Patterns of acquired HIV-1 drug resistance mutations and predictors of virological failure in Moshi, northern Tanzania

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Abstract

Drug resistance is a public health concern. Profiles of HIV drug resistance mutations (HIVDRM) and virological failure (VF) are not extensively studied in Tanzania. This study aimed to determine HIVDRM and predictors of VF in HIV-infected individuals failing first-line HIV drugs in Moshi, northern Tanzania. A case-control study was conducted at KCMC, Mawenzi, Pasua and Majengo health facilities with HIV-care and treatment clinics in Moshi from October, 2017 to August, 2018. Cases and controls were HIV-infected individuals with VF and viral suppression (VS) respectively. HIV-1 reverse transcriptase and protease genes were amplified and sequenced. Stanford University's HIV drug resistance database and REGA HIV-1 Subtyping tool 3.0 determined HIVDRM and HIV-1 subtypes respectively. Odds ratios with 95% confidence intervals investigated predictors of VF. P-value <5% was considered statistically significant. A total of 124 participants were recruited, of whom 63 (50.8%) had VF and 61 (49.2%) had VS. Majority (66.1%) were females. Median [IQR] age and duration on ART were 45 [35-52] years and 72 [48-104] months respectively. Twenty five out of 26 selected HIV-1 RNA samples from cases were successively sequenced. Twenty four samples (96%) had at least one major mutation conferring resistance to HIV drugs, with non-nucleoside reverse transcriptase inhibitor (NNRTI) associated mutations as the majority (92%). Frequent NNRTI resistance mutations were K103N (n=11), V106M (n=5) and G190A (n=5). Prevalent nucleoside reverse transcriptase inhibitors mutations were M184V (n=17), K70R (n=7) and D67N (n=6). Dual-class resistance was observed in 16 (64%) samples. Thirteen samples (52%) had at least one thymidine analogue-associated resistance mutation (TAM). Three samples (12%) had T69D mutation with at least 1 TAM. Age was independently associated with VF [aOR 0.94 (0.90-0.97) p<0.001]. In conclusion, HIV drug resistance is common among people failing antiretroviral therapy and resistance testing will help to guide switching of HIV drugs.

Introduction

Human Immunodeficiency Virus (HIV) infection is still a public health problem with an estimation of 37.9 million people living with it worldwide by the end of 2018. Among the people living with HIV (PLHIV) globally, 36.2 million are adults, 1.7 million are children below 15 years and 18.8 million are women aged 15 years and above. Every day, 5000 people are infected with HIV globally, and more than a half of these new infections occur in sub-Saharan Africa (sSA). Eastern and Southern Africa alone harbours more than a half of the world burden of HIV-infections with 20.6 million of PLHIV by the year 2018 [1]. The incidence-prevalence ratio (IPR) of the worldwide HIV-infection has declined considerably from 11.2% in 2000 to 4.6% in 2018. In the same year, sSA was also observing a decline in HIV-infection IPR to about 3.9% and 5.5% in Eastern and Southern Africa; and Western and Central Africa respectively. Despite the decline in IPR, HIV-infection is still a public health problem in sSA due to unacceptably high number of PLHIV in this setting (approximately 68% of the global burden) with substantial new HIV-infections [1].

Introduction of highly active antiretroviral therapy (HAART) in sSA countries including Tanzania has significantly helped to reduce HIV/AIDS related mortality from around 900,000 in 2010 to 470,000 in 2018 [1] with improved quality of life [2]. The globe further committed that by the year 2020, 90% of PLHIV should know their HIV status, 90% of PLHIV who know their HIV status should be on ART, and also 90% of PLHIV on ART should enjoy the sustainable viral suppression, this is alias known as 90-90-90 target [3]. In the race to achieve the second UNAIDS '90' target, about 51% of PLHIV are on HAART in Western and Central Africa (WCA); and 67% in Eastern and Southern Africa (ESA) respectively. The proportions of HIV-infected individuals on treatment with an enjoyment of viral suppression is better in WCA (79%) compared to ESA (58%) respectively, however, the

numbers are below the third UNAIDS '90' target [1]. One of the key challenges which partly explain the failure to achieve the third UNAIDS '90' in sSA is the emergence of HIV drug resistance (HIVDR).

HIVDR is defined by World Health Organisation (WHO) as presence of one or more mutations in HIV drug targeted genes that compromise the ability of a specific drug or combination of drugs to block replication of HIV [4]. HIVDR reverses the gains of HAART in HIV-infected individuals on treatment for at least 6 months. HIV-infected individuals on failed first-line HAART regimens are reported to have 50 to 97% of non-nucleoside reverse transcriptase inhibitors (NNRTI) resistance world-wide [4]. In sSA, more than 80% of HIV-infected individuals with VF on first-line HAART have HIV-drug resistance [5]. In addition, sSA has been reported to accommodate tenofovir resistance in more than a half of people with first-line HAART failure on tenofovir-based regimens. Cytosine analogue resistance was also highly evident in sSA as compared to Western Europe. Eastern Africa was more commonly reported to have lamivudine and emtricitabine resistance than NNRTI resistance [6].

Tanzania introduced antiretroviral therapy (ART) program in 2004. As of 2016, the total number of people on ART was 839,544, and the current recommended first-line HAART for adults and adolescents is tenofovir + lamivudine + dolutegravir. In case of first-line HAART failure, the recommended second-line HAART for adults and adolescents is zidovudine/lamivudine + ritonavir-boosted atazanavir or tenofovir/emtricitabine + ritonavir-boosted atazanavir [7]. In Tanzania, the markers for treatment failure are based on immunological, clinical and WHO recommended virological criteria [8]. Clinical and immunological criteria were extensively described to be less sensitive and less effective [9] and may reduce early notifications of VF. Tanzania continues to scale up HIV viral load

(HVL) testing which is currently done at few selected settings. Of more public health importance is that HIV-infected individuals confirmed to have VF are switched to second-line HAART without programmatic HIV drug resistance testing and monitoring, a practice which may transfer cross-resistance patterns to newly switched drugs and limit the choices for few available treatment options.

Recently, few studies in Tanzania have reported a wide range of first-line HIV drug resistance mutations (HIVDRMs) including the thymidine analogue associated mutations which compromise susceptibility to multi-NRTIs [10–12]. In order to complement the efforts to unearth the burden of HIVDR and preserve the integrity of limited second-line HIV drugs in Tanzania, this study aimed to test genotypically for drug resistance in the reverse transcriptase (RT) and protease genes from individual with VF on first-line HAART in Moshi municipality, northern Tanzania. The study also explored the independent predictions for VF.

Materials and methods

Study design and settings

Between October, 2017 and August, 2018, unmatched case-control study was done in four HIV/AIDS care and treatment clinics (CTC) in Moshi municipality, situated in Kilimanjaro region, northern Tanzania. Moshi municipality is one of the seven districts of Kilimanjaro region. The municipality has a total of 18 health facilities with CTC. Four out of 18 CTCs with high client volume were purposively selected to participate in the study. The CTCs included were that of Kilimanjaro Christian Medical Centre (KCMC), Mawenzi regional referral hospital, Majengo and Pasua health centres. KCMC provides tertiary care hospital services to around 6.8 million people living in the northern zone of Tanzania (Tanga, Kilimanjaro, Arusha and Manyara) and other referrals from nearby settings. Mawenzi

regional referral hospital provides referral medical services to around 1.6 million people in Kilimanjaro region.

At the time of recruitment of study participants, the management of HIV-infected individuals was done in-line with national guidelines for management of HIV and AIDS of 2017 [8]. The CTCs routinely provided HIV counselling and testing services, ART care and treatment, treatment monitoring, laboratory investigations and treatment adherence support to HIV-infected individuals. The recommended first-line HAART for adults and adolescents at the time of the study was tenofovir + lamivudine + efavirenz (TLE), given as a single dose formulation. Prescriptions of antiretroviral drugs were done by clinicians and to some points by trained nurses in the health centres. HVL testing was routinely done to all HIV-infected individuals who have been on ART for at least 6 months. Those found with HVL > 1,000 copies/ml were offered enhanced adherence counselling (EAC) by trained health care providers. EAC was provided on monthly bases for three months at the CTCs. After 3 months of EAC, retesting of HVL was done and HIV-infected individuals found still to have HVL > 1,000 copies/ml were classified as having VF and followed by further actions to change their regimens to second-line. The clinic visits for adolescents and youths were scheduled at different times from adults to maximize adherence counselling and support. Adherence level of $\geq$ 95% and < 95% was regarded as good and poor respectively [8].

Study population

The study population was HIV-infected individuals attending the selected CTCs for routine care and were on first-line HAART treatment for a year or more. HIV-infected individuals with VF and viral suppression (VS) were included into study as cases and controls respectively. The cases were defined as HIV-infected individuals with >1000 copies/ml of HIV plasma RNA confirmed by <0.5 logarithmic difference between initial and

147 second HVL with EAC in between. Controls were the HIV-infected individuals attending the
148 same CTCs along with cases but they have viral suppression (HVL < 1000 copies/ml). The
149 cases found to have less than three EAC were excluded from the study. The study further
150 excluded the cases with significant difference HVL measured at the date of interview and that
151 measured before starting EAC (HIV-1 plasma RNA viral $\log_{10}$ drop greater than 0.5 at three
152 month interval with 3 EAC in between).
153

154 Sample size determination

155 We assumed that the study had 80% power and the proportion exposed in the control
156 group was 20%. With equal number of cases and controls (ratio of 1:1), we could manage to
157 detect odds ratio (OR) of 3.0 or greater with the following sample size calculation as
158 described by Charan and Biswas [13].
159

160 $$n = (\frac{r+1}{r}) \frac{(\bar{p})(1-\bar{p})(Z_{\beta} + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

161

162 n = sample size in each group, r = ratio of controls to cases, $\bar{p}$ = a measure of variability
163 (average proportion exposed), p_1 = proportion exposed in cases, p_2 = proportion exposed in
164 controls, $p_1 - p_2$ = Effect size (the difference in proportions), $Z_{\alpha/2}$ = level of two-tailed
165 statistical significance, Z_{β} = standard normal variate for power of the study. Our study power
166 was 80%, Z_{β} = 0.84; Level of significance was 0.05, Z_{α} = 1.96; r = 1 (equal number of cases
167 and controls), OR = 3.0 and p_2 = 20% or 0.2.

168 $$p_1 = \frac{OR \times p_2}{p_2(OR-1)+1} = \frac{3.0 \times 0.2}{0.2(3.0-1)+1} = 0.43$$

$$\bar{p} = \frac{(p_1 + p_2)}{2} = \frac{(0.43 + 0.2)}{2} = 0.31$$

$$\therefore n = \frac{2 \times (0.31) \times (1 - 0.31) \times (0.84 + 1.96)^2}{(0.43 - 0.2)^2} = 63$$

Therefore, the study planned to recruit 63 study participants with first-line VF and 63 with VS making a total sample size of 126.

Enrolment and study procedures

Study participants who met the inclusion criteria and consented to participate were interviewed to collect demographic and clinical information. After the interviews, 8-10 ml of EDTA whole blood was collected and centrifuged at 2200 revolutions per minute for 10 minutes with brake-offs to separate plasma from buffy coat and red blood cells within 4 hours post-collection. HVL was enumerated in-vitro from plasma by reverse transcription-polymerase chain reaction as per Abbott m2000rt system and was expressed in copies/ml of plasma.

Due to financial constraints, 26 out of 63 samples of cases were selected for sequencing. Selection was based on having HVL $\geq$ 14,000 copies/ml. Plasma HIV-1 RNA was extracted using PureLink® Viral RNA/DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, USA) as per manufacturer's instructions. RT and protease genes of the extracted HIV-1 RNA was reversely transcribed into complementary DNA (cDNA) and subsequently subjected into nested polymerase chain reactions (PCR) according to manufacturer's

191 instructions prescribed in HIV-1 genotyping kit: Amplification module (Applied Biosystems,
192 Life Technologies, Warrington, UK). The amplified cDNA was purified using ExoSAP-IT™
193 PCR product clean-up reagent (Applied Biosystems, Thermo Fischer Scientific, Inc.).
194 Reactions were performed using HIV-1 genotyping kit: Cycle sequencing module (Applied
195 Biosystems, Life Technologies, Warrington, UK) based on Sanger sequencing method using
196 BigDye™ Terminator v3.1 cycle sequencing kit (Applied Biosystems, Thermo Fischer
197 Scientific, Inc.). Sequencing was done using 3500xl genetic analyzer (Applied Biosystems)
198 with a 24 capillary 50 cm array. The study profile was described in Fig **1**.

199 **Fig 1. Study profile**

200

201 # Data analysis

202 ## Sequence data analysis

203 The raw sequence data were assembled, aligned and edited using automated base
204 calling software, RECall, available at (http://pssm.cfenet.ubc.ca) to generate individual
205 consensus sequences. The sequence data were submitted to GenBank and obtained accession
206 numbers MT347616 – MT347640. HIVDRMs were analysed using HIV drug resistance
207 database of Stanford University available at
208 (http://hivdb.stanford.edu/pages/algs/sierra_sequence.html) and HIVDRM mutation list from
209 2019 updates of drug resistance (AIDS Society). To get HIV-1 subtypes, generated consensus
210 sequences were analysed using REGA HIV-1 Subtyping tool 3.0 available at
211 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/). REGA
212 confirmed a subtype after clustering with a pure subtype in a database by > 800 base pairs
213 with a bootstrap confidence of more than 70% in absence of recombination in the boot scan.

214

Statistical analysis

Data analysis was performed using STATA version 14.0 (College Station, Texas 77845-4512, USA). Categorical variables were summarized using frequency and proportion while mean or median with their respective measure of dispersion were used to summarize numerical variables. A Chi-square test was used to compare the differences in proportions between groups. Odds ratios (OR) and 95% confidence interval (CI) for predictors of virological failure were estimated using multivariable logistic regression model. A p-value <0.05 (2 tails) was considered statistically significant.

Ethics Consideration

The study was approved by the Kilimanjaro Christian Medical Research Ethics Review Committee in 2017 with ethical clearance certificate number 2028. Approvals to conduct the study were further granted by respective authorities in four study sites. Patient information sheet was given to every potential study participant to ensure informed consenting process before and after commencement of the study, potential participants discussed it with the researcher. The sheet explained in detail the aim of the study, purpose, confidentiality, benefits, unconditional withdrawal from the study and risks of taking part into the study. Every study participant consented/assented in writing to take part in the study. The HIV drug resistance testing results were shared with the respective CTCs for further management of the HIV-infected individuals.

Results

Demographic characteristics of study participants

A total of 124 study participants were recruited in this study. Out of the 124, 63 (50.8%) of study participants had virological failure and 61 (49.2%) had viral suppression. The age of the 124 participants ranged from 15 to 79 years, with median [IQR] age of 45 [35-52] years. Of the 124; 82 (66.1%) were females and 89 (71.8%) were employed in either formal or informal sector (Table **1**).

Table 1. Demographic characteristics of study participants (N=124)

	Overall	VF	VS
Characteristic	**n (%)**	**n (%)**	**n (%)**
Age (years)			
15-34	29 (23.4)	26 (41.3)	3 (4.9)
≥35	95 (76.6)	37 (58.7)	58 (95.1)
Median age [IQR] *a*	45 [35-52]	41 [21-49]	48 [43-54]
Sex			
Male	42 (33.9)	26 (41.3)	16 (26.2)
Female	82 (66.1)	37 (58.7)	45 (73.8)
Education level			
Primary or none	84 (67.7)	36 (57.2)	48 (78.7)
Secondary and above	40 (32.3)	27 (42.8)	13 (21.3)
Marital status			
Married/cohabiting	40 (32.3)	18 (28.6)	22 (36.1)
Single	84 (67.7)	45 (71.4)	39(63.9)
Occupation			
Employed	89 (71.8)	36 (57.1)	53 (86.9)
Unemployed	35 (28.2)	27 (42.9)	8 (13.1)
CTC enrolled			
KCMC	53 (42.7)	38 (60.3)	15 (24.6)
Majengo HC	19 (15.3)	7 (11.1)	12 (19.7)
Mawenzi RRH	29 (23.4)	14 (22.2)	15 (24.6)
Pasua HC	23 (18.6)	4 (6.4)	19 (31.1)

VF = Virological failure, **VS** = Viral suppression; **a** = Expressed as median [Interquartile range]

Clinical characteristics of study participants

The median [IQR] time on ART for the 124 participants was 72 [IQR 48 - 104] months, 92 (74.0%) had good adherence, 65 (52.4%) were in non-tenofovir based HAART and 66 (53.2%) were in WHO clinical stage III/IV (Table **2**). The median CD4 count (cells/µL) at follow up was higher in VS participants 518 [IOR: 326-741] than in VF group 334 [IQR: 134-549]. Likewise 100% of VS participants reported good adherence to ART compared to 49% of VF participants (Table **2**).

Table 2. Clinical characteristics of the participants (N = 124)

	Overall	VF	VS
Characteristic	n(%)	n(%)	n(%)
Duration of ART intake (months)			
12 – 36	20 (16.1)	7 (11.1)	13 (21.3)
>36	104 (83.9)	56 (88.9)	48 (78.7)
Median [IQR]	72 [48-104]	84 [60-120]	72 [48-96]
Adherence status			
Good	92 (74.2)	31 (49.2)	61 (100.0)
Poor	32 (25.8)	32 (50.8)	0 (0.0)
Ever switched ART			
Yes	54 (43.5)	30 (47.6)	24 (39.3)
No	70 (56.5)	33 (52.4)	37 (60.7)
HAART initiated			
TDF based	39 (31.5)	12 (19)	27 (44.3)
None TDF based	85 (68.5)	51 (81)	34 (55.7)
HAART on use			
TDF based	59 (47.6)	22 (34.9)	37 (60.7)
Non TDF based	65 (52.4)	41 (65.1)	24 (39.3)
Baseline CD4 count (cells/µL)			
<100	37 (29.8)	17 (27)	20 (32.8)
≥100	87 (70.2)	46 (73)	41 (67.2)
Median CD4 count [IQR]	173 [72-276]	179 [68-256]	169 [73-282]
Follow up CD4 count (cells/µL)			
<100	13 (10.5)	12 (19)	20 (32.8)
≥100	111 (89.5)	51(81)	41 (67.2)
Median [IQR]	444[215-628]	334 [134-549]	518 [326-741]
WHO clinical stage			
I/II	58 (46.8)	26 (41.3)	32 (52.5)
III/IV	66 (53.2)	37 (58.7)	29 (47.5)
Time from HIV diagnosis to ART initiation (months)[a]		3.5 [1.4 – 17.9]	2.6 [0.9 – 14.0]

TDF= Tenofovir Disoproxil Fumarate, **VF**= Virological failure **VS**= Viral suppression; **a**= Expressed as median [Interquartile range]

Profiles of acquired HIV drug resistance in RT and protease genes

Genotyping of RT and protease genes of HIV-1 was successful on 25 out of 26 selected samples from participants with VF. Of the 25, almost all (n=24) had at least one major mutation conferring resistance to HIV drug (Table 3). Out of 25, 23 (92%) samples had at least one non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance associated mutations (Fig **2**). The most frequent NNRTI mutations were K103N (44%), V106M (20%), and G190A (20%) (Fig **2**), all of these three mutations confer high level resistance to efavirenz and nevirapine.

Fig 2. Profiles of NNRTI resistance mutations in participants with virological failure

In addition, 17 samples (68%) had at least one mutation associated with nucleoside reverse transcriptase inhibitors (NRTI) resistance. The most frequent NRTI mutations were M184V (68%), K70R (28%), and D67N (24%) (Fig **3**). M184V mutation was associated with high level resistance to emtricitabine, and lamivudine; while K70R and D67N are among thymidine analogue-associated resistance mutations (TAM) reducing the susceptibility of all current NRTI on use.

Fig 3. Profiles of NRTI resistance mutations in participants with virological failure

Characteristics of successfully genotyped samples

The characteristics of successfully genotyped samples have been displayed in Table **3**. Dual class resistance was observed in 16 (64%) samples. In general, thirteen samples (52%) had at least one TAM while three samples (12%) had T69D mutation together with at least 1 TAM. Identified TAMs were T215YF (n=9), K219QE (n=7), K70R (n=7), D67N (n=6), M41L (n=4), and L210W (n=3). Presence of T69D mutation along with at least 1 TAM reduces the susceptibility of all currently NRTIs on use. Of more scientific interest is that one sample was fully susceptible (no detectable HIV drug resistance mutation) despite having VF and substantial high HVL at the date of interview.

Table 3. Characteristics of successfully genotyped samples (N=25)

SN	Sample ID	Age (years)	Duration on HAART (years)	HAART at date of interview	Viral Load at date of interview	NRTI mutations	NNRTI Mutations	PI Mutations	HIV-1 Subtype
1	K03	18	10	AZT+3TC+EFV	389,006	M184V, D67T, K70R,T215AITV, K219E	Y188L, K238T	None	A1
2	K11	55	6	ABC+3TC+LPVr	24,463	D67N, K70R, M184V,K219E, T215F	None	L10F,K20T, M46I,I47V, N88G	C
3	K12	29	6	AZT+3TC+EFV	20,805	M184V	K103N,V108I, K238T	None	CRF 10_CD
4	K15	49	12	AZT+3TC+NVP	81,123	None	None	None	A1
5	K16	44	3	TDF+3TC+EFV	23,354	None	K103N	None	A1
6	K17	15	7	AZT+3TC+NVP	25,879	None	G190A	None	A1
7	K19	17	6	ABC+3TC+EFV	296,646	M41L, D67N, K70R, M184V, T215Y, K219Q, L74I,T69D	A98G,K103N, P225H,F227F, 238T	None	C
8	K21	20	13	ZVD+3TC+EFV	33,887	M184V,T215F	K103N,Y188F, M230L	None	C
9	K25	19	10	AZT+3TC+EFV	37,794	None	V106M, G190A	None	C
10	K29	34	9	AZT+3TC+EFV	14,431	None	K103S, V106M	None	C
11	K32	41	4	TDF+3TC+EFV	161,220	A62V, K65R, M184V,K219E	K101E,V106M, Y181C, G190A	None	C
12	K36	29	10	AZT+3TC+EFV	23,056	M184V	L100I, K103N	None	A1
13	K37	15	9	AZT+3TC+NVP	20,540	D67N, K70R, M184V,T215F, K219Q	A98G, K101E, G190A	None	C
14	K38	46	13	ABC+3TC+ATVr	1,635,827	M41L, E44D, D67N, T69D, M184V,L210W, T215Y, K219R	K101E, E138K, Y181C, G190A, H221Y	L24I, L33F, M46I, I54V, Q58E,V82A , N88S	CRF 10_A1D
15	M02	35	5	AZT+3TC+NVP	124,714	D67N, T69D, K70R, M184V	G190A	None	D
16	M03	69	9	AZT+3TC+NVP	36,600	M41L, E44A, M184V,L210W, T215Y, K219N	Y181C, H221Y	None	A1
17	M05	53	10	AZT+3TC+NVP	65,638	D67G, K70R, M184V,T215FV, K219E	K103S, E138Q	None	A1

18	M07	52	4	ABC+3TC+EFV	66,486	M184V	K103N	None	A1
19	M10	41	8	AZT+3TC+NVP	45,819	D67N, K70R, M184V,K219Q	K103N, V179L, K238T	None	A1
20	MJ01	48	9	AZT+3TC+EFV	14,065	M184V,L210W, T215Y	K103N	None	C
21	MJ03	36	9	TDF+3TC+EFV	176,378	None	K103N	None	C
22	MJ05	45	5	TDF+FTC+EFV	222,227	None	L100I, K103N	None	C
23	MJ06	51	4	TDF+3TC+EFV	14,711	K70E, M184V	K103N,V108I, H221Y, F227L	None	D
24	P02	54	8	AZT+3TC+EFV	235,443	None	V106M,V179D	None	C
25	P03	45	5	TDF+3TC+EFV	128,781	M41L, K70S, L74I, M184V, T215Y	V106M,E138Q, F227L	None	C

HIV-1 diversity in the RT and protease gene

The HIV-1 diversity in the RT and protease genes is shown in Table **4**. HIV-1 subtype C was the most prevalent subtype (48%) followed by A1 (36%), D (8%) and recombinants (8%). The recombinants identified were A1D and CD. All the recombinants were circulating recombinant forms (CRF).

Table 4. HIV-1 diversity in RT and protease genes (N=25)

Rega assignment	Number of sequences	Percentage
HIV-1 Subtype C	12	48
HIV-1 Subtype A (A1)	9	36
HIV-1 Subtype D	2	8
Recombinant of A1, D	1	4
Recombinant of C, D	1	4
Total	**25**	**100**

Predictors of virological failure

The predictors of virological failure (VF) are shown in Table **5**. In bivariate analysis, participant age, occupation, HAART initiated and HAART on use were significantly associated with virological failure. In the multivariable logistic regression analysis, only age remained to be independent predictor of VF. We found that, one unit increase in participant age (year) was associated with 6% lower odds of VF [aOR 0.94 (0.90-0.97) p<0.001].

Table 5. Predictors of HIV virological failure (VF) (N=124)

Characteristic	VF		Crude analysis		Multivariable analysis	
	No (%)	Yes (%)	cOR (95% CI)	p-value	aOR (95% CI)	p-value
Age (years)*	48 [43-54]	41 [21-49]	0.93 (0.90-0.96)	<0.001	0.94 (0.90-0.97)	0.001
Sex						
Male	16 (26.2)	26 (41.3)	1		1	
Female	45 (73.8)	37 (58.7)	0.51 (0.24-1.08)	0.079	0.56 (0.21-1.51)	0.254
Marital status						
Married	22 (36.1)	18 (28.6)	0.71 (0.33-1.51)	0.373	-	-
Single	39(63.9)	45 (71.4)	1			
Occupation						
Employed	53 (86.9)	36 (57.1)	0.2 (0.08-0.49)	<0.001	0.35 (0.12-1.04)	0.059
Unemployed	8 (13.1)	27 (42.9)	1		1	
Duration of ART intake (months)						
12 - 36	13 (21.3)	7 (11.1)	1			
>36	48 (78.7)	56 (88.9)	2.17 (0.80-5.87)	0.128	-	-
Switched ART						
Yes	24 (39.3)	30 (47.6)	1.4 (0.69-2.86)	0.353	0.89 (0.31-2.53)	0.827
No	37 (60.7)	33 (52.4)	1		1	
HAART initiated						
TDF based	27 (44.3)	12 (19)	0.3 (0.13-0.66)	0.003	0.26 (0.05-1.27)	0.096
Non-TDF	34 (55.7)	51 (81)	1		1	
HAART on use						
TDF based	37 (60.7)	22 (34.9)	0.35 (0.17-0.72)	0.005	0.85 (0.25-2.85)	0.795
Non-TDF	24 (39.3)	41 (65.1)	1		1	
Baseline CD4 count (cells/µL)						
<100	20 (32.8)	17 (27)	0.76 (0.35-1.64)	0.481	0.86 (0.35-2.11)	0.745
≥100	41 (67.2)	46 (73)	1		1	
WHO clinical stage at interview						
I/II	32 (52.5)	26 (41.3)	1		1	
III/IV	29 (47.5)	37 (58.7)	1.57 (0.77-3.19)	0.213	1.04 (0.44-2.46)	0.931

*** Median age [IQR] ; cOR: Crude odds ratio; aOR: Adjusted odds ratio**

Discussion

The present study aimed at determining acquired HIV drug resistance mutations (HIVDRMs) and predictors of VF in HIV-infected individuals failing to respond to first-line ART in Moshi municipality, northern Tanzania. High profiles of HIVDRMs conferring acquired HIV drug resistance to NRTIs, NNRTIs and PIs were found. Age was a significant factor associated with VF.

In the present study, most (96%) of the successfully sequenced samples had at least one major mutation conferring resistance to HIV drugs. NNRTI resistance mutations profiles (92%) were at the lead compared to NRTIs resistance mutation profiles (68%) consistently with findings elsewhere [6]. The most frequent NNRTI mutations were K103N, V106M, and G190A and they are implicated to confer high level resistance to efavirenz and nevirapine [14]. NNRTIs were previously reported to have low genetic barrier and hence highly vulnerable to resistance [15]. These findings further support the Tanzanian programmatic intervention to replace efavirenz with dolutegravir which has proven to have high genetic barrier to resistance [16]. This study further reports frequent NRTI mutations as M184V, K70R, and D67N in line with other studies [10]. M184V associates with high level resistance to abacavir, emtricitabine, and lamivudine; while K70R and D67N are among thymidine analogue-associated resistance mutations (TAM). More than a half of sequenced samples had at least one TAM. Existence of TAMs reduce the susceptibility of all available NRTIs with an exception of emtricitabine and lamivudine [17].

In addition, this study reports co-existence of T69D mutation with at least one TAM in three samples, two of these samples had M41L and T215Y TAMs, a combination which further fuel cross-resistance to all US FDA approved NRTIs, a phenomenon known as Multi-NRTI resistance [14,18]. This means that the Tanzanian AIDS control program is at risk of remaining with fewer options of main-stream HIV drugs both in first and second-line regimens. Presence of T69 insertions along with TAMs in northern Tanzania further advocates the need for implementation of HIV drug resistance testing and monitoring before and after switching HAART. Continuous programmatic monitoring of HIV drug resistance in HIV-infected individuals failing to respond to first-line HIV drugs will preserve the integrity of few NRTI options in second-line. However there is a need to consider deployment of NRTIs with substantial genetic barrier to resistance.

In the other hand, this study describes full genotypic susceptibility of prescribed HAART to a 49 years old individual with ID K15, on ART for 12 years and with HIV plasma viral load 81,123 copies/ml. At the interview, he reported good adherence to the prescribed HAART. This may be explained by presence of HIV drug resistance mutations away from the *pol* gene and hence necessitating the need for sequencing the whole viral genome in this sample.

A significant association between age and VF concurs with findings from Cameroon [19], Senegal [20], Ethiopia [21], Swaziland [22], Kenya [23,24], Rwanda [25] and Uganda [26]. HIV infected adolescent and young adults on HAART were previously reported to have inconsistence in adhering to antiretroviral medication due to anxiety, depression, forgetfulness, fear of disclosure, ART adverse events and abandoning medication when they feel better [27], the consequences which contributes to VF. Therefore, special care and treatment to the adolescent and young adults is of paramount with emphasis on health education regarding the importance of disclosure and adhering to medications. In addition, assessment and treatment of cognitive and mental health problems is also needed.

Although not statistically significant, this study reports the association between advancement of WHO clinical stage of AIDS and VF, a finding which was consistent with those reported by Jobanputra et al in (2015). Virological failure in individuals with advanced HIV/AIDS can be explained by the severe state of immunodeficiency which attracts opportunistic infections (OIs) and replicative fitness of HIV [28]. Early diagnosis of HIV, effective treatment and monitoring of HIV/AIDS and related OIs to patients on HAART is highly recommended [9].

Tenofovir (TDF) based combination antiretroviral therapy is one of the recommended first-line antiretroviral of choice to the adolescents and adults living with HIV worldwide [9]

as well as in Tanzania [8]. Although not statistically significant, being on tenofovir based first-line HAART was protective against VF, this was consistent with studies previously done in the north America [29], Thailand [30] and in the systematic review [31]. On top of that, TDF based regimens were extensively reported in previous randomized clinical trials to be more effective than other combination therapies in bringing favourable virological and immunologic outcomes [32,33]. The possible explanation of this fact is that, compared to other back-borne antiretroviral, TDF based therapy is consumed once daily with minimal treatment related toxicity [32,34], more tolerable [35] and can be easily adhered by PLHIV [36]. Therefore we complement the prescription of TDF based HAART in PLHIV initiating HAART as the strategy of reducing VF.

Limitations of the study

One of the limitations of this study is to rely on self-reporting of adherence to HAART and pill counting at the clinic visit. It is certain that some of the study participants might have provided imprecise information about adherence. However, this was the common practice prescribed under the Tanzanian HIV and AIDS treatment and management guideline of October 2017. In addition, recall biases are certain to some of the information requested from the VS group, for example it was difficult to report when was the last day to miss taking pills.

Finally, the profiles of HIVDRMs should not be generalized as some of the samples were not sequenced due to financial meltdowns. However, compared to the data in this study, similar high proportions of HIVDRMs are anticipated in the samples yet to be sequenced.

Conclusion

There is high rate of HIVDRMs in HIV-infected individuals failing to respond to first-line HAART in northern Tanzania. This happens when there is no programmatic monitoring of HIV drug resistance in individuals with VF. Prompt intervention are required to safeguard the limited number of second-line HIV drug options, one of them is implementation of HIV drug resistance monitoring before and after switching HAART. In addition, consideration to deploy HIV drugs with higher genetic barrier is of importance.

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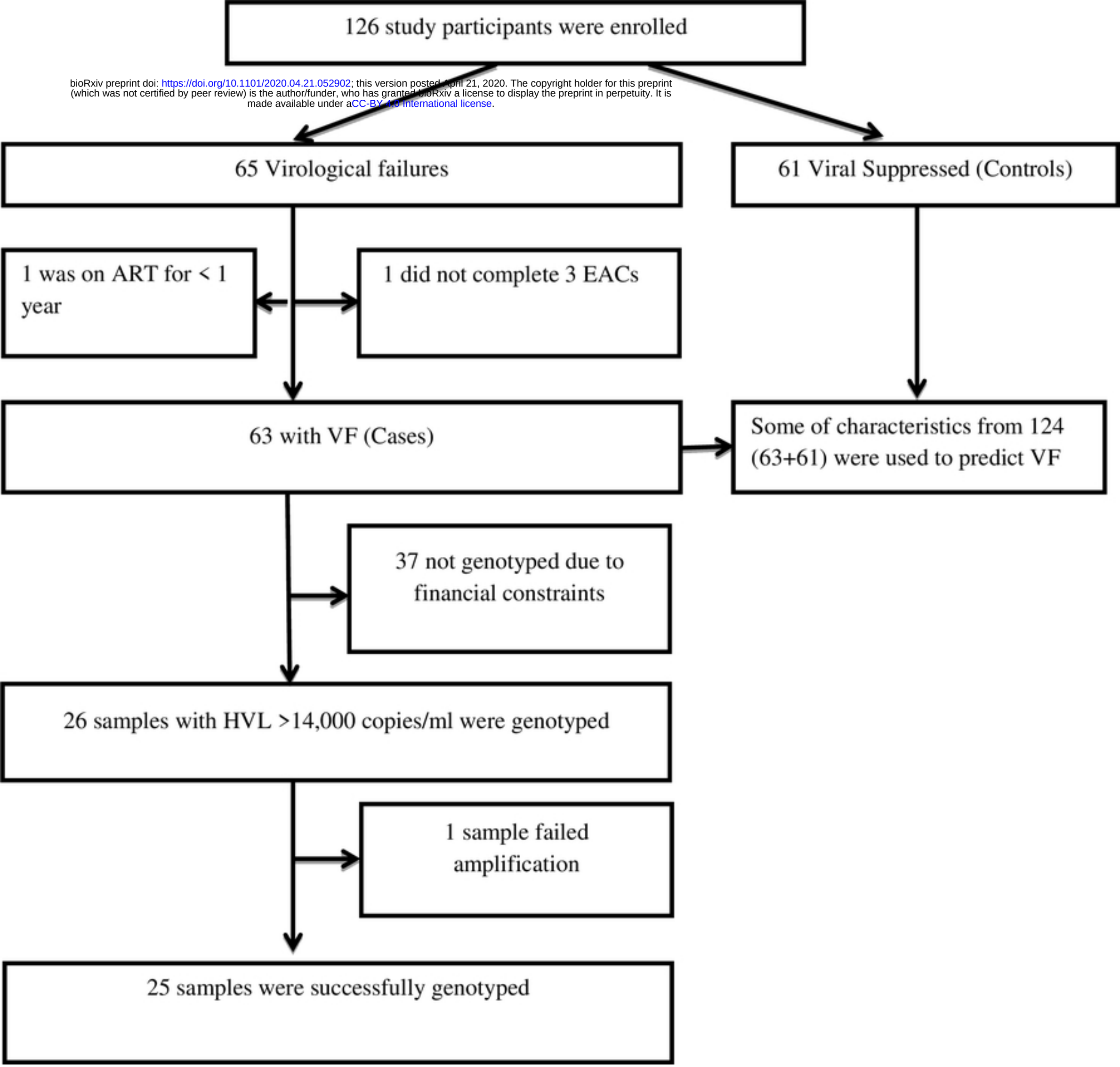
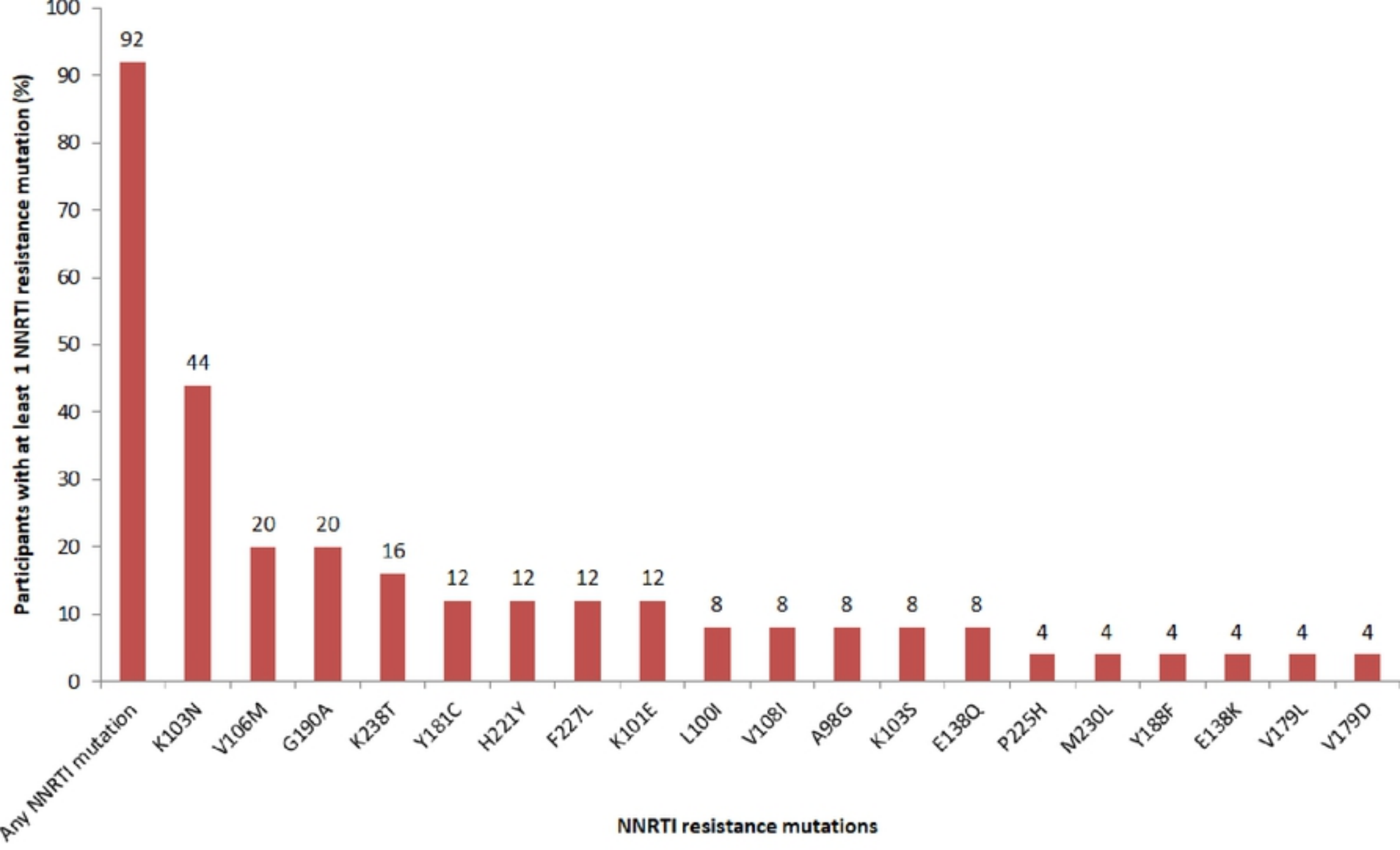
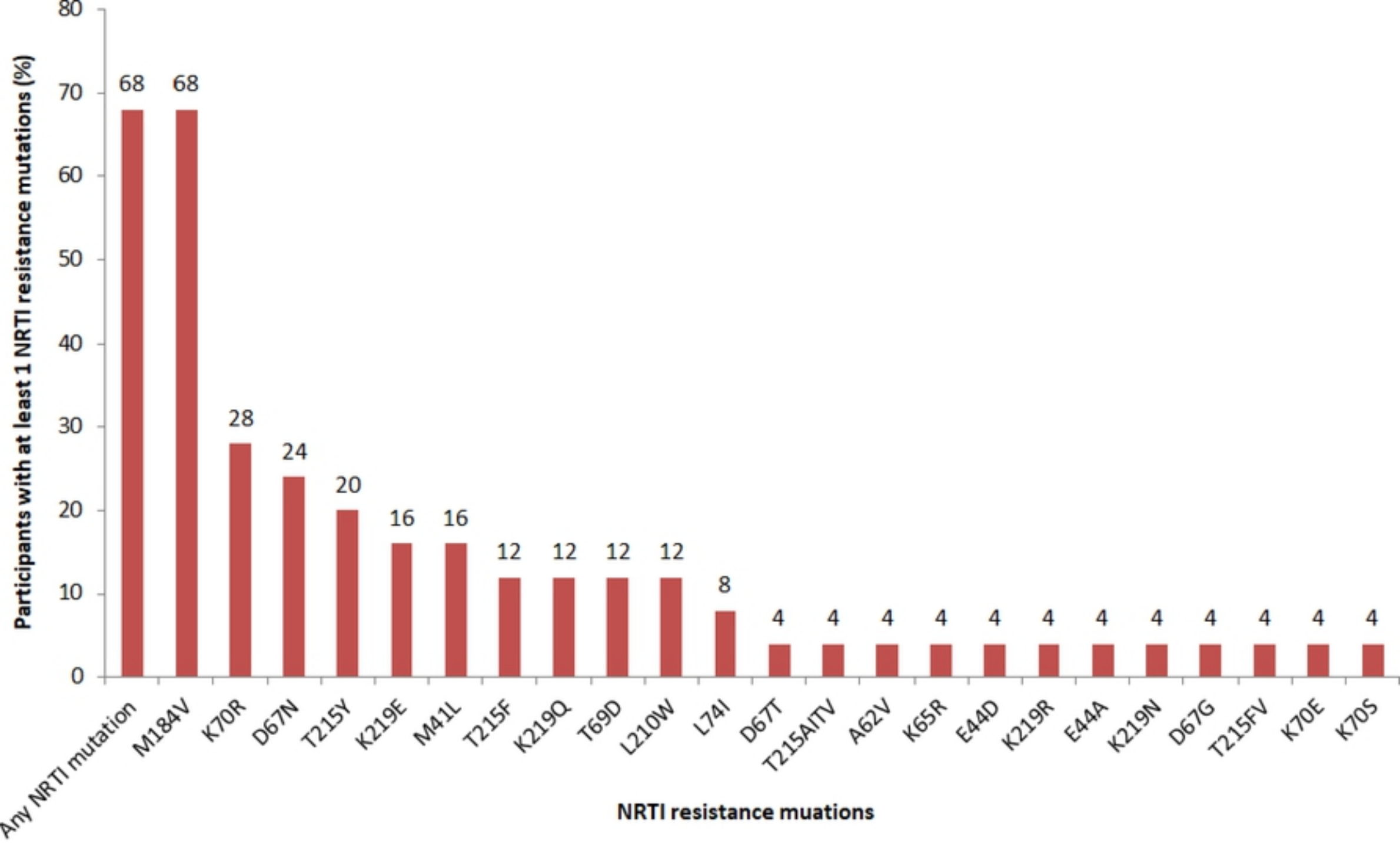
S1 Dataset. Demographic and clinical data



Figure


Participants with at least 1 NNRTI resistance mutation (%)
100
90
80
70
60
50
40
30
20
10
0
92
44
20
20
16
12
12
12
12
8
8
8
8
8
4
4
4
4
4
4
Any NNRTI mutation
K103N
V106M
G190A
K238T
Y181C
H221Y
F227L
K101E
L100I
V108I
A98G
K103S
E138Q
P225H
M230L
Y188F
E138K
V179L
V179D
NNRTI resistance mutations

Figure


Participants with at least 1 NRTI resistance mutations (%)
0
10
20
30
40
50
60
70
80
68
68
28
24
20
16
16
12
12
12
12
8
4
4
4
4
4
4
4
4
4
4
4
4
Any NRTI mutation
M184V
K70R
D67N
T215Y
K219E
M41L
T215F
K219Q
T69D
L210W
L74I
D67T
T215AITV
A62V
K65R
E44D
K219R
E44A
K219N
D67G
T215FV
K70E
K70S
NRTI resistance muations

Figure