HIV virological failure and drug resistance in a cohort of Tanzanian HIV-infected adults

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Objectives: There are few data on ART failure rates and drug resistance from Tanzania, where there is a wide diversity of non-B HIV subtypes. We assessed rates and predictors of virological failure in HIV-infected Tanzanians and describe drug resistance patterns in a subgroup of these patients.

Methods: ART-naïve, HIV-1-infected adults enrolled in a randomized controlled trial between November 2006 and 2008 and on ≥ 24 weeks of first-line NNRTI-containing ART were included. Population-based genotyping of HIV-1 protease and reverse transcriptase was performed on stored plasma from patients with virological failure (viral load >1000 copies/mL at ≥ 24 weeks of ART) and at baseline, where available.

Results: A total of 2403 patients [median (IQR) age 37 (32–43) years; 70% female] were studied. The median (IQR) baseline CD4+ T cell count was 128 (62–190) cells/ μ L. Predominant HIV subtypes were A, C and D (92.2%). The overall rate of virological failure was 14.9% (95% CI 13.2%–16.1%). In adjusted analyses, significant predictors of virological failure were lower CD4+ T cell count ($P=0.01$) and non-adherence to ART ($P<0.01$). Drug resistance mutations were present in 87/115 samples (75.7%); the most common were M184V/I (52.2%) and K103N (35%). Thymidine analogue mutations were uncommon (5.2%). The prevalence of mutations in 45 samples pre-ART was 22%.

Conclusions: High levels of early ART failure and drug resistance were observed among Tanzanian HIV-1-infected adults enrolled in a well-monitored study. Initiating treatment early and ensuring optimal adherence are vital for the success and durability of first-line ART in these settings.

Introduction

An estimated 6.2 million HIV-infected patients are receiving ART in sub-Saharan Africa (SSA) as a result of intensive national and international efforts to scale up HIV care and treatment services in low- and middle-income countries.¹ The successful rollout has resulted in significant reductions in HIV-related morbidity and mortality and high rates of treatment success comparable to those in resource-rich settings.

Despite effective therapies, virological failure (VF) is inevitable in some HIV-infected patients on treatment. In SSA, rates of ART failure vary between 11.1% and 24% in the first 12 months after ART initiation and $\sim 71\%$ – 90% of patients with ART failure have evidence of resistance.^{2–5} Common causes of treatment failure in these settings include poor adherence to medication, drug toxicities and use of antiretrovirals with lower genetic barriers.

The Government of Tanzania has been providing ART to HIV-infected populations since 2004; in 2012 $>399\,886$ people were receiving treatment.¹ In Tanzania there have been few studies of treatment failure and resistance, and risk factors associated with treatment failure have been largely unexplored. Routine virological monitoring in Tanzanian HIV programmes is still uncommon and genotypic testing is rarely available, making any robust assessment of ART failure and resistance difficult to conduct. Findings from other treatment failure studies in SSA cannot necessarily be extrapolated to the Tanzanian setting since a wide diversity of non-B HIV subtypes exist, which may influence treatment response.⁶ In the largest study to date of treatment failure in HIV-infected adults from Tanzania, VF was reported in 25/212 (11.8%) after a median follow-up of 22.3 months, and 82% of patients with viral load (VL) >1000 copies/mL had at least one clinically relevant mutation. However, the numbers of patients

who were successfully genotyped was small ($n=22$) and pre-ART HIV drug resistance (DR) was not assessed.⁷

In this study, we assessed rates of ART failure in Tanzanian HIV-infected adults enrolled in a large clinical trial with access to routine HIV virological monitoring. We characterized DR mutations (DRMs) in a subset of patients prior to and after ART initiation, and examined predictors of VF and resistance.

Methods

Study population

This retrospective cohort study was conducted among HIV-1-infected adults who were enrolled in HIV treatment clinics supported by the Management and Development for Health (MDH) HIV Care and Treatment programme, Dar es Salaam, Tanzania, and participating in a randomized, controlled Trial of Vitamins Study (TOV3). The MDH programme (formally a collaboration between the Muhimbili University of Health and Allied Sciences, Harvard School of Public Health and Dar City) was established in 2004 and provides technical support with US President's Emergency Plan for AIDS Relief (PEPFAR) funds to 100 private and public healthcare HIV care and treatment facilities, including district and tertiary referral hospitals, healthcentres and dispensaries. The TOV3 trial was conducted during 2006–09 and compared multivitamins (vitamins B complex, C and E) in high amounts with standard amounts of the recommended dietary allowance (RDA) in HIV-infected women and men on ART (www.clinicaltrials.gov: NCT00383669). Details of the study are described elsewhere.⁸ The study enrolled 3418 HIV-infected, non-pregnant, non-lactating female and male adults (≥ 18 years) who were initiating ART at seven MDH-supported care and treatment sites. Patients eligible for ART and meeting inclusion criteria were enrolled in the TOV3 study at their ART initiation visit. All patients enrolled in TOV3 were included in this ancillary study.

Patients were considered eligible for first-line therapy if they were HIV positive and met criteria for initiation of ART in adults according to the National AIDS Control Program Guidelines for the Management of HIV and AIDS.⁹ ART initiation criteria at the time of the study were: all patients at WHO stage 4 regardless of CD4; all patients at WHO stage 3 with CD4 ≤ 350 cells/ μ L; and all patients with CD4 < 200 cells/ μ L, regardless of clinical symptoms. Patients were followed up by a health-care provider every month for clinical assessment and monitoring of ART adherence, response to therapy, management of toxicities and outcome status (i.e. new opportunistic infection, death, loss to follow-up, stopped treatment, transferred out). Management of patients enrolled in the TOV3 study followed the usual standards of care in Tanzania. First-line HIV treatment in study patients at the time of the study was with generic drugs purchased by the Government of Tanzania, including stavudine, lamivudine and nevirapine. Zidovudine was substituted for stavudine for patients who had peripheral neuropathy or could not tolerate stavudine, and efavirenz was substituted for nevirapine for patients who were intolerant of nevirapine.

Determination of absolute count of T cell subsets (FACSCalibur flow cytometer, Becton Dickinson, SA), complete blood cell count (AcT5 Diff AL Analyzer, Beckman Coulter, SA) and ALT (Cobas Integra 400 Plus Analyzer, Roche Diagnostics Systems, SA) was conducted every 4 months. Plasma VL assessment was performed at enrolment and every 4 months, subject to the availability of reagents (Cobas Amplicor HIV-1 Monitor Test Version 1.5, Roche Diagnostics Systems, SA).

Analysis of DRMs

Genotype and subtype testing was performed retrospectively on baseline and follow-up specimens in a subset of patients with VF. VF was defined as VL > 1000 copies/mL after ≥ 24 weeks on first-line ART; however,

genotyping was only performed on patients with VL ≥ 2000 copies/mL because of the limited success in amplifying samples from other parts of Africa with VL < 2000 copies/mL using ViroSeq. HIV genotyping and subtyping were performed at the Kanki Laboratory in the Department of Immunology and Infectious Diseases at the Harvard School of Public Health in Boston, MA, USA. Samples were analysed using the ViroSeq HIV Genotyping (GT) Kit Version 2 (Abbott Molecular, Abbott Park, IL, USA) on an automated sequencer (ABI 3100 series, San Francisco, CA, USA) according to manufacturer instructions. Briefly, HIV RNA was isolated from plasma using a Qiagen kit (Valencia, CA, USA) according to the manufacturer's instructions. RNA was converted into cDNA and the cDNA was later amplified to increase the copy number of the DNA. After amplification, DNA was purified and put into a cycle sequencing reaction and later a genetic analyser to generate sequences. ViroSeq software (Abbott Park, IL, USA) was used to analyse the sequences and subtypes were established by construction of a genetic tree using subtype-specific reference sequences.

The following clinical and demographic data were extracted from the existing TOV3 dataset on study patients at baseline and until last follow-up: patient demographics (age, sex, date of scheduled and unscheduled clinic visits); WHO stage; prior and new AIDS defining illnesses; ART history (date of initiation, time on therapy, first-line ART regimen); scheduled clinic visits; patient outcome including death, loss to follow-up and transfer out; CD4+ T cell count, haemoglobin, HIV RNA, HIV subtype and genotype testing (the last only in a subset of patients).

Outcome measures and statistical analysis

The primary outcomes of this study were the probability of VF and DR [defined as the presence of one or more mutations in the HIV genome that has been demonstrated to be associated with the ability of the virus to replicate in the presence of drugs that are used as standard first- and second-line regimen(s) (<http://hivdb.stanford.edu>)]. The denominator used to calculate probabilities of failure was based upon all patients who had a VL measured after 6 months of ART ($n=2403$). The prevalence of DR among patients with VF after 6 months of ART was calculated as the proportion of any major antiviral DRM among those with VF and DR. A descriptive analysis of mutational patterns identified in these samples from patients with antiretroviral resistance mutations at ART failure and at baseline was performed.

Risk factors for VF were assessed using generalized estimating equations (GEEs) defined by Proc genmod in SAS with a robust variance estimator for binary outcome with the log link function. Potential risk factors with a P value ≤ 0.2 in the unadjusted model were included in the adjusted model. Missing indicators were created and included in the adjusted model for covariates with $> 5\%$ missing values. Potential baseline predictors included in the model were district, age, sex and BMI, stage of disease (WHO clinical stage), marital status, education level, ART non-adherence, laboratory indices (CD4, haemoglobin, ALT) and TOV3 study randomization arm (high-dose multivitamins or RDA multivitamins). ART non-adherence was defined as $\geq 5\%$ non-compliance with scheduled ART pick-up visits. Patients were considered non-adherent if their visit was > 3 days beyond their recorded appointment date or if the inter-visit interval was ≥ 35 days in cases where there was no record of a future appointment date. Adherence to multivitamins was performed by pill counts each month. For ordinal variables, P values for trend were calculated by use of a median score trend test, in which the median value for each category was included in models as a continuous variable.

The study was approved by the institutional review boards of the Harvard School of Public Health, Muhimbili University of Health and Allied Sciences, CDC Tanzania and USA, Tanzania Food and Drugs Authority and the National Institutes of Medical Research. All patients provided written informed consent to participate in this study.

Results

Baseline characteristics of TOV3 cohort (n = 2403)

The median (IQR) age was 37 (32–43) years and 1682 (70%) of the patients were female (Table 1). The median (IQR) CD4+ T cell count at ART initiation was 128 (62–190) cells/ μ L. One thousand six hundred and sixty-four (69%) were on a first-line ART regimen of stavudine, lamivudine and nevirapine or efavirenz.

Table 1. Baseline characteristics at ART initiation (n = 2403)

	Median (IQR) or n (%)
Male	721 (30)
Age (years)	37 (32–43)
Age categories (years)	
<30	372 (15)
30 to <40	1134 (47)
40 to <50	682 (28)
≥ 50	215 (9)
Marital status	
married	1109 (49)
single	279 (12)
widowed/divorced/separated	857 (38)
BMI (kg/m ²)	20.7 (18.6–23.4)
BMI categories (kg/m ²)	
<17.0	244 (10)
17.0 to <18.5	327 (14)
18.5 to <25.0	1435 (60)
≥ 25.0	382 (16)
Haemoglobin (g/dL)	10.2 (8.8–11.7)
WHO disease stage	
I	141 (6)
II	416 (19)
III	1429 (64)
IV	259 (12)
CD4+ T cell count (cells/ μ L)	128 (62–190)
<50	462 (20)
50 to <100	440 (19)
100 to <200	932 (40)
≥ 200	478 (21)
HIV RNA (log copies/mL) (n = 1075)	5.32 (4.94–5.64)
ART regimen ^a	
stavudine, lamivudine and nevirapine	1423 (59)
stavudine, lamivudine and efavirenz	241 (10)
zidovudine, lamivudine and nevirapine	183 (8)
zidovudine, lamivudine and efavirenz	556 (23)
Multivitamin supplementation	
high dose	1185 (49)
standard dose	1218 (51)

Numbers may not add up to 2403 due to missing values.

^aMedian (IQR) time on ART = 26.1 (16.6–35.2) months.

The median (IQR) overall time on ART was 26.1 (16.6–35.2) months (Table 1). HIV subtyping was performed in 64 (2.7%) patients. Subtype distribution was A (17, 26.6%), B (1, 1.6%), C (24, 37.5%), CRF01_AE (4, 6.3%) and D (18, 28.1%).

Incidence of VF and predictors

VF occurred in 14.9% (95% CI 13.2%–16.1%) patients overall and 11.0% (95% CI 9.6%–12.4%) within 12 months after ART initiation. In multivariate analyses, significant predictors of VF were lower CD4+ T cell count [relative risk (RR) 1.36 (95% CI 0.97–1.92), 1.57 (95% CI 1.13–2.19) and 1.16 (95% CI 0.84–1.59) for patients with CD4 <50, 50 to <100 and 100 to <200 cells/ μ L versus CD4 ≥ 200 cells/ μ L, respectively (*P* for trend = 0.01)] and non-adherence [RR 1.47 (95% CI 1.14–1.91), *P* = 0.004]. There was a trend towards a higher risk of VF in age group <30 years versus 30 to <40 years [RR 1.15 (95% CI 0.88–1.51)] and lower risk among older age groups [40 to <50 years versus 30 to <40 years, RR 0.89 (95% CI 0.70–1.14) and ≥ 50 years versus 30 to <40 years, RR 0.86 (95% CI 0.59–1.27)] (*P* for trend = 0.09). The risk of VF did not differ significantly by gender or ART regimen (Table 2).

Genotyping testing

Of 358 study patients with VF and a VL ≥ 2000 copies/mL, 106 had genotyping performed at the time of antiretroviral failure (number of samples = 115). Thirty-three patients had a VL between 1000 and 2000 copies/mL and were not sequenced; samples from 46 patients failed to be amplified during the sequencing process and samples from 173 patients were not available. Forty-three of 358 study patients had genotyping performed on a baseline sample (number of samples = 45). Eighteen patients had sequencing performed on both baseline and ART failure samples. Of the 115 samples genotyped at follow-up, 97 had ART regimen data: 77 on stavudine-containing regimens and 20 on zidovudine-containing regimens. Patients with VF who had genotyping performed had more advanced WHO stages than patients with VF who did not have genotyping performed (*P* = 0.04). There was no difference in other baseline characteristics between the two patient groups.

Prevalence of DRMs at VF

DRMs were present in 87/115 (75.7%) of samples sequenced from 106 patients with VF and 10/45 (22.2%) of samples sequenced from 43 patients at baseline. Major and accessory NRTI and NNRTI mutations were present in 66/115 (57.4%) and 80/115 (69.6%) patients, respectively. Dual-class resistance to both NRTIs and NNRTIs occurred in 59/115 (51.3%).

The most common NRTI mutations observed were M184V/I (60, 52.2%). K65R was present in 2 (1.7%) patients: 1 patient with HIV subtype D and 1 with unknown subtype. Both patients were on stavudine. The majority of patients had only one significant NRTI mutation. One or more thymidine analogue mutations (TAMs) were present in only 6 (5.2%) samples from six patients: M41L (*n* = 2, 33.3%), D67N (*n* = 4, 66.7%), K70R (*n* = 4, 66.7%), L210W (*n* = 1, 16.7%), T215Y/F (*n* = 3, 50%) and K219Q/E (*n* = 4, 66.7%). Accessory TAMs were present in 6 samples (5.2%). No patients had mutations conferring multidrug resistance (T69ins, Q151M) (Figure 1). The number of overall NRTI DRMs did not differ significantly between zidovudine- and stavudine-containing

Table 2. Risk factors for VF (n=2403)

Variable	Univariate		Multivariate ^a	
	RR (95% CI)	P	RR (95% CI)	P
District		0.13		0.13
Ilala	1.00		1.00	
Kinondoni	1.07 (0.85–1.34)		1.05 (0.84–1.32)	
Temek	0.82 (0.63–1.06)		0.81 (0.62–1.05)	
Sex		0.85		
male	1.00			
female	0.98 (0.80–1.21)			
Age (years)				0.09
<30	1.15 (0.89–1.49)	0.01	1.15 (0.88–1.51)	
30 to <40	1.00		1.00	
40 to <50	0.82 (0.64–1.04)		0.89 (0.70–1.14)	
≥50	0.80 (0.55–1.17)		0.86 (0.59–1.27)	
Marital status, % ^b		0.68		
married	1.00			
single	1.10 (0.81–1.48)			
widowed/divorced/separated	0.95 (0.76–1.18)			
BMI (kg/m ²)		0.52		
<17.0	1.02 (0.73–1.41)			
17.0 to <18.5	1.05 (0.79–1.40)			
18.5 to <25.0	1.00			
≥25.0	1.14 (0.88–1.47)			
Haemoglobin (g/dL) ^b		0.99		
<7	1.04 (0.66–1.64)			
7.0 to <8.5	0.97 (0.67–1.40)			
8.5 to <12.0	1.11 (0.85–1.43)			
≥12.0	1.00			
WHO disease stage ^b		0.13		0.18
I	1.00		1.00	
II	0.88 (0.59–1.31)		0.85 (0.57–1.27)	
III	0.69 (0.48–1.00)		0.70 (0.49–1.02)	
IV	0.85 (0.55–1.31)		0.83 (0.53–1.29)	
CD4+ T cell count (cells/μL)		0.02		0.01
<50	1.30 (0.94–1.78)		1.36 (0.97–1.92)	
50 to <100	1.49 (1.09–2.03)		1.57 (1.13–2.19)	
100 to <200	1.14 (0.85–1.52)		1.16 (0.84–1.59)	
≥200	1.00		1.00	
ALT >40 U/L	1.04 (0.76–1.43)	0.8		
ART regimen containing zidovudine	1.00 (0.81–1.23)	0.99		
ART regimen containing nevirapine	1.01 (0.82–1.24)	0.93		
Calendar year of ART initiation, %		0.38		
2006	1.00			
2007	1.04 (0.70–1.56)			
2008	0.93 (0.61–1.42)			
Season of ART initiation, %		0.98		
April–May (long rainy)	1.00			

Continued

Table 2. Continued

Variable	Univariate		Multivariate ^a	
	RR (95% CI)	P	RR (95% CI)	P
June–September (long dry)	0.99 (0.77–1.28)			
October–November (short rainy)	1.04 (0.81–1.33)			
December–March (short dry)	1.00 (0.73–1.36)			
Non-adherence ^c	1.48 (1.15–1.90)	0.003	1.47 (1.14–1.91)	0.004
Multivitamin supplementation				
high dose	0.97 (0.80–1.18)	0.77		
standard dose	1.00			

^aVariables with $P < 0.20$ in univariate analysis were selected for multivariate analysis.

^bVariables with $> 5\%$ data missing.

^cDefined as $\geq 5\%$ non-compliance with scheduled ART pick-up visits.

regimens ($P = 0.71$). However, there were significantly more TAMs in patients on zidovudine than on stavudine regimens ($P = 0.054$). All of the patients with three or more TAMs were on zidovudine-containing regimens (Table S1, available as Supplementary data at JAC Online).

The most common NNRTI mutations were K103N [40 (35%)] and Y181C/I [28 (24.3%)]. The G190A/S mutations were present in 20 (17.4%). Other mutations that occurred at moderate frequency included K101E (9, 7.8%), V108I (5, 4.3%), P225H (2, 1.7%) and Y188C/H/L (5, 4.3%) (Figure 2). K103N was more frequent in samples from patients on efavirenz compared with those on nevirapine [9 (50%) versus 26 (33%), $P = 0.17$], while Y181C was more frequent in samples from patients on nevirapine compared with those on efavirenz [19 (24.7%) versus 1 (5.0%), $P = 0.08$]. Overall, the number of NNRTI mutations did not differ significantly between patients on nevirapine- and efavirenz-containing regimens ($P = 0.93$) (Table S2). The number of NRTI and NNRTI mutations did not differ significantly between multivitamin arm. There was no difference in the proportion of NNRTI or NRTI mutations or major mutations (M184V, K103N, Y181C) between HIV subtypes A, C and D (Table S3).

PI mutations

A total of 29 PI mutations were observed in 27 patients. Twenty-five patients had a single non-polymorphic or polymorphic accessory mutation, none of which has been shown to be associated with high levels of phenotypic PI resistance (A71T, $n = 4$; L10I, $n = 12$; L10V, $n = 6$; L33F, $n = 2$; and Q58E, $n = 1$). One patient had both K43T and I54S and the other patient both V82T and L24I.

Pre-ART HIV DR

NNRTI and/or NRTI DRMs prior to ART initiation were present in 10/45 samples (22.2%). The most common mutation was M184V, which was present in 6 (13.3%) samples. L10I occurred in 5 (11.1%) samples. Y181C (3, 6.7%) and G190A (3, 6.7%) were the most frequent NNRTI mutations. The number of TAMs was 1 (2.2%). Major DRMs were present in 4/18 patients who had both baseline and failure samples sequenced. Three of these patients had major DRMs present in both samples. In two patients the M184V observed at

Figure 1. Prevalence of acquired NRTI mutations ($n = 115$).

Figure 2. Prevalence of acquired NNRTI mutations ($n = 115$).

baseline persisted at the time of failure. G190A was present at baseline in two patients, but at the time of failure K103N was observed (Table 3).

Discussion

This paper describes virological outcomes and DR patterns in HIV-infected adults with multiple non-B subtypes, enrolled

Table 3. DRMs at baseline and follow-up (n=18 patients)

Patient	Baseline mutations	Mutations at VF
1	none	Y181C, V108I, M184V, K103N, E44D
2	none	M184V, K101E, G190A
3	none	M184V, L10V, K103N
4	L10I	Y181C, M184V, L10I
5	none	none
6	L10I	none
7	none	Q151L, K103N
8	none	L10V
9	none	M184V, K103N
10	none	K219N, K219Q, K103N
11	none	none
12	none	K103N
13	G190A, L10I, M184V	M184V, K103N
14	L10V, M184V, Y181C	none
15	A98G, K103N, M184V, N88D, P225H, V82A	K103R
16	none	L10V
17	none	Y181C, K103N, K65R
18	G190A, K101E, M184V, M230L, T215S, T215Y	M184V, K103N

in one of the largest HIV care and treatment programmes in Tanzania. A relatively high incidence of VF (14.9%) and DR was observed in this cohort despite close laboratory and clinical monitoring and follow-up. Rates of mutations conferring extensive DR were low, however, suggesting that the activity of second-line ART in Tanzania would be preserved provided treatment switches are made early.

A number of studies in SSA have examined virological outcomes in HIV-infected patients on first-line ART, with various results. In a recent multisite study, by Aghokeng *et al.*,³ the overall frequency of VF was 11.1% and 12.4% at 12 and 24 months of ART, respectively, with rates ranging from <5% (Burkina Faso, 24 months) to 26% (Togo, 24 months). In another multisite study of 19 SSA cohorts, by Barth *et al.*,² the overall proportion of VF was 15%–19%, similar to rates in our study. Higher rates of VF have been reported in SSA HIV cohorts with longer follow-up and use of immunological criteria with or without targeted HIV RNA testing to define failure.^{10,11}

In this study, both non-adherence and lower CD4+ T cell count at baseline were found to be independently associated with VF, similar to findings in several other SSA and Tanzanian cohorts.^{2–14} Although not assessed directly, we hypothesize that non-adherence in this study was due to increased toxicity and more complex dosing associated with zidovudine and stavudine. In 2010, the WHO recommended use of tenofovir instead of stavudine for first-line ART where possible.¹⁵ However, in many countries in SSA, stavudine has not been completely phased out and there is still wide variability in patterns of use. In Kenya, Uganda and Zambia, stavudine-based first-line treatment has been successfully reduced to <15% of all patients. However other countries, including Zimbabwe and Malawi, are still reporting stavudine in up to 70%–80% of patients on first-line regimens.¹⁶ Zidovudine continues to be recommended

as an alternative in first-line regimens and therefore is still widely used. In both the USA and SSA, tenofovir-containing ART has been found to be associated with better adherence and fewer side effects compared with zidovudine- or stavudine-containing regimens.^{17,18} Therefore, improving access to tenofovir, particularly in areas where the risk of VF is high and where routine VL monitoring is limited, is a priority.

Although not statistically significant, we also observed a trend towards higher rates of VF in younger age groups, again similar to findings from other SSA HIV cohorts.^{19,20} Data from both the USA and SSA have shown that young adults face a number of challenges that may impact adherence to ART, including peer-related stigma, disclosure, and sexual, reproductive and gender health concerns.¹⁸ Interestingly, in contrast to the current literature,^{21,22} we found no association between gender and risk of VF. However, this could be due to the relatively smaller proportion of men enrolled in this cohort.

The most common mutations in this study (M184V, K103N, Y181C and G190A) occurred at similar frequencies to those reported in resistance studies from other SSA and Tanzanian settings.^{4,23–28} There were no significant differences in mutations between the most common types of HIV subtype (A, C and D). These subtypes were distributed similarly to other Tanzanian HIV-infected populations.^{29–31} All of the observed mutations are known to be associated with commonly used antiretrovirals in SSA with a low genetic barrier to resistance (lamivudine, nevirapine and efavirenz). The low prevalence of TAMs and K65R and absence of multidrug-resistance mutations (T69ins, Q151M) is reassuring. In other SSA HIV cohorts, prevalence rates of TAMs have ranged between 8.5% and 50.9%, with the highest rates reported in cohorts without access to routine virological monitoring.^{4,32} In a recent study from rural Tanzania, the prevalence of TAMs was 28%.⁷ The low frequency of mutations conferring extensive resistance in this study is likely due to the timing of genotyping, which was performed relatively soon after ART initiation in most patients, and more stringent monitoring, which allowed earlier detection of resistance. Routine virological monitoring is critical for the prevention of widespread DR in SSA; a higher prevalence of DR and more complicated mutations have been observed in programmes where less-sensitive criteria have been used to define ART failure.^{3,7,25,27,33–36} Minor PI DRMs were observed in 27 patients, similar to what has been observed in other Tanzanian settings.³⁷ Only one of the PI mutations (V82T/S) is known to be associated with reduced susceptibility to PIs (atazanavir, indinavir, lopinavir and tipranavir) and potentially could have impacted second-line therapy in this patient. To our knowledge, none of the patients had received any ART regimen prior to enrolment into the parent TOV3 study, suggesting that these PI mutations were transmitted rather than acquired.

A final important finding in this study, although limited by the small numbers, was the high prevalence of pre-ART DR in a small subgroup of patients at baseline. Rates in this study (22%) exceed rates reported in other SSA studies conducted at a similar stage of ART rollout and in similar settings (0%–15%),^{5,38–45} although it should be noted that in this study baseline genotyping was performed only in patients who went on to develop antiretroviral failure. Although a high prevalence of baseline resistance was seen in this study, only 2/18 patients with genotyping at both baseline and the time of ART failure retained any of the same mutations at follow-up (M184V in both cases) and most patients in this

small cohort developed new DRMs by the time of first-line failure. This is similar to observations from a recent study, by Hamers *et al.*,⁴ where 96% of mutations at failure were newly acquired during the first year of ART. It is possible that these baseline mutations persisted at low frequency during follow-up, but that the sequencing methods used in this study were not sensitive enough to pick up mutations that had faded over time. One patient reverted to WT virus, which could have been due to an interruption in therapy, but this could not be confirmed. Several studies have demonstrated an association between pre-therapy resistance and an increased risk of ART failure,^{46–49} and thus efforts to minimize transmission of drug-resistant HIV through more enhanced surveillance should be undertaken.

There were several limitations to this study. First, due to missing samples ($n=173$) and amplification failures ($n=46$), we were only able to perform genotyping on a subset of patients with VF. However, there were no significant differences in baseline characteristics between those that did and did not have genotyping performed, reducing the possibility of selection bias. Second, we were unable to accurately calculate the duration of treatment failure and conduct a time-to-event analysis of VF, given the large amount of missing VL data. Third, we were unable to assess the effects of pretreatment resistance or subtype on VF because patients identified for baseline sequencing and subtype testing were selected from patients initially identified with VF. Fourth, the definition of VF used in this study may have led to overestimates of the rates of VF compared with the standard WHO definition (VL >1000 copies/mL based on two consecutive VL measurements after 3 months, with adherence support). However, in routine care many patients failed to return for consecutive VL testing within the 3 month period, and therefore this definition could not be applied. Finally, population-based sequencing is unable to detect minority variants, thus potentially underestimating the true prevalence of resistance in this population. To our knowledge this is the largest description of pre-ART and acquired DR mutations in a government programme in Tanzania to date. Additional strengths of the study include the large number of patients with DR data compared with other cohorts in SSA and the inclusion of patients enrolled in a closely monitored clinical trial, minimizing high rates of attrition.

In conclusion, our study findings highlight the critical importance of VL monitoring in SSA HIV programmes where stavudine and zidovudine are still being used. As demonstrated in this study, routine virological monitoring can help detect VF earlier before extensive multidrug resistance develops, which can in turn render second-line ART ineffective. Initiating ART early and ensuring optimal adherence are vital for the success and durability of first-line regimens in these settings. Additional studies are needed to confirm our findings of high levels of pre-ART DR, which may also have important implications for the success of first-line therapies.

Sequence data

GenBank accession numbers KP681703 to KP681817.

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Transparency declarations

None to declare.

Author contributions

C. H. contributed to protocol design and development, data analysis and writing of the first draft of the manuscript. N. U. contributed to protocol design and development, genotypic analysis, laboratory data collection and writing of the manuscript. E. L. contributed to data management, statistical analysis and review of the manuscript. S. A. contributed to protocol design and development, laboratory data collection and review of the manuscript. F. M. contributed to protocol design and development, and review of the manuscript. G. C. contributed to study site monitoring, patient care, ethical oversight of the parent TOV3 study and review of the manuscript. D. S. contributed to study site monitoring, patient care and data collection, and review of the manuscript. E. A. contributed to study site monitoring, patient care and data collection, and review of the manuscript. D. C. contributed to protocol design and development, and review of the manuscript. W. F. contributed to protocol design and development, oversight of the overall TOV3 parent study and writing of the manuscript. All authors read and approved the final version.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

Supplementary data

Tables S1 to S3 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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