), NFV, and APV show the lowest potency, respectively [54]. The data on TPV are however controversial, with other studies showing several fold lower potency when compared to LPV, SQV, and DRV [55, 56]. Once protease inhibitor-(PI-) based ART starts, this background of minor mutations may result in rapid acquisition of a multi-PI resistance phenotype [45, 46].

In HIV-1, PI resistance is associated with the accumulation of four or more resistance mutations in the protease gene, though major mutations can cause substantial resistance on their own [57]. HIV-1 and HIV-2 have similar PI resistance mutations [33, 43, 45, 46, 53], with a few mutations unique to HIV-2 [20, 45, 46]. The presence of certain natural polymorphisms in HIV-2 can reduce the time to resistance in some cases [34, 46]. For instance, I47A and V32I are associated with high-level resistance to LPV/ritonavir (LPV/r) in HIV-1 [58–60], and V47A is associated with phenotypic resistance to LPV/r in HIV-2 [61]. In HIV-1, the emergence of the LPV/r mutation I47A is a two-step process (I → V → A), whereas in HIV-2 it can occur in a single step from V → A [61]. In addition, V321 is present naturally in HIV-2. Therefore while LPV/r resistance in HIV-1 requires the acquisition of V32I and a two-step process to acquire I47A, only a one-step change in HIV-2 is required, making the development of this mutation easier and faster in HIV-2 [34].

2.2. Potential Options for Standardized First- and Second-Line Regimes in Resource Poor Settings. Until recently, most studies reporting antiretroviral use in HIV-2 patients were from European cohorts, and often involved mono- or dual-therapy and multiple heterogeneous regimens [28, 32, 33, 40, 62]. Due to the recent availability of ART in West Africa, data from the use of standardized first-line ART regimens in these cohorts are now appearing [34, 35, 63], although the numbers are still relatively small when compared to the HIV-1 literature. Given the lack of utility of NNRTIs in HIV-2, a key issue in choosing first-line ART regimes in HIV-2 infection is the question of whether triple NRTI regimens are a viable, safe, and efficacious option. The appeal of this approach lies in its lower pill burdens and reservation of PIs for second-line therapy, maintaining parallels with HIV-1 protocols. Prior to the development of a heat stable formulation of ritonavir, and in settings where this is not yet available, cold chain requirements also argue for a PI-sparing regimen. Unfortunately studies to date suggest that these regimes, including those with TDF, perform poorly in HIV-2 [28, 62, 64] and in our opinion should be avoided, although in certain specific circumstances they may represent the best balance of risk and benefit (see special populations, below). One case of a patient achieving viral suppression on a quadruple NRTI regimen (d4T/3TC/ABC/TDF) has been reported [62], although clearly more evidence is needed to conclude that such a regimen is superior to using triple NRTIs in HIV-2. The principal challenge of the PI-sparing nucleoside regimens in HIV-2 is the rapid development of the Q151M pan-NRTI resistance mutation [36]. Unlike the case in HIV-1 where this typically arises only after multiple other resistance mutations have developed, in HIV-2 it is one of the earliest and most common NRTI mutations (after those at the M184 locus), especially after mono-/dual-/triple-NRTI treatment [28, 32, 33, 40, 62] and compromises the entire regimen [36]. Triple nucleotide regimes containing ABC (in the absence of TDF) have also been shown to rapidly select for K65R in HIV-2 patients [64].

Our experience at the MRC Gambia [34] and that of others [62, 65] suggest that the combination regimen of AZT/3TC and LPV/r has a reasonable chance of success as a first-line regime for HIV-2 infection [34, 62, 65]. The use of an AZT/3TC backbone with unboosted IDV, however, has been shown to result in a high proportion of ART failures and accumulation of resistance mutations in a Senegalese cohort [35, 56]. ABC/3TC, TDF/FTC, and ddI-based regimens have the advantage of daily dosing and show potential for success, although in our opinion there is currently insufficient experience with those combinations in HIV-2 to draw firm conclusions. Moreover the inclusion of appropriate PIs in HIV-2 regimes will necessitate twice daily dosing in most circumstances, reducing the benefit of once-daily nucleoside analogue dosing. Didanosine also has a rather unique set of advantages and disadvantages as part of ART regimes. It should generally be taken on an empty stomach while other antiretrovirals, in particular TDF, should be taken with food, adding to regimen complexity. We believe ddI is less well tolerated than ABC, AZT, TDF, or 3TC and that this could threaten patient adherence to the overall regimen. Its use *with* TDF is relatively contraindicated because of the negative impact this combination has on CD4 cell counts and the increased risk of viral failure [66], even at the appropriate 250 mg dose [64]. Although no head-to-head comparisons have been performed in HIV-2-infected individuals, the HIV-1 literature suggests that an NRTI backbone of TDF/FTC (or 3TC) may, on the grounds of efficacy and tolerability, be a better choice than AZT/3TC [67, 68] or ABC/3TC [69, 70]. On that

basis, TDF may be desirable in first-line treatment in spite of its greater cost when compared with AZT (see Table 2), although the low yet measurable risk of renal toxicity with TDF use, particularly in settings where renal monitoring may be limited, is grounds for concern [71]. Tolerability issues should also be considered. If a patient does not tolerate AZT in first-line treatment other alternatives (including d4T) could be used, following the same protocols as are used for AZT intolerance in HIV-1-infected individuals; however an equivalent substitute for TDF in the face of resistant virus is not easy to find in the event of TDF intolerance. Given that the prevalence of HLA B*5701 is low in black African individuals [72], with HLA*B5703 being the only B57 subtype found in populations in Guinea-Bissau [73], the risk of ABC hypersensitivity, if ABC/3TC is used first line without the ability to determine HLA type, may not be of great concern in sub-Saharan Africa.

As mentioned above, PI options are constrained in HIV-2 as a result of natural polymorphisms that support PI resistance. In addition, unboosted PI regimens should be avoided as they tend to perform poorly [29, 35, 40, 56]. While good clinical outcomes with LPV/r have been observed [34, 65], *in vitro* data [54] suggests that SQV/r would be a reasonable first-line PI [54] too, while IDV/r may also be effective [28]. DRV/r would appear to be reliable based on *in vitro* data, although at present there is insufficient data to justify its use as the preferred first-line PI for HIV-2 given its higher cost (see below). Boosted ATV cannot be recommended in HIV-2 [54, 55], and given the conflicting results on the use of boosted TPV [54–56], it also cannot be recommended for use until further studies confirm its efficacy.

Second-line therapy should be considered in drafting treatment guidelines for first-line ART, as initial regimen choices narrow later treatment options. In the absence of viral load monitoring, resistance should be anticipated at the time of regimen change, and we make the assumption that resistance test results will not generally be available. Two fundamentally different strategies in ART are to increase potency up-front in order to minimize failure rates, or to hold potent antiretrovirals in reserve in order to mitigate the impact of failure of the first-line. Knowledge of typical mutations selected for during failure allows one to optimize sequential treatment, although HIV-2 is much less well studied in terms of the frequency with which various sequential regimens select for resistance mutations.

With regards to NRTIs, extensive resistance should be assumed to include the Q151M, K65R, and M184V, depending on the NRTIs employed in the first-line ART. While ABC is probably an option where only the Q151M mutation is present, it would be compromised in the presence of K65R and M184V [30]. Older NRTIs including AZT and ddI are not likely to have much residual potency in the face of these mutations; however TDF and d4T might retain sufficient potency in this setting [30]. The argument that the M184V mutation carries a substantial fitness cost has not been demonstrated as clearly in HIV-2 as it has in HIV-1 [75], nonetheless as it occurs in the highly conserved YMDD motif within the reverse transcriptase's active site [76], we believe it is likely to affect fitness similarly, and we recommend continued exposure to 3TC or FTC in order to maintain the M184V.

Based on the resistance data described earlier, recommended first-line boosted PIs for HIV-2 in resource limited settings are LPV/r, SQV/r, and possibly IDV/r. It appears that HIV-2 V47A mutants, selected for by failure on a LPV/r regimen, retain susceptibility to other PIs and are in fact hypersusceptible to ATV and SQV [61]. We find that this makes SQV/r an attractive choice for second-line therapy to follow up LPV/r-based ART in HIV-2.

Given the more limited range of effective antiretrovirals, both biologically and as a consequence of HIV-2's disproportionate prevalence in the resource-limited settings of West Africa, second-line treatment in HIV-2 becomes markedly challenging. Going back to the broader question of strategy, we support a boosted PI in the first regimen because we believe that failure rates on triple NRTI regimens are unacceptable. Nonetheless we recommend TDF be held in reserve to lend potency to second-line treatment.

2.3. HIV-1/HIV-2 Dual Infection. Co-infection with both HIV-1 and HIV-2 occurs in countries where both viruses circulate. Although progression, as implicated by higher viral loads, is driven by HIV-1 in the majority of dually infected individuals [77], this is not always the case [34]. Treatment of dually infected individuals should be carried out using an HIV-2 regimen, to ensure that the drugs used can effectively treat both viruses [34, 78]. Given that the HIV-2 plasma viral load is usually undetectable or low in dual infections, it might seem reasonable to treat and monitor only HIV-1 (discussed in [79]). In our opinion this represents a dangerous strategy, as even with an undetectable baseline HIV-2 VL, the risk exists that as HIV-1 is controlled and CD4+ T-cell targets expand, the potential for HIV-2 replication will also increase [78]. In addition, we have successfully treated eight dually infected individuals on an HIV-2 regimen of AZT/3TC/LPV/r achieving complete suppression of both viruses for more than three years [34].

Taken together, Tables I(a) and I(b) show possible combinations that would be likely to optimize control of HIV-2 in mono- and dual-infections, across two regimens, with the authors' preference given in bold.

2.4. Other Agents. While some newer agents developed for use against HIV-1 show no activity against HIV-2 and other products are currently unrealistic options in resource poor settings, they warrant discussion even if they lie far outside the protocols and budgets of West African treatment programs currently. With potentially increasing numbers of HIV-2 infected patients with first-line (and perhaps second-line) regimen failures in West Africa, increasing experience with the use of newer agents in salvage therapy in European settings and, hopefully, the costs of newer agents dropping over time, HIV-2 ART guidelines will require frequent reconsideration and updates.

Two types of entry inhibitors, fusion inhibitors (FI) and coreceptor binding inhibitors, have been approved for

Table 1

(a) Potential first- and second-line NRTI backbones for HIV-2 and dual infection

First-line	Second-line
AZT/3TC	**TDF/AZT/3TC or FTC**
TDF/FTC or 3TC*	TDF/AZT/3TC or FTC
ABC/3TC	TDF/AZT/3TC or FTC

* FTC and 3TC are assumed to be essentially equivalent in the table, despite FTC's possible superiority and 3TC's possibly lower cost.

(b) Potential first- and second-line PIs for HIV-2 and dual infection

First-line	Second-line
LPV/r	**SQV/r** or DRV/r
SQV/r	LPV/r or DRV/r
IDV/r	LPV/r or SQV/r or DRV/r

Table 2: Representative daily costs of selected antiretrovirals in West Africa in 2010 [74]. All values represent amounts paid since 01/01/2010 in West Africa except where otherwise noted, in which case the nearest equivalent in terms of year of purchase and income was used.

Drug	Cost per day[a]
AZT/3TC	\$0.28–\$0.36
d4T/3TC	\$0.12
ABC/3TC	\$1.38[b]
ddI (400 mg buffered)	\$0.79
3TC	\$0.08–\$0.10
TDF	\$0.72[c]
TDF/FTC	\$0.87–\$0.88[c]
LPV/r	\$1.24–\$1.56[d]
IDV	\$0.96[d]
SQV	\$7.20[e]
DRV	\$22.12–\$28.40[f]
Ritonavir (100 mg bd)	\$0.22–\$0.96[d]

[a] Costs are given in US dollars for standard doses given twice daily or daily in the case of TDF, ddI, ABC/3TC, and TDF/FTC
[b] Dominican Republic; 2008
[c] Republic of South Africa, Somalia
[d] 2009
[e] Egypt
[f] Bulgaria, Jamaica; 2009.

HIV therapy. Enfurvitide (T20), a fusion inhibitor currently licensed for use in HIV-1, has been found to have no activity against HIV-2 [8] which is not surprising given that HIV-1 and HIV-2 only share an amino acid sequence similarity of less than 30%–40% in the Env protein [80]. Maraviroc, a coreceptor binding inhibitor, works by blocking the CCR5 receptor, thereby inhibiting the virus from further conformational changes that will allow fusion with the host membrane. The activity of maraviroc against HIV-2 has not been formally tested, but since this drug binds to the CCR5 receptor, it should work against R5-tropic HIV-2 viruses [81, 82]. A recent case report demonstrates the inclusion of maraviroc in a regime used successfully to control resistant HIV-2 infection [83]. However, the ability of HIV-2 efficiently to utilize other coreceptors may limit the effectiveness of these antagonists in HIV-2 treatment [84]. Another potential concern is the switch or emergence of X4-tropic viruses, which is associated with faster disease progression [84]. Although R5 to X4 switch has only been reported in a few HIV-2-infected individuals [85], a limited number of X4-tropic viruses have been isolated from symptomatic patients [80].

Integrase inhibitors (INIs) work by interfering with the insertion of HIV DNA into host DNA and raltegravir (RAL), the first licensed INI for HIV-1 therapy, appears to be safe and efficacious in both ART naïve [86] and ART-experienced patients [87]. Despite the 40% heterogeneity in HIV-1 and HIV-2 integrase genes, the functionally important motifs (the catalytic triad DDE, the HHCC, and RKK) are 100% conserved in HIV-1 and HIV-2 [88, 89]. *In vitro* susceptibility of 14 clinical HIV-2 isolates, as well as HIV-2 ROD, to RAL, has showed similar activity for HIV-1 and HIV-2 [88]. *In vivo* studies on highly treatment-experienced HIV-2-infected individuals, two with group A [88, 90] and one with group B [91], showed promising results, with viral loads reduced to undetectable results, when RAL was used in combination therapy. HIV-2 resistance to RAL *in vivo* occurs via the N155H mutation [91] which is also associated with phenotypic resistance against RAL in HIV-2 [92]. However, these HIV-2 N155H mutants, like the M184V mutation in the reverse transcriptase, are much less fit than the wild type [75, 92]. This loss in replicative capacity can be exploited when viral suppression is no longer a realistic goal of therapy, and maintaining these mutations through continued selective pressure can slow disease progression.

3. Special Circumstances

3.1. Pregnancy. While the risk of HIV-2 transmission in pregnancy only reaches about 4% (including breast milk transmission) [93], clinical and *in vitro* data would suggest that AZT monotherapy as part of a prevention of mother to child transmission program poses a considerable threat to the mother, and to the child in the event of infection, of selecting for the Q151M mutation with subsequent pan-NRTI resistance [36]. Boosted PI-based ART through the latter two trimesters of pregnancy and the breastfeeding period should be the mainstay of vertical transmission prevention; however boosted PIs may result in greater nausea or insulin resistance in a small number of patients [94].

Dosing of PIs in pregnancy is not well validated, with evidence of reduced plasma concentrations with several agents, especially when used unboosted [95, 96]. Recent findings suggest that in the absence of TDM, LPV/r dose should be increased 50% in the second and third trimesters of pregnancy [97]. SQV/r is probably effective at its standard dose of 1000/100 mg twice daily [98, 99] and IDV/r at its standard dose of 800/100 mg twice daily may be adequate, but further clarification is required [100]. As with HIV-1, concerns exist about the use of TDF as part of the nucleoside backbone during pregnancy potentially interfering

with bone mineralization, although it has not as yet been associated with congenital abnormalities [101].

3.2. Tuberculosis (TB) Coinfection. TB is endemic in West Africa, and the problematic drug interactions between PIs and rifampin are well known, with induction of the cytochrome P450 system by rifampin resulting in accelerated metabolism of PIs, making effective dosing of the PIs more difficult to achieve. Provision of rifabutin as part of TB therapy for HIV-2 co-infected patients would therefore be ideal, allowing for the more predictable pharmacokinetic interactions between LPV/r and rifabutin. However TB treatment protocols, particularly where TB and HIV treatments are managed by different health care providers, might not adopt rifabutin as a result of cost or other considerations. In this case, where ART cannot be safely deferred, a PI-sparing regimen may represent the best balance of safety and efficacy for HIV-2/TB co-infected patients. Increasing the PI dose, for example doubling the dose of LPV/r, may be an alternative, although achieving therapeutic drug levels with tolerable dosing of LPV/r appears challenging [102, 103]. If a triple or quadruple NRTI regime is used, ART should be reassessed once TB treatment is completed and the patient switched to a boosted-PI regimen.

3.3. Chronic Hepatitis B (HBV) Co-Infection. Chronic HBV infection is common in West Africa, with prevalence rates of 8%–20% [104–106]; as a consequence many individuals infected with HIV-2 can be expected to have chronic HBV co-infection and a substantial proportion is likely to have high HBV viremia. Unlike the epidemiology in Western countries, most HBV transmission occurs between children and is not due to shared risk factors for transmission as between sexually active or intravenous drug using adults [107]. Screening protocols for comorbidities in HIV care settings in HBV endemic countries should include HBsAg, either for all new patients or at a minimum for those with evidence of liver disease, such as transaminitis. Where chronic HBV is present, ideally TDF/FTC or TDF/3TC should be in the first-line ART regimen [108], although this recommendation may be difficult to follow where HBV diagnostics are limited. Moreover it introduces another layer of complexity into protocol-based sequential ART. Clearly these issues would be addressed if TDF/FTC (or 3TC) were adopted as the preferred NRTI backbone, although it may be necessary to maintain them in succeeding regimens, regardless of the addition of other agents, to avoid the risk of HBV "flare" arising with their discontinuation [109].

3.4. Childhood. Children with HIV-2 infection present many of the same challenges as those with HIV-1 infection, such as concerns about dosing, formulations, and specifically TDF toxicity [110]. The principal differences, that vertical HIV-2 transmission is distinctly less common and that it is not rare for perinatal HIV-2 infections to present in teenagers, do not argue for any specific differences in their management compared to children with HIV-1, beyond their antiretroviral regimen being appropriate for HIV-2 as described above for adults.

4. Operational Issues

Endemic HIV-2 brings with it complications in terms of program management in West Africa beyond the necessary alterations in the antiretroviral therapy protocols, specifically that it complicates HIV testing and management of both stocks and staff.

4.1. Diagnosis. Testing to distinguish HIV-1 from HIV-2 and dual infection can be complicated and expensive due to the presence of cross-reactive antibodies and strain differences [111, 112]. Screening tests need high sensitivity for HIV-2, while confirmatory testing may require multiple steps in order to reliably distinguish between HIV-1, HIV-2, and HIV-1/HIV-2 dual infection, detailed review of which is beyond the scope of this paper. The alternative to these more demanding and elaborate testing protocols is misdiagnosis, primarily over-diagnosis of HIV-1/HIV-2 dual infection, resulting in HIV-1 monoinfected people going onto more expensive and cumbersome PI regimens. Diagnostic clarity therefore is a trade-off between higher upfront costs in testing and savings over the longer term in pharmaceuticals, although no rigorous analysis of costs has yet been made in this context. Misdiagnosis that results in HIV-2 and dually infected patients going on treatment that ignores their HIV-2 carries a greater risk, as discussed earlier.

4.2. When to Start. Compared with HIV-1, more patients with HIV-2 will be long-term nonprogressors or slow progressors. Although this could be used to argue for a later CD4-driven initiation of ART, it has been demonstrated that immunological recovery on ART is worse in HIV-2 compared with HIV-1 [113] and excessive delay in initiating ART may carry long-term negative immunological consequences. While the authors support initiating treatment for HIV-2 below a CD4 count of 350/mm^3 or possibly higher, instead of below 200/mm^3, it may be operationally awkward to apply different CD4 cut-offs for starting ART in HIV-1 and HIV-2 where CD4-driven initiation of therapy has not yet advanced to the earlier thresholds.

4.3. Monitoring. There is little evidence to suggest that monitoring of patients on ART should be any different for HIV-2 than for HIV-1. In practice the lack of a commercially available viral load assay [114] makes viral load measurements harder to obtain for HIV-2. CD4 recovery has been found to be blunted in HIV-2 [113]; combined with the more limited treatment options for HIV-2 this argues against considering a lack of substantial CD4 gains on ART to be a failure. Other immunologic criteria, including a drop from peak or a return to baseline CD4, may not be any worse for monitoring response to treatment in HIV-2 than in HIV-1.

4.4. Stock Management. In terms of stock management, the more complicated the program, the more difficult it

will be to avoid stock shortages and wastages. Endemic HIV-2 complicates the program. The numbers of HIV-2 patients needing ART are harder to estimate and may vary with changes in testing algorithms (see Section 4.1). As HIV-2 patients on ART will represent a small minority of a program or project's total number of HIV patients, small fluctuations in their number result in disproportionately large fluctuations in utilization rate, a situation that is further exacerbated for second-line treatment and pediatric cohorts. Stock management for pediatric cohorts with their heterogeneity in terms of weight, physical maturity, and ability to swallow pills is particularly difficult, and pediatric HIV-2 cohorts are likely to be extremely small. Partial standardization across both HIV-1 and HIV-2 regimens, for example using the same NRTIs regardless of HIV type or using the same PI for HIV-2 first-line treatment that one uses for HIV-1 second-line treatment, may simplify stock management and reduce shortages and wastage.

Another factor affecting ART choices in West Africa is cost. While costs can be expected to vary over time and between countries or regions, representative daily costs for several combinations, primarily from West Africa in 2010, are given in Table 2, although neither the costs nor the ratios of costs that follow should be presumed to be static. Compared with AZT, TDF and ddI are 2-3 times and ABC 4-5 times as expensive, while d4T costs half to one-third as much. The most expensive part of the regimen is the boosted PI, and this is also the main source of cost differences between regimens. Compared to coformulated LPV/r, SQV/r is 5–7 times and DRV/r 14–23 times as expensive, while IDV/r is approximately of the same cost. Comparisons of costs should also take efficacy into account. Depending on the model and assumptions this may result in medicines with a higher daily cost being more cost-effective, as has been recently shown for TDF in first-line ART in India [115].

4.5. Training and Protocol Development. The differences in recommendations between HIV-1 and HIV-2 and the dosing complications, particularly with TB cotreatment and in late pregnancy, pose further challenges to front line staff involved in program implementation in the HIV-2 endemic areas of West Africa. More complicated protocols call for more detailed training of staff. Greater diagnostic ambiguity and a broader range of ART regimens require more complete medical records. Finally patients getting information from various sources, especially long-term nonprogressors, need additional counseling to understand their disease.

5. Summary Recommendations

West African and other programs faced with HIV-2 patients need locally adapted protocols for testing, treatment, monitoring, and stock management in order to be effective. With regards to treatment, the delivery of optimal therapy should be a program goal, and although more complicated, it is achievable within a public health framework, with nurse-led clinics, even where infrastructural or staffing deficits may exist. For adults with HIV-2 or HIV-1/HIV-2 dual infection without access to ART susceptibility testing, optimal antiretroviral therapies for first- and second-line treatment are suggested in Tables I(a) and I(b). It is hoped that these recommendations will rapidly become obsolete as other agents and drug classes come into wider use in West Africa, and prospective randomized controlled trials of ART in HIV-2 provide more reliable indications of the suitability of specific regimens.

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Research Article

HIV Suppression among Patients on Treatment in Vietnam: A Review of HIV Viral Load Testing in a Public Urban Clinic in Ho Chi Minh City

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Background. There are few reports of HIV viral load (VL) testing among patients on ART in Vietnam. *Methods*. From a public clinic in Ho Chi Minh City (HCMC), we reviewed cases of VL measurements from adults on ART. *Results*. We identified 228 cases. Median age was 30 years (27–34), 85% were male, and 77% had a history of IDU. The mean ART duration was 26 months (95% CI 25–27); d4T/3TC/NVP was the most common regimen. Viral suppression was seen in 160/228 (70%). Viremia (>1000 copies/mL) was associated with prior ART exposure (OR 5.68, $P < .0001$) and immunologic failure (OR 4.69, $P = .0001$). Targeted testing accounted for 13% of cases, only half of which yielded viremia. *Conclusion*. We demonstrate a high HIV suppression rate among patients on ART in HCMC, Vietnam. In this setting, routine testing detects viremia missed by targeted testing.

1. Introduction

Vietnam is a country with one of the highest prevalences of HIV in Southeast Asia. With an estimated prevalence of 293 000 people in 2007 (approximately 0.5% of the general population), the HIV epidemic is primarily concentrated in urban areas among key high-risk populations, the majority of which are injection drug users (IDU), and to a lesser extent, female sex workers (FSWs) and men who have sex with men (MSM) [1, 2]. Efforts to confront the HIV epidemic in Vietnam face a high burden of patients with comorbid substance abuse and limited resources.

International efforts to scale up antiretroviral therapy (ART) have greatly improved funding for treatment in Vietnam, allowing for approximately 14 969 people to receive ART as of 2007 [3]. The success of ART programs has been documented in resource-limited settings throughout the world [4]. However, cohorts examining the effectiveness of ART programs in low-income countries traditionally consist of countries with a low prevalence of IDU, the majority of which are in Africa [5, 6]. Thus, there is less information regarding ART scale-up efforts in resource-limited settings with a high burden of comorbid IDU. Only since 2009 has the first report of ART among IDU in Vietnam been documented [7].

Virologic suppression is the measure of successful antiretroviral therapy. The cost of viral load monitoring, however, has been prohibitive in resource-limited settings.. The World Health Organization (WHO) has recommended an algorithm using clinical and immunologic criteria to assess treatment failure in the absence of viral load testing which has become standard of care in many resource-limited settings [8]. The concern has been raised that delayed recognition of treatment failure may lead to prolonged use of failing regimens and amplification of drug resistance [9]. This concern may be most important in populations at highest risk for failure including those with active substance abuse.

At the An Hoa Outpatient Center (OPC), a public urban HIV clinic located in District 6 (D.6) of Ho Chi Minh City (HCMC), Vietnam, viral load (VL) testing has been available in a limited capacity since 2005. Virologic testing has been used principally as a confirmatory test targeted at patients suspected of failing based on WHO criteria of clinical or immunologic failure. In December 2007, as part of a quality improvement process, a program of routine viral load surveillance was implemented at the An Hoa OPC. The goals of this study were to document ART efforts in a resource-limited setting with high prevalence of IDUs, to identify high-risk groups for failure who may benefit from more frequent monitoring or other interventions and to assess the potential for delayed diagnosis of treatment failure when using targeted testing based on clinical and immunologic criteria.

2. Methods

2.1. Site and Population. Located in District 6 (D.6), An Hoa Outpatient Center (OPC) is funded by USAID and the US President's Emergency Plan for AIDS Relief (PEPFAR) and has been operated by the French nongovernmental organization Médicins du Monde (MdM) since 2003. An Hoa Outpatient OPC is one of 75 PEPFAR clinical sites, with additional oversight by the Vietnamese Ministry of Health (MOH), that provides ART- and HIV-related services to one of the HCMC's 18 inner city districts.

Available first-line three-drug ART regimens are consistent with WHO standards which include two nucleoside reverse transcriptase inhibitors (either stavudine (d4T) + lamivudine (3TC) or zidovudine (AZT) + lamivudine (3TC)) with one non-nucleoside reverse transcriptase inhibitor (either nevirapine (NVP) or efavirenz (EFV)) [8]. All patients preparing to initiate ART through the An Hoa OPC must undergo pretherapy adherence counseling sessions. Once initiated, patients are scheduled to undergo clinical assessments with a physician and nursing at weeks 2, 4, 8, 12, 16, 24, 32, 40, and 48. Routine visits are scheduled every 2 to 6 months afterwards. Per MOH guidelines, once stable on ART, patients must come to the OPC monthly to receive their ART supply. Additionally, patients are seen by an adherence counselor at weeks 2, 4, 8, 24, and 48. CD4 counts are routinely obtained 12, 24, and 48 weeks and every 6 months afterwards.

Treatment failure is suspected if after one year of therapy, patients exhibit immunologic failure (fall to baseline level CD4 count, 50% fall in CD4 count from posttreatment peak, or CD4 counts persistently $<100/mm^3$) and/or clinical failure (new or recurrent WHO stage IV condition) according to WHO criteria [10]. Switching to second-line regimen involves a thorough committee review by specialists at the Hospital for Tropical Disease in Ho Chi Minh City.

Viral load (VL) testing is available at the An Hoa OPC in a limited capacity. During the dates from 12/1/2007 to 2/28/2009 VL testing was performed according to two distinct approaches the following.

(1) Targeted testing to confirm virologic failure among those suspected of treatment failure and who were in consideration for 2nd-line therapy. This approach was done with funding from the US CDC based on the Vietnamese Ministry of Health and is available to all PEPFAR sites in HCMC.

(2) Routine testing to screen for subclinical virologic failure for those on ART for greater than one year. This approach was internally funded through MdM and has been in practice since 12/1/07. Under this approach, patients established on ART for greater than one year received one-time VL testing as part of a routine visit (as stated above).

All VL samples were sent to an offsite facility at the Pasteur Institute of HCMC, where they were assessed based on real-time reverse transcriptase PCR assay (Generic HIV viral load assay, Biocentric, Bandol, France) with a threshold for detection of VL of 250 copies/mL.

2.2. Data Collection. We reviewed cases of VL testing performed at the An Hoa OPC between the dates of 12/1/07 and 2/28/09. Included for review were adult patients (>18 years) on ART for greater than one year while actively registered at the An Hoa OPC. We excluded patients younger than 18 years of age, and those who had been on ART for less than one year in duration from date of registration to An Hoa OPC.

An onsite database managed by trained nursing staff was used to identify cases for review. In situations where multiple VL measurements were sequentially performed on a single patient, the initial date of VL testing was used for case identification.

Medical records were reviewed and abstracted for demographic data (e.g., age and sex), self-reported history of IDU, date of registration to the AnHoa OPC, date of ART initiation, 1st-line ART regimen, duration of ART at time of VL testing, baseline CD4, CD4 at time of VL testing, prior ART exposure, adherence, history of immunologic and clinical failure, switch to 2nd-line regimen, and approach used to perform VL testing, that is, targeted or routine (nontargeted). Current and prior history of IDU was not distinguished.

Significant viremia was designated as a VL of greater than 1000 copies per milliliter. Virologic failure, as defined by the WHO, was designated as VL greater than 10 000 copies/mL.

Prior ART exposure was defined as prior ART usage not under the supervision of any MOH monitored site. Immunologic failure was determined according to WHO standards, that is, fall to baseline level CD4, 50% fall posttreatment peak, or levels persistently $<100/mm^3$ after one year of ART. Clinical failure was determined by new or recurrent WHO stage IV condition. Adherence was designated as "Good" if patient had >95% self-reported adherence rate in the preceding month, and "Poor" if the patient had <95%.

Descriptive statistics were generated for demographic and clinical parameters. Univariate associations between demographic and clinical factors and the presence of significant viremia were tested by means of chi-square statistics.

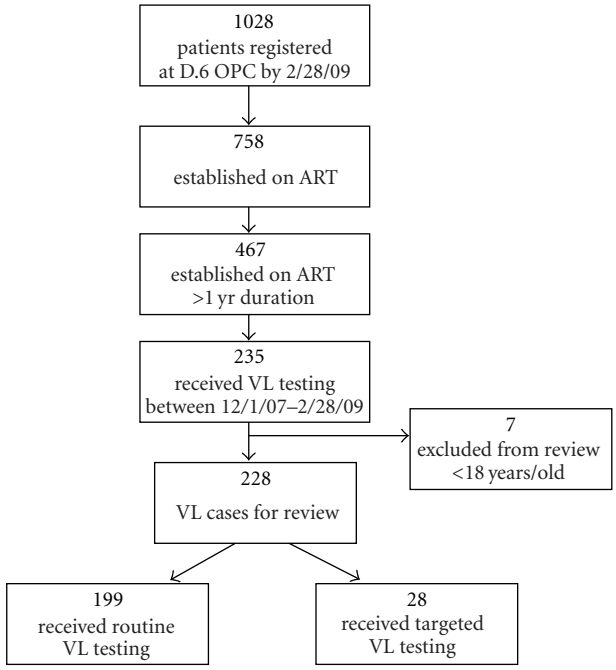

Figure 1: Flow chart of adult patients on ART >1 yr who received VL testing and enrolled for review.

Multivariate logistic regression was used to control for potential confounding factors.

This paper was approved by the Provincial AIDS Committee (PAC), Ministry of Health (MOH) of Ho Chi Minh City, Vietnam.

3. Results

As of the end of February 2009, a total of 1028 patients were registered as active at the An Hoa OPC. Seven hundred and fifty-eight patients were receiving ART, and 467 of whom had been on ART for greater than one year's duration. We identified 235 unique cases of viral load measurements for review. Seven were excluded because of age less than 18 years yielding a total of 228 for our study (Figure 1).

Composite baseline characteristics are outlined in Table 1. The majority of patients were male (85%) and had a history of IDU (77%). The median age was 30 years (interquartile range 27–34). The regimen of d4T/3TC/NVP was the most common 1st-line regimen utilized (65%), and 74% had documented Good adherence. Thirty percent had prior ART exposure not under the supervision of a MOH monitored site. The median CD4 count at time of registration to the An Hoa OPC and time of VL testing was 57 cell/μL and 239 cells/μL, respectively. The mean duration of ART prior to VL testing was 26 months (95% CI 25–27), and undetectable virus was exhibited in 160 of 228 cases (70%). Measurements below our designated threshold of significant viremia (1000 copies/mL) were seen in 175 of 228 cases (77%).

In univariate analysis the odds of significant viremia were higher for those with prior ART exposure ($P < .0001$), preceding immunological failure ($P < .0001$), clinical failure ($P = .024$), and female sex ($P = .0428$) (Table 2). In multivariate analysis, two factors remained strongly associated with significant viremia: prior ART exposure (OR 5.69, $P < .001$) and history of immunologic failure (OR 4.69, $P = .0001$). There was an observed trend towards increased viremia among women, but not enough women were enrolled to find a significant difference ($P = .066$).

A comparison of targeted and routine testing is seen in Table 3. Of all cases yielded for review, 13% (29) came as a result of targeted testing. Only half (48%) of cases targeted for testing yielded significant viremia (>1000 copies/mL), and all but one of which had virologic failure (>10,000 copies/mL). The sensitivity of targeted testing in detecting significant viremia was 26% with a positive predictive value of 50%. Approximately 80% of those targeted had immunologic failure, 72% had prior ART

Table 1: Baseline characteristics of patients on ART >1 yr who received VL testing, $N = 228$.

Characteristic	
Male (%)	193 (85)
Female (%)	35 (15)
Age at study, median (interquartile range), years	30 (27–34)
Age at ART initiation, median (interquartile range) years	27 (24–31)
IDU (%)	175 (77)
Prior ART exposure (%)	30 (13)
Duration of ARV at time of viral load testing, mean (95% CI), months	26 (25–27)
Adherence: "Good" (%)	169 (74)
Adherence: "Poor" (%)	59 (26)
First-line regimen	
AZT/3TC/EFV	4 (2)
AZT/3TC/NVP	14 (6)
D4T/3TC/EFV	61 (27)
D4T/3TC/NVP	149 (65)
CD4 cell count, median (interquartile range) cells/μL	
at registration to An Hoa OPC	57 (18–146)
at viral load testing	240 (144–366)
Documented immunologic failure (%)	39 (17)
Documented clinical failure (%)	14 (6)
Switch to 2nd line* (%)	13 (6)
VL testing	
according to targeted approach (%)	29 (13)
according to routine approach (%)	199 (87)

IDU: injection drug use, VL: viral load.
ART: antiretroviral therapy.
AZT: zidovudine, D4T: stavudine, EFV: efavirenz.
NVP: nevirapine, 3TC: lamivudine.
*Second-line regimen: tenofovir (TDF)/lamivudine (3TC)/lopinavir (LPV).

Table 2: Characteristics of patients on ART >1 year according to result of VL testing, threshold VL 1000 copies/mL.

Characteristics	VL < 1000 ($N = 175$)	VL > 1000 ($N = 53$)	OR (95% CI)	P value	
				Univariate	Multivariate
Gender: male (%)	153 (87)	40 (75)	0.44 (0.2–0.95)	—	—
Gender: female (%)	22 (13)	13 (25)	2.26 (1.05–4.88)	.043	.066
IDU (%)	133 (175)	42 (79)	1.2 (0.57–2.55)	.34	—
Prior ART exposure (%)	12 (7)	18 (34)	6.99 (3.09–15.8)	<.0001	<.0001
Adherence: "Good" (%)	132 (75)	37 (70)	0.75 (0.38–1.49)	.419	—
Adherence: "Poor" (%)	43 (25)	16 (30)	1.33 (0.67–2.62)	—	—
CD4 cell count, median (interquartile range) cells/uL					
at time of registration to An Hoa OPC	59 (20–143)	50 (12–149)	—	—	—
at time of viral load testing	267 (171–374)	167 (78–260)	—	—	—
Documented immunologic failure (%)	19 (11)	18 (34)	4.2 (2.0–8.9)	<.0001	.0001
Documented clinical failure (%)	7 (4)	7 (13)	3.6 (1.2–10.9)	.024	—

IDU: injection drug use, ART: antiretroviral therapy.
OPC: outpatient center, VL: VIRAL load.

Table 3: Characteristics of patients on ART >1 yr—targeted testing and routine testing.

Characteristic	Targeted testing ($N = 29$)	Routine testing ($N = 199$)
CD4 cell count, median (interquartile range) cells/μL		
at time of registration to An Hoa OPC	45 (14–107)	59 (18–149)
at time of VL testing	78 (53–93)	267 (179–400)
Documented immunologic failure (%)	24 (83)	14 (7)
Documented clinical failure (%)	6 (21)	8 (4)
Switch to 2nd line (%)	9 (31)	3 (2)
Undetectable or VL < 1000 copies/mL (%)	15 (52)	160 (80)
Significant viremia (copies/mL)		
High viremia: VL > 10000 (%)	13 (45)	14 (7)
Moderate viremia: 10000 > VL > 1000 (%)	1 (3)	25 (13)

ART: antiretroviral therapy.
OPC: outpatient center, VL: viral load.

exposure, and 21% had clinical failure, compared to 7%, 11%, and 4% of those who had routine testing, respectively.

4. Discussion

In our study, viral load testing was performed on 49.7% of adult patients on ART for greater than one year attending the An Hoa OPC, and 77% of whom had a history of IDU. The mean duration of ART was 26 months, and the rate of undetectable virus and viremia below 1000 copies/mL was 70% and 77%, respectively.

The detectable threshold of our assay, 250 copies/mL, is one that is not commonly used in studies evaluating ART efficacy. A detectable threshold of 400 copies/mL is what has been traditionally used in assays from studies conducted in resource-limited settings. In studies from Africa, the viral load suppression rate has ranged between 66% and 82% for patients on ART for duration of 26–48 weeks [11–13]. Our study found a comparable suppression rate at a longer mean ART duration, 26 months, despite using a lower level of detection (i.e., more sensitive assay), in a predominantly IDU population. Since our study excluded patients on ART for less than one year, there may have been a selection bias towards patients more tolerant of and more compliant with ART which could partially explain the relatively high viral suppression rate observed.

Injection drug users have often been associated with lower rates of virologic suppression [14, 15]. This is traditionally attributed to incomplete adherence, and the psychosocial instability that comes with drug-seeking behavior [16, 17]. International treatment cohorts which have documented the efforts of ART programs in low income countries (ART-LINC) have excluded resource-limited countries with higher rates of IDU [5, 6]. Amongst developing

nations, China and Russia have been estimated to have the highest rates of IDU [18]. Recently, the first study examining viral load suppression rates among 8 ART programs in China revealed a VL suppression rate of 67% for patients on 24 months of ART [19]. However, IDUs may have been underrepresented in this study, as IDU associated transmission was reported in only 8% of patients. Studies documenting the VL response of ART programs in Russia and Eastern Europe remain to be seen.

The program at the An Hoa OPC demonstrates a successful campaign of viral suppression among an HIV population with a high prevalence of IDU. The viral suppression rate in the current study is similar to that seen in a cohort of HIV positive drug users on ART in Hanoi, Vietnam [7]. It is notable however that IDU history was not statistically associated with viremia in our study. This observation perhaps is due to the lack of distinction between current and former IDU activity in our patients. Former IDUs have been shown to have similar VL suppression rates to non-IDU patients on ART [14].

The observed trend towards increased viremia among women is somewhat surprising in our study. It has been noted that the HIV epidemic among women in Vietnam has been greatly under-reported and under-recognized. Institutional efforts focus primarily on young male injection drug users, leaving women not only less likely to get tested, but also less likely to receive optimal care [20]. A combination of several cultural factors, including stigma directed against HIV-infected patients, poor education, and reluctance to seek medical care, subordinate gender roles, may also work to create a significant barrier to the optimization of medical care for HIV-infected women in Vietnam [21–23]. However, given the relatively low number of women in our cohort, our findings need to be interpreted with caution. Further studies are needed to adequately address variables such as gender differences in patterns of clinical utilization that may be contributing to failure among HIV-infected Vietnamese women on ART.

Prior unmonitored ART exposure was shown to be a significant risk factor for treatment failure in our study. Patients in developing countries with a history of unmonitored ART usage are at risk of improper administration of medications as well as exposure to substandard or counterfeit drugs. These patients are at risk not only for harmful side effects but also for the development of HIV drug resistance. Our data adds to the previous studies which suggest that patients with prior ART should be more closely targeted for suspected ART failure [24].

The significant association of immunologic failure and viremia in our study is not surprising. The history of HIV shows that immunologic failure naturally follows progressive sustained viremia. Virologic failure predates immunologic failure, which is followed by clinical failure. Thus, strictly using immunologic and clinical monitoring, that is, the WHO algorithm, as a method to identify ART failure will invariably miss early virologic failure thus allowing for extended viral replication under drug pressure and promote drug resistance.

Multiple studies have shown that the application of the WHO algorithm is a poor substitute for viral load testing, lacking sensitivity and specificity for detecting treatment failure in resource-limited settings [12, 24–29]. Furthermore, in settings with no capacity for viral load testing, strictly using the WHO algorithm also leads to potential misclassification of treatment failure and premature switch to second-line regimens [26, 30]. A recent study evaluating immunologic monitoring in a resource-limited setting, found that only 42% of patients qualifying for immunologic failure had detectable virus [26]. Our study also found a potential for misclassification as only 48% of those targeted had significant viremia.

The goal of targeted VL testing, as adopted by the Vietnamese MOH in HCMC, is to reduce the likelihood of premature switch to valuable second-line regimens. However, as our study shows, given the insensitivity of the criteria used for targeted testing, treatment failure may be under-diagnosed and opportunities to intervene early will be lost.

Our review suggests that routine testing has the potential to identify patients with significant viremia not identified by targeted testing programs and thus prevent delayed recognition of treatment failure. Implementing routine testing, however, poses a number of logistical barriers [31]. Decisions regarding the use of virologic monitoring need to consider the cost, frequency, and availability of follow-up testing along with the risk of reducing access to treatment or other necessary health services. Other factors that need to be considered are the threshold for change in regimen and the role of adherence interventions.

A major limitation in our study was the usage of single viral load measurements. Low level viremia in our review is difficult to distinguish from "blips" clinically insignificant episodes of nonsustained, transient low level viremia [32]. We designated a level of 1000 copies/mL to represent significant viremia, as levels above this threshold have been shown to be frequently associated with resistance, often leading to therapy changes [31, 33]. However, using this threshold, we potentially excluded those who may have been experiencing persistently low viremia, thus possibly under-diagnosing treatment failure.

Additionally, single viral load measurements are challenging to interpret without the appropriate infrastructure for followup, that is, subsequent testing, resistance analysis, and resources to target adherence. Our study was not designed to assess followup, but data from our review may be used in designing a protocol for follow-up testing and targeted interventions. Targeted adherence interventions have been shown to be successful in reducing viral load breakthrough to undetectable levels in a resource-limited setting [34].

5. Conclusions

In summary, we demonstrate a successful campaign of HIV viral load suppression in HCMC, Vietnam, a resource-limited area with a high prevalence of IDUs. We found that An Hoa OPC patients well established on ART experienced

a high viral load suppression rate comparable to that seen in other studies. Significant viremia was strongly associated with immunologic failure and prior ART exposure. A trend towards increased viremia was observed among women on ART that warrants further investigation. Targeted testing, based on the WHO algorithm, was a poor predictor of virologic failure. Routine screening is better able to identify patients on ART who experience significant viremia. This approach however requires a comprehensive structure for followup and intervention; the costs of which need to be considered on a site by site basis in resource-limited areas.

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Research Article

Associations between HIV Antiretroviral Therapy and the Prevalence and Incidence of Pregnancy in Rakai, Uganda

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Background. Use of antiretroviral therapy (ART) may be associated with higher pregnancy rates. *Methods*. The prevalence and incidence of pregnancy was assessed in 712 HIV+ pre-ART women of reproductive age (WRA) (15–45) and 244 HIV+ WRA initiating ART. Prevalence rate ratios (PRR), incidence rate ratios (IRR), and 95% confidence interval (CI) were assessed. *Results*. The incidence of pregnancy was 13.1/100 py among women in pre-ART care compared to 24.6/100 py among women on ART (IRR = 0.54; 95% CI 0.37, 0.81, $p < 0.0017$). The prevalence of pregnancy at ART initiation was 12.0% with CD4 counts 100–250 compared with 3.2% with CD4 <100 (PRR = 3.24, CI 1.51–6.93), and the incidence of pregnancy while on ART was highest in women with a good immunologic response. Desire for more children was a very important factor in fertility. *Conclusion*. ART was associated with increased pregnancy rates in HIV+ women, particularly those with higher CD4 counts and good immunologic response to therapy, suggesting a need to strengthen reproductive health services for both women and their partners that could address their fertility decisions/intentions particularly after ART initiation.

1. Introduction

The HIV/AIDs epidemic remains a serious public health challenge, as well as a social dilemma especially among women of childbearing age [1] who were 46% of the HIV global burden [2]. HIV infected women face difficult decisions regarding childbearing. HIV-infection compromises their immunity which further aggravates their chances of conception as well as sustaining a pregnancy to term. Reduced fertility among HIV-positive women has been reported in sub-Saharan Africa [3–7]. Fertility is not impaired during early HIV infection [8, 9] but declines with disease progression, and the reduction is greatest with onset of AIDS [10]. The mechanisms through which fertility rates are reduced by HIV are not fully understood, but higher viral load and decreased CD4 counts with advanced HIV disease are likely to be implicated [11]. Also, progression to AIDS leads to decreased general health and well-being that may be associated with reduced sexual activity. However, the advent of ART has improved the general health and may increase sexual activity, and improved survival prospects may increase the desire for more children [12–15]. Desire for more children may also be due to improvements in pregnancy care that have reduced the risk of vertical transmission, especially if pregnant women receive timely ART or if they deliver by caesarean section and avoid breastfeeding when recommended. However, even in the era of HIV/AIDS other predictors of fertility including age, marital status, level of education, and socioeconomic status still play a significant role in determining fertility [16–20]. A combination of all these factors may be associated with the currently observed increase in pregnancy rates among women on ART [21, 22]. Although HIV-infected women's fertility desires have been studied, there is limited information on the possible role

of the improving immunological and virological outcomes on the actual fertility among women initiated on ART. Understanding the effects of ART on women's fertility is critically important for health service providers to be able to protect the reproductive rights of HIV-infected women through creating increased access to sexual and reproductive health services and sexuality education, so that women may decide freely and responsibly on their family size through spacing and timing of their pregnancies [23] In this paper we therefore assess the association between HIV infection, ART use, and pregnancy rates, taking into account immunological and virological status, in a community-based HIV care program in Rakai district, Uganda.

2. Methods

2.1. Study Setting, Population, and HIV Treatment and Care. Since June 2004, with funding from the President's Emergency Plan For AIDS Relief (PEPFAR), the Rakai Health Sciences Program (RHSP) in Uganda has offered HIV care and ART free of charge. HIV care, offered to all HIV-infected persons, includes cotrimoxazole prophylaxis, treatment for opportunistic infections, reproductive health services, treatment of opportunistic infections, cotrimoxazole prophylaxis, provision of a basic care package, health education, and HIV counseling. Individuals are initiated on ART at a CD4 cell count ≤250 cells/mm^3 or WHO Stage IV disease. General HIV care and ART is provided via 17 mobile community-based out-patient clinics, called Suubi or "Hope" clinics. All patients are offered ongoing HIV counseling, health education including family planning, prevention of mother to child transmission of HIV, and counseling on reduction of risky sexual behaviors for example, multiple sexual partners. At each visit, general health status is evaluated, and use of family planning methods are ascertained.

At the time data were collected for these analyses, individuals on HIV care but not yet started on ART were seen every 3 to 6 months depending on CD4 cell count. Patients initiating ART were followed weekly for the first month, biweekly for the subsequent 2 months, monthly until one year on treatment, and then every 2 months thereafter. CD4 counts were assessed every 3 or 6 months prior to ART, at time of ART initiation, and every 3 months thereafter. Viral load was tested at time of ART initiation and then at 6 monthly intervals. Two weeks prior to ART initiation, women were interviewed regarding their and their sexual partner's fertility desires and intentions; the interview was repeated at 6 months and annually thereafter. Women provided written informed consent for interview, and were part of the ongoing Rakai community cohort surveillance (RCCS). The RCCS study was approved by the Science and and Ethics Committee of the Uganda Virus Research Institute, the Ugandan National Council for Science and Technology, and the Western Institutional Review Board.

2.2. Definition of Key Variables. Pregnancy was detected through self-report and hCG urine test for those uncertain of their pregnancy status or whose last normal menstrual period (LNMP) was more than 30 days prior to interview, with the exception of women using Depo-Provera or norplant, and those who were postmenopausal or had lactational amenorrhoea <2 months, who were excluded from this analysis. Prevalent pregnancies were defined as those detected at or within two-weeks of enrolment into HIV care or ART initiation. Incident pregnancies were defined as those first detected more than 2 weeks after entry in HIV care or ART initiation. (The two-week window was included because, on average, using the pregnancy kits available to this program, hcg could only be detected in a pregnant woman's urine after at least 10 days.) If a woman had more than one pregnancy during follow-up, only the first pregnancy was considered for the incidence analysis, and subsequent observation time was censored.

All women initiating ART had CD4 counts of ≤250 cells/mm^3 and were categorized into CD4 counts <100 or 100–250 cells/mm^3. Desire for more children was categorized as both partners wanting a child, only the male or the female partner wanting a child, or neither wanting a child.

We also grouped responses to desire for more children into four categories as (i) *both partners did not* want a child if neither wanted more children, (ii) *only male partner* wanted *a child,* (iii) o*nly the woman* wanted more children, (iv) *both wanted (more) children*.

2.3. Statistical Analysis. The two key outcomes for this analysis were prevalent pregnancy defined as a pregnancy at enrolment into HIV care for the pre-ART period, or at ART initiation or within the first 2 week of follow-up for those initiated on ART. Incident pregnancy was defined as the first pregnancy during subsequent visits starting at the 2nd week followup following ART initiation and at 3 or 6 months visits after enrolment in HIV care prior to ART initiation, among women who were not pregnant at time of enrollment in care or initiation of ART.

2.3.1. Analyses of Incident Pregnancy during HIV Care prior to ART and during ART. The prevalence of pregnancy at two time points (at the time of HIV care initiation and at ART initiation) was determined as the number of women pregnant at time of HIV care or ART initiation divided by the total number of sexually active women of childbearing age (15–45 years) starting HIV care or ART (premenarche women and women using depo-provera or norplant, and those who were post menopausal or had lactational amenorrhoea <2 months were excluded from analysis). Prevalence risk ratios (PRR) and 95% confidence intervals (CI) were estimated using generalized linear models (glm) with family of binomial and log link. Models for adjusted PRR included baseline CD4 and other factors statistically significant at $p < 0.15$ in univariate analyses or potential confounders.

The incidence of new pregnancies detected between the second week up to the 48th week during two time periods (after initiation of HIV care and after initiation of ART) was estimated as the number of first pregnancies in each time period divided by the total person-years (py) accrued in that time period before pregnancy detection or before censoring at week 48. Incidence rate ratios (IRR) of first pregnancy and

95% confidence intervals (CI) were estimated using Poisson regression with observation time as an offset.

Covariates assessed in the pre-ART period pregnancy incidence included CD4 at screening for enrolment into HIV care, categorized as 251–350 and 351+ cells/mm^3, age at enrolment, marital status, family planning use, and breastfeeding in the past 12 months. In multivariable models, we adjusted for factors statistically significant at $p < 0.15$ or potential confounders. The main factor of interest in this analysis was CD4 counts at enrolment into HIV care.

For the ART treatment period, covariates included change in CD4 counts between baseline (the time of ART initiation) and week 12 of treatment, divided into three categories: baseline and week 12 CD4 were both <100 cells/mm^3, baseline CD4 was <100, week 12 CD4 was 100+ cells/mm^3 and both baseline CD4 and week 12 were 100+ cells/mm^3, age at ART initiation, marital status, baseline desire for children, body mass index (BMI) calculated as (Weight in Kilograms/(Height in Meters) $\times$ (Height in Meters)) at week 12, family planning use at baseline, and HIV viral load at week 24. In multivariable models, we adjusted for factors which were statistically significant at $p < 0.15$ in univariate analyses or potential confounders. Using Kaplan-Meier survival curves, we also assessed the association between CD4 counts and the cumulative probabilities of incident pregnancies comparing women in the three categories of change in CD4 between baseline and week 12. The log-rank test was used to assess differences in the cumulative probabilities of pregnancy between these CD4 change categories.

In both, the generalized linear models (glm), for estimation of the PRR and IRR, we adjusted for clustering of observation at the mobile clinic (hub) levels, because women attending specific mobile clinics were more likely to be similar in various characteristics than those attending others.

3. Results

Table 1 shows the characteristics of women who enrolled into pre-ART HIV care in the RHSP. The majority were aged 25–34 years (54.8%), currently married (48.2%), and those with no sexual partners in the past 6 months (64.4%). About 40% had parity of 4 or more children, and 37% were prime gravidae. About 18.4% used medications to prevent pregnancies, but injectables were the most commonly reported pregnancy prevention medication among users 187/278 (67%). Among those with available data on measures of health indicators, about 10% had been bedridden in the past 30 days, 20.4% with WHO stage of 3/4, and about a third (32%) were eligible for ART initiation by CD4 count of ≤250 cells/mm^{3}as per the RHSP criteria.

3.1. Prevalence and Incidence of Pregnancy during HIV Care prior to ART. Table 2 shows the prevalence and prevalence risk ratios of pregnancy among HIV+ women at enrollment into HIV care prior to ART. The prevalence of pregnancy at enrolment into HIV care was 7.2% (109/1514). Factors significantly associated with higher prevalence of pregnancy were CD4 >350 cells/mm^3 (adj. PRR = *4.71*; 95% CI *1.41, 15.80*), being currently married (adj. PRR = 3.82; 95% CI 1.83, 7.97). Factors associated with lower pregnancy prevalence were older age 25–34 years (adj. PRR = *0.55*; 95% CI *0.34, 0.88*) or 35–45 years (adj. PRR = *0.31*; 95% CI *0.15, 0.67*) compared to women aged 15–24 years, those breastfeeding in the past 12 month (adj. PRR = *0.16*; 95% CI *0.08, 0.30*), or those using medication to prevent pregnancy (use of family planning) in the past 12 month (adj. PRR = *0.09*; 95% CI *0.02, 0.33*).

Table 3 shows the incidence of pregnancy and incidence rate ratio of pregnancy prior to ART. The overall incidence of pregnancy during the first year in HIV care was 13.1/100 py, 95% CI (10.14, 16.75). Being currently married as compared to never married tended to have higher incidence of pregnancy, but this did not reach statistical significance, adj. IRR = 5.90 (0.87, 40.04) nor was being divorced/separated/widowed. On the other hand, the incidence of pregnancy was significantly reduced among women reporting use of medication to prevent pregnancy (nonuse of family planning) in past 12 months, adj. IRR = 0.22 (0.08, 0.61), older age (35–45 years) compared to young age (15–24 years), adj. IRR = 0.15 (0.07, 0.34), and parity of 1–3 children compared to prime gravids, adj. IRR = 0.43 (0.22, 0.86). The level of CD4 counts at entry into HIV care prior to ART initiation was not associated with incidence of pregnancy 13.2.0/100 py 95% CI (10.02,17.23) for the 351+ cells/mm^3 compared to 12.4/100py 95% CI (5.68,23.60) for the 251–350 cells/mm^3.

3.2. Prevalence and Incidence of Pregnancy during ART Use. Table 4 shows the prevalence of pregnancy and the prevalence rate ratios at the time of ART initiation. The prevalence of pregnancy was 10.1% (7.7, 12.9). The prevalence of pregnancy was higher among women with baseline CD4 of 100–250 (12.0%), compared to those with CD4 <100 (3.2%, adj. PRR = 3.24; 95% CI 1.51, 6.93). Also, the prevalence of pregnancy was significantly associated with desire for more children especially when both the woman and her spouse desired a (more) child compared to women in which both partners did not want a (more) child (adj. PRR = 2.27; 95% CI 1.04, 4.97), among currently married women (adj. PRR = 1.33; 95% CI 1.00, 1.76) compared to those not in union, and older women aged 35–45 years compared to the younger women aged 15–24 years (adj. PRR = 0.47; 95% CI 0.25, 0.91).

Among the 566 women with data at the time of ART initiation, the overall mean (SD) CD4 counts were 159.0 cells/mm^3 (SD = 72.7). The CD4 counts were significantly higher among the 57 pregnant women 184.0 (57.5) cells/mm^3 compared to the 509 nonpregnant women (156.2 cells/mm^3, $p = 0.0013$). Among the pregnant women, 24.6% (14/57) reported that only the male spouse desired to have a (another) child compared to 1.8% (1/57), where only the female did ($p = 0.0003$).

A total of 244 women were eligible for the analysis of incident pregnancy after ART initiation. The incidence of pregnancy was 24.6/100 py; 95% CI 18.1, 32.6. The incidence tended to be higher among women whose CD4 counts at the time of ART initiation were either higher than 100 cells/mm^3

Table 1: Characteristics of women enrolling into pre-ART HIV care.

Characteristics	Number of women*	Proportion, %
Overall	1514	100
Age (years)		
15–24	199	13.1
25–34	830	54.8
35–45	485	32.0
Marital status		
Never married	155	10.2
Divorced/separated/widowed	629	41.6
Currently married	730	48.2
Sexual partner past 6 months		
None	970	64.4
One	493	32.7
2+	43	2.9
Fertility and family planning		
Pregnancy status		
Not pregnant	1405	92.8
Pregnant	109	7.2
Parity (ever live-births)		
Prime gravid	545	36.9
1–3	353	23.9
4+	580	39.2
Use of any medication to prevent pregnancy		
Not taking any	1236	81.6
Taking some medications	278	18.4
Medications used to prevent pregnancy		
Not taking any	1236	81.7
Injectables	187	12.4
Tablets	56	3.7
Others	34	2.3
Breastfeeding		
Not breastfeeding	1286	84.9
Yes breastfeeding	228	15.1
General health		
Bed-ridden in past 30 days		
No	473	89.8
Yes	54	10.3
WHO stage at initial screening		
I	476	48.3
II	309	31.3
III	151	15.3
IV	50	5.1
CD4 at initial screening		
<100	167	11.0
100–250	312	20.6
251–350	173	11.4
351+	862	56.9

Note *some totals do not add up to 1514 because of missing information on variables.

Table 2: Prevalence of pregnancy, unadjusted and adjusted prevalence risk ratios among HIV+ women prior to ART initiation.

Characteristics	Pregnant/total (%)	Unadjusted PRR (95% CI)	Adjusted PRR (95% CI)
Overall	109/1514 (7.2)		
Age (years)			
15–24	31/199 (15.6)	1.0	1.0
25–34	61/830 (7.4)	**0.47 (0.30, 0.74)**	**0.55 (0.34, 0.88)**
35–45	17/485 (3.5)	**0.23 (0.11, 0.47)**	**0.31 (0.15, 0.67)**
Marital status			
Never married	5/155 (3.2)	1.0	1.0
Divorced/separated/widowed	15/629 (2.4)	0.74 (0.27, 2.5)	0.80 (0.28, 2.22)
Currently married	89/730 (12.2)	**3.78 (1.81, 7.90)**	**3.82 (1.83, 7.97)**
Sexual partner past 6 months			
None	63/970 (6.5)	1.0	
One	44/493 (8.9)	1.37 (0.92, 2.04)	
2+	2/43 (4.7)	0.72 (0.17, 3.07)	
Parity (ever live-births)			
Prime gravid	41/545 (7.5)	1.0	
1–3	26/353 (7.4)	0.98 (0.57, 1.67)	
4+	41/580 (7.1)	0.94 (0.64, 1.38)	
Use of any medication to prevent pregnancy			
Not taking any	106/1236 (8.6)	1.0	1.0
Taking some medications	3/278 (1.1)	**0.13 (0.03, 0.47)**	**0.09 (0.02, 0.33)**
Breastfeeding			
Not breastfeeding	103/1286 (8.0)	1.0	1.0
Yes breastfeeding	6/228 (2.6)	**0.33 (0.17, 0.64)**	**0.16 (0.08, 0.30)**
WHO stage at initial screening			
I	46/476 (9.7)	1.0	
II	14/309 (4.5)	**0.47 (0.26, 0.84)**	
III	4/151 (2.7)	**0.27 (0.13, 0.58)**	
IV	5/40 (10.0)	1.03 (0.53, 2.00)	
CD4 at initial screening			
<100	3/167 (1.8)	1.0	1.0
100–250	16/312 (5.1)	2.85 (0.89, 9.13)	2.74 (0.85, 8.82)
251–350	9/173 (5.2)	2.90 (0.95, 8.83)	2.22 (0.71, 6.98)
351+	81/862 (9.4)	**5.23 (1.54, 17.74)**	**4.71 (1.41, 15.80)**

or were improved beyond 100 cells/mm^3 by week 12 post-ART initiation (Table 5). Factors associated with high incidence of pregnancy but which did not reach statistical significance include being currently married when compared to never married (adj. IRR = 1.41; 95% CI 0.76, 2.59) and CD4 counts of 100 or higher by week 12 while on ART. Older age 35–45 years was significantly associated with lower incidence of pregnancy (adj. IRR = 0.27; 95% CI 0.15, 0.50). Pregnancy incidence tended to increase when both women and their spouses desired more children, but this increase was not significant (adj. IRR = 1.07; 95% CI 0.43, 2.63). If only the woman wanted (more) children, the incidence of pregnancy tended to be lower (adj. IRR = 0.50; 95% CI 0.09, 2.81) compared to when both partners did not want (more) children. The first viral load suppression data were available at week 24 after initiating ART. Although women with undetectable viral load had higher pregnancy rates, this was not significantly different from women with detectable viral loads, adj. IRR = 1.52; 95% CI (0.72; 3.22; results not shown in Table 5). Use of family planning methods, BMI at week 12, and WHO stage at week 12 were not associated with incident pregnancy. Figure 1 shows the Kaplan-Meier cumulative probability of incident pregnancy in the first 48 weeks while on ART treatment, by CD4 counts level at week 12. The cumulative probability of incident pregnancy increased overtime and was higher among women with CD4 count ≥100+ compared to those with CD4 counts level of less than 100 cells/mm^3, but this difference was not statistically significant (log rank $\chi^2 = 2.32$; $p = 0.3133$). The incidence of pregnancy prior to ART 13.1/100 py was significantly lower compared to incidence after ART initiation, 24.6/100 py (IRR = 0.54, 95% CI 0.37, 0.81, $p < 0.0017$).

Table 3: Incidence of pregnancy, unadjusted and adjusted incidence rate ratios among HIV+ women prior to ART initiation.

Characteristics	Number of women	Incident pregnancy/pyrs	Incidence/100 pyrs (95% CI)	IRR (95% CI)	Adjusted IRR (95% CI)
Overall	712	65/494.6	13.1 (10.14, 16.75)		
CD4 at screening					
251–350	130	9/72.4	12.4 (5.68, 23.60)	1.0	1.0
351+	582	56/422.1	13.2 (10.02, 17.23)	1.07 (0.57, 2.01)	0.95 (0.44, 2.05)
Age (years)					
15–24	84	14/54.5	25.7 (14.0, 43.31)	1.0	1.0
25–34	389	44/268.4	16.4 (11.91, 22.00)	0.64 (0.36, 1.12)	0.73 (0.45, 1.19)
35–45	239	7/171.7	3.5 (1.28, 7.60)	**0.16 (0.06, 0.40)**	**0.15 (0.07, 0.34)**
Marital status					
Never married	60	2/40.1	5.0 (0.60, 18.03)	1.0	1.0
Divorced/separated/widowed	310	20/217.2	9.2 (5.62, 14.22)	1.84 (0.45, 7.53)	3.83 (0.55, 26.77)
Currently married	342	43/237.2	18.1 (13.12, 24, 42)	3.62 (0.84, 15.74)	5.90 (0.87, 40.04)
Use of any medication to prevent pregnancy					
Not taking any	485	57/325.8	17.5 (13.25, 22.67)	1.0	1.0
Taking some medications	227	8/168.8	4.7 (2.05, 9.34)	**0.27 (0.10, 0.73)**	**0.22 (0.08, 0.61)**
Breastfeeding					
Not breastfeeding	572	49/397.9	12.3 (9.11, 16.28)	1.0	1.0
Yes breastfeeding	140	16/96.7	16.5(9.46, 26.87)	1.34 (0.95, 1.89)	0.43 (0.48, 1.06)
Parity (ever live-births)					
Prime gravid	301	38/211.1	18.0 (12.74, 24.71)	1.0	1.0
1–3	134	8/93.1	8.4 (3.71, 16.93)	**0.48 (0.27, 0.83)**	**0.43 (0.22, 0.86)**
4+	267	18/184.5	9.8 (5.78, 15.42)	**0.54 (0.30, 0.99)**	**0.73 (0.39, 1.36)**
WHO stage at initial screening					
I	249	20/174.5	11.5 (7.00, 17.70)	1.0	
II	133	7/90.6	7.7 (3.11, 15.92)	0.67 (0.28, 1.62)	
III/IV	35	1/22.5	4.4 (0.11, 24.76)	0.39 (0.06, 2.56)	

4. Discussion

We found that the incidence of pregnancy was lower prior to initiation of ART and significantly increased while on ART. Pregnancy prevalence was significantly reduced with lower CD4 counts and tended to increase among women with viral suppression and good CD4 cell response while on ART. The incidence of pregnancy also tended to increase when both women and their spouses desired more children and decreased with older age. These findings are consistent with other studies which showed increased fertility after ART initiation [21, 22].

The significantly higher incidence of pregnancy after initiating ART compared to prior to ART could be due to improved immune status or reduced HIV viral load. An ethnologic study in Nigeria showed that improvements in health status after initiating ART enabled women to reassess their childbearing [24], while in another study people living with HIV (PLHIV) viewed having children as making them look forward to the future thus providing them a reason to live [1, 25]. Such views are important to be integrated in reproductive health service components of the ART programs as a way of empowering women to make appropriate reproductive health decisions. Previous studies suggest that women on HAART are significantly more likely to use contraceptives [25–27], but treatment optimism affects their fertility intentions [28–30]. In Rakai, the provision of Prevention of Mother to Child Transmission (PMTCT) interventions,

Table 4: Prevalence of pregnancy, unadjusted and adjusted PRR among HIV women at time of initiating ART.

Characteristics	Pregnant/total (%)	Unadjusted PRR (95% CI)	Adjusted PRR (95% CI)
Overall	57/566(10.1)		
Baseline CD4			
<100	4/124 (3.2)	1.0	1.0
100–250	53/442 (12.0)	**3.72 (1.37, 10.08)**	**3.24 (1.51, 6.93)**
Age			
15–24	8/46 (17.4)	1.0	1.0
25–34	35/304 (11.5)	0.66 (0.34, 1.34)	0.73 (0.39, 1.34)
35–45	14/216 (6.5)	**0.37 (0.17, 0.84)**	**0.47 (0.25, 0.91)**
Desire for more children			
Both do not want	28/387 (7.2)	1.0	1.0
Only male partner wants	14/102 (13.7)	**1.90 (1.04, 3.47)**	**1.54 (0.81, 2.94)**
Only female wants	1/18 (5.6)	0.77 (0.11, 5.34)	0.78 (0.10, 5.90)
Both want	14/59 (23.7)	**3.28 (1.84, 5.86)**	**2.27 (1.04, 4.97)**
Marital status			
Not in union	22/308 (7.1)	1.0	1.0
In union	35/258 (13.6)	**1.90 (1.14,3.15)**	**1.33 (1.00, 1.76)**
HIV status disclosure			
Not to anybody	5/98 (5.1)	1.0	1.0
Yes, to somebody	52/468 (11.1)	2.18 (0.89,5.31)	1.86 (0.87, 3.99)
*FP use at ART initiation			
None	52/452 (11.5)		
Only condoms	0/8 (0)		
Other methods	0/7 (0)		

*Total less than 566 because some do not have data available.

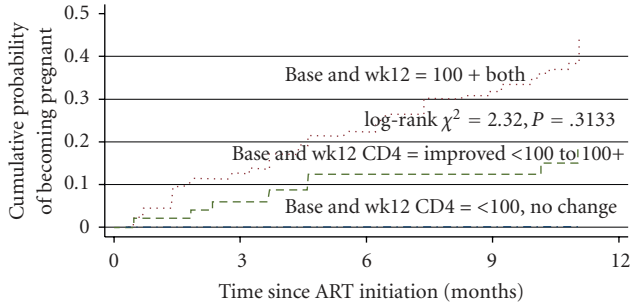

Figure 1: Probability of pregnancy while on ART among women, 15–45 years.

including infant formula to HIV-exposed babies, might also have affected HIV+ women fertility intentions.

Desire for (more) children, being currently married, and younger age are known to be associated with increased pregnancy. A recent study in Kenya has shown that HIV/AIDS patients have increased the desire for children and increased fertility partly due to increased infant/child mortality and reduced breastfeeding [31]. Social expectations are also associated with increased desire for more children [28], and ART is associated with higher fertility desires and intentions [28–30]. In our study, a significantly higher proportion of women reported that their spouses desired more children compared to the women. This finding was consistent with previous studies in various cultural settings [32, 33]. Men's higher desire for (more) children or their future reproductive intentions may partly be explained by their knowledge about the positive effects of PMTCT on infant health [1]. This finding suggests that reproductive health services, especially educative health messages about reproductive issues, should also be extended to involve men rather than focusing only on the needs of the female.

In summary, our data and findings from other previous studies [21, 22] show that women's fertility increases while on ART either as a result of their improved health and well-being leading to a reevaluation of their intentions and decisions regarding childbearing, or through improvement of their immunological and virological outcomes that may increase the probability of conception. Therefore, ART programs need to broaden their counseling on reproductive health services to follow the WHO (2006) *Guidelines on care, treatment and support for women living with HIV/AIDS and their children in resource-constrained settings* which recommend that the selection of ART regimen for women should consider the possibility of a planned or unintended pregnancy.

Women in the ART programs need to be equipped with information on effective contraceptive methods to prevent

Table 5: Incidence of pregnancy, unadjusted and adjusted IRR among HIV+ women after initiating ART.

Characteristics	Number of women	Incident pregnancy/pyrs	Incidence/100 pyrs (95% CI)	IRR (95% CI)	Adjusted IRR (95% CI)
Overall	244	48/195.5	24.6 (18.1, 32.6)		
Baseline and week 12 CD4					
Both <100	18	1/14.69	6.8 (0.17, 37.92)	1.0	1.0
Both 100+	182	39/145.50	26.8 (19.06, 36.64)	3.94 (0.68, 22.70)	4.12 (0.75, 22.50)
Base CD4 <100, wk12 100+	44	8/35.38	22.6 (9.76, 44.55)	3.32 (0.50, 2.26)	3.24 (0.46, 22.60)
Age (years)					
15–24	19	6/14.31	41.9 (15.39, 91.26)	1.0	1.0
25–34	133	34/103.94	32.7 (22.65, 45.71)	0.78 (0.44, 1.39)	0.82 (0.38, 1.77)
35–45	92	8/77.31	10.3 (4.47, 20.39)	**0.25 (0.13, 0.46)**	**0.27 (0.15, 0.50)**
Desire for more children					
Both do not want	179	34/144.52	23.5 (16.29, 32.88)	1.0	1.0
Only male wants	35	6/28.88	20.8 (7.62, 45.23)	0.88 (0.33, 2.33)	0.81 (0.36, 1.84)
Only female wants	8	1/6.13	16.3 (0.41, 90.89)	0.69 (0.12, 4.12)	0.50 (0.09, 2.81)
Both want	22	7/16.04	43.6 (17.55, 89.92)	*1.85 (0.84, 4.10)*	1.07 (0.43, 2.63)
Marital status					
Not married	142	23/117.13	19.6 (12.45, 29.46)	1.0	1.0
Married	102	25/78.44	31.9 (20.63, 47.05)	1.62 (0.81, 3.25)	1.41 (0.76, 2.59)
HIV status disclosure					
Not to anybody	58	15/45.69	32.8 (18.37, 54.15)	1.0	1.0
Yes, to somebody	186	33/149.88	22.0 (15.16, 30.92)	0.67 (0.38, 1.19)	0.69 (0.36, 1.32)
*Family planning method					
None	69	18/51.83	34.7 (20.58, 54.89)	1.0	
Condom only	45	12/35.45	33.8 (17.49, 59.13)	0.97 (0.47, 2.00)	
Other	11	2/8.34	24.0 (2.91, 86.67)	0.69 (0.16, 3.08)	
*BMI at week 12					
Under weight, <18.5	24	5/18.11	5 (8.96, 64.43)	1.0	
Normal weight	139	28/113.83	24.5 (16.35, 35.55)	0.89 (0.29, 2.72)	
Overweight/obese	33	5/27.23	18.4 (5.96, 42.85)	0.67 (0.14, 3.16)	
*WHO stage at week 12					
I & II	94	19/75.81	25.1 (15.09, 39.14)	1.0	
III & IV	48	6/39.27	15.3 (5.61, 33.26)	0.61 (0.23, 1.63)	

* Data not available on all the 244 women.

pregnancy, if so desired, as well as information on the potential drug interactions with hormonal contraceptives to enable them make informed decisions on childbearing.

The other potential program/policy challenge is that pregnancy is an indicator of engagement in unprotected sex. If women and their spouses still desire to have a (another) child they will have unprotected sex, which can result in increased risk of HIV transmission to sexual partners, in addition to acquisition of multiple HIV virus strains to the already infected woman on ART. Such scenarios can lead to failure on first-line regimen further exposing the women to increased risk of morbidity and mortality.

On the other hand, ART programs that are providing or advising women to use hormonal contraceptives should provide information on the potential risk of interaction between ARVs and some hormonal contraceptives [34] which alter the safety and effectiveness of both the hormonal contraceptives and the antiretroviral drugs. Also ART

programs need to be aware of the potential risk of pill burden of both ARV drugs and contraceptives that may compromise adherence to the contraceptives or the HIV-related medication if method of contraceptive choice is pills.

Therefore, ART programs should fully engage women and their partners whenever possible, to make them aware of such considerations while selecting contraceptive methods if they desire to use them, or the potential consequences of having unprotected sex if they still desire to have a (another) child. Full reproductive health services integration into HIV care program including family planning as well as health education should involve both men and women to help them make informed decisions on their reproductive and sexuality needs.

This study had limitations especially in the depth and scope of data that could be used to address some key issues. For example, detailed data on socioeconomic status as well as contraceptive methods and history of child bearing were not collected at the time of enrolling in HIV care. Also, the available sample size for this analysis was limited resulting in wide confidence intervals for measures of association used. However, our findings are still consistent with results from other larger studies addressing the incidence pregnancy and enrolment into ART programs.

5. Conclusion

In conclusion, fertility is increased among HIV-infected women after initiation of ART, and the desire by both men and women to have (more) children is still high reinforcing the need for increased reproductive health services including family planning as well as educative health messages to enable HIV-infected women and their spouses make informed decisions about their reproductive and sexuality needs.

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Research Article

CD8$^+$ T-Cell Responses before and after Structured Treatment Interruption in Ugandan Adults Who Initiated ART with CD4$^+$ T Cells <200 Cell/μL: The DART Trial STI Substudy

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Objective. To better understand attributes of ART-associated HIV-induced T-cell responses that might be therapeutically harnessed. *Methods*. CD8$^+$ T-cell responses were evaluated in some HIV-1 chronically infected participants of the fixed duration STI substudy of the DART trial. Magnitudes, breadths, and functionality of IFN-γ and Perforin responses were compared in STI ($n = 42$) and continuous treatment (CT) ($n = 46$) before and after a single STI cycle when the DART STI trial was stopped early due to inferior clinical outcome in STI participants. *Results*. STI and CT had comparable magnitudes and breadths of monofunctional CD8$^+$IFNγ^+ and CD8$^+$Perforin$^+$ responses. However, STI was associated with significant decline in breadth of bi-functional (CD8$^+$IFNγ^+Perforin$^+$) responses; $P = .02$, Mann-Whitney test. *Conclusions*. STI in individuals initiated onto ART at <200 CD4$^+$ T-cell counts/μl significantly reduced occurrence of bifunctional CD8$^+$IFNγ^+/Perforin$^+$ responses. These data add to others that found no evidence to support STI as a strategy to improve HIV-specific immunity during ART.

1. Introduction

Previous studies correlated ART uptake with diminution of HIV-specific responses [1–3], whilst others linked it with restoration of these responses [4–6]. Uptake of ART has also presented challenges such as high pill burden, drug resistance, cost, and drug-induced toxicities. Structured treatment interruption has been widely evaluated in an effort to decrease costs and side effects of ART uptake and to investigate associated immunological and clinical outcomes. Consequently, several large clinical trials have explored whether minimizing dose [7–10], or duration of ART exposure [11, 12] and therefore, their toxic effects would be beneficial.

The cellular arm of the immune system has been associated with protection from HIV disease progression [13]. This has been remarkably demonstrated in CD8$^+$ T-cell depletion studies in macaque models [14–16] as well as in acute infection studies correlating the emergence of virus specific CD8$^+$ T cells with control of viraemia [17]. In addition, correlation between slow HIV-1 disease progression and protective HLA allele-induced CTL responses has been observed [18], and associations between viral escape in targeted HIV epitopes and elevation of plasma viral loads have been demonstrated [19]. Despite this body of evidence, consistent quantitative and qualitative correlates of protection remain elusive. Evaluations comparing breadths and magnitudes of CD8$^+$ T-cell responses in infected persons

have sometimes failed to show any association between these parameters and viral load [18, 20, 21]. Moreover, despite the preservation and increase in HIV-specific $CD8^+$ T-cell responses in individuals who initiated ART during acute infection [22, 23], attempts to boost T-cell responses, for example, through autovaccination during ART interruptions in chronic HIV infection, have proven disappointing [9, 19, 24–26].

The potential for STI to boost HIV-specific immunity through controlled autologous virus exposure has been previously evaluated in chronically infected HIV patients [27–29] and has been reviewed in [30]. In some of these studies, $CD8^+$ T-cell responses were boosted by re-exposure to autologous virus, although increases in virus-induced $CD4^+$ T-cell responses were transient. Marked interpatient heterogeneity and small cohort sizes in previous studies yielded inconclusive and inconsistent results [26, 31–33]. While functional attributes of $CD8^+$ T-cell responses have accounted for differential disease outcomes observed in chronic HIV infection [34–36], the extents to which differences in $CD8^+$ T-cell functionality occur during STI remain unclear.

In this study, we evaluated a proportion of DART trial participants [37] who enrolled into the DART STI substudy [38] in order to better understand the possible immunological outcomes of STI in individuals initiated onto ART with advanced HIV disease (<200 $CD4^+$ T cells/μL). Participants randomized to STI or to continuous treatment (CT) at one clinical centre (MRC/UVRI, Entebbe, Uganda) were compared in order to reevaluate the hypothesis that STI would allow regeneration of HIV-specific responses through cyclical viral antigen exposure whilst CT would not allow viral antigen exposure and consequent regeneration of immune responses.

2. Methods

2.1. Study Design and Population. The multisite DART main trial (ISCRTN 13968779-DART) [37] recruited 3316 chronically HIV-infected, ART-naïve adults (except for ART exposure during pregnancy for prevention of mother-to-child transmission) with WHO stage 2, stage 3, or stage 4 symptomatic disease and $CD4^+$ counts $\leq$200 cells/μL at screening to primarily compare clinical driven monitoring with laboratory monitoring plus clinical monitoring as strategies of ART delivery. A fixed duration STI randomization substudy (12 weeks on ART and 12 weeks off ART per STI cycle) was nested within the DART trial to primarily evaluate whether STI was clinically noninferior to continuous treatment. Participants, who had attained $\geq$300 $CD4^+$ T cells/μL by 48 or 72 weeks after DART trial entry, underwent a second randomization at 52 or 76 weeks to either STI ($n = 408$) or CT ($n = 405$), with intention to follow up 8 STI cycles. At the time of STI/CT randomization, we consecutively recruited 60 STI and 60 CT participants from each arm. Heparinised blood (10 mls) was collected at the beginning and end of one STI cycle corresponding to week 0 and week 12, respectively. By the time the trial was stopped early following a DSMC review, 42 STI and 46 CT participants had completed one full STI cycle. None of the 88 evaluated subjects (60 women) received any prior exposure to ART even for prevention of mother-to-child transmission.

2.2. $CD4^+$ T-Cell Count Quantification and Timing of T-Cell Response Evaluations. Scheduled 12 weekly $CD4^+$ T-cell counts were performed within the main DART trial using FACScount (Becton Dickinson) according to manufacturer's protocols. The beginning of the first STI/CT randomization cycle was timed to occur 4 weeks after the last prerandomization $CD4^+$ count. The next $CD4^+$ count occurred 8 weeks after the start of the cycle and 8 weeks after restarting ART in the STI group. To evaluate HIV-induced $CD8^+$ T-cell responses, additional blood specimens were collected in both groups at the beginning of an STI cycle and when ART was restarted in the STI group.

2.3. HIV Peptides and Preparation of Pools. Uganda is predominantly infected with HIV-1 clades A and D as well as recombinants of these [18, 39–41]. We therefore attempted to work with peptide pools that were matching these strains as much as possible. Peptides were obtained through the National Institute of Health, AIDS Research Reference Reagent programe (https://www.aidsreagent.org/Index.cfm). Unfortunately, peptides available from this source were only representative of clade B with the exception of peptides corresponding to the Gag region which matched the strains found in our study population. Individual peptides consisted of 20-mer peptides overlapping by 11 amino acids and spanning the HIV-1 consensus clade A (92UG037) and D (94UG114) Gag sequences, as well as 15-mer peptides overlapping by 11 amino acids spanning the HIV-1 clade B Nef, Tat, Vif, Rev, Vpr, Vpu, and Pol consensus sequences. Individual peptides were grouped together into pools according to HIV protein. Each individual peptide within a pool was used at a final concentration of 2 μg/ml. Due to limitations in blood volume, it was not possible to map individual responding T-cell epitopes; consequently, $CD8^+$ T-cell responses to complete HIV-1 protein pools are presented.

2.4. Intracellular Cytokine Staining Procedure. Activation and processing of peripheral blood mononuclear cells (PBMCs) for intracellular cytokine staining analysis was performed as previously described [42]. Briefly, activation reagents (HIV peptide pools and 1 μg/mL costimulatory CD28 and CD49d antibodies (BD Biosciences)) were added to 1 mL of fresh whole heparinised blood and incubated at 37°C, in a 5% CO_2 in air atmosphere for 1 hour, followed by further 5 hours in the presence of a secretion inhibitor (Golgi Plug, 10 μg/ml, BD Biosciences). Red blood cells were subsequently lysed with FACS lysis solution (BD Biosciences). The PBMCs were fixed and permeabilised with FACS permeabilising buffer (BD Biosciences) according to the manufacturer's protocol and then simultaneously stained for 1 hour in the dark with surface antibodies CD3-FITC, $CD8^+$-PerCP and intracellular antibodies IFN-γ-APC and Perforin-PE (BD Biosciences) at room temperature. Stained cells were washed and fixed

with Cellfix (BD Biosciences). At least 200,000 PBMCs were acquired on a FACSCalibur flow cytometer (BD Biosciences). Negative controls (backgrounds) were autologous PBMCs that were not stimulated with peptides but had otherwise been treated identically. Positive controls were specimens that had been stimulated with 10 μL of 1 μg/μL of Staphylococcal Enterotoxin B (SEB). Flow cytometry data was analysed using CellQuest (BD Biosciences). Criterion for evaluating positive responses was $\geq$0.03% of $CD8^+$ T cells responding to any of the HIV peptide pools after subtracting the background cytokine production. Response to the two Gag pools (clade A and D) was evaluated collectively as the mean Gag response. Monofunctional T cells were defined as those secreting either IFN-γ or Perforin alone; bifunctional T cells were defined as cells that simultaneously released both Perforin and IFN-γ. Breadth of response was defined as the number of HIV protein pools targeted by each participant. The frequency of HIV-induced response was defined as the proportion (%) of $CD8^+$ T cells inducing HIV-specific IFN-γ or Perforin responses or both. The frequency of responders was defined as the proportion of participants in which HIV-specific T-cell responses were induced.

2.5. Statistical Analysis. Medians and interquartile ranges (IQR) were used for all summary presentations of $CD4^+$ T-cell counts and T-cell responses. Kruskal-Wallis rank and Mann-Whitney tests were used to compare medians. Proportions were compared using Pearson's chi-square test. Median alterations in response for each patient were compared by evaluating the increase or decrease in $CD8^+$ T-cell response at the beginning and end of a 12-week cycle. This approach provided a more powerful test of differences between CT and STI, since it allowed each patient to act as their own control therefore reducing variability. Graph Pad 5.0 and Excel were used for graphical presentations of the data. All statistical analyses were performed using Stata v8.0 (Stata Corp, Texas).

3. Results

3.1. Study Population and Baseline Characteristics. Of the 120 participants recruited into this substudy, 88 subjects (42 STI and 46 CT) had completed one cycle of STI by the time the Trial Steering Committee (TSC) followed the recommendation of the DSMC to terminate the STI trial due to observed inferior clinical outcomes among participants randomized to the STI group. Similar to the overall demographics seen in the main DART trial, our study evaluated significantly more females ($n = 60$) than males ($n = 28$). There was no significant difference in the median age of CT (38; 33–44 years) and STI (38; 32–44 years) subjects. The proportion of STI and CT subjects with pre-STI/CT randomization ART exposure duration of either 52 (28/46 versus 22/42, resp.) or 76 weeks (18/46 versus 20/42, resp.) was also comparable.

3.2. Comparison of $CD4^+$ T-Cell Counts. In the current study subjects, the median $CD4^+$ counts at ART initiation within the main DART trial were comparable between STI (124; IQR 87–177 $CD4^+$ T cells/μL) and CT subjects (129; IQR 77–159 $CD4^+$ T cells/μL). Inline with the findings of the main DART STI trial [38], we did not find any difference between $CD4^+$ T-cell counts of STI (391; 334–449 cells/μL) and CT subjects (391; 333–449 cells/μL) at STI/CT randomization. Additionally, $CD4^+$ T-cell counts were comparable among subjects that were randomized after 52 weeks (397; 334–457 cells/μL) or 76 weeks of ART initiation (385; 330–434 cells/μL). These data suggest that an additional 24 weeks of ART exposure prior to STI/CT randomization did not significantly influence the $CD4^+$ T-cell counts anymore at this stage.

3.3. Frequency of HIV-Specific $CD8^+$ T-Cell Responses at STI/CT Randomization. We evaluated the frequency of HIV Gag (clade A and D), Nef, Tat, Vif, Rev, Vpr, and Vpu (all clade B)-induced T-cell responses as the proportion of subjects with detectable virus-specific $CD8^+$ T-cell IFN-γ or Perforin. Overall, HIV-specific $CD8^+$ T-cell responses were detected against all the seven evaluated HIV proteins as follows: Gag- (85%), Nef- (67%), Tat- (51%), Vpr- (53%), Vpu- (58%), Rev- (52%) and Vif- (48%). At STI/CT randomization, 99% of the participants had the potential to induce IFN-γ while 31% completely lacked the intrinsic potential to induce Perforin (as evaluated by stimulation with SEB) (Figures 1(a) and 1(b), resp.)

We then evaluated the relationship between the duration of ART uptake before STI/CT randomization, $CD4^+$ count at ART initiation and the frequency of virus-specific $CD8^+$ T-cell responses at STI/CT randomization. Both HIV-specific IFN-γ (Figure 1(c)) and Perforin T-cell responses (Figure 1(d)) did not significantly differ between participants who received ART for 52 or 76 weeks. Similarly, there was no correlation between the $CD4^+$ count at ART initiation and the frequency of virus-induced T-cell responses (data not shown). Taken together, these findings suggest that the additional 24 weeks of ART in individuals who underwent STI/CT randomization at 76 weeks did not significantly modify the proportion of subjects with detectable HIV-induced $CD8^+$ T-cell recognition.

3.4. Breadth of HIV-Induced $CD8^+$ T-Cell Responses at STI/CT Randomization. Breadth of response was defined as the number of HIV protein pools targeted by each subject. Overall, breadths of $CD8^+IFN\gamma^+$, $CD8^+Perforin^+$ and $CD8^+IFN\gamma^+Perforin^+$ at STI/CT randomization lacked correlation with the nadir $CD4^+$ count and did not differ in subjects that took ART for either 52 or 76 weeks before STI/CT randomization (Figure 2). These data suggest that the additional 24 weeks of ART uptake before STI randomization did not influence the breadth of virus-specific T-cell responses.

3.5. Magnitude of HIV-Induced T-Cell Responses at STI/CT Randomization. Magnitude of T-cell response was defined as the proportion (%) of $CD3^+CD8^+$ T cells inducing release of either IFN-γ, Perforin or both, following stimulation with HIV-1 Gag, Nef, Tat, Vpr, Vpu, Rev and Vif peptide

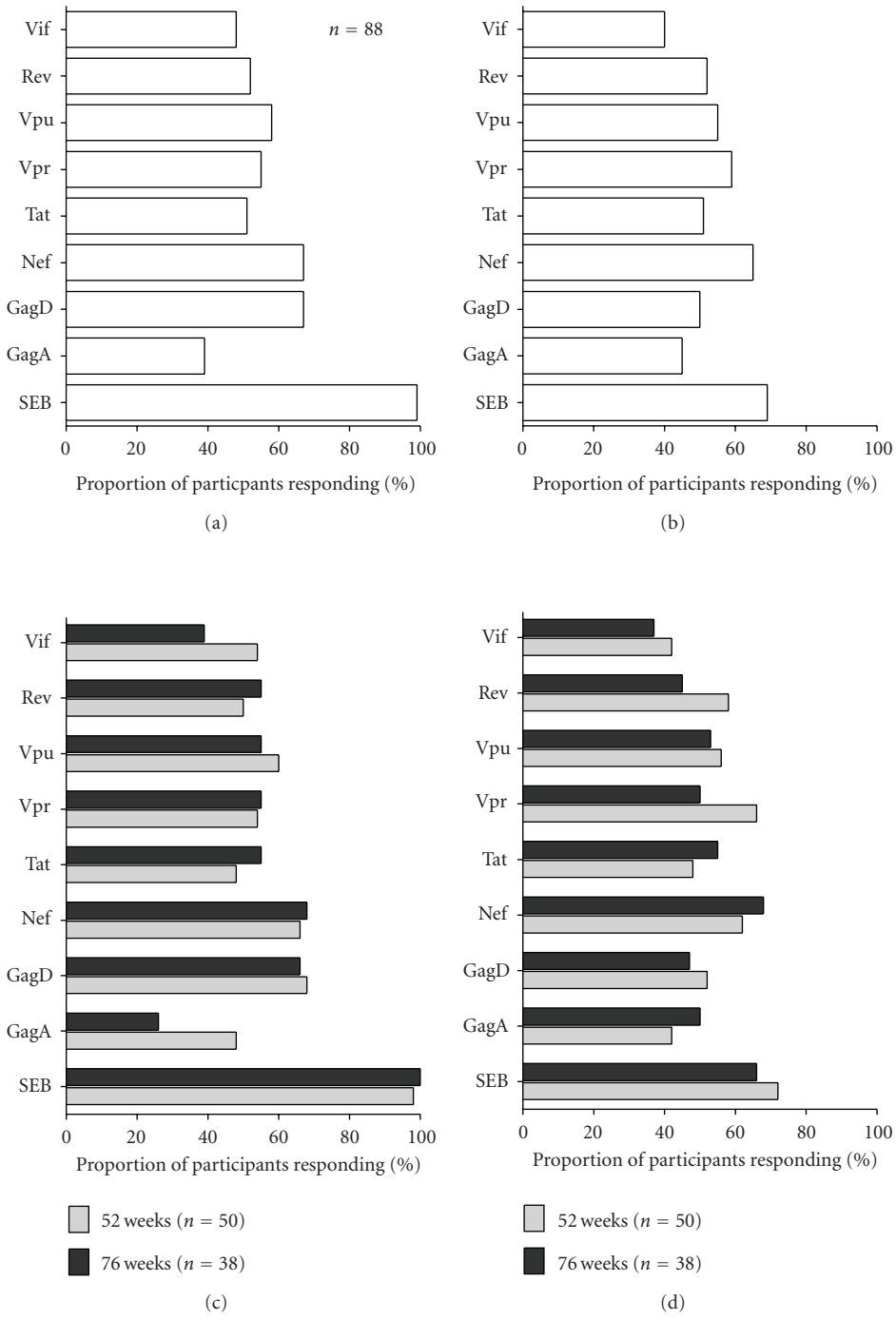

Figure 1: HIV-specific $CD8^+$ T-cell responses at STI/CT randomization. This figure compares the proportions (%) of participants ($n = 88$) inducing (a) HIV-specific IFN-γ or (b) Perforin responses at the time point of STI/CT randomization and proportions of participants inducing (c) HIV-specific IFN-γ or (d) Perforin responses at STI/CT randomization initiated 52 or 76 weeks after ART.

pools. Gag data is presented as the average of the two Gag pools. At STI/CT randomization, the inherent magnitude of T-cell responses as evaluated using Staphylococcal Enterotoxin B (SEB) was significantly lower for Perforin (0; 0–0.12% $CD3^+CD8^+$ T cells) compared to IFN-γ (5; 3–9% $CD3^+CD8^+$ T cells), respectively; $P < .0001$, Mann-Whitney test. Thus, it may be seen that HIV-1-specific $CD8^+$ T-cell responses in this cohort were mostly comprised of IFN-γ-secreting cells at the time point of STI/CT randomization. We therefore used the IFN-γ data to evaluate the relationship between STI and the pattern of virus-specific T-cell recognition. Overall, Nef and Gag (mean of the two Gag pools) induced significantly higher magnitude of $CD8^+$ T-cell responses than Tat, Vpr, Vpu, Rev, and Vif in both CT

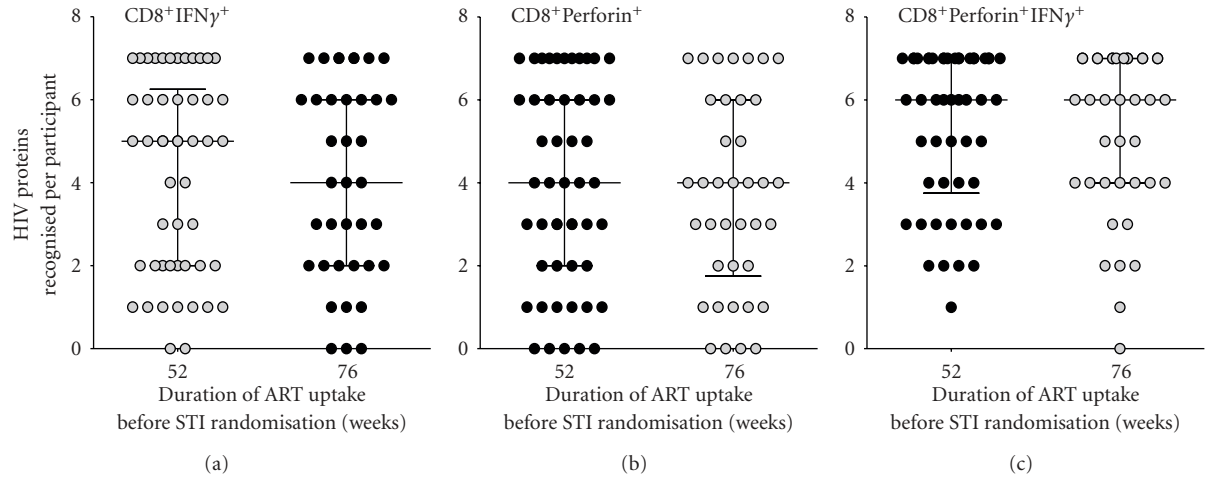

Figure 2: Relationship between breadth of HIV-specific CD8+ T-cell recognition at STI/CT randomization and the duration of pre-ART randomization. This figure evaluates whether ART uptake for either 52 weeks or 76 weeks preceding randomization had any influence on the breadths of HIV-specific (a) CD8+IFNγ+, (b) CD8+Perforin+, or (c) CD8+IFNγ+Perforin+ T-cell responses. Individual HIV peptides were grouped together in pools according to HIV protein. HIV-specific T-cell recognition was evaluated against these pools that were based on consensus sequences of HIV-1 Gag clades A (92UG037) and D (94UG114); and consensus sequences of HIV-1 clade B (Nef, Tat, Vif, Rev, Vpr, Vpu, and Pol). HIV-specific responses to Gag (clades A and D) were considered concomitantly. Breadth was defined as the number of HIV protein pools recognised per individual. Horizontal bars represent medians and interquartile ranges.

and STI participants; magnitudes of Gag- and Nef-induced responses did not significantly differ (Figures 3(a) and 3(b)).

3.6. Magnitudes after One STI Cycle (12-Week On/12-Week Off). The median change in Nef-, Tat-, Vpr-, Vpu-, Rev-, and Vif-induced IFN-γ magnitudes remained comparable among CT and STI subjects although there was significant increase in magnitude of Gag-induced IFN-γ (Figure 3(c)). Despite the apparent improvement in magnitudes of Gag-induced IFN-γin STI subjects, the magnitude of Perforin responses remained significantly lower compared to IFN-γ even after 12 weeks of CT or STI, and the ability (proportion of individuals) of the STI arm to induce simultaneously release of both IFN-γ and Perforin did not improve even for Gag (data not shown). Taken together, these data suggest a preexisting functional impairment of CD8+ T cells in this cohort, mainly characterised by diminution of Perforin-inducing potential, which remained unchanged after 12 weeks of STI.

3.7. Breadths after One STI Cycle (12-Week On/12-Week Off). We evaluated the relationship between one STI cycle and the breadth of HIV-induced CD8+ T-cell responses (number of HIV proteins recognised per subject). Recognition of the two Gag pools was analyzed concomitantly to represent the average response to the Gag protein. Median change in breadth was defined as the increase or decrease in number of HIV pools recognised after completing one STI cycle. After 12 weeks of STI or CT, the median change in breadth of CD8+IFNγ+ (Figure 4(a)) and CD8+Perforin+ (Figure 4(b)) did not significantly differ between STI and CT participants. However, STI was associated with a significant reduction in breadth of bifunctional CD8+IFNγ^-Perforin+ responses (median -1, IQR 0 to -3.3 protein pools) compared to CT, (median 0, IQR -2.0 to 3.0 HIV protein pools) (Figure 4(c)). Similarly, there was no difference in the breadth of monofunctional IFN-γ responses targeted (Figures 4(d) and 4(e)). However, 12 weeks of STI resulted in significantly lower bifunctional T-cell responses, (median 1, IQR 0–3 protein pools) compared to a similar timeframe on continuous treatment (median 3, IQR 1–4 protein pools); $P = .027$ (Figure 4(f)), Mann-Whitney test. These data suggest that STI was associated with degeneration rather than restoration of functional CD8+ T-cell responses in this cohort.

4. Discussion

Better understanding of HIV-induced CD8+ T-cell responses in chronic HIV-1 infection may be important for the development of preventive or therapeutic approaches designed to enhance T-cell-mediated immunity. In this study, we used structured treatment interruption that was initiated within the DART trial [38] as a model to reevaluate the hypothesis that STI would allow for regeneration of HIV-specific responses through cyclical viral antigen exposure. The DART cohort differed from previous studies that assessed individuals initiated on ART at an earlier HIV disease state [8, 10, 43]. The principle findings of this study were firstly that magnitudes of CD8+ T-cell responses did not significantly differ following STI or CT for an identical timeframe, secondly that breadths of monofunctional CD8+ T-cell responses were comparable between STI and CT, and thirdly that STI was apparently associated with significant loss of bifunctional CD8+ T-cell responses.

Because the overall potential to induce Perforin was apparently impaired in this cohort initiated on ART at

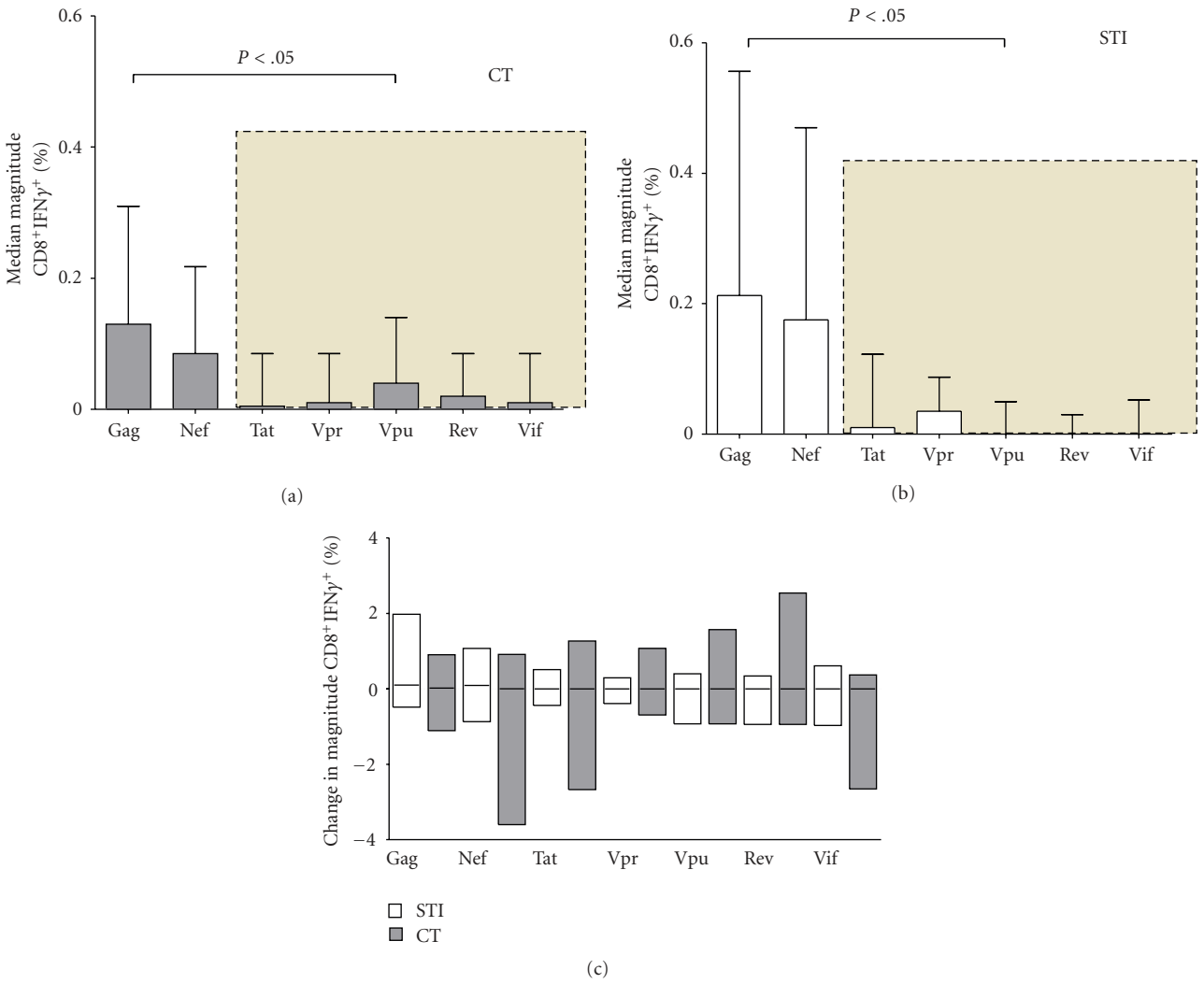

Figure 3: Magnitudes of $CD8^+$ T-cell responses associated with STI. This figure compares the median magnitudes of HIV-induced $CD8^+$ T-cell IFN-γ responses in (a) STI and CT participants after one cycle of STI or matching time on CT. Bars represent medians, while error bars represent interquartile ranges. The shaded areas represent HIV proteins that induced significantly lower magnitudes of $CD8^+$IFN-γ^+ than Gag or Nef. (c) illustrates the median change in magnitudes of HIV-specific IFN-γ responses after one cycle of STI or matching time on CT. Horizontal bars represent medians.

around 125 (85–173) $CD4^+$ T cells/μL, we used the IFN-γ data to evaluate the relationship between STI and the profile of $CD8^+$ T-cell responses. Overall, Gag and Nef induced significantly higher magnitude of IFN-γ responses than other HIV proteins tested at STI/CT randomization in both STI and CT participants; this finding was consistent with others that reported relative immunodominance of Gag and Nef in this and other populations [17, 18]. However, there were some limitations related to the reagents used to evaluate $CD8^+$ T-cell responses, and the Gag results need to be interpreted with caution. The peptides used to stimulate $CD8^+$ T cells matched the infecting HIV strains only in the Gag region, whereas clade B peptides were used to evaluate responses to the other proteins. Consequently, virus-specific $CD8^+$ T-cell responses to other HIV proteins may not have been detected as efficiently as responses to Gag, where the infecting viral sequences were better represented. Indeed, several studies have shown that interclade cross-reactivity response rates tend to be lower than clade-specific responses [18, 44, 45] and that use of variant sequences can result in reduced T-cell recognition.

HIV-specific T-cell responses are common in infected adults, become progressively dysfunctional during chronic virus persistence, and exhibit rapid decay during ART uptake [1, 46–48]. Previous studies suggested that several functional attributes of HIV-specific $CD8^+$ T cells influence the differential disease outcome in chronic infection [34–36, 49, 50]. Continuous use of potent ART has been shown to significantly suppress viral replication [51–53], thereby potentially allowing for limited T-cell functional restoration. In this cohort, STI significantly correlated with diminution of $CD8^+$IFNγ^+Perforin$^+$ bifunctional T cells. This was consistent with previous reports of STI-associated functional impairment of T cells [19]; attribution of the prognostic

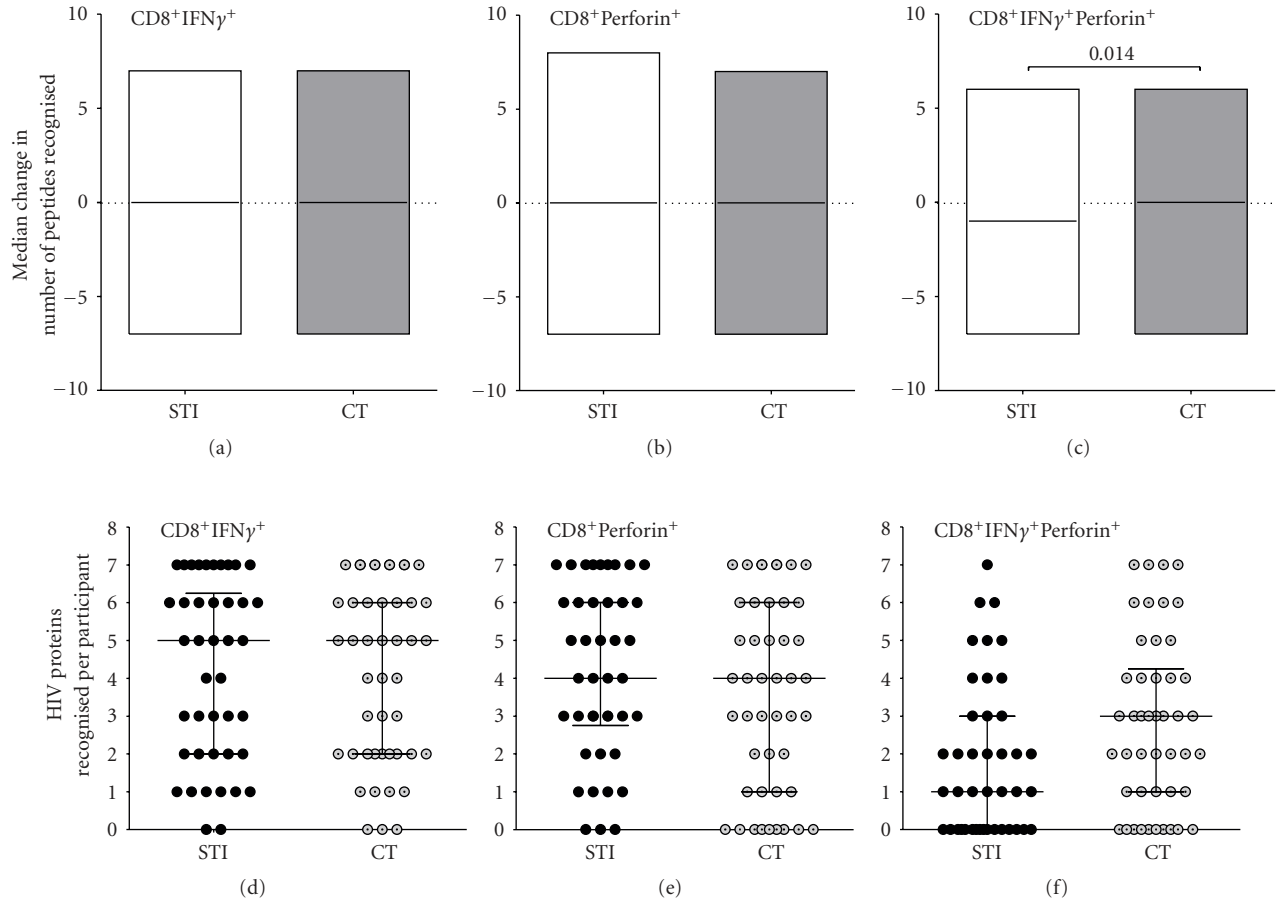

Figure 4: Breadths of $CD8^+$ T-cell responses after 12 weeks of STI and matching time point on CT. Study participants were evaluated for $CD8^+$ T-cell responses to complete peptide pools corresponding to Gag, Nef, Tat, Vpr, Vpu, Rev and Vif HIV proteins. Response to the two Gag pools was analysed concomitantly to represent response to the Gag protein. Breadth of $CD8^+$ T-cell response was defined as the number of HIV pools recognised by an individual. Median change in breadth was defined as the increase or decrease in number of pools recognised. Positive readouts indicate increase while negative readouts indicate decrease. The figure demonstrates changes in the breadth of $CD8^+IFN\gamma^+$ (a), $CD8^+Perforin^+$ (b) and $CD8^+IFN\gamma^+Perforin^+$, (c) responses among CT and STI participants; and compares the median number of peptide pools targeted with the induction of $CD8^+IFN\gamma^+$ (d), $CD8^+Perforin^+$ (e), and $CD8^+IFN\gamma^+Perforin^+$ (f) at the end of one STI cycle. Horizontal lines represent medians.

outcomes of STI to a defined virus-specific $CD8^+$ T-cell functional profile [50] and to findings that indicated that continued use of potent ART could defer this impairment [46, 54].

In this study, an extra 24 weeks of therapy in individuals that were randomized to STI or CT at 76 weeks did not result in any apparent change in profile of HIV-induced $CD8^+$ T-cell responses when compared to those that were randomized at 52 weeks. Consequently, these data suggest that the observed difference in breadth of the bifunctional $CD8^+$ T cell responses may be possibly attributed to STI rather than to the restoration of functionality by continued treatment. These data collectively suggest an STI-associated impairment of $CD8^+$ T-cell functionality and that the quality rather than the quantity of the HIV-specific $CD8^+$ T-cell responses correlated with the dynamics of STI.

There were other limitations to this study. First of all, we evaluated a population that was initiated onto ART at a very advanced stage of HIV disease potentially masking possible positive effect that others observed in individuals who initiated ART much earlier in disease [55, 56]. The benefits and risks of antiretroviral therapy can vary considerably with stage of disease, mainly due to irreversible destruction of the immune system that occurs as HIV infection progresses. Treatment during acute HIV infection may preserve and reconstitute HIV-specific immune function. Alternatively, the primary goal of late-stage disease treatment is to control viral replication leading to decreased morbidity and increased survival. While our sample size was not big enough to evaluate the effect of disease stage, exploration of the data revealed no correlation between the $CD8^+$ T-cell responses and the original $CD4^+$ count before ART initiation. Secondly, we evaluated only one cycle of STI; consequently, we could not extrapolate on possible longer-term effects of STI that have been reported in studies that used a longitudinal approach to evaluate several STI cycles [31]. Lastly, this study was limited to collect 10 mls of blood from each subject at each study visit. Subsequently, individual peptides were

grouped according to HIV protein and evaluated as pools. As a result, it was not possible to map individual responding T-cell epitopes in order to determine whether it was responses specific for a particular epitope that disappeared during an STI or all epitopes were affected equally.

In summary, the current study found no evidence to support the hypothesis that STI would enhance regeneration of HIV-induced T-cell responses in individuals starting ART with advanced HIV disease state. Instead, our findings are inline with previous reports that indicated ART-associated improvement of $CD8^+$ T-cell functional markers [52, 57], preferential destruction of HIV-infected $CD4^+$ T cells during treatment interruptions [58], and viral burden-induced deterioration of the quality of $CD8^+$ T-cell response even when HIV-specific IFN-γ responses were still apparent [59]. This study found no evidence to support STI as a strategy for ART delivery in African patients starting therapy with $CD4^+$ counts <200 cells/μL.

Disclaimer

This manuscript has not been published in its current form or a substantially similar form. There are no financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest.

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Research Article

Protease Inhibitor Resistance Is Uncommon in HIV-1 Subtype C Infected Patients on Failing Second-Line Lopinavir/r-Containing Antiretroviral Therapy in South Africa

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Limited data exist on HIV-1 drug resistance patterns in South Africa following second-line protease-inhibitor containing regimen failure. This study examined drug resistance patterns emerging in 75 HIV-1 infected adults experiencing virologic failure on a second-line regimen containing 2 NRTI and lopinavir/ritonavir. Ninety six percent of patients ($n = 72$) were infected with HIV-1 subtype C, two patients were infected with HIV-1 subtype D and one with HIV-1 subtype A1. Thirty nine percent ($n = 29$) of patients had no resistance mutations in protease or reverse transcriptase suggesting that medication non-adherence was a major factor contributing to failure. Major lopinavir resistance mutations were infrequent (5 of 75; 7%), indicating that drug resistance is not the main barrier to future viral suppression.

1. Introduction

The South African antiretroviral roll out programme consists of a non-nucleoside reverse transcriptase inhibitor (NNRTI) based first-line regimen and a ritonavir-boosted protease inhibitor (PI) containing second-line regimen. Standard genotype analyses of first-line failure samples from South Africa have shown that the majority of patients remain susceptible to the second-line regimen of zidovudine (AZT), didanosine (ddI), and lopinavir/ritonavir (LPV/r) [1–3]. With over one million patients on antitretroviral therapy (ART) in the South African programme, a rise in second-line regimen failures is expected. Currently, little is known about treatment options after second-line failure or the frequency of protease inhibitor resistance in HIV-1 subtype C.

Patients with HIV-1 subtype B who experience virologic failure on an initial regimen containing LPV/r infrequently have major protease (PR) mutations detected [4]. Resistance to LPV generally requires the accumulation of several mutations in the PR gene although rare, single mutations can reduce susceptibility [5, 6]. Several studies have identified naturally occurring polymorphisms in subtype C PR that may facilitate the development of PI resistance, but their clinical significance is uncertain [7, 8]. The current study assessed the occurrence of known HIV-1 drug-resistance mutations in PR and RT in patients with second-line ART failure and the remaining treatment options.

2. Methods

2.1. Patient Samples. Plasma samples from 75 patients on a failing second-line regimen (LPV/r and 2 NRTI) were sent for HIV-1 drug-resistance testing from clinics at two large state hospitals in Johannesburg, South Africa.

Virologic failure was defined as having confirmed (two consecutive measurements) of plasma HIV-1 RNA greater than 5000 copies/mL. Because pretherapy samples were not available from these patients, PR sequences were compared with those from 226 LPV/r naïve patients on failing first-line ART from the same two clinics [1]. The work conducted on these samples was with the understanding and the consent of the human subjects.

2.2. Population Genotype Analysis. Population-based genotyping was performed using an in-house drug-resistance assay. Briefly, a 1.7 kb amplicon was generated by RT-initiated polymerase chain reaction encompassing the entire PR and partial RT-coding regions. The amplicon was sequenced using five primers that ensure bidirectional coverage from codons 1–99 of PR and codons 1–230 of RT. Sequencing was performed with either an ABI Prisms 3730 or an ABI Prism 3100-*Avant* Genetic Analyzer (Applied Biosystems, USA).

2.3. Data and Statistical Analyses. Sequences were assembled, manually edited using Sequencher v4.5 software (Gene codes, Ann Arbor, MI), and submitted to the ViroScore database, which uses the IAS-USA mutation list to identify HIV-1 drug resistance mutations (http://www.iasusa.org/). The frequency distribution of PR mutations was also compared in LPV/r-naïve and -exposed patients. The REGA HIV-1 subtyping tool was used to determine HIV-1 subtype of each patient sample (http://www.bioafrica.net/subtypetool/html). The chi-squared test was used to determine if the frequency of naturally occurring polymorphisms in LPV/r-naive and -exposed patients differed significantly. A P-value of <.01 was considered significant to adjust for the multiple comparisons across PR.

3. Results

A total of 75 plasma samples were available from patients experiencing virologic failure on a second-line LPV/r-based regimen (Table 1). The mean age was 34 years (IQR 29–40), 69% were female ($n = 52$), and the average time on second-line therapy was 16 months (range 4–54 months; Table 1). At the time of failure, median CD4+ T-cell count and mean HIV-1 RNA were 141 cells/mm^{3}and 184,779 copies/mL, respectively. There was no difference in HIV-1 RNA or CD4 levels observed in patients with and without PI mutations ($P = .36$ and $P = .57$, resp.).

Ninety six percent of patients ($n = 72$) were infected with HIV-1 subtype C, two patients were infected with HIV-1 subtype D, and one with HIV-1 subtype A1.

Twenty nine of the 75 patients (39%) had no major mutations present in PR or the RT region examined that would confer resistance to PI, NRTI, or NNRTI, suggesting medication nonadherence to the second-line regimen. A further 19 (25%) had NNRTI resistance mutations without PI or NRTI mutations. Only five of 75 patients (7%) had major LPV resistance mutations (Table 1). The major LPV resistance mutations detected were M46I, L76V, and V82A, occurring alone or in combinations of up to 5 mutations.

Sixty seven (89%) patients had one or two minor lopinavir (LPV) resistance mutations (Figure 1). These minor mutations would not be expected to impact the efficacy of boosted PIs [9]. Nevertheless, we compared all 75 sequences from LPV/r-exposed patients with those from 226 LPV-naïve patients to assess if any of the minor mutations detected at failure were likely to have been selected by LPV/r. There were no statistically significant differences between LPV-naïve and -exposed patients in the frequency of changes in PR.

Forty-one (55%) patients had mutations in the RT region only. Nineteen had mutations conferring resistance to NNRTI alone, and 22 had resistance to both NRTI and NNRTI. The most common NNRTI mutations observed were K103N ($n = 16$; 21%) and V106M ($n = 9$; 12%; Figure 2). Thirteen of the 75 patients (17%) had NNRTI resistance mutations associated with reduced susceptibility to etravirine, but only 2 of the 13 had a weighting score of greater than 2.5 predictive of a poor virologic response to etravirine. Of the 30 patients with NRTI resistance mutations, 15 had the M184V mutation, 10 had TAMs (Table 1), 1 had Q151M complex, and none had K65R. Eight percent ($n = 6$) of all patients had two or more TAMs or other resistance mutations that would cause broad NRTI cross-resistance (Q151M).

4. Discussion

Almost half (29 of 75 [39%]) of the patients in this study on failing second-line therapy (LPV/r and 2 NRTIs) did not have detectable resistance to NRTI, NNRTI, or PI. This suggests that medication nonadherence contributed to some of the virologic failure observed. In support of this, a recent study by Pulido et al. [10] showed that loss of viral suppression on a LPV/r regimen was linked to a low baseline CD4 count or hemoglobin levels and medication nonadherence. Nonadherence may be linked to side effects arising from the combination of ddI, AZT, or LPV/r [11], which was the most common second-line regimen prescribed ($n = 53$; 71%). Indeed, frequent toxicity was observed in a Ugandan study of second-line therapy containing LPV/r [12].

In patients who did have evidence of drug resistance (46 of 75 [61%]), major LPV/r mutations were infrequent (5 of 46 [11%]). Overall, only 7% (5 of 75) of the patients in this study had major LPV/r resistance mutations. The lack of accumulation of major mutations in PR is similar to that seen in other subtype C-infected patients [13]. Thus, differences in subtypes may influence the emergence of LPV resistance. In addition, our results are very similar to a recent report of 6% PI resistance in patients with viremia on second-line therapy from Soweto, South Africa [14].

Minor LPV mutations were found in 89% (67 of 75) of patients in this study, but comparison with 226 sequences from LPV/r-naïve patients revealed that all are likely to be naturally occurring subtype C polymorphisms. Several PR polymorphisms in subtype C that could affect

Table 1: Patient characteristics and mutations at failure of second-line therapy.

Variable	Median (inter quartile range)	Median (inter quartile range)	
		No PI major mutations ($n = 70$)	PI major mutations ($n = 5$)
Age (years)	34 (29–40)		
CD4+ T-cells/mm^3	141 (75–245)	138 (80–229)	246 (194–254)
HIV-1 RNA (copies/mL)	184,779 (8790–166,300)	61000 (15000–155000)	3260 (2200–33000)
Time on second-line (months)	16 (7–18)		
Regimens	*n*		
LPV/r, AZT, ddI	53		
LPV/r, 3TC, AZT	8		
LPV/r, 3TC, TNF	4		
LPV/r, 3TC, ABC	2		
LPV/r, AZT, d4T	2		
LPV/r, ddI, TNF	2		
LPV/r, 3TC, ddI	1		
LPV/r, 3TC, EFV	1		
LPV/r, AZT, EFV	1		
LPV/r, FTC, TNF	1		
Resistance Mutations	*n* (%)		
NRTI mutations	26 (35%)		
M184V	15 (20%)		
K65R	0 (0%)		
Q151M	1 (1%)		
TAMs	10 (13%)		
NNRTI mutations	39 (52%)		
K103N	16 (21%)		
V106M	9 (12%)		
Any PR mutations (major and minor)	67 (89%)		
MajorLPV mutations	5 (7%)		
M46I, L76V	1		
M46I	1		
L33F, I54S, V82A, I84V	1		
L33F, M46I, I54V, I84V, L90M	1		
M46I, I54V, L76V	1		

PI susceptibility have been noted by others [8]. A recent study by Champenois and colleagues showed that there was no association between these polymorphisms and the slope of viral RNA decline or time to undetectable virus in patients receiving initial PI-based therapy, indicating that these polymorphisms probably have little impact on treatment response. Mutations outside of PR, at gag cleavage sites, can reduce susceptibility to PIs but infrequently occur without PR mutations [15].

Twenty seven percent of patients (20 of 75) had both NRTI and NNRTI mutations. The high percentage of K103N and V106M is likely the result of first-line failure of a NNRTI-containing regimen. M184V and TAMs were likely selected by NRTI in either the first- or second-line regimens. However, only 15% of patients had multiple TAMs or other multi-NRTI resistance mutations (Q151M) indicating that NRTI could be used again in the majority of patients.

The mutation profiles observed indicates that several third-line options are available after second-line failure. The large majority of patients (93%) would have virus that is likely to be susceptible to other boosted PI. The 4 patients with M46I would be expected to have a decreased susceptibility to indinavir, nelfinavir, fosamprenavir, and atazanavir, and the 2 patients with L76V would likely have a reduced susceptibility to indinavir, fosamprenavir, and darunavir. The sole patient with V82A would have reduced susceptibility to indinavir. The virus with I54S would be interesting to study further as the exact effect of this mutation on PI resistance is not well understood. In addition, the second-generation NNTRI etravirine could be effective in the majority of the patients (73 of 75 [97%]), although NNRTI mutations, including etravirine resistance mutations, may have declined to undetectable levels without NNRTI exposure in the second-line regimen. Additional

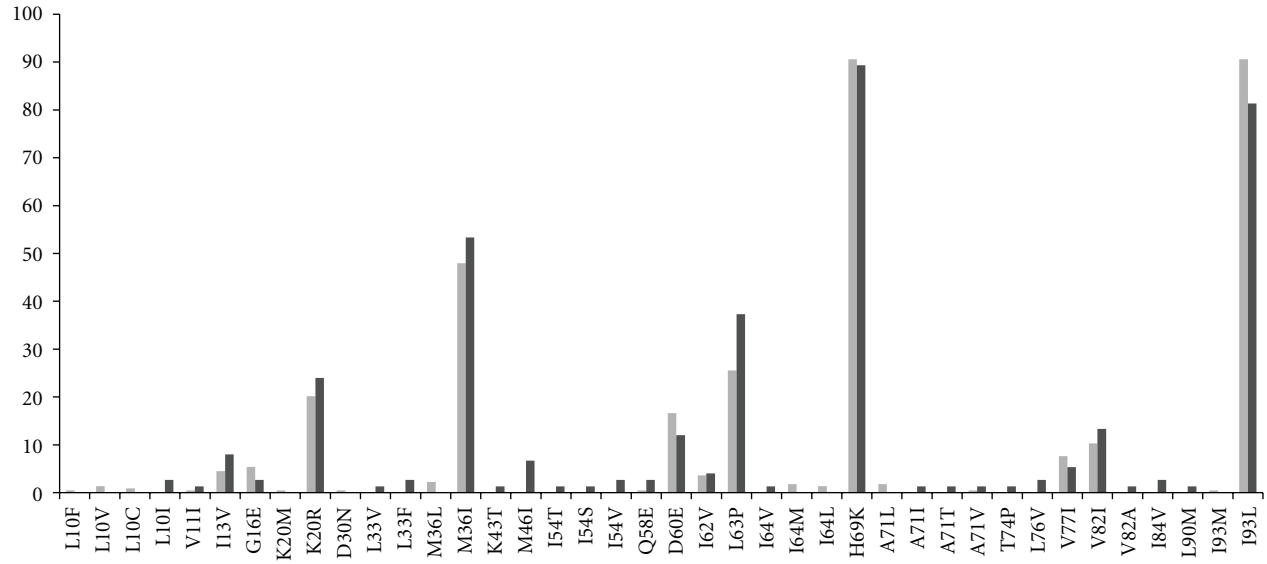

Figure 1: Comparison of changes from HXB2 reference between 45 lopinavir/r-exposed (light gray bars) versus 226 LPV-naïve patients (dark gray bars). Only L63P was significantly more frequent in lopinavir/r-exposed than -naïve patients ($P = .0435$).

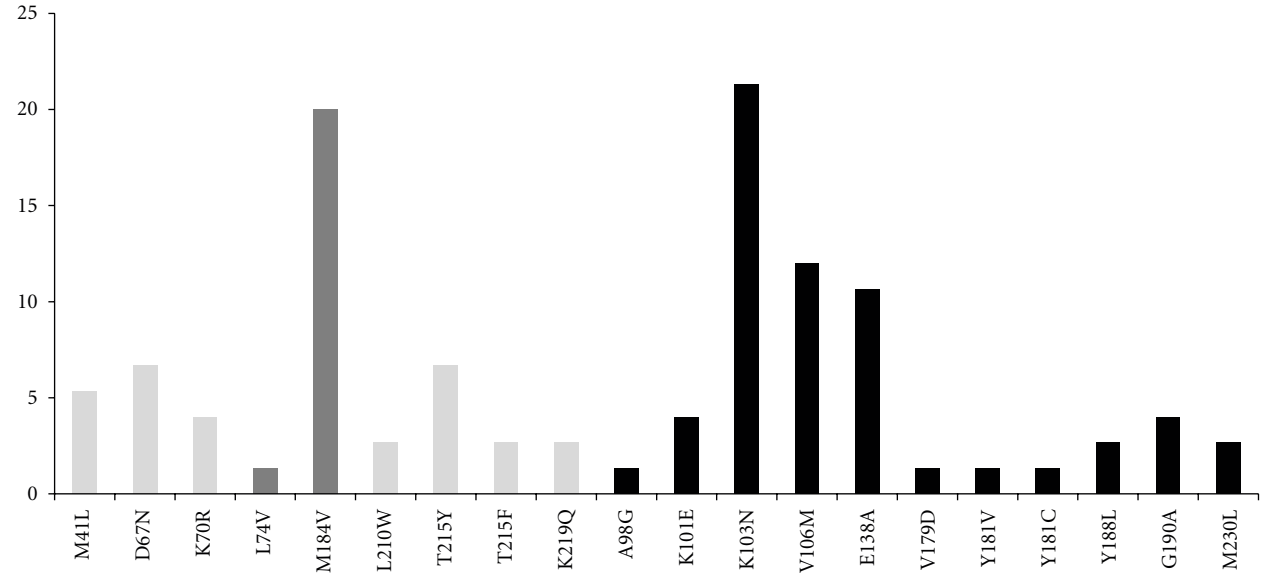

Figure 2: Frequency of mutations occurring in the RT region of patients failing second-line therapy. Light gray bars indicate thymidine analog mutations (TAMs), dark gray bars other NRTIs mutations, and black bars NNRTIs mutations.

studies would be necessary to exclude the existence of minor populations of etravirine-resistant variants.

5. Conclusion

Major LPV resistance mutations were infrequent among patients on a failing second-line regimen containing LPV/r and 2 NRTI in the South African roll-out programme. Most ritonavir-boosted PIs would be a good option for subsequent therapy, possibly in combination with etravirine and NRTIs. Alternatively, the integrase inhibitor raltegravir could be used in combination with a boosted PI although comparative data are needed. The absence of HIV-1 drug resistance in approximately half of the patients suggests that better tolerated regimens and improved adherence could achieve virus suppression without the use of new classes of antiretrovirals.

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Review Article

Monitoring Antiretroviral Therapy in HIV-Infected Children in Resource-Limited Countries: A Tale of Two Epidemics

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Twenty-nine years into the HIV epidemic, several advances have been made; however, there remain several challenges particularly with pediatric HIV in resource-limited countries. The obstacles facing pediatric antiretroviral therapy (ART) delivery in resource-limited countries are multifaceted: lack of health care infrastructure, limited availability of pediatric drug formulations, lack of early HIV diagnostic and monitoring techniques, limited manpower with expertise in pediatric HIV care, limited donor funding, and competing public health priorities with limited health care budget. In this paper, the challenges with various ART monitoring tools in resource-limited countries are discussed. Noninvasive (e.g., patient, clinical events outcome, and adherence) and invasive (e.g., immunologic and virologic) monitoring tools are discussed. Several cheap and technically less complex laboratory tests for monitoring are becoming available. Funding agencies and country programs should invest in validating the use of current technologies to optimize pediatric HIV care in resource-limited countries.

1. Introduction

The current state of the HIV epidemic can be likened to the description of the setting of Charles Dickens's novel, "A Tale of Two Cities"—"it was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity…" Twenty-nine years into the HIV epidemic, several advances have been made; however, there remain several challenges with regard to access and management of antiretroviral therapy (ART), particularly in resource-limited countries. While the birth of an HIV-infected child is rare in resource-rich countries, mother-to-child transmission (MTCT) of HIV continues to fuel the HIV epidemic in resource-limited countries [1]. Two sentinel advances in the pediatric HIV epidemic were (1) an initial 67% reduction in perinatal HIV transmission with the administration of zidovudine (AZT) during pregnancy and peripartum period [2] and (2) a subsequent reduction of perinatal transmission of HIV by 98%-99% in resource-rich countries with the use of highly active antiretroviral therapy (HAART) during pregnancy [3]. Despite these successes, progress has not been uniform worldwide and care for HIV-infected children continues to lag behind. About 2 million of the 2.1 million HIV-infected children live in sub-Saharan Africa, where there is still limited access to antiretroviral drugs even with the unprecedented global effort at scaling up ART [4]. About 1000 children are infected with HIV each day worldwide. At the end of December 2008, only 38% of HIV-infected children less than 15 years of age in resource-limited countries needing ART were on therapy (Table 1) (http://www.who.int/hiv/topics/paediatric/data/en/index.html). The disparity in global coverage of ART, as illustrated in Table 1, underscores the need to scale up pediatric ART delivery. The obstacles facing pediatric ART delivery in resource-limited countries are multifaceted: lack of health care infrastructure, limited availability of pediatric drug formulations, lack of early HIV diagnostic and monitoring techniques, limited manpower with expertise in pediatric HIV care, limited donor funding, and competing public health priorities with limited health care budget [5–7].

The hallmark of HIV infection is progressive CD4+ T cell depletion leading to an increased risk for the development of opportunistic infections, acquired immune deficiency syndrome (AIDS), and death [8–10]. The advent of HAART

Table 1: Antiretroviral therapy coverage among HIV-infected children less than 15 years of age in resource-limited countries, December 2008.

Geographical region	Number on ART	Number needing ART (range)	Percent of coverage (range)	Percent of total need
Eastern and Southern Africa	195 100	440 000 (340 000–540 000)	44% (36%–57%)	61%
Western and Central Africa	29 800	200 000 (140 000–260 000)	15% (11%–22%)	27%
Latin America	13 700	17 000 (14 000–20 000)	82% (70%– >95%)	2%
The Caribbean	2500	4600 (3400–5800)	55% (43%–72%)	1%
East, South, and South-East Asia	30 000	58 000 (41 000–78 000)	52% (38%–73%)	8%
Europe and Central Asia	4200	4900 (2700–7500)	85% (56%– >95%)	1%
North Africa and the Middle East	400	6700 (3400–11 000)	6% (4%–12%)	1%
Total	275 700	730 000 (580 000–880 000)	38% (31%–47%)	100%

Adapted from (http://www.who.int/hiv/topics/paediatric/data/en/index.html).

in 1996 significantly reduced the morbidity and mortality in HIV-infected children in both resource-rich countries [11, 12] and resource-limited countries [13–17]. However, the treatment of HIV infection is a life-long undertaking, and therapeutic benefit can be limited by the evolution of drug-resistant virus and long-term toxicity resulting in treatment failure [18, 19]. There is the need to monitor treatment to early detect and avoid the untoward effects of HAART. In this paper, the successes at monitoring antiretroviral treatment in HIV-infected children in resource-limited countries and the challenges that remain are discussed.

2. Monitoring the Response to Antiretroviral Therapy

The goal of HAART is to suppress HIV viral replication and restore immune function. Successful treatment results in virologic suppression, a quantitative increase in the number of CD4+ T cells, and improvement in the clinical well-being of the individual, manifesting as weight gain and resolution or control of opportunistic infections. In resource-limited countries, the World Health Organization (WHO) recommends initiating ART for (i) HIV-infected infants diagnosed in the first year of life, irrespective of CD4 count or WHO clinical stage, (ii) HIV-infected children between 12 and 24 months of age irrespective of CD4+ T cell count or WHO clinical stage, (iii) HIV-infected children between 24 and 59 months of age with CD4+ T cell count of $\leq$750 cells/mm^3 or %CD4+ $\leq$25, whichever is lower, irrespective of WHO clinical stage, (iv) HIV-infected children more than 5 years of age with a CD4+ T cell count of $\leq$350 cells/mm^3 (as in adults), irrespective of WHO clinical stage, (v) HIV-infected children with WHO clinical stages 3 and 4, irrespective of CD4 count, and (vi) any child less than 18 months of age who has been given a presumptive clinical diagnosis of HIV infection [20]. Despite the limited armamentarium of first-line antiretroviral drugs in resource-limited countries, national HIV/AIDS programs continue to report good treatment outcomes similar to those in resource-rich countries—with 1 and 2 year survival estimated at 93%–95% and 91%, respectively [14, 15, 21–25]. In resource-rich countries, the standard of care for monitoring treatment in HIV-infected children is the routine laboratory monitoring of CD4+ T cell percentage or count and HIV viral load [26]. In USA, CD4+ T cell percentage or count and HIV viral load are measured at the time of diagnosis of HIV infection and at least every 3-4 months thereafter (http://AIDSinfo.nih.gov/). Due to the lack of accessible and affordable laboratory services, these tests are not routinely available in many resource-limited settings [27]. The WHO highly recommends national programs to develop the laboratory capacity for monitoring ART. However, in the absence of laboratory capacity, clinical parameters are used for monitoring ART. In this paper, the challenges associated with various ART monitoring tools in resource-limited countries are discussed. Noninvasive (e.g., patient, clinical events outcome, and adherence) and invasive (e.g., immunologic and virologic) monitoring tools are discussed.

2.1. Patient Monitoring of Antiretroviral Therapy. Direct patient monitoring is the most affordable and cost-effective measure and forms the backbone of clinical care, treatment, and prevention. The WHO has published technical guidelines outlining ART care of infants and children to guide health care delivery programs in resource-limited countries [20]. These guidelines provide a list of essential minimum standards of HIV care and ART monitoring data to be collected at each clinic visit. The collection, collation, and analysis of data on patients over time will be useful for evaluation of both local and national programs. Standardization of the data collection process will also make it easy to identify strengths and weaknesses of programs. An overarching advantage will be the ease of forming ART care coalitions in resource-limited countries to inform best practices parallel to lessons learned from the Pediatric AIDS Clinical Trials Group (PACTG) in resource-rich countries.

Good record keeping continues to be a perennial problem in most resource-limited countries. Many health care providers are not proficient in basic data management skills, and the few who are proficient in data management are overloaded with work such that data management is not their priority. There is a need for national programs to invest in training data management personnel to assist the providers in collecting, storing, and analyzing data.

2.1.1. Clinical Events Monitoring of Antiretroviral Therapy. Clinical events monitoring provides a noninvasive and low-cost measure for following patients on HAART. Since the ultimate goal of HAART is to restore immune function and halt HIV disease progression, the presence or absence of certain clinical events can be used as surrogates for monitoring the efficacy of HAART. Children starting ART in resource-limited countries are usually severely immunocompromised and very sick [22–25]. The immediate clinical benefit from ART initiation is improvement in the overall well-being and functional status of the patient; subjective and objective measures of these at follow-up visits can be employed to monitor treatment success. Screening questions such as (i) has the patient returned to school or play or is the patient able to engage in activities of daily living without help? (ii) Is the patient ambulating or bedridden? An improvement score could be devised as a quick and subjective assessment of improvement or "return of energy" since starting ART. Using a scale of one to 10, with 10 being "most improvement" and "return of energy" since starting ART, the patient could be asked to score his/her state of health. The answers to these questions will provide a quick measure of the efficacy of ART. However, providers have to be aware of the presenting symptoms of immune reconstitution inflammatory syndrome (IRIS) in their patient cohort as these could confound the functional state assessment [28]. A semiquantitative approach to the clinical assessment that one can explore is to use changes in WHO clinical staging at clinic visits after initiating ART. Studies are needed to determine how long it takes for one to move to the next higher clinical stage after initiating ART and to determine if these changes will accurately predict virologic outcome.

The majority of children starting ART in resource-limited countries have growth deficiencies. Growth failure in HIV-infected children is a multifaceted problem; it is partly due to the underlying HIV infection itself, with its lack of virologic and immunologic control prior to ART, HIV-associated opportunistic infections, and food deprivation resulting from poverty. Several studies have demonstrated weight gain in children after initiating ART [29, 30]. On average, the weight gain is about 1.8–3.6 kg in the first year of ART. The mean weight-for-age z scores also increase substantially, by about 1 SD from −2 SD or below at baseline [21, 31–34]. In a study of 212 HIV-infected children initiating ART, they continued to catchup in growth and height through the first five years on ART [35]. In these studies, weight gain was significantly associated with virologic control. Where viral load determinations are not feasible, weight gain could be used as part of an algorithm to predict virologic responders.

Clinical algorithms based on signs, symptoms, and simple laboratory assays are being used to identify and treat patients needing ART in resource-limited countries [36, 37]. The Pediatric AIDS Clinical Trials Group (PACTG) 219 Study Team developed and validated a simple Pediatric AIDS Severity Score (PASS) based on baseline weight percentile, WHO clinical stage, symptoms, a general health rating, total lymphocyte count, packed cell volume (hematocrit), and a measure of albumin to guide decisions on initiation of ART in resource-limited countries [38]. The PASS system provided a statistically significant alternative to CD4+ T cell percent and HIV viral load in deciding when to start ART [38]. Such a measure could be adapted in resource-limited countries. Other clinical indices that could provide easy measure of treatment outcome are resolution or decreased frequency or reduction in the severity of HIV-associated illnesses. It has been shown that the incidence of diarrheal diseases, pneumonia, and hospitalization among HIV-infected children decreases substantially on ART [13, 14, 39].

2.1.2. Adherence Monitoring of Antiretroviral Therapy. Adherence to treatment regimens is a prerequisite for the efficacy and durability of any antiretroviral therapy regimens [40, 41]. In resource-limited countries, where second-line regimens are limited, keeping a patient on a first-line regimen as long as possible is an important goal of ART. Patients starting ART should, therefore, be counseled on the need for adherence, and adherence should be monitored at every encounter with the patient. Several studies from resource-limited countries have reported prevalence of adherence among HIV-infected children similar or better than that achieved among those in resource-rich countries [25, 39, 42–45]. There are several measures of adherence, for example, self-report or sophisticated microelectronic monitors that record bottle openings and reconstruct complex pill-taking patterns [46, 47]. Due to resource constraints, self-reporting adherence is used predominantly in resource-limited countries.

One study compared the relative performance of various low-cost adherence measures: caregiver recall, pill counts at scheduled visits, and unannounced pill counts at home visits. The proportion of patients who achieved perfect adherence (i.e., >95%) was 72%, 89%, and 94% when measured by unannounced pill count, caregiver report, and pill count at scheduled visits, respectively [45]. In a study from South Africa, investigators sought to determine the accuracy of adherence assessments for predicting and detecting virologic failure and to compare the accuracy of adherence-based monitoring with CD4+ T cell monitoring in HIV-infected adults on ART [48]. Pharmacy-based time-to-refill of HAART was used as a measure of adherence [49]. Adherence was calculated as the number of months for which ART claims were submitted to the pharmacy, divided by the number of complete months from ART initiation to the date of study endpoint, and the results multiplied by 100. Adherence values were found to provide statistically significant accuracy for detecting virologic failure at 6 and 12 months compared to CD4+ T cell count changes (AUCs of 0.79 versus 0.68 at 6 months and 0.85 versus 0.75 at 12 months) [48]. This finding implies that in resource-limited countries where CD4+ T cell counts are not readily available, a comprehensive monitoring of pharmacy refill data could be used to identify patients with a high probability of virologic failure. Grossberg et al. determined the validity and utility of pharmacy-based time-to-refill measure of antiretroviral therapy adherence in a cohort of 110 HIV-infected adults [49]. The viral load of study individuals decreased by 0.12 log copies/mL

(95% confidence interval [CI] 0.01–0.23 log copies/mL) for each 10% increase in pharmacy-based time-to-refill defined adherence as compared with 0.05 log copies/mL (95% CI: −0.14–0.25 log copies/mL) for the self-reported adherence measure. The key to the validity of using adherence measures as surrogates for monitoring depends on meticulous acquisition, maintenance, collation, and analysis of the data and making pharmacy data available at each patient encounter. Country programs will have to devise a comprehensive and an easy-to-access pharmacy medication refill database.

Though the importance of adherence is universally recognized, there is no consensus on how to measure adherence. Several programs have adopted adherence measures based on availability of resources. For example, in Malawi, patients are given a 30-day supply of 60 antiretroviral (ARV) pills, and a pill count is carried out at each visit (every 28 days), and patient adherence is said to be >95% if a patient has eight pills or fewer at each visit [41]. Columbia University's MTCT-plus program sites use a 7-day patient recall of number of pills taken, and adherence is categorized according to response as none, very few, about half, most, and all of the pills [20]. The WHO provides a guide for estimating adherence: adherence is said to be "good adherence" (i.e., missing ≤3 doses in a month, ≥95%), "fair adherence" (i.e., missing 4–8 doses in a month, 85%–94%), and "poor adherence" (i.e., missing ≥9 doses in a month, <85%) [20]. It is therefore feasible for all programs to measure adherence as a surrogate of treatment outcome. The irony is that the best measure of adherence is virologic assessment. Therefore, funding agencies and country programs should strive to institute viral load measurement as part of all HIV care programs.

2.2. Immunologic Monitoring of Antiretroviral Therapy. CD4+ T cell count remains the single most important parameter in monitoring ART in HIV-infected individuals [26, 50–52]. CD4+ T cell monitoring is more appropriate than virologic monitoring because a decreasing CD4+ T cell count is a better predictor of disease progression [53]. Table 2 illustrates the immunologic and virologic outcomes of antiretroviral therapy in HIV-infected children in resource-limited countries obtained from selected papers published in the last 5 years [22–25, 54–57]. The immunologic outcome of ART in HIV-infected children in resource-limited countries is comparable to that of children in resource-rich countries. However, due to resource constraints and technological challenges, CD4+ T cell count determination is still not available in all HIV care programs in resource-limited countries, and, where available, frequent determinations are not feasible as illustrated by several missing data points in Table 2. In these studies, on average the children doubled their baseline CD4+ T cell count after six months on therapy. The CD4+ T cell count continued to increase at the same rate over the second 6 months and slowed thereafter. The gain in CD4+ T cell count at 18 months on therapy was not very different from that at 12 months. In the Zambian study, where CD4+ T cell values were available for some of the patients at 24 months, there was no significant increase between the 12- and 24- month CD4+ T cell values [24]. Studies of HIV-infected adults have shown that CD4+ T cell recovery reaches a plateau after 4 to 5 years of HAART despite complete viral suppression [58–60]. Could we take advantage of this observation to determine when and how often to monitor CD4+ T cell counts in HIV-infected children on ART in resource-limited countries?

Because of the well-known large natural decline and variation in absolute CD4+ T cell numbers in early childhood [63, 64], the percentage of CD4+ T cell is used for monitoring HIV disease progression in children particularly in those less than 5 years of age [65]. As shown in Table 2, it is interesting to note that the changes in the percentage of CD4+ T cells with treatment are similar to that of absolute CD4+ T cell count. This is consistent with our recent finding that absolute CD4+ T cell count had similar utility as CD4+ T cell percentage in monitoring HIV infection in a pediatric cohort in the US, regardless of age [66]. Others have found that absolute CD4+ T cell counts have less prognostic value in younger children than CD4+ T cell percentage [67]. In resource-limited countries, most low-cost assays available for enumeration of CD4+ T cells mainly provide the absolute counts but not the percentages [27]. There is a need for further studies to examine whether absolute CD4+ T cell counts could be used for children of all ages, especially in situations where available instruments provide only absolute CD4+ T cell counts.

2.3. Virologic Monitoring of Antiretroviral Therapy. HIV viral load is a useful tool for initiation and monitoring of ART [51]. It is not a part of the WHO recommendation for routine monitoring of ART in resource-limited countries. HIV-infected children are less likely than infected adults to achieve full viral suppression on ART [68–70]. The cut-off for undetectable viral load differs among studies from resource-limited countries (Table 2); however, the proportion of patients who achieved virologic success is comparable to that in resource-rich countries. Only six of the studies had a viral load for at least two time points. This is most likely due to the cost and the need for sophisticated equipment for determination of viral load. Viral load is a valuable tool for detecting early treatment failure, and it is all the more important in resource-limited countries where second-line regimens are limited. For HIV-infected individuals on ART, it has been found that the CD4+ T cell count and viral load after six months of ART are the strongest predictors of disease progression and death [71, 72]. In resource-limited countries, perhaps viral load determination could also be done less frequently. The critical assay could be the one performed at six months of ART, and the frequency of testing, thereafter, would depend on accessibility, affordability, and the clinical condition of patient.

3. Future of Laboratory Monitoring of Antiretroviral Therapy: The Age of Wisdom

Immunologic and virologic measures are the state of the art for monitoring antiretroviral therapy and should be made

Table 2: Immunologic and virologic outcomes of antiretroviral therapy in HIV-infected children in resource-limited countries.

Study	1	2	3	4	5	6	7	8	9	10	11	Average of all studies
Number of children	2928	67	151	29	107	212	285	274	67	250	78	—
Country [ref]*	Zambia [24]	India [57]	South Africa [25]	Kenya [54]	Thailand [55]	Cambodia [23]	Haiti [22]	Thailand [56]	Kenya [39]	Uganda [61]	Cote d'Ivoire [62]	—
Median age (years)	6.75	6.28	5.3	8.5	7.7	6	6.3	7	4.4	9.2	6.5	6.7
WHO clinical staging III or IV (%)	72.4	49.3	70.2	62.1	72	64.5	98	65	82	89	na	72.5
Median CD4 count at baseline (cells/mm^3)	284	225	na	182.3	72	100	608	na	288	272	na	253.9
CD4 gain at 6 months	280	478	na	203	226	na	na	na	210	na	na	279.4
CD4 gain at 12 months	351	516	na	334	332	490	na	na	na	na	na	404.6
CD4 gain at 18¶ or 24 months	427¶	493¶	na	na	532	na	na	na	na	na	na	460¶
Median CD4% at baseline	12.9	12	7.4	na	3	6	12	5	5.8	8.6	7.5	8.02
CD4% gain at 6 months	10.8	8	10.2	na	12	na	na	7	9.4	na	4.6	8.86
CD4% gain at 12 months	14.1	11	16.2	na	17	17	10.3	na	na	na	11.1	13.8
CD4% gain at 18¶ or 24 months	15.1¶	13¶	na	na	21	na	na	18	na	na	16	19.5
Median viral load (VL) at baseline (Log)	na	na	na	5.11	5.4	na	5.3	na	6.1	5.3	5.37	5.43
Proportion with undetectable VL at 6 months (%)	na	na	84	~50	53	na	na	na	67	na	52.1	61.2
Proportion with undetectable VL at 12 months (%)	na	na	80.3	na	69	81	56	na	na	74	49.3	68.26
Proportion with undetectable VL at 18 months (%)	na	na	na	na	76	na	na	na	na	na	47.5	61.75
Frequency of CD4 determination (months)	6	3–6	6	3	6	6	6	na	3–6	3–6	6	—

* Reference to papers from which figures were extracted.
¶Values available at 18 months.
na: not available.

available in all settings where HIV infection is treated. With the current ARV scale-up campaign, there is an unprecedented call on scientists to develop simpler, more robust, low-maintenance, and cost-efficient laboratory technologies for monitoring antiretroviral therapy in resource-limited countries. Posterity will not forgive modern science if it does not deliver its promise to help mankind in its dire needs [73]. Moreover, the global community sees this as a moral duty, leading to an unusual public-private sector partnership in encouraging and funding the next generation of innovations.

3.1. CD4+ T-Cell Testing. HIV primarily targets CD4+ T cells, and CD4+ T cells play an essential role in HIV pathogenesis [74]. Gut-associated lymphoid tissue (GALT) contains about 60% of the body's total CD4+ T cell pool, and it is an important site of HIV viral replication during acute HIV infection leading to significant CD4+ T cell depletion [75]. Chronic HIV infection affects both quantitative and qualitative function of CD4+ T cells. CD4+ T cell recovery during ART is biphasic: an increase of about 100–200 cells/mm^3 during the first year of virologic suppression on ART, followed by a gradual increase [76]. Given the central role of CD4+ T cells in HIV pathogenesis, CD4+ T cell determination during the course of HIV disease is one of the most reliable predictors of prognosis [26].

Despite the global effort to make CD4+ T cell determination available in all HIV treatment centers, there are

still quite a number of centers in resource-limited countries with no access to reliable CD4+ T cell enumeration. The challenge has been to develop a technology easy to operate, able to withstand the tropical environment that could be hostile to equipments, compatible with intermittent electric power delivery, and affordable [77]. Several relatively cheap and technically less complex devices have been developed for CD4+ T cell testing. These include the FACScount system (Becton Dickinson Sciences, California), the Guava Easy CD4 Assay (Guava technologies, Hayward California), the Cyflow (Partec, Germany), and the panleucogating (PGL) CD4 technique [78].

Many programs in resource-limited countries are using these affordable technologies; however, not all the devices are designed for pediatric HIV management as they do not measure the percentage of CD4+ T cells. The percentage of CD4+ T cells, rather than the absolute number, has been used as a marker of HIV disease progression in children [65]. This is due to the natural decline and variation in absolute CD4+ T cell numbers in early childhood [63, 64]. There are limited data on the long-term utility of the absolute CD4+ T cell counts to guide treatment changes in pediatric HIV. We recently reported that absolute CD4+ T cell count had similar utility as CD4+ T cell percentage in monitoring HIV infection in a pediatric cohort in USA, regardless of age [66]. There is a need for further research to determine whether absolute CD4+ T cell count can be used in place of CD4+ T cells percentage in managing HIV-infected children of all ages especially in areas where available CD4+ T cell enumeration devices do not measure the CD4+ T cells percentage.

3.2. Viral Load Testing. Viral load testing that has been shown to optimize HIV care in resource-rich countries is currently unavailable in most resource-limited countries. HIV RNA assays used in resource-rich countries—Abbott m2000 test, the Roche COBAS Taqman test, bioMérieux NucliSens HIV-1 QT Assay, and Versant HIV-1 RNA 3.0 Assay (bDNA)—are not accessible in resource-limited countries due to costly technical equipment, complex technology, expensive reagents, poor laboratory infrastructure, and prohibitive maintenance cost. Unfortunately, the available commercial assays for viral load determination are very expensive (between $50 and $100 per test) making it unaffordable to many centers in resource-limited countries [79]. CD4+ T cell count may be insensitive for detecting early treatment failure as CD4+ T cells may take several months to drop significantly after virologic failure. Moreover, there are instances of discordant virology and immunologic responses, that is, persistently low or declining CD4+ T cell counts despite complete virologic suppression, or increasing CD4+ T cell count during increasing viremia. Viral load determination is therefore an essential complementary test to CD4+ T cell count [80].

There are several cheaper viral load assays being developed and evaluated for use in resource-limited countries. Examples of these assays are the ultrasensitive p24 assay (a signal-amplification boosted ELISA for HIV-1 p24 antigen in plasma after heat-mediated immune complex dissociation), the ExaVir Load reverse transcriptase activity test (an assay that quantify virion-associated reverse transcriptase enzyme), "home-made" real-time PCR HIV-1 RNA assays, the Liat HIV RNA analyzer (http://www.iquum.com/products/analyzer.shtml), and the SAMBA assay (http://www.haem.cam.ac.uk/ddu/). The performances of these assays in comparison to assays used in resource-rich settings have received mixed reviews [81, 82]. Efforts are being made to optimize these assays to improve their sensitivities and specificities. Even when these assays are validated for clinical use, not all HIV clinics will be able to access them locally as resources vary significantly within countries. To make testing available to all clinics, issues surrounding specimen collection, processing, and transport have to be taken into consideration. The use of dried blood spots may be an ideal alternative for specimen collection in rural settings, where there are no laboratories, as it can be transported to testing sites at ambient temperature [81].

3.3. What Do We Do in the Interim? There are preponderance of evidence to suggest that the use of CD4+ T cell enumeration and viral load determination in resource-limited countries is the way to go as the use of clinical algorithms are confounded by the protean of infections and diseases that can masquerade as HIV- or AIDS-associated conditions. The arguments against their routine use are sustained more by consideration of cost, technical expertise, and lack of infrastructure [78]. Country programs should make the effort to adopt available low-cost technologies in clinical care of HIV patients. A question that remains unanswered is: that is it necessary to have frequent determination of CD4+ T cells and viral load as the practice in resource-rich countries is currently? From the data reviewed herein (Table 2), there is little if anything to be gained in measuring CD4+ T cells and viral load more frequently than every 6–12 months. There is a need to come out with appropriate testing intervals which will reduce the overall cost in managing a patient and at the same time sensitive enough to capture patients most likely to fail treatment. Research is needed to investigate and validate monitoring algorithms that utilize all the available tools, that is, more frequent noninvasive measures and invasive measures in a timely and informed fashion.

4. Conclusion

Great times and innovative technologies are on the horizon for HIV care, particularly for pediatric HIV care in resource-limited countries. The goal is not to abandon the tried and tested monitoring modalities such as CD4+ T cell count and viral load determinations but to develop technologies that will make these affordable, accessible, and acceptable in resource-limited countries. Funding agencies and country programs should invest in validating the use of current technologies to optimize pediatric HIV care in resource-limited countries.

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Research Article

Forecasting the Population-Level Impact of Reductions in HIV Antiretroviral Therapy in Papua New Guinea

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Papua New Guinea (PNG) recently did not secure external funding for the continuation of its antiretroviral treatment (ART) programs meaning that supplies of HIV drugs for the estimated 38,000 people living with HIV in PNG could be completely depleted during 2010. Using a mathematical model of HIV transmission calibrated to available HIV epidemiology data from PNG, we evaluated the expected population-level impact of reductions in ART availability. If the number of people on ART falls to 10% of its current level, then there could be an approximately doubling in annual incidence and an additional 12,848 AIDS-related deaths (100.7% increase) over the next 5 years; if ART provision is halved, then annual incidence would increase by ~68%, and there would be an additional ~10,936 AIDS-related deaths (85.7% increase). These results highlight that maintenance of ART and associated services through external funding is essential for the health and well-being of HIV-positive people in PNG.

1. Introduction

Papua New Guinea (PNG) is a low income country that has experienced a rapidly expanding HIV epidemic [1–3]. It has the highest HIV prevalence and incidence rate in the Pacific region [1] with 28,294 HIV infections reported by December 2008 since the first diagnosed case in 1987 [2]. The vast majority of HIV cases have been due to heterosexual transmission with similar numbers of diagnoses in men and women [2, 3]. Fortunately, recent estimates suggest there has been a leveling out of HIV prevalence in PNG at approximately 1% [4]. The reasons for this leveling of prevalence are currently unknown but could be due to the saturation of HIV in particular at risk population groups or geographic areas, or reflect the impact of the roll-out of intervention programs in recent years and the successful scale-up of antiretroviral therapy (ART) services across the country. ART first became available in PNG in 2004 and the PNG National Department of Health recently estimated that more than 70% of people requiring treatment were receiving it in 2009 [2, 4].

Despite experiencing high levels of economic growth, mainly from the natural resources sector, PNG has limited resources available for its HIV programs and treatment services [5, 6]. In addition, the distribution of these programs and services is limited by a lack of infrastructure and transport facilities due to PNG's mountainous and rugged terrain; over 85% of the population live in rural areas with only 3% of roads paved and many villages can only be accessed by foot [6]. Almost all of its resources for HIV services in PNG rely on external sources and in early 2010 PNG did not secure funding from the Global Fund for the continuation of its ART programs. This could have resulted in the complete depletion of ART. Fortunately, the PNG government provided resources to cover the costs of PNG's ART programs until the next round of funding in 2011 but has not been able to continue expansion of ART rollout. Without this government intervention, which impacts on an already limited and constrained health budget, the availability of life-sustaining ART would likely have had a large impact on the health and well-being of HIV-infected people in PNG and potentially result in a worsening epidemic.

To investigate the potential population-level impact of a rapid fall in ART supplies in PNG, we developed a mathematical model to describe the HIV epidemic in PNG incorporating country specific demographic, epidemiological, behavioral, and clinical data. This model was calibrated to accurately reflect the estimated HIV incidence and prevalence, the number of recorded HIV diagnoses, and the number of people on ART in PNG. The model was then used to forecast the epidemic trajectory and number of AIDS deaths over the next 5 years under scenarios that the number of people receiving ART: (i) continues to increase following current trends, (ii) is maintained at the 2009 number, (iii) decreases by 50%, and (iv) decreases by 90%. We also investigated scenarios where there was a decrease by 50% and 90% for a two-year period before the re-establishment of the current roll–out; this coincides with the next round of possible funding from the Global Fund. Considering the limited antiretroviral stockpiles in PNG and reliance on foreign funds, scenarios involving substantial declines in ART access are not unrealistic.

2. Materials and Methods

A mathematical HIV transmission model was developed to describe the history of the HIV epidemic in PNG and to forecast potential epidemic trends in the future due to reductions in ART availability. All model simulations were executed with Matlab R2009a.

2.1. Model Specifications. The HIV transmission model describes the movement between twelve population compartments within both urban and rural sexually active populations. Urban HIV susceptible individuals (S_u) may become infected based on probabilistic rates that inform a deterministic "force of infection". Newly infected individuals enter into the undiagnosed chronic/asymptomatic stage (C_u^u). Chronically infected individuals can progress to advanced HIV/ AIDS (A_u^u) and people in chronic or advanced HIV stage can become diagnosed with their infection (C_u^d, A_u^d). Only diagnosed advanced HIV patients can begin ART (T_u). The same model applies to the rural population with similar disease stages, denoted by S_r, C_r^u,C_r^d, A_r^u, A_r^d, and T_r. A schematic diagram of the model is shown in Figure 1. In the model, individuals can migrate between urban and rural settings while remaining in the same disease stage and sexual mixing occurs among people in the same (urban or rural) setting. It is assumed that ART patients remain at their current location for treatment services.

The overall population-level transmission and disease progression of HIV is described by the following set of equations:

$$\begin{aligned}
\frac{dS_x}{dt} &= \pi_x - (\Lambda_x + \mu + \nu_x)S_x + \nu_y S_y,\\
\frac{dC_x^u}{dt} &= \Lambda_x S_x - (\gamma + \delta_x^c + \mu_c + \nu_x)C_x^u + \nu_y C_y^u,\\
\frac{dC_x^d}{dt} &= \delta_x^c C_x^u - (\gamma + \mu_c + \nu_x)C_x^d + \nu_y C_y^d,\\
\frac{dA_x^u}{dt} &= \gamma C_x^u - (\mu_a + \delta_x^a + \nu_x)A_x^u + \nu_y A_y^u,\\
\frac{dA_x^d}{dt} &= \delta_x^a A_x^u + \gamma C_x^d + \omega T_x - (\tau_x + \mu_a + \nu_x)A_x^d + \nu_y A_y^d,\\
\frac{dT_x}{dt} &= \tau_x A_x^d - (\omega + \mu_t)T_x,
\end{aligned} \tag{1}$$

where π_x and Λ_x denote the entry rate into the population and force of infection, respectively,

$$\pi_x = \rho\left(S_x + C_x^u + C_x^d + A_x^u + A_x^d + T_x\right), \tag{2}$$

$$\Lambda_x = \frac{\beta\left(C_x^u + C_x^d + \theta_a\left(A_x^u + A_x^d\right) + \theta_t T_x\right)}{S_x + C_x^u + C_x^d + A_x^u + A_x^d + T_x}, \tag{3}$$

and the subscripts $x, y = u, r$ denote urban and rural populations. The definition of each parameter in these equations is given in Table 1.

To evaluate the impact of HIV treatment on transmission and the number of advanced HIV-related deaths, we combine sexual behavior and HIV transmission into a single parameter β that represents the rate of HIV infection for susceptible individuals with chronically infected partners. Due to a lack of data distinguishing sexual behavior in urban and rural areas, we assume the same value of β for urban and rural areas. Multiplicative factors θ_a and θ_t are used to take into account the increased infectiousness of advanced HIV patients and the decreased infectiousness of people on ART, as described in (3). We assume identical population growth rates, HIV-related death rates, rates of disease progression to advanced HIV, and ART drop-out rate for both urban and rural areas. The values used for these rates are based on the best available international evidence for comparable settings and are presented in Table 1.

Model parameter values associated with ART are important in this analysis. Specifically, it was assumed that ART reduces the infectiousness of HIV-infected people by 92%. This is based on a recent meta-analysis [9] and a prospective cohort study [10] which each calculated a 92% reduction in HIV transmission rates from heterosexual men and women who are treated compared with those who are untreated. We also assumed that in PNG 5% of people on ART die per year, compared with 30% of people with advanced HIV disease [11, 12].

2.2. Model Fitting and Calibration. The epidemic model is calibrated against available data on HIV prevalence, HIV incidence, the number of HIV diagnoses, and the number of people on ART for PNG from 1990 to 2008 [2, 4]. To fit data, the values of β, δ_u^c, δ_u^a, δ_r^c, δ_r^a, τ_u, and τ_r were varied over time while the other parameters remained constant (Table 1). The population growth rate ρ and the urban-rural migration rates ν_u and ν_r were fixed for 1990–2008 to match World Bank estimates and PNG Census data [13, 15].

The overall force of infection for HIV in PNG (Λ) was estimated by adding the change in the number of

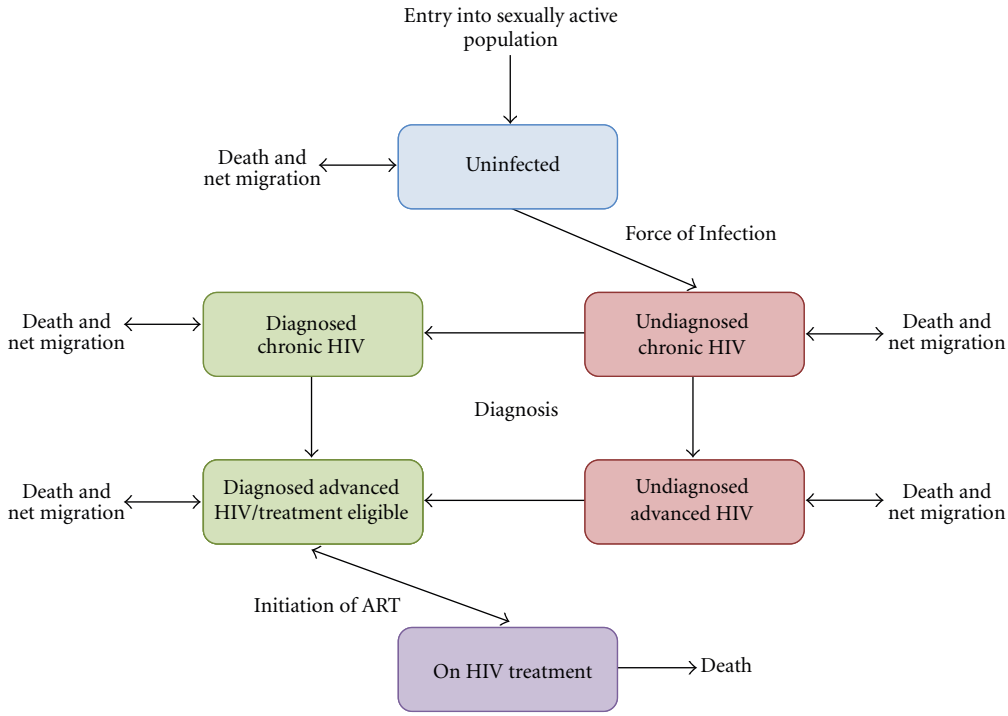

Figure 1: Schematic diagram of the stages of HIV infection described by the model and the progression of people through these stages due to infection, disease progression, diagnosis, and initiation of treatment. These stages are replicated for the urban and rural population in PNG with migration (except for people on treatment) between corresponding infection stages. People enter the uninfected population when they become sexually active and leave the population due to natural or HIV-related mortality.

HIV-infected patients to the estimated number of HIV-related deaths and dividing by the overall size of susceptible population (3). The fitted force of infection was then used to determine the value of β using (3) and the fact that $\Lambda(S_u + S_r) = \Lambda_u S_u + \Lambda_r S_r$. The resulting fitted value of β has a value of 0.256 at the start of 1990, reaches a peak value of 0.443 just prior to 2000, and then rapidly falls to a minimum value of 0.064 by the end of 2008. The parameter β refers to the probability of transmission occurring per year in a long-term discordant partnership in which the HIV-infected person is in the chronic stage; a range of 0.05–0.25 was reported among different stratifications of discordant partners in an African settings suggesting that our estimates are not unrealistic [7].

The only diagnoses data available was the annual diagnoses for all of PNG. We assumed values for the proportion of diagnoses that occur in urban areas ϕ_u and the proportion of diagnoses that occur in advanced HIV patients ϕ_a. Until 1996 the only location for HIV testing in PNG was at the Port Moresby General Hospital [3]. Since then, numerous testing sites have been established across the country, but urban residents still have more accessibility to HIV testing facilities. This suggests that there should be no diagnoses of HIV in rural areas until 1996; however, expert opinion and anecdotal evidence suggests that many people travelled to Port Moresby for HIV testing. Therefore, for fitting and simulation purposes, we assume 20% of HIV/AIDS infected patients are from urban areas (slightly higher than the proportion living in urban areas). In addition, we assume that 75% of HIV diagnoses occur when an infected person has developed advanced HIV symptoms. These assumptions result in diagnoses rates that slowly increase until 2003 before rapidly increasing until the end of 2008, matching the actual rapid expansion in HIV testing across PNG in recent years [3]. The model-fitted diagnoses rates were generally 2-3 times higher in urban areas and 2–4 times higher in the advanced HIV population groups, which are consistent with the perceived situation in the country. The fitted testing parameters values at the end of 2008 correspond to an undiagnosed advanced HIV patient taking on average from 5 to 16 months to be diagnosed in urban and rural areas, respectively.

Rates at which diagnosed chronic patients go onto treatment (τ_u, τ_r) are calculated based on available data on the number of people starting ART in PNG since 2004 when ART first became available. As there are no available data for the location of residence for patients on ART, we assumed that the rate at which diagnosed AIDS patients initiate ART in urban areas is twice that in rural areas. The rates of initiating treatment rapidly increase from zero in 2004 and match the roll-out of ART services that cover an estimated 80% of treatment-eligible people in 2009 [2, 4].

Due to gaps in data and the absence of an effective and systematic reporting system, there are large uncertainties surrounding HIV epidemiological data in PNG. To incorporate these uncertainties, we assume a range of ±10%

Table 1: Definitions and value ranges for input and fitting parameters used in our HIV transmission model.

Parameter	Description	Value (Range)	Footnote/ Reference
	Demographic parameters		
N_0	Initial size of sexually active population	2,317,648	(a)
ρ	Population growth rate	4.8% (4.7–4.9%)	
α_0	Initial proportion of population in urban areas	15%	(b)
ν_u	Migration rate from urban to rural settings	2.25% (2–2.5%)	
ν_r	Migration rate from rural to urban settings	0.2% (0.18–0.23%)	
$1/\mu$	Average duration that a person remains in the sexually active population	45 years	Assumed
	HIV Biological parameters		
β	Rate at which susceptible individuals with partners that are chronically infected become infected with HIV	fitted (±10%)	(c)
θ_a	Multiplicative increase in HIV transmission probability for partners with AIDS	5 (4–6)	[7, 8]
θ_t	Multiplicative decrease in HIV transmission probability for HIV-infected partners on effective ART	0.08 (0.05–0.13)	[9, 10]
γ	Disease progression rate at which individuals chronically infected with HIV progress to AIDS	0.075 (0.06–0.09)	[7, 9]
μ_c	Death rate per year for a person chronically infected with HIV	3%	[11, 12]
μ_a	Death rate per year for individuals with AIDS	30%	[11, 12]
μ_t	Death rate per year for HIV-infected individuals on ART	5%	[11, 12]
	Clinical parameters		
δ_u^c	Diagnosis rate for urban individuals with chronic infection	fitted (±10%)	(e)
δ_u^a	Diagnosis rate for urban individuals with AIDS	fitted (±10%)	
δ_r^c	Diagnosis rate for rural individuals with chronic infection	fitted (±10%)	
δ_r^a	Diagnosis rate for rural individuals with AIDS	fitted (±10%)	
τ_u	Rate at which urban individuals diagnosed with AIDS begin treatment	fitted (±10%)	(f)
τ_r	Rate at which rural individuals diagnosed with AIDS begin treatment	fitted (±10%)	
$1/\omega$	Average duration at which individuals remain on ART	10 years (6.7–20 years)	(g)

[(a)]To calibrate the model to the HIV epidemic in PNG from 1990 to 2010, the sexually active population size was assumed to be equal to the 15–49-year-old adult population estimated by the World Bank [13]. From the initial 1990 population, the value of ρ (which is assumed to be the same for urban and rural areas) is set to match the growth of the 15–49-year-old population seen in PNG from 1990 to 2008 [13].

[(b)]Data from the World Bank gives the proportion of the population that is living in urban areas to be approximately 15%. However, the proportion of the population in urban areas has been declining very slightly from 1990 to 2008 [13]. The migration rates between urban and rural areas are set to follow this trend after the urban proportion is set to be initially 15%.

[(c)]This rate incorporates the number of sexual partners, condom usage, effect of STIs, and other factors that affect HIV transmission. As we are focused on the impact of ART reductions, we group these factors into a single rate which is then fitted to the annual prevalence and incidence estimates for PNG. The value of β is likely to be different for urban and rural areas; however, there is limited data available for these population categories so we have assumed the same value for fitting purposes.

[(d)]The value and range for γ have been used to match the estimates for the number of people who require ART in PNG. The disease progression rate we use corresponds to an average duration of being chronically infected equal to 11–16.6 years which is much longer than established durations for low resource settings [14]. Due to the simplicity of our HIV transmission model, which only has one infection stage prior to AIDS, a longer average time period is required to match the actual epidemiology.

[(e)]The rate at which infected people are diagnosed is fitted to match the overall number of diagnoses recorded in PNG [2].

[(f)]The rate at which individuals diagnosed with AIDS begin treatment is fitted to match the overall number of people who have started treatment since 2003, the estimated number of people who require treatment, and the ART coverage in PNG [2, 4].

[(g)]The average duration at which urban and rural people remain on ART is likely to be different due to supply and logistical issues. However, there is very little data on the length of time people remain on ART in PNG; hence, we have assumed the same value and range for urban and rural areas.

for each of the fitted parameter values (see Table 1). One hundred parameter sets were used in the analysis, sampled (through Latin hypercube sampling implemented in the SaSAT computer program [16]) from the parameter ranges specified in Table 1 (Figure 2).

2.3. ART Reduction Scenarios. To understand the future impact of ART reductions in PNG, we considered four scenarios. These were simulated by our model for the next five years for each of our parameter sets. These scenarios are specified by the number of people on ART. The scenarios are as follows:

(i) continual roll-out of ART which extend current trends in PNG. This is the baseline scenario used for comparison with other scenarios and is simulated

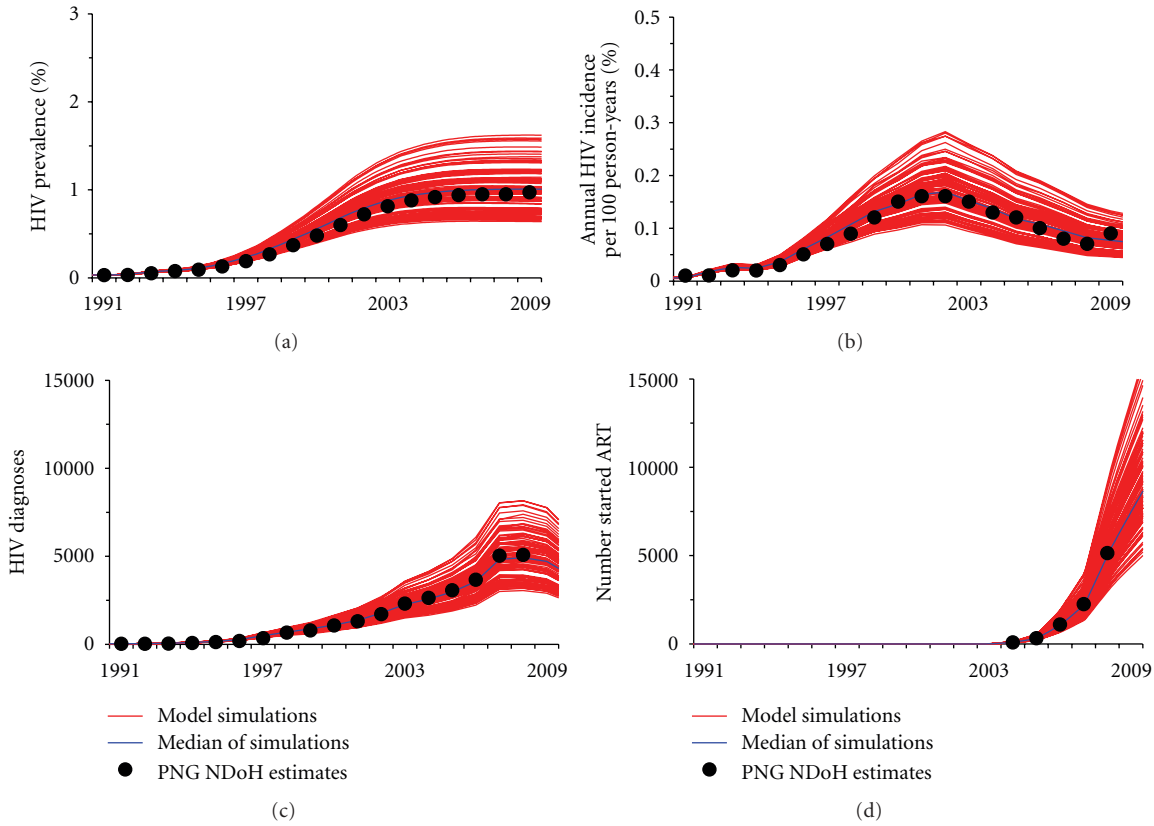

Figure 2: Fitting and calibration of the HIV transmission model to PNG epidemiological data from 1990 to 2009. In each figure, estimates or data from the PNG National Department of Health (black discs, data) are compared with 100 model-based simulations (red) and median simulations (blue) over the period from 1990 to 2009. (a) HIV prevalence in adult population. (b) HIV incidence each year per 100 persons. (c) Number of HIV diagnoses each year. (d) The cumulative number of HIV-infected people who have started antiretroviral treatment by the end of each year.

by extending the period of simulation with the parameters fixed to their values at the end of 2009,

(ii) fixing the number of people on ART to a constant value as at the end of 2009. This represents a consistent supply of ART in PNG that sustains a fixed number of people on ART into the future,

(iii) reducing the number of HIV-infected people on ART by 50% by 2015,

(iv) reducing the number of HIV-infected people on ART by 90% by 2015.

The variation in number of people on ART from the end of 2009 for each of these scenarios is shown in Figure 3(a). We also investigated two scenarios where the reduction in ART services only lasted for two years before the re-establishment of the current ART roll-out (potentially due to a successful funding application in the next Global Fund round in 2011). In these two scenarios, the parameter values were set to the values in scenario (iii) and (iv), respectively, before returning to their current values after two years. The number of people on ART follows the same trend as the (iii) and (iv) scenarios for two years before rapidly increasing towards the expected number under current conditions.

3. Results and Discussion

Using our mathematical model we evaluated the impact of reductions in ART roll-out in PNG by forecasting the change in expected HIV incidence and advanced HIV deaths in PNG over the 5 years for each scenario. Our results show that a reduction in ART availability in PNG is likely to have a large and significant impact on the HIV epidemic.

3.1. Increase of Annual HIV Incidence. Our model predicts that a continual roll-out of ART in PNG would result in a declining trend in HIV incidence. The annual HIV incidence is predicted to reduce from a median of 2,794 infections (0.074 per 100 person-years, IQR 2,227–3,360) in 2009 to 2,674 infections in 2014 (0.062 per 100 person-years, IQR 2,109–3,239) (Figure 3(b)). If the continual roll-out of ART is interrupted in 2010, then a large rise in annual HIV incidence is expected (Figure 3(b)). For the scenario where the number of ART patients remains at a constant level after 2009, the expected number of new HIV infections in 2014 will be 4,253 (0.099 per 100 person-years, IQR 3,264–5,242) which is a 59% increase from the baseline level. If the number of people on ART is reduced by 50% or 90%, then incidence is forecasted to increase to 4,490 (0.10% per 100 person-years,

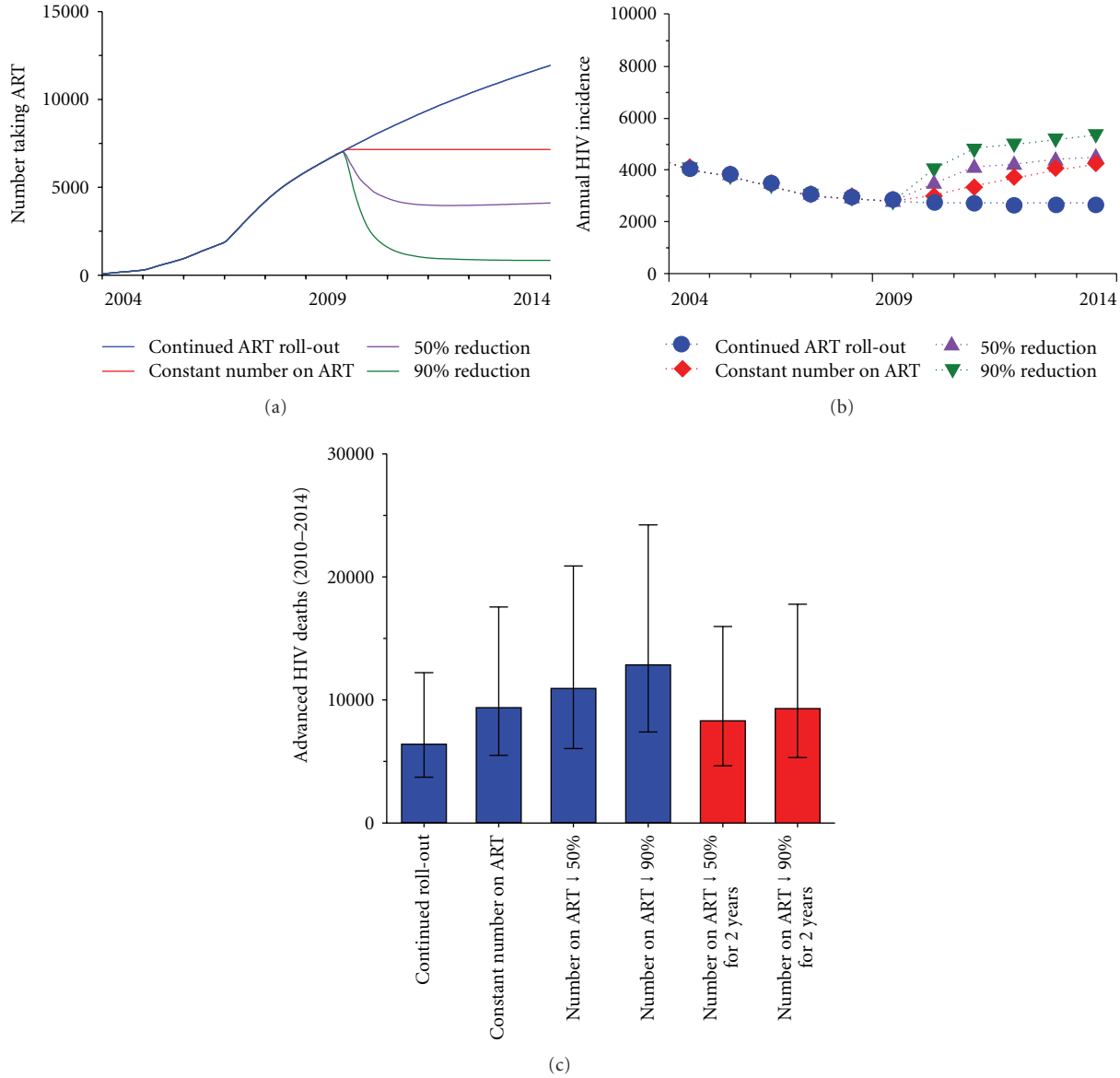

Figure 3: The impact of reductions in ART availability. (a) Median number of HIV-infected people receiving ART for scenarios from (i) to (iv) in the text. These scenarios were a continuation of the ART roll-out after 2009, maintaining ART services so that the number of people who receive ART remains constant, a reduction in the number of people receiving treatment of 50% from the level at the end of 2009, and a reduction in the number of people receiving treatment of 90% from the level at the end of 2009. (b) Median annual incidence per 100 person-years after 2009 for 100 simulations of each of the scenarios considered. (c) Total number of advanced HIV deaths for the period 2010–2014 for all the scenarios considered. The blue bars represent the median number of deaths for scenarios from (i) to (iv) in the text with the black error bars showing the minimum and maximum number of deaths for 100 model simulations of each scenario. Similarly, the red bars represent the median number of deaths for the scenarios where ART programs and services are interrupted for two years.

IQR 3,382–5,599) and 5,323 (0.12% per 100 person-years, IQR 4,035–6,610) in 2014, which, correspond to 67.9%, and 99.1% increases, respectively. Cumulatively, for scenarios (ii), (iii), and (iv), 4,871, 7,181, and 10,934 additional HIV cases (compared to baseline) are estimated to occur during 2010–2014 for these scenarios. Further, if ART availability remains low beyond 2015, higher incidence levels will likely result. If the 50% and 90% reductions in ART are only in effect for two years, there is still a substantial increase in the expected incidence over the two-year period before declining to a level slightly above forecasts for current conditions. According to these two scenarios, it could be expected that ~3,298 and 5,342 additional HIV cases (compared to baseline) will occur during 2010–2014 period, respectively.

This increase in incidence is likely to be an upper bound as the sexual behavior and prevalence of other sexually transmitted infections (STIs) affecting HIV transmission are assumed to be constant in our model; however, these factors are implicitly incorporated in our epidemiological fitting routine. In reality, sexual behavior could change

due to the continuation of other education and prevention programs or because people are sick with advanced HIV. Furthermore, the level of STIs in the population is expected to change with the expansion of voluntary counseling and testing services and targeted treatment programs. On the other hand, many of these programs could also be affected by decreases in HIV funding potentially leading to larger increases in incidence. By only focusing on ART, our results show the relative impact of reductions in ART availability on the PNG HIV epidemic. The effect of other factors that could also change requires separate evaluation; however, the direction and impact of decreases in funding on these factors are unknown.

3.2. Increase of Advanced HIV-Related Deaths. With the continuation of the current ART roll-out, the total number of advanced HIV deaths in PNG over 2010–2014 is forecasted to be 6,402, (range 3,714–12,221) which corresponds to an average annual death rate of 1.52% in the population of people living with HIV. Figure 3(c) highlights the severe increase in the number of advanced HIV deaths resulting from a reduction in ART availability. Our model predicts an additional 2,975 (range 1,783–5,333) advanced HIV deaths between the beginning of 2010 and the end of 2014 if the supply of ART is maintained at the current level. This corresponds to a 46.5% increase in the number of advanced HIV deaths in comparison with the continuous ART roll-out scenario. Further, for the scenarios where the number of patients on ART reduces to 50% and 10% of the 2009 level, the median number of cumulative deaths over the period 2010–2014 will increase to 10,936 (range 6,047–20,891) and 12,848 (range 7,392–24,232), which correspond to 70.8% and 100.7% rise, respectively. This lifts the advanced HIV death rates of HIV-infected individuals to 2.5% and 2.9% per year. When ART supplies are only interrupted for two years the median number of cumulative deaths over the period 2010–2014 is projected to increase to 8,300 (range 4,651–15,969) and 9,299 (range 5,323–17,786) for the 50% reduction and 90% reduction scenarios, respectively. A large reduction over this short period results in almost as many deaths as maintaining the supply of ART at the current level (Figure 3(c)).

4. Conclusions

The HIV epidemic in PNG has been in a large expansion phase over the past 15 years. Although the prevalence of HIV has likely started to reach a plateau, prevention and control measures require renewed efforts. Thus far, the epidemic has resulted in an estimated 38,000 people becoming infected with HIV in PNG with over 28,000 of these diagnosed. To remain healthy, these people require clinical care and management of their infection and access to effective combination antiretroviral therapy. As a lower income country, PNG has not been able to finance the required antiretroviral drugs for its citizens and has depended on external funding for its ART programs. Unfortunately, PNG recently did not secure external funding for the continuation of these vital programs and the Papua New Guinean government had to allocate funding from already constrained health budgets to continue the roll-out of ART. Without funding, ART availability in PNG would not increase according to scale-up plans and could decrease very substantially.

In this study, we used a mathematical model to demonstrate that if ART supplies are not funded and declined, then substantial population-level impacts could be expected in PNG. Specifically, if the number of people on ART falls to 10% of current level, then over the next 5 years there could be an approximate doubling in annual incidence and an additional 12,848 AIDS-related deaths. Even if ART provision is halved then annual incidence would increase by ~68% and there would be an additional ~10,936 AIDS-related deaths.

The model we developed was calibrated to accurately reflect the unique epidemiology of PNG at the overall population-level and was based on the best data available; however, it is a relatively simple model and could not capture the full degree of complexity and heterogeneity that exists within the population. In particular, the model did not capture the movement of HIV-positive people accurately, did not investigate the impact on particular at risk population groups such as commercial sex workers, and did not consider treatment failure and the possible increased emergence of drug-resistant strains of HIV. Regardless, the model is useful for obtaining the population-level relative change in new incident cases and AIDS-related deaths that could result due to a drug shortage in PNG. These results highlight the large degree of crisis for people at risk of or living with HIV in PNG and their families and community. This crisis also affects other concerning health issues such as tuberculosis; the HIV prevalence in tested tuberculosis patients is ~3.5% in PNG [2]. A similar population-level relative impact could be expected for other low and middle income settings that rely on external funding for ART supplies if their ART funding were to reduce significantly.

The majority of funds for HIV prevention, care, and treatment in PNG are externally funded from the Global Fund, international government development organizations, and nongovernment organizations. While PNG's economy has experienced relatively strong growth, many international organizations suffered a reduction in funding after the 2008 global financial crisis which could lead to impacts on HIV/AIDS epidemics [17, 18]. A judicious mix of funding sources and disbursement channels could be important for responding to HIV epidemics in PNG [19]. This includes the PNG government which may need to consider taking greater responsibility for funding HIV programs to reduce exposure of these programs to external volatility, especially since Government expenditure for HIV has decreased in recent years. Irrespective of funding sources, it must be acknowledged that there could be a state of emergency for people living with HIV in PNG. People with late-stage HIV infections do not have access to effective ART and are at real risk of premature death. Therefore, it is of very high importance that funding for HIV programs are obtained and that appropriate administration processes are put in place to handle any funds procured and manage the logistics

of channeling ART for effective distribution. In addition, improvements in infrastructure are required to reach and care for people with HIV in PNG. These complex factors need to be addressed in PNG and in other similar low-resource settings in the process of reaching a major goal of achieving universal treatment access for all people living with HIV.

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Research Article

Estimating the Impact and Cost of the WHO 2010 Recommendations for Antiretroviral Therapy

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In July 2010, WHO published new recommendations on providing antiretroviral therapy to adults and adolescents, including starting ART earlier, usually at a CD4 count of 350 or lower, specific regimens for first- and second-line therapies, and other recommendations. This paper estimates the potential impact and cost of the revised guidelines by first, calculating the number of people that would be in need of antiretroviral therapy (ART) with different eligibility criteria, and second, calculating the costs associated with the potential impact. Results indicate that switching the eligibility criterion from CD4 count < 200 to < 350 increases the need for ART in low- and middle-income countries (country-level) by 50% (range 34% to 70%). The costs of ART programs only to increase coverage to 80% by 2015 would be 44% more (range 29% to 63%) when switching the eligibility criterion to CD4 count < 350. When testing and outreach costs are included, total costs increase by 62%, from US$26.3 billion under the previous eligibility criterion of treating those with CD4 < 200 to US$42.5 billion using the revised eligibility criterion of treating those with CD4 < 350.

1. Introduction

In July 2010, the World Health Organization (WHO) published new recommendations on providing antiretroviral therapy (ART) to adults and adolescents in resource-limited settings that revised the guidelines previously published in 2006. The new recommendations encourage starting ART earlier, usually at a CD4 count of 350 or lower, specifies regimens for first and second line therapies, and contains other recommendations regarding laboratory monitoring and other elements [1]. The revised guidelines were developed based on systematic reviews of the evidence, consultation with key stakeholders, and consideration of the impact and cost of potential changes. This paper describes the model and analysis prepared to examine the potential impact and cost of the revised guidelines.

2. Materials and Methods

The analysis consists of two parts: first, we construct a model to calculate the number of people that would be in need of ART with different eligibility criteria, in order to calculate the potential impact of the new guidelines. Second, we calculate the costs associated with the potential impact in order to evaluate the financial implications of the new guidelines.

The model tracks the HIV+ population by CD4 count using an approach similar to one used in South Africa recently to estimate the need for treatment (see Figure 1) [2]. The values and sources for all of the parameters described below can be seen in Supplementary Material available online at doi:10.1155/2011/738271 Annex A.

We assume that all newly infected people start with CD4 counts above 500, and that their CD4 counts decline over time. The transition probabilities $\lambda 1$, $\lambda 2$, $\lambda 3$, and $\lambda 4$ represent the probability of progressing from one CD4 category to the next; the derivation of these probabilities is discussed in detail below. In each category there is some probability of death from HIV-related causes, designated as $\mu 1$, $\mu 2$, $\mu 3$, $\mu 4$, and $\mu 5$ as well as a chance of death from non-AIDS causes, $\mu 0$ (not shown in the figure). The probability of HIV-related death increases as CD4 counts decrease.

The number of people in the different CD4 count categories represents the HIV-infected population that is not on ART. The number of people eligible for treatment is

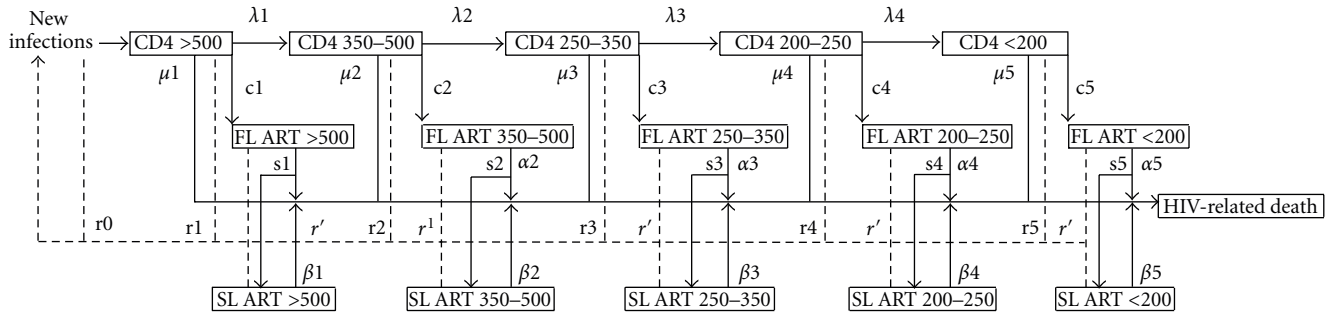

Figure 1: Model of HIV-Infected Population, Eligibility for ART and HIV-related Mortality. Notes: (1) FL ART = First line ART, SL ART = Second line ART, (2) The population receiving ART is categorized according to CD4 count at the initiation of ART, (3) The population in each box is also subject to non-AIDS mortality, and (4) Solid lines indicate flows of people, dashed lines indicate flows of information.

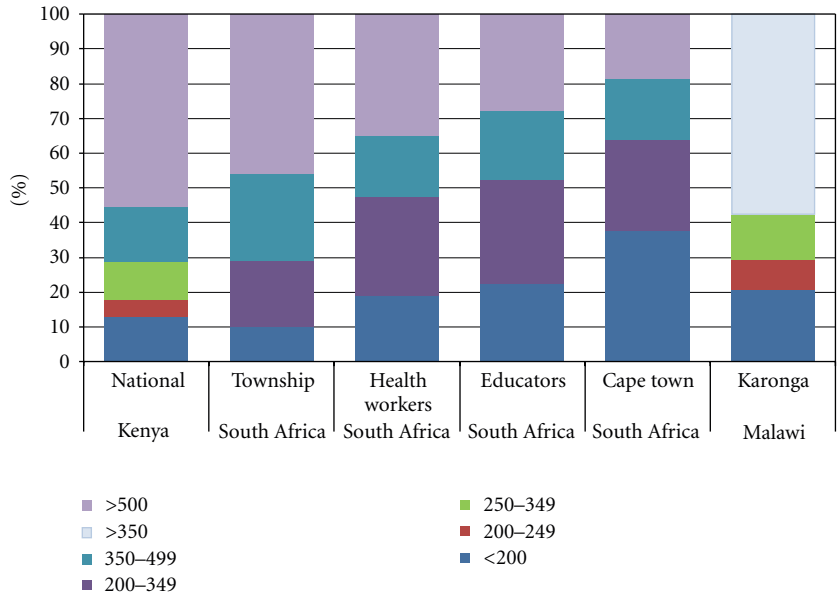

Figure 2: Distribution of HIV+ Population not on ART by CD4 Count.

the number in each CD4 count category that is below the recommended level for initiating ART.

Depending on the eligibility criterion and the level of first-line ART coverage a percentage of those eligible for treatment will start first-line ART (c1, c2, c3, c4, c5). Those on ART are categorized by their CD4 count at the initiation of treatment. The model does not track the temporal decline of CD4 counts of those on treatment. Those on first-line ART have a probability of failure depending on their CD4 count at initiation, $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, and $\alpha 5$.

The number starting ART each year is determined by the assumed coverage and the number of people eligible for treatment. We assume that those starting on ART will be distributed among the eligible CD4 categories such that an equal percentage of people in each eligible CD4 category initiate treatment.

Those failing on first line ART will either start on second line ART (according to second line coverage s1, s2, s3, s4 and s5) or die from HIV-related causes. Those on second line have some probability of dying from HIV-related causes each year ($\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\beta 5$).

The number of HIV-related deaths each year is the sum of HIV-related deaths from those not on ART and those on ART.

The historical annual number of new infections is exogenous to the model and is based on a Spectrum projection using historical surveillance and survey data to determine HIV prevalence and incidence trends [3]. The future number of new infections is also based on the Spectrum projection but can be modified by expanding treatment. For those not on ART infectiousness varies by CD4 count (as a result of variations in viral load) as indicated by r1, r2, r3, r4, and r5. Infectiousness is high during primary infection, r0, low during the asymptomatic period (r1, r2, r3, and r4) and high during the symptomatic period, r5. Those on ART have reduced infectiousness, r′. As a result the future number of new infections can be influenced by the dynamics of CD4 decline and the coverage of ART.

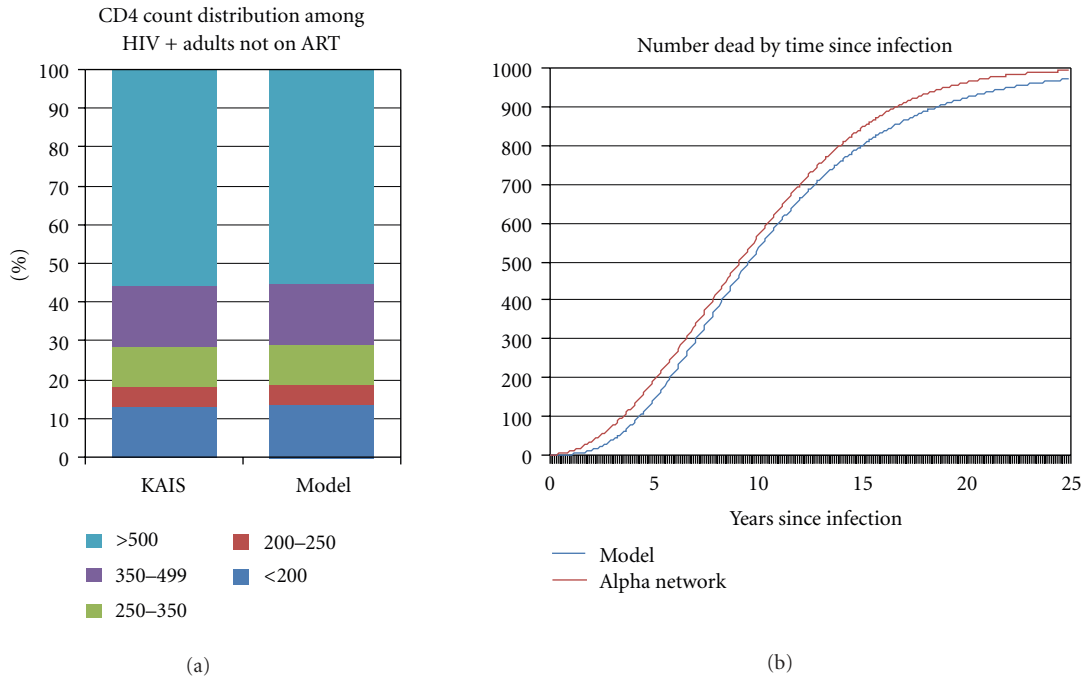

Figure 3: Model results compared to CD4 count distributions in Kenya in 2007 and progression from infection to death compared to ALPHA network analysis.

3. Transition Probabilities

We have estimated the transition probabilities by fitting the model to data on the distribution of the HIV-infected population by CD4 count and the pattern of progression from HIV infection to AIDS death. Data on the distribution of the HIV-infected populations by CD4 count are available from studies in a township near Johannesburg, South Africa (community-based survey of 1000 men and women aged 15–49 [4]), health care workers in Gauteng, South Africa (all 2032 professional and support staff at two hospitals [5]), educators in South Africa (national survey of 21,669 public school educators from all provinces of South Africa [6]), Cape Town, South Africa (observational cohort from two public sector clinics consisting of 2086 patients [7]), Karonga, Malawi (demographic surveillance site studying all adults aged 18–59 and including about 150 HIV-positive individuals [8]), and Kenya (nationally representative sample of adults 15–64 [9]). The distribution of these populations by CD4 count category is shown in Figure 2.

Data are also available from several cohort studies on the overall progression from HIV infection to HIV-related death. The Analysing Longitudinal Population-based HIV/AIDS data on Africa (ALPHA) network has conducted a pooled analysis using data from several cohorts to estimate the proportion surviving by the number of years since infection [10]. Only the Kenya data set is a nationally representative sample, and it is the only one that provides information on all CD4 categories of interest. Thus we have estimated the parameter values using only the Kenya data set, along with the age-adjusted, net survival curve based on the East and Southern Africa cohorts from the ALPHA network, but checked the results against the other data sets.

We fit the model to both sets of data simultaneously. One version of the model was set up for Kenya and used the Spectrum estimates of the number of new infections from 1980 to 2007 and the reported number of people on ART from 2000 to 2007. We compared the data on distribution by CD4 count from the Kenya AIDS Indicator Survey (KAIS) with the model projection for 2007. Another version of the model followed a cohort of 1000 new HIV infections as they progress through the various CD4 categories and to death. The resulting proportions surviving were compared with the ALPHA network survival curve for East and Southern Africa. We searched for the single set of transition probabilities that provided the best fit in both cases. The model used a time step of one-tenth of a year in order to accommodate the short duration in the 200-250 category that could be less than one year. The fits are shown in Figures 3(a) and 3(b). The resulting parameters are shown in Supplementary Annex Table A1.

The fit of the model to Karonga (Malawi) and Orange Farm (South Africa) data sets using the parameter values derived from the fit to the Kenya data and the annual number of new infections in Malawi and South Africa is shown in Figures 4(a) and 4(b).

4. Costs

Four categories of cost are considered: antiretroviral (ARV) drugs, laboratory costs, service delivery costs, and identification (outreach and testing). The cost of ARV drugs is

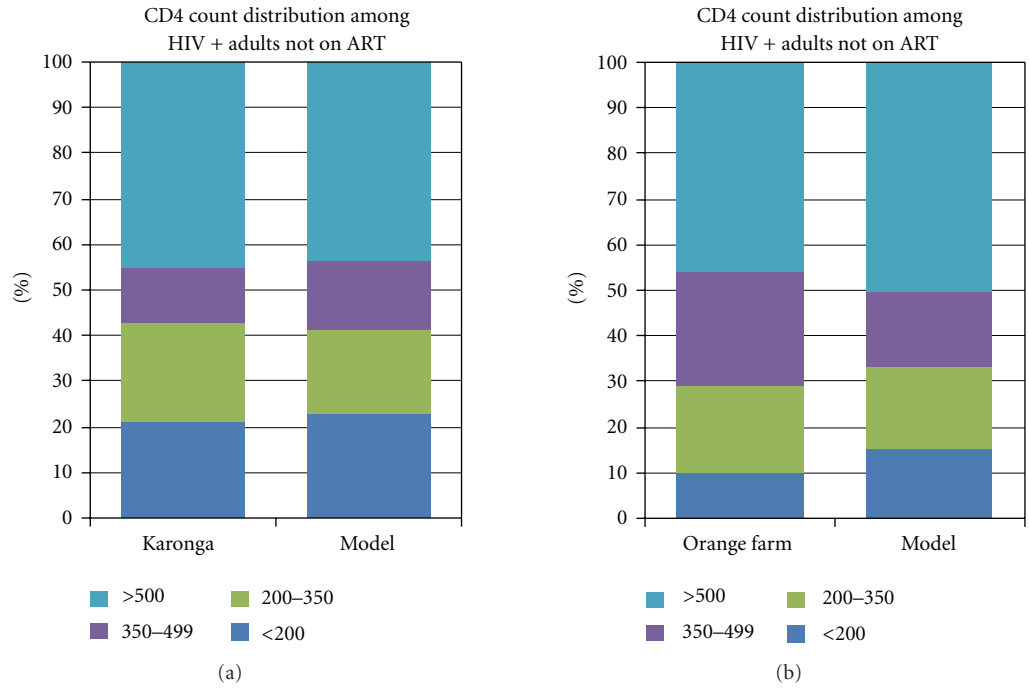

Figure 4: Model results compared to CD4 count distributions for Malawi and South Africa.

determined from the number of people on first and second line, the distribution of patients by regimen and the costs of each regimen. Following previous work, we examine two sets of alternative regimens: one that contains a fast phase-out of d4T, and another that contains a slower phase-out of d4T [11].

Drug costs may be different for patients in low and middle income countries. Current costs are based on WHO and Clinton Foundation reports (Table 1).

Laboratory costs are calculated separately for new and continuing patients and can vary by regimen. Currently, laboratory costs are calculated as the annual median cost for lab tests across recent literature. Recent studies in various countries (Cote d'Ivoire, Ethiopia, Mexico, Nigeria, South Africa, Thailand, Uganda, Zambia) are used as the basis [12–20]. The median cost is $250 per year for new patients and $190 per patient per year for continuing patients.

Service delivery costs are based on a standard number of inpatient days and outpatient visits per patient per year and country specific costs for inpatient days and outpatient visits. For this analysis we used the same studies referenced above for laboratory costs (with the exception of Cote d'Ivoire and the addition of another South Africa study [21]) to calculate the median number of outpatient visits per year as 9.5. Only three of these studies also had data on the number of inpatient days for ART patients [12, 14, 21]; we used these to calculate the median number of inpatient days for ART patients per year as 1.56. The country-specific costs per inpatient day are the costs of one bed day at a primary-level hospital as reported in the WHO-CHOICE database of service delivery costs [22]. The cost of an outpatient visit is for a 20-minute outpatient visit at a health centre, from the same WHO database. Representative regional costs are shown in Table 2.

Outreach and testing costs vary primarily by the type of population reached. The model considers 10 population categories for testing:

(1) patients with symptoms of HIV,
(2) sexually-transmitted Infection (STI) patients,
(3) tuberculosis patients,
(4) pregnant women,
(5) other health system contacts,
(6) sex workers,
(7) men who have sex with men (MSM),
(8) injecting drug users (IDU),
(9) voluntary counseling and testing (VCT),
(10) general population.

Due to lack of data, those coinfected with hepatitis B are not included here.

The unit cost of VCT services average about $16 per client. We have used this cost also for provider-initiated testing and counseling. No additional testing and counseling costs are included for pregnant women since the costs of testing and counseling are already covered in the Prevention of Mother-To-Child Transmission (PMTCT) programs. Similarly we assume that outreach and counseling for sex workers, IDU, and MSM are already covered in prevention programs for those populations, and add only $1 for the costs of the test itself. For general population testing we have

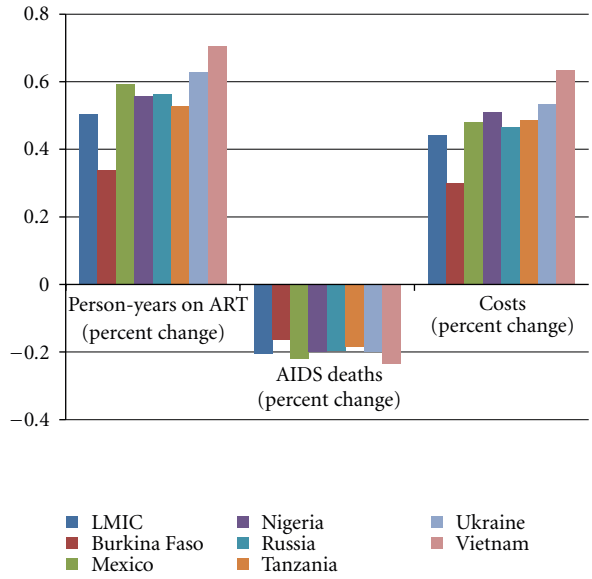

Figure 5: Comparison of results for changing eligibility criterion to CD4 count <350 between LMIC and country-level calculations.

Table 1: Antiretroviral costs per patient per year for low- and middle-income countries.

Regimen	Low income countries	Middle income countries
d4T + 3TC + NVP	$89	$88
AZT + 3TC + NVP	$149	$226
AZT + EFV + 3TC	$220	$281
TDF + 3TC + EFV	$210	$268
TDF + FTC + EFV	$255	$325
TDF + FTC + NVP	$190	$243
TDF + 3TC + LPV/r	$590	$1070
AZT + 3TC + LPV/r	$585	$1150

Sources: WHO, UNAIDS and UNICEF, 2009, Towards Universal Access: Scaling up priority HIV/AIDS interventions in the health sector; Clinton Foundation Antiretroviral Price list, August 2009, available at http://www.clintonfoundation.org/files/chaiarvpricelistaugust2009english.pdf, accessed 1 June 2010.

doubled the personnel costs associated with VCT to allow for additional outreach programs in addition to the testing and counseling costs. The resulting cost is $23 per person tested.

The number of tests for each population group will depend on the eligibility criterion and the coverage. We assume that patients with symptoms who are found to be HIV+ will be in the lowest CD4 count category. We assume that those who are found to be HIV+ in the other population groups will be distributed by CD4 count according to the distribution of all HIV+ people excluding those <200.

5. Results and Discussion

The model has been applied to all low- and middle-income countries (LMIC) and to seven countries individually: Burkina Faso, Mexico, Nigeria, Russia, Tanzania, Ukraine, and Vietnam. The number of new infections each year and the number of people on ART through 2008 were taken from the Spectrum projections for each country. Estimates of the population sizes are based on national estimates prepared as part of the effort to estimate global resource needs [23].

Table 3 displays the results for LMIC for the additional cost and impact of scaling up ART coverage to reach 80% by 2015, assuming that the criterion for eligibility to treatment switches from a CD4 count 200 to 350 in 2010. In addition, the financial implications of two ARV regimens are presented; first with a slower phase-out of d4T, and second with a fast phase-out of d4T. In order to compare across countries and across scenarios, all cost and impact figures are discounted to 2010 using an annual discount rate of 3 percent.

Our estimates suggest that switching the eligibility criterion from CD4 count <200 to <350 increases the number of person-years of ART from 40.7 million to over 61 million, a 50% increase. There is a concomitant reduction in the number of AIDS deaths, with the number decreasing by 21% when the eligibility criterion switches to CD4 count <350. The number of new HIV infections is also reduced, due to the lower infectivity that occurs when people receive ART; new HIV infections are reduced by 11% when the eligibility criterion changes.

The financial costs of providing ART to meet the new need from increasing the eligibility criterion also increase in a similar way to the increase displayed in the number of person-years of ART. The costs of providing ART would be 44% higher with a switch to providing ART to those with CD4 count <350. Although the overall costs are higher with the fast phase-out of d4T relative to the slower phase-out of d4T, the difference is quite small.

Note that the slightly lower percentage increase in costs versus the number of person-years on ART reflects the relatively greater numbers of people on first-line therapy with the increase in eligibility criterion. When the additional testing costs incurred in order to identify the new patients are included, however, total costs increase relatively more than the number of person-years of ART. Total costs increase from US$26.3 billion (US$27.0 billion) to US$42.5 billion (US$43.5 billion) if the eligibility criterion is CD4 count <350 and there is a fast (slower) phase-out of d4T, an increase of 62% (61%). Combining the results for incremental costs and deaths averted suggests that the cost per AIDS death averted is approximately US$9,700 if the eligibility criterion switches to CD4 count <350.

In order to perform a sensitivity analysis, we vary the costs of laboratory testing and service delivery costs using the interquartile distribution of laboratory testing costs from the studies cited above. Using the first quartile function result, laboratory and service delivery costs are reduced by 31%, while using the third quartile function result increases laboratory and service delivery costs by 64%. Overall, this translates to a range in total costs (not presented here) of US$42.5 billion to US$55.5 billion for the scenario with a slower phase-out of d4T, and a range in total costs of US$37.2 billion to US$56.5 billion for a fast phase-out of d4T.

Table 2: Representative service delivery costs by region.

Regional service delivery costs for ART patients	Annual cost of inpatient Days (ART patient)	Annual cost of outpatient visits (ART patient)	Total annual service delivery cost (ART patient)
Sub-Saharan Africa	$18.43	$53.62	$72.05
East Asia	$36.48	$64.36	$100.84
Oceania	$56.33	$77.62	$133.94
South and South-East Asia	$29.20	$64.77	$93.98
Eastern Europe and Central Asia	$52.07	$71.82	$123.89
Western and Central Europe	$106.23	$239.38	$345.61
North Africa and Middle East	$63.44	$73.68	$137.12
Caribbean	$58.92	$70.52	$129.45
Latin America	$59.34	$72.91	$132.25

Source: WHO-CHOICE database, available at http://www.who.int/choice/en/.

Table 3: Global results when ART eligibility is switched from CD4 count <200 to CD4 count <350 in 2010 while increasing coverage to 80% by 2015, by different d4T phase-out scenarios (2010–2015).

LMIC	CD4 < 200	CD4 < 350	Difference	% Change
Person years of ART	40,752,534	61,292,374	20,539,839	50%
AIDS deaths	8,180,609	6,501,483	−1,679,126	−21%
Life years of PLHIV	162,032,903	163,012,351	979,448	1%
New HIV infections	11,198,013	9,946,912	−1,251,101	−11%
Slower phase-out of d4T				
ART costs (Millions of US$)	$25,027	$36,072	$11,045	44%
Testing costs (Millions of US$)	$1,282	$6,480	$5,198	406%
Total costs (Millions of US$)	$26,309	$42,552	$16,243	62%
Fast phase-out of d4T				
ART costs (Millions of US$)	$25,678	$37,047	$11,369	44%
Testing costs (Millions of US$)	$1,282	$6,480	$5,198	406%
Total costs (Millions of US$)	$26,960	$43,527	$16,567	61%

Source: Authors' calculations.

In order to compare results for different epidemic types and different regions, we performed the analysis for seven countries: Burkina Faso, Mexico, Nigeria, Russia, Tanzania, Ukraine, and Vietnam. Results indicate that there is not a great deal of variation across countries (see Figure 5). While the average percentage increase in the number of person-years on ART for LMIC was 50% when the eligibility criterion switched from CD4 count <200 to <350, this varies across countries from a low increase of 34% in Burkina Faso to a high increase of 70% in Vietnam. A similar pattern can be observed for AIDS deaths; the country level results range from a reduction of 16% in Burkina Faso to a reduction of 23% in Vietnam when the eligibility criterion switches to CD4 count <350. Finally, the changes in the country-level additional ART costs associated with changing the eligibility criterion mirror the changes in the results for LMIC; for LMIC, the additional ART costs increase by 44% when the eligibility criterion switches to CD4 count <350, while the increases at the country level vary from 30% (Burkina Faso) to 63% (Vietnam).

6. Conclusions

In this paper, we model both the impact and cost of the new 2010 WHO recommendations for providing antiretroviral therapy to adults and adolescents in resource-limited settings. We examine the impact of changing the eligibility criterion for antiretroviral therapy from CD4 count <200 to CD4 count <350 on the number of person-years on ART, the number of AIDS deaths averted, and the costs of the change including the costs of additional tests and recruitment costs. We also examine the financial impact of switching away from d4T towards other recommended regimens.

We find that, although the total costs for providing ART increase, the percentage increase is slightly less than the increase in number of person-years on ART. The number of

person-years on ART increases for LMIC by 50%, varying between 34% and 70% at the country level, while the cost of providing ART increases by 44% for LMIC, varying between 30% and 63% at the country level when the eligibility criterion changes to CD4 count <350. There is minimal impact on the incremental cost when phasing out d4T either fast or more slowly when the eligibility criterion varies. When testing and outreach costs are included, total costs increase by 62%, from US$26.3 billion under the previous eligibility criterion of treating those with CD4 <200 to US$42.5 billion using the revised eligibility criterion of treating those with CD4 <350.

In addition, the number of AIDS deaths decreases at the global level by 21% when the eligibility criterion switches to CD4 count <350, with country-level results varying between decreases of 16% and 23%. Combining the data results in a cost per AIDS deaths averted varying between approximately US$7,100 and US$9,700 (US$4,800 and US$14,000 along with US$6,400 and $16,300) depending on the change in eligibility criterion.

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Clinical Study

Long-Term Outcome of an HIV-Treatment Programme in Rural Africa: Viral Suppression despite Early Mortality

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Objective. To define the long-term (2–4 years) clinical and virological outcome of an antiretroviral treatment (ART) programme in rural South Africa. *Methods*. We performed a retrospective observational cohort study, including 735 patients who initiated ART. Biannual monitoring, including HIV-RNA testing, was performed. Primary endpoint was patient retention; virological suppression (HIV-RNA < 50 copies/mL) and failure (HIV-RNA > 1000 copies/mL) were secondary endpoints. Moreover, possible predictors of treatment failure were analyzed. *Results*. 63% of patients (466/735) have a fully suppressed HIV-RNA, a median of three years after treatment initiation. Early mortality was high: 14% died within 3 months after treatment start. 16% of patients experienced virological failure, but only 4% was switched to second-line ART. Male gender and a low performance score were associated with treatment failure; immunological failure was a poor predictor of virological failure. *Conclusions*. An "all or nothing" phenomenon was observed in this rural South African ART programme: high early attrition, but good virological control in those remaining in care. Continued efforts are needed to enrol patients earlier. Furthermore, the observed viro-immunological dissociation emphasises the need to make HIV-RNA testing more widely available.

1. Introduction

Sub-Saharan Africa houses most of the 33 million people who are human immunodeficiency virus- (HIV-) infected globally. The scale-up of antiretroviral treatment (ART) roll-out that has taken place in this region over the last decade is impressive. Consequently, an increasing number of treatment-outcome data from African ART programmes has been published. In spite of frequently observed high early mortality rates, ART provides clear benefits for most HIV-infected patients. Typically, improvements in clinical, immunological, and virological outcome measurements are seen within months after starting treatment. However, long-term data (more than one year of followup) are less readily available [1, 2]. Moreover, most available data come from urbanized areas, and for many ART programmes regular HIV-RNA monitoring is not feasible. Clinical or immunological decline is frequently used to predict treatment failure in low-income countries (LICs). However, several studies portray limited correlation between such parameters and virological failure [3–5]. Furthermore, recently it has been shown that virological monitoring results in switching treatment earlier and at higher CD4 counts [6].

Ndlovu Medical Centre (NMC) is located in rural South Africa. Since the start of its ART programme, prospective virological monitoring has been done for all patients. Previously, we reported favourable short-term (one-year followup) results of patients receiving ART at NMC [7]. The objective of this report was to describe the long-term (2 to 4 years of followup) outcome. Due to consequent monitoring, longitudinal virological data could be analyzed. Furthermore, possible predictors for treatment failure were analyzed.

2. Methods

2.1. Cohort Description and Data Collection. Ndlovu MC is located in a poor, rural area of Limpopo, a province in the northeast of the Republic of South Africa. Details

on NMC and its ART programme have been described elsewhere [7]. For the current study, all adults were included who started first-line ART at least 24 months before data-collection. A WHO clinical stage IV and a CD4+ T-cell count below 200 cells/mm^3 were used as criteria for treatment eligibility. Treatment consisted of efavirenz or nevirapine plus stavudine or zidovudine and lamivudine. In case of virological failure, second-line ART was available. To this end, a boosted protease inhibitor (PI, lopinavir/ritonavir) was added to the nucleoside reverse transcriptase inhibitor (NRTI) backbone.

Data were retrospectively collected from the medical charts. Body mass indices (BMI, weight/height^2) are calculated at each visit. CD4+ T-cell counts (FACSCount system, Becton Dickinson Biosciences, San Jose, CA) and plasma HIV-RNA levels (system 340 bDNA analyzer, Bayer AG, Leverkusen, Germany, detection limit 50 copies/mL) are measured before treatment initiation and regularly thereafter (four times in the first and biannually during consecutive years). Patients are seen by HIV-counsellors during each clinic visit in order to provide information and to stimulate treatment adherence.

2.2. Outcome Definitions. Patient retention-rate was defined by the proportion of patients remaining in care at NMC, combined with the proportion of patients who were transferred to other clinics. Patient attrition was compiled of all-cause mortality and people who were lost to followup.

Achieving an HIV-RNA below 50 copies/mL was used to define virological suppression. An HIV-RNA over 1000 copies/mL after at least three months of ART was considered to be an indicative of virological failure.

Patient attrition and virological failure were combined to define treatment failure.

Immunological failure was defined according to the WHO guidelines as either (1) a CD4+ T-cell count after six months of therapy below 100 cells/mm^3 or below the pre-therapy count or (2) a 50% decline from the on-treatment peak CD4+ T-cell count value [8].

2.3. Statistical Analysis. Patient retention-rate was the primary endpoint. Secondary outcomes were virological suppression and treatment failure.

The negative and positive predictive values (NPV and PPV, resp.) of immunological failure, to determine virological failure, were calculated. Moreover, to define predictors of treatment failure, we compared the distribution of several determinants (Gender; Age; baseline BMI, CD4+ T-cell count, Karnofsky score and log HIV-RNA; (N)NRTIs used; employment status and years since treatment initiation) between patients with treatment success and those experiencing treatment failure. To this end, all patients who started ART were included in analyses. The same determinants were used to define predictors of virological failure. In order to minimize the influence of early mortality, only patients with more than three-month followup were included in these analyses.

Continuous data were compared with the Student's t-test or the paired t-test as appropriate. Proportions were compared with the χ^2 test, and data that were not normally distributed were analyzed via the Mann-Whitney U or Wilcoxon test. Kaplan-Meier survival analysis was used to estimate the time from initiation of antiretroviral therapy to virological suppression and the time from initial suppression to subsequent virological failure. Odds ratios and 95% confidence intervals (95% CI) were calculated using logistic regression analysis. The Cox proportional-hazards model was used to identify independent predictors of the endpoints. To this end, determinants that were associated with the outcome in univariate analysis (defined as a P-value $<.1$) were included in multivariate analysis. A P-value $\leq .05$ was considered statistically significant. Data were processed and statistical analyses were done using SPSS version 15.

3. Results

3.1. Patient Characteristics. The first 735 adults who started ART at NMC were included in analysis. Median duration between treatment initiation and data collection was 35 months (range 24–59). Women predominated (526/735, 72%). Unemployment rate was high at 70%; 24% of patients had less than six years school attendance. The median baseline CD4+ T-cell count (68 cells/mm^3, IQR 20–140) and BMI (19.8 kg/m^2, IQR 17.4–23.0) demonstrate that many patients were at an advanced stage of disease when initiating treatment. Most individuals initially received a stavudine-containing ART regimen (579/735, 79%); in 26% (151/579) of those patients, stavudine was subsequently switched to zidovudine, as they showed signs of neuropathy or lipodystrophy. Efavirenz was the most commonly prescribed NNRTI (58%. 426/735).

3.2. Clinical and Virological Outcome. Patient retention-rate at end of followup was 65% (476/735). Most (429/476, 90%) of these patients were still in care and on treatment at the NMC, but 10% (47/476) patients were transferred out to other clinics. The remaining 35% (259/735) of patients had either died or were lost to followup. Mortality was the main cause for attrition (171/259, 66%). Attrition typically occurred soon after treatment initiation; within three months, one out of five persons was not in care anymore ($n = 152$, Figure 1).

562/735 patients (76%) achieved virological suppression on first line ART. Suppression was established at a median of 12 weeks after treatment initiation. Not achieving virological suppression was mainly due to early attrition (133/173, 77% within three months after treatment start).

Longitudinal virological monitoring showed continued virological control on first-line ART in most patients (466/735, 63%). This proportion increased to 80% (466/583) if only those with more than 3-month followup were included. Virological failure was observed in 117/735 patients (16%: 20%{117/583} of those with >3 months followup). Median duration between initial response and subsequent failure was 18 months. Only 4% (31/735) of all patients who started first-line ART were switched to second-line ART. Most of them achieved virological suppression

after switching (25/31, 81%). Virological outcome data are summarized in Figure 1.

Ongoing viral replication was limited in patients receiving first-line ART. During complete followup, less than 10% of patients who remained in care had an HIV-RNA >1000 copies/mL and the vast majority of patients (71–82%) showed complete, virological suppression (HIV-RNA <50 copies/mL, Figure 2).

3.3. Predictors of Failure. Ninety-five patients showed evidence of immunological failure (13%). On the basis of immunological parameters, virological failure would have been missed in 66/117 patients (56%) and 44/95 patients (46%) would mistakenly have been classified as experiencing virological failure. Immunological failure therefore showed a PPV and NPV for virological failure of 54% and 87%, respectively.

Overall, treatment failure (attrition and/or virological failure) occurred in 337 patients (46%) during followup. Of all baseline factors that were associated with treatment failure in univariate analysis (low BMI and CD4+ T-cell count, Karnofsky score ≤50, Zidovudine-use and gender), only a low performance (Karnofsky) score (OR 3.6, 95% CI 1.8–7.1) and male gender (OR 1.7, 95% CI 1.2–2.3) remained independently associated with the outcome after multivariate analysis (Table 1).

In patients with more than three-month followup, baseline CD4+ T-cell count ($P = .03$) and age ($P = .07$) were associated with virological failure in univariate analysis. Neither remained independently associated with this outcome after multivariate analysis.

Table 1: Predictors of treatment failure.

Determinant	Treatment Success* $n = 398$	Treatment Failure** $n = 337$	Univariate (P-value)	Multivariate (P-value)
Male gender n (%)	95 (24)	114 (34)	< **.01**	**.01**
Mean Age (years)	35.3	35.3	1.0	—
Mean BMI (kg/m_2)	21.1	19.9	< **.01**	.4
Karnofsky score ≤50 n (%)	12 (3)	35 (11)	< **.01**	< **.01**
Median CD4 ($cells/mm_3$)	84	54	< **.01**	.2
Mean 10log HIV-RNA	4.9	4.9	.8	—
Time since start ART n (%)			.3	—
(i) 2-3 years	217 (55)	162 (48)		
(ii) 3-4 years	121 (30)	111 (33)		
(iii) >4 years	60 (15)	60 (18)		
Efavirenz use n (%)	223 (56)	204 (61)	.2	—
Stavudine use n (%)	324 (82)	253 (76)	**.05**	.3
Unemployed n (%)	275 (74)	237 (79)	.1	—

Treatment success* patients who remained in care during followup and showed a good, virological response. Treatment failure**: a combined endpoint of patient attrition and virological failure. *n*: number of patients. %: percentage of patients. BMI: body-mass index. CD4: CD4+ T-cell count. ART: antiretroviral treatment.

4. Discussion

A median of three years after treatment initiation, half of the patients receiving ART in this rural South African clinic is still in care and has a fully suppressed HIV-RNA. Nearly a quarter of patients died, mostly early on. Sixteen percent of patients showed virological failure; only few of them were switched to second-line treatment. Apart from a low Karnofsky score at baseline, male gender was predictive of treatment failure. The correlation between immunological and virological failures on the other hand was minimal.

The high early mortality rate is probably largely attributable to the advanced clinical stage patients are in when seeking care, as has been described for other African cohorts [1, 9]. Regression analysis indeed shows a low performance score to be associated with treatment failure. Thus, continued efforts are needed to enrol patients earlier, before clinical illness becomes evident. Male gender also remained as an independent predictor of treatment failure in our analysis. Similar associations have been observed previously [10, 11], but others did not find gender to be of influence on ART outcome [12, 13]. In contrast, the CD4+ T-cell count did not remain significantly associated with treatment failure, suggesting that starting ART prior to clinical illness is of greater importance than merely avoiding a low CD4+ T-cell count.

Drug-resistance development is a major concern when treating HIV. The virus can select drug-resistance mutations in the presence of suboptimal drug levels. In case of continued ART use in spite of ongoing viral replication, accumulation of resistance can occur [14–16]. Mortality was the main cause of treatment failure in our cohort. In addition, continued virological control was observed in the majority of patients who did remain in care. Due to this observed "all or nothing" phenomenon, the number of people experiencing ongoing viral replication in the presence of ART was limited.

Regular HIV-RNA testing is not feasible in many LICs. In accordance with WHO-guidelines, treatment changes are frequently based on immunological criteria alone [8, 17]. Immunological failure was a poor predictor for experiencing virological failure in our cohort. Basing treatment decisions on immunological parameters would lead to unnecessary treatment switches in a substantial number of patients, as has been described previously [4, 5]. We however show, that the proportion of patients that would mistakenly continue a failing regimen if treatment decisions were merely based on immunological parameters is even larger. Such viro-immunological dissociation highlights the need for virological monitoring for all patients receiving ART.

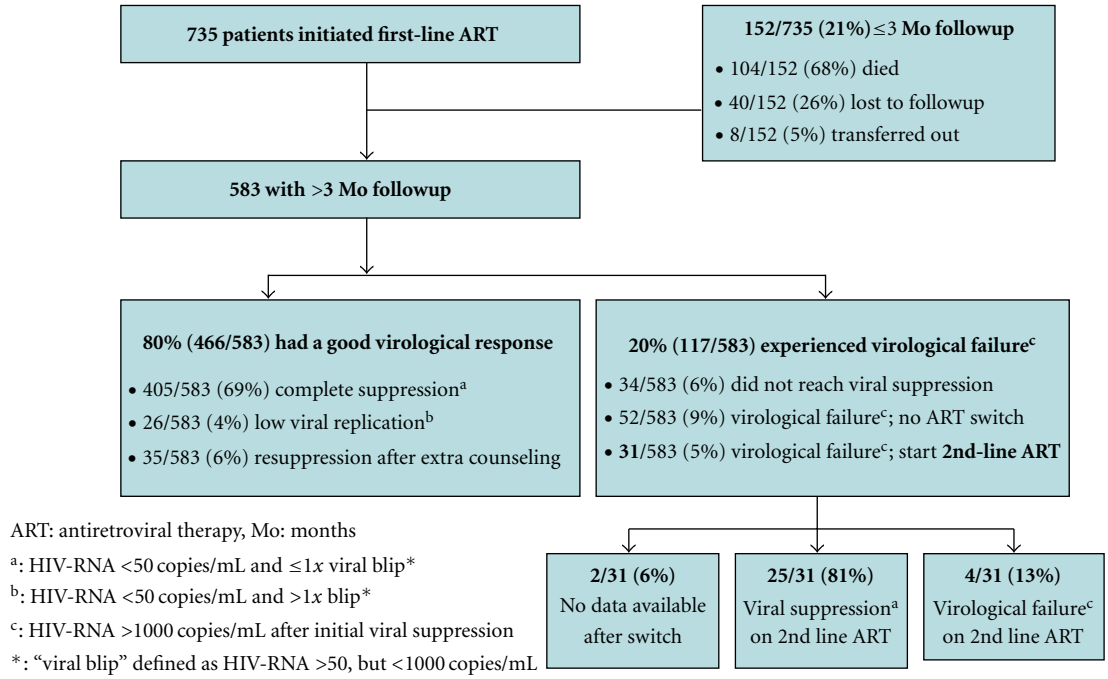
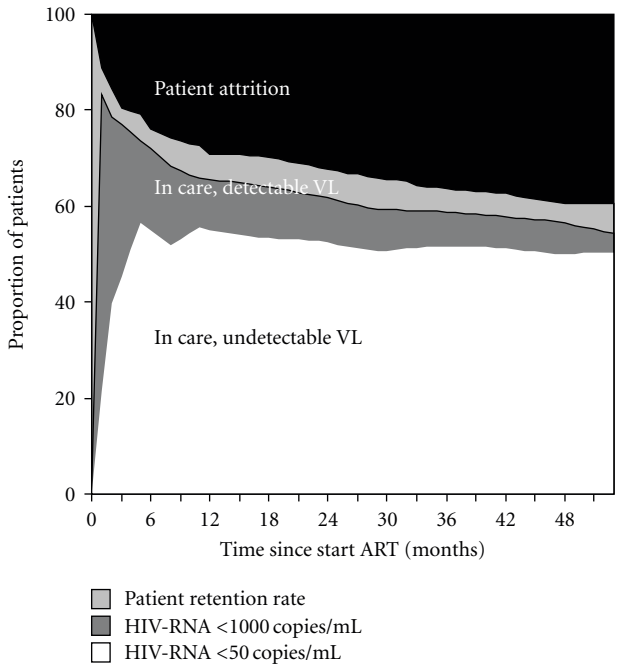


Figure 1: Virological treatment outcome.



% Retention:	100	80	76	71	70	68	66	64	63	61
No at risk:	735	583	547	504	486	464	322	207	133	67
% VL <1000:	1	93	93	90	91	91	90	92	92	93
% VL <50:	0	58	71	76	76	78	77	81	82	82

Figure 2: Viral replication during 1st line ART.

In the current study, regular (bi-annual) virological monitoring was performed. Still, a substantial number of patients continued their first-line ART after showing the first evidence of virological failure. The delay between virological failure and treatment switch is probably due to care-givers focusing on extra adherence counselling, prior to switching to the only available second-line regimen. On the basis of virological failure alone, 16% of patients in our cohort had an indication for a treatment switch. A recent review reported nearly twice as many patients in low and middle income countries needing a second-line regimen (26–32%, [17]), but others reported similar numbers to those observed in our cohort [18–20]. As most patients on second-line ART achieved virological suppression again, the number of people with ongoing viral replication may be reduced even further if treatment switches are made earlier. However, future cost-benefit analyses will have to determine the optimal time to switch.

There are some limitations to our study. Diagnostics for underlying illnesses and documentation of adverse events were limited. Causes of attrition were therefore generally unknown. Moreover, due to the retrospective nature of our study, bias due to unmeasured determinants cannot be ruled out.

In conclusion, in this rural South African ART programme with access to virological monitoring, early attrition was the main cause for treatment failure. Virological failure was limited in those remaining in care. Continued efforts are needed to enrol patients earlier into care. Furthermore, the observed viro-immunological dissociation emphasises the need to make HIV-RNA testing more widely available.

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