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HIV-1 clade C resistance genotypes in naïve patients and after first virological failure in a large community ART programme

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Abstract

Objectives—To evaluate HIV drug resistance pre-treatment, and in those failing first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART), in South Africa.

Design—An observational cohort.

Methods—Genotypic resistance testing was performed on naïve individuals and those failing first-line ART (confirmed HIV RNA >1000 copies/ml) from public sector clinics in the Cape Town (2002 – 2007). Resistance profiles and mutations relative to timing of known virological failure were examined.

Results—230 patients (120 naïve; 110 with virologic failure) were included; 98% had clade C virus. Among naïve patients, prevalence of primary resistance was 2.5% (95% CI:0.0%-5.3%). Three patients had 1 significant reverse transcriptase (RT) mutation: K65R, Y181C and G190A. Among non-naïve patients, 95 individuals (86%) had therapy-limiting NNRTI mutations including K103N (55%), V106M (31%) and Y181C (9%). The M184V mutation was the most common mutation seen in 86 patients (78%). Ten patients (9%) had the K65R mutation. More individuals tended to develop thymidine analogue mutations (TAMs) when sampling occurred after 6 months of detected therapy failure [10/31 individuals (32%)], compared to those who had genotyping before 6 months [15/79 patients (19%)] (p=0.246).

Conclusion—Prevalence of primary resistance in a sample of ART-naïve clade C HIV-infected individuals in South Africa was low during the study period. Patients failing first-line ART most often developed resistance to NNRTIs and nucleoside reverse transcriptase inhibitors, the two drug classes used in first-line therapy. Viral load monitoring in this setting is critical and individual genotypes in those failing first-line therapy should be considered.

Keywords

antiretroviral therapy; Africa; clade C; resistance; genotype

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Introduction

Over 3 million individuals now have access to antiretroviral therapy (ART) in low and middle income countries (LMIC).[1] Delivery of ART on this scale has required utilisation of a public health approach in which standardised, rather than individualised, regimens are prescribed to very large numbers of HIV-1-infected individuals.[2] At present, the majority of individuals in these countries are initiating first-line therapy with a non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTI).[3] In addition to those receiving ART for treatment, many women receive nevirapine and/or zidovudine for prevention of mother-to-child HIV transmission (PMTCT).[4]

Second-line ART, based on a boosted protease inhibitor with two nucleoside reverse transcriptase inhibitors (NRTIs), is several-fold more expensive than the first-line regimens.[1]Although the proportion of patients receiving second-line therapy is presently estimated to be 4%, this is increasing by 3% per annum.[5] In LMIC the decision of when to change to a second-line regimen is frequently delayed, as it is often based on clinical or immunological criteria in the absence of viral load measurement.[3,6] Rational choice of the NRTI component of second-line therapy should be based on patterns of resistance developed during first-line therapy.[7]

Much concern was expressed during the initial phase of expanded access to ART that “antiretroviral anarchy and viral mayhem” might follow the widespread use of ART in LMIC.[8] However, despite the large scale of PMTCT and ART roll out, there has been little published data detailing resistance either prior to or within large scale ART programs. The impact of the widespread use of single-dose nevirapine for PMTCT on primary resistance patterns of those entering ART programs has not yet been widely characterised.[9]

Further, most data on viral mutations developing in patients on ART are from HIV-1 subtype B prevalent industrialised countries, whereas viral subtypes in LMIC are frequently non-B, and non-B subtypes may have different pathways to viral resistance.[10–12] Data on resistance patterns in both naive and treatment-exposed clade C subtype are limited.[13–21] Our objective was to describe the resistance genotype patterns in both ART-naive individuals and in those with first virological breakthrough while on first-line NNRTI therapy in the public-sector ART programme in South Africa.

Methods

Study sample

Naive samples—Staff at the Desmond Tutu HIV Centre in Cape Town, South Africa, drew 30 samples per annum for genotype from naive HIV-positive individuals between 2003 and 2006, resulting in 120 samples available for the current analysis. These subjects were from 2 peri-urban resource poor communities in Cape Town. HIV-infected individuals attending HIV-clinics at either of the 2 sites were asked to donate a sample on a first-come first-serve basis beginning in April each year until 30 samples had been collected. None of these subjects had been exposed to any ART, including pMTCT, at the time of sampling.

Non-naive samples—All samples were from people failing first-line therapy in public sector ART clinics in the greater Cape Town area between 2002 and 2007. Eight clinics provided samples. HIV-positive people in these clinics may access ART with a CD4 count < 200 cells/uL or with WHO stage 4 clinical disease. First-line ART consists of stavudine (d4T) and lamivudine (3TC), with a NNRTI (efavirenz or nevirapine).[6] Pregnant women

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who do not yet qualify for ART are commenced on zidovudine (AZT) at 34 weeks and given a single dose of nevirapine in early labour.[4] For this study virological breakthrough was defined as the first time a viral load was noted to be > 1000 copies/ml while on ART, and virological failure was defined as two consecutive viral loads above 1000 copies/ml.

Laboratory testing

Viral load (VL) and CD4 cell counts were monitored 4 to 6-monthly according to local protocol.[4,6]. Viral load assays were done using the branch DNA hybridisation technique (Bayer HIV-1 RNA 3.0 assay (branch DNA)). Genotypic analyses of the reverse transcriptase and protease sequences of the HIV-1 DNA were completed using either the Bayer Health Care Trugene® HIV-1 or Monogram Biosciences GeneSeq™. The International AIDS Society (IAS-USA) list of mutations was used to determine which mutations may be related to drug resistance.[22] Mutations noted in the IAS-USA listing which were not noted in these data group were not listed in the results. The Stanford University HIV Resistance Database Genotypic Resistance Interpretation Algorithm was used to determine the possible drug resistance patterns per genotype. (http://hivdb6.stanford.edu/asi/deployed/hiv_central.pl?program=hivdb&action=showMutationForm) [23]

The majority of samples were sourced from the Hannan Crusaid Treatment Centre; this cohort has been described previously.[13–15] Excess plasma from the 4-monthly scheduled visits was frozen and stored. All individuals who reached a viral load of greater than 1000 copies/ml and who had an available stored plasma sample at the time of confirmation of virological failure were analysed for HIV genotype. The dates of treatment initiation, first observed virological breakthrough (>1000 copies/ml) and confirmation of virologic breakthrough (the date of the sample used for genotype analysis) were recorded.

Other samples were sourced from other ART sites in the Western Cape. Clinicians were asked to refer patients with a previously noted viral load of >1000 copies/ml on first-line therapy to the study site for one off genotype sampling. The dates of treatment initiation, first virological breakthrough (>1000 copies/ml) and confirmation of virologic breakthrough were recorded.

Demographic data (age, gender, disease stage) were recorded for all individuals with genotype results, as was viral load and CD4 cell count at the time of genotypic sampling. Mutations considered related to the function of the HIV reverse transcriptase and protease enzymes were recorded.

Statistical Analysis

Demographic and baseline data were described using medians and proportions as appropriate. Baseline characteristics described by interval data were compared using non-parametric statistics for data not normally distributed. 95% confidence intervals around resistance mutation prevalence were constructed using the normal approximation of the binomial distribution. Among patients failing ART we examined the association between timing of genotyping and resistance profile.

Results

Naïve patient samples

Samples from 120 ART-naïve HIV-infected individuals were included in the current analysis (Table 1). The median age of the cohort was 31 years (IQR 25–38) and 63% were women. The median CD4 count at the time of sampling was 262 cells/uL (IQR 149–405

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cells/uL) and the median viral load was 4.88 log (IQR 4.29 – 5.23 log). One hundred seventeen samples (98%) were clade C; the other 3 were clade B and were known to be collected from men who have sex with men (MSM).[24]

The individual genotype results for the naïve sample is group are shown in Table 2. There was very little variation in the reverse transcriptase (RT) gene. One individual (0.8%) had a K65R mutation, denoting probable reduced sensitivity to tenofovir and abacavir, and 3 had the V118I mutation (2.5%). Despite this cohort having no prior NNRTI exposure, there were two individuals with single NNRTI mutations (1.7%), one Y181C and one G190A, In contrast to the RT, in the protease inhibitor region there were a number of mutations which occurred frequently, though these were not expected to cause drug resistance. The most frequent protease mutations were L89I/M (89%), H69K (88%), L63P (52%) and M36I (87%). In addition, more than 10% had mutations at loci 20 (17%), 74 (10%) and 77 (18%).

Non-naïve patient samples

119 individuals taking first-line therapy experienced virological breakthrough from the Hannan Crusaid Treatment Centre between September 2002 and December 2007. Six individuals who had failed a protease-inhibitor based first-line regimen were excluded from the analysis. Stored samples were not available for 34 individuals and genotype results were obtained for the remaining 79 individuals. Samples from an additional 31 individuals with the same failure criteria were received from seven other public sector antiretroviral clinics bringing the total number of genotypes available from individuals failing first-line therapy to 110.

The demographics of the non-naive group were similar to that of the naïve cohort. Their median age was 32 years (IQR 28 –35 years) and 70% were women. The median CD4 count was significantly lower than in the naïve group, at 192 cells/uL (IQR 128 – 288 cells/uL, $p=0.003$) and the median log viral load at time of sampling was significantly lower at 4.02 log (IQR 3.61 – 4.76 log, $p<0.001$). The median time from treatment start date to initial detected virological breakthrough (>1000 copies/ml) was 271 days (IQR 177 – 525 days), and that from first detected virological breakthrough to the time of confirmation and sampling for genotype was 97 days (IQR 31 – 195 days). Seventy-nine people (72%) had their repeat sample within 180 days of their initial raised viral load and 39 people (28%) after 180 days.

There were many more RT mutations in the non-naive samples than in the naïve samples [Table 3]: 91 individuals (83%) had one or more mutations limiting susceptibility of NRTIs and 97 individuals (88%) had one or more therapy-limiting NNRTI mutations. The M184V, conferring resistance to 3TC and emtricitabine (FTC), was the most common single mutation (n=86, 78%) and emerged rapidly in failure (Figure 1a). According to the genotypic resistance interpretation algorithm four (4.0%) more people were likely to have intermediate resistance to 3TC and FTC due to the presence of K65R (Table 4).[23] Seven (6.4%) of the samples had no RT mutations.

A total of 10 individuals (9.0%), all taking d4T, had developed the K65R mutation, limiting future use of ddI, tenofovir and abacavir, without having had exposure to any of these medications (Table 4). In this group, those with the K65R did not have a significantly higher mean viral load than those without.[25] Six (5.5%) individuals had both the K65R and the M184V mutations.

Twenty-five individuals (23%) had a total of 33 thymidine analogue mutations (TAMs). Those with TAMs had a median viral load of 4.32 log (IQR 3.47 – 4.71 log) compared to a median of 4.01 log (IQR 3.47 – 4.71 log) in those without TAMS at the time of genotype

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sampling (p=0.896).[26] Only five individuals (4.5%) had more than one TAM. Figure 2 describes the proportion of those with non-TAM resistance that had also developed TAMs in the reverse transcriptase gene. There were relatively few individuals with resistance to either 3TC, i.e. presenting with the M184V mutation (n=6, 5.4%), or NNRTIs alone (n=20, 18%). These individuals also had few TAMs, and no sample had more than a single TAM. Over two thirds of individuals (n=75, 68%) had a combination of 3TC and NNRTI resistance. Twenty-one of these had TAMs (28%) and it was only in these samples that 2 or 3 TAMs were noted.

More TAMs were noted in those individuals who had failure confirmed more than 180 days after initial virological breakthrough (Figure 1b). Seventeen TAMs were noted in 15 of 79 (19%) individuals whose genotype was completed within 6 months, compared to 16 TAMs noted in 10 of 31 (32%) individuals whose genotype was completed after 6 months (p=0.246). Multivariate logistic regression modelling of factors associated with acquiring a TAM demonstrated that for every 20 unit increase in CD4 count at time of genotyping, the reduction in risk of developing TAMs was 9% (OR 0.91, CI 0.83–0.99, p=0.035). Age, gender, time from failure to sample, viral load and NRTI used did not affect the acquisition of TAMs. There was no significant difference in the number of TAMs generated by the specific thymidine analogue taken, whether AZT (4 in 13 individuals, 31%) or d4T (28 in 97 individuals, 29%, p=0.917). Susceptibility to AZT and D4T remained high in this group (Table 4).

Development of NNRTI resistance occurred rapidly and these mutations were the most common noted in this group. (Figure 1c). Seventy of 79 individuals (88%) whose genotype was completed within 6 months had NNRTI mutations, compared to 27 of 31 individuals (87%) whose genotype was completed after 6 months (p=0.956). In total, 97 individuals (88%) had one or more therapy-limiting NNRTI mutations (table 3), including K103N (55%), V106M (31%) and Y181C (10%) and probable drug susceptibility according to the genotypic interpretation algorithm was poor (Table 4).[23] Thirty-one individuals (28%) had 1 NNRTI mutation, 46 (42%) had 2 NNRTI mutations, 16 (15%) had 3 NNRTI mutations and 3 (3%) samples had as many as 4 NNRTI mutations. One individual (0.9%) had 6 NNRTI mutations. The Y181C emerged more frequently in those failing nevirapine (8 of 25, 32%) than efavirenz (3 of 85, 3.5%, p=0.0004). There was no significant difference in the emergence of K103N whether nevirapine (9 of 25, 36%) or efavirenz (51 of 85, 60%) was taken (p=0.229).[27]There was no significant difference in the emergence of V106M by drug. This mutation was seen in 8 of 25 (32%) people on nevirapine and 26 of 85 (31%) people on efavirenz (p=0.923). The ratio of V106M/K103N in patients failing EFV therapy was 0.5.

The protease gene had similar mutations to those noted in the naïve population. The median number of mutations was four (IQR 3–5). The most frequent protease mutations remained M36I (86%), L63P (60%), H69K (94%) and L89I/M (82%), similar to the consensus sequence noted for clade C (differing amino acids compared to clade B subtypes at positions M361, R41K, H69K AND L89M).[26] As in the naïve cohort, more than 10% had mutations at loci 20 (27%), 74 (13%) and 77 (15%). According to the genotypic resistance algorithm, those with mutations at point 74 (n=14, 13%) have possible low level resistance to nelfinavir (Table 4).[23] Two individuals (1.8%) with mutations at point 33 had possible low level resistance to fosamprenavir and tipranavir, and 1 individual (0.9%) had multiple protease inhibitor resistance due to mutations at points 73 and 82 (Table 4).

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Discussion

With increasing access to ART in LMIC, increased numbers of patients are failing first-line therapy and thus being switched to second-line therapy.[15] In higher income countries, both choice of initial therapy and switch to second-line are done with the use of individual genotypes.[7] In LMIC, a public health approach, with more limited first and second-line treatment options, has been used. In this setting it is critical to understand the evolution of resistance patterns, and whether the current treatment regimens are adequate.

The majority of virus in this South African sample, in both naive and non-naïve patients, was clade C.[24] In both the naïve and non-naïve group there were a number of mutations in the protease enzyme, possibly indicating divergence from clade B virus. The impact of these on viral drug susceptibility is uncertain, but many, including L10I/V, K20R, M36I, L63P, A71V/T and V77I, are not expected to cause major drug resistance. [28] These mutations are similar to those noted in other African clade C virus and clade C consensus sequences. [16–18, 29] Other mutations that are more likely to impact on the future use of a protease inhibitor, including D30N, M46I, I47V, I50V and V82A/F, were present, but in a minority of individuals, with only a single mutation noted per individual. The effect of such single mutations on the use of lopinavir in second line is not clear in our population.

There were very few mutations noted in the reverse transcriptase enzyme in the naive group. The two mutations that are likely to reduce susceptibility to NRTIs if transmitted, T215C and M41L, were not seen in either the naïve or non-naïve groups. [28] The multiplicity of NNRTI mutations seen in the non-naïve group make NNRTI resistance the most likely to be transmitted in our population. Although no K103N mutation, expected to have the most impact on the use of NNRTIs, was seen in the naïve samples, there were 2 individuals (1.6%) each with a single mutation (Y181C and G190E) that would have some impact on NNRTI susceptibility. Although the currently recommended treatment regimens for first-line remain appropriate, ongoing surveillance of NNRTI-resistant virus remains important. [3,6]

The samples in the non-naïve group were taken from 110 individuals failing initial NNRTI therapy in the South African public sector. Previous data have shown that the rate of confirmed virological failure in this cohort was 5.6% at 32 months.[15] Adherence is monitored by tablet count in all public-sector clinics in South Africa and any viral load increase should initiate a “stepped-up” adherence package including counsellor-driven re-education sessions, more regular clinic visits with emphasis on the use of a pill-box as a reminder system, as well as a home visit to assess living circumstances where the resources are available for this service.[15] Seventy-five percent of those with an initial viral load breakthrough of >1000 copies/ml again achieved suppression after structured adherence interventions.[15] For those in whom failure was confirmed with a second specimen >1000 copies/ml, the median time from treatment commencement to noting initial virological breakthrough was 9 months.

The focus on adherence may explain the relatively small number of individuals with virological failure who did not have a significant drug-resistant mutation. Only seven individuals (6.4%) had wild type virus at genotype, a smaller proportion than seen in the DART study (10%).[20] Most of these individuals with confirmed failure had resistance mutations which would exclude use of two of the antiretrovirals used in first-line regimens, i.e. 3TC (83%), a similar proportion to that seen in the DART study (70%), and the NNRTIs (86%).[20] Resistance to both NNRTIs and 3TC (M184V) develops rapidly after initial virological breakthrough.

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Resistance to the third drug in the regimen, the thymidine analogue, occurred more slowly. While 23% of the group had at least one TAM, relatively few had two or three TAMs and the majority of individual remained susceptible to both AZT and d4T. A trend towards TAM accumulation with prolonged time on failing therapy was noted, but was not-significant. People with lower CD4 counts at the time of genotype were also more likely to have acquired a TAM, perhaps indicative of a longer time on failing therapy than noted here, due to the length of time between viral loads in this cohort. Thymidine analogues are currently recycled in second-line therapy in South Africa (AZT, ddI and lopinavir/ritonavir), so TAM accumulation may reduce the efficacy of this therapy. However, if failure is identified before acquisition of TAMs, second-line therapy may remain more efficacious.

The increased presence (9%) of the K65R mutation in the non-naive samples was unexpected, given the absence of abacavir or tenofovir in the South African treatment regimens. There is emerging evidence that non-subtype B virus may have a propensity to develop the K65R more readily compared to subtype B.[10,25,26] Doualla-Bell noted that d4T also selected for K65R in subtype C virus in Botswana, and that the mutation developed within 3 months of tenofovir therapy, unlike in subtype B where the K65R tends to emerge slowly in a small proportion of individuals on tenofovir.[10] It is also possible that 3TC may select for the K65R mutation as recently described.[22]With the registration of tenofovir in South Africa in 2007, there is a push for the widespread use of this agent to replace d4T, initially in those experiencing adverse effects, but with the view to broad-spectrum first-line use. The likely rapid emergence of resistance to tenofovir in clade C virus should be of concern for treatment programmes, as the presence of this mutation reduces susceptibility to all NRTIs except AZT, and thus would limit the choice of NRTIs for second-line therapy.[30]

A limitation of this study is the 4 to 6 month window between viral load samples in the South African antiretroviral programme. Some individuals may have failed within weeks of their last suppressed viral load and others within days of their first raised viral load. Time from first virological breakthrough to time of genotype may therefore be an underestimate.

Resistance to the reverse transcriptase enzyme after exposure to NNRTI-containing first-line therapy follows a pattern that is predictable and similar to that of clade B: initial resistance to antiretrovirals that require a single point mutation, followed by slower development of resistance to drugs with a higher genetic barrier to resistance, such as the thymidine analogues.

Had second-line treatment been commenced within 6 months of initial virological breakthrough in the non-naïve group in this study, the likelihood of accumulating TAMs may have been reduced, with a potential increase in the efficacy of the recycled thymidine analogue in second-line therapy. Identification of and rapid response to virological failure is thus important to maintain the full benefit of second-line therapy. This would suggest clinical value to regular viral load testing to identify virologic failure soon after it occurs, in contrast to a recently published model.[31] Due to the unexpected emergence of the K65R mutation in a substantial proportion of the cohort, tenofovir should be introduced cautiously with careful assessment of its impact on the emergence of resistance.

This study suggests that, at present, it is not critical in the context of the South African National ART programme to have routine access to genotypes at baseline, as the vast majority of naïve samples continue to be wild type. In contrast, the development of extensive resistance in those failing first-line therapy suggests that viral load monitoring is critical and there may well be a role for individual genotypes in those failing first-line therapy, particularly if second-line therapy is likely to be compromised by resistance to first-

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line therapy. Increased availability of low cost assays for identifying resistance in patients in South Africa would be clinically valuable.

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Figure 1a: Proportion with the M184V mutation.

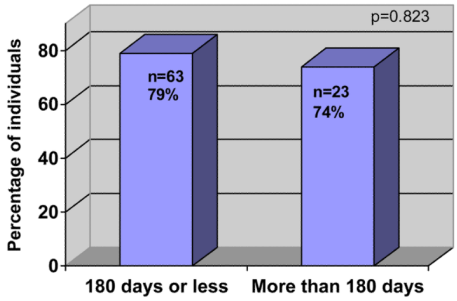

Figure 1b: Proportion with TAMs.

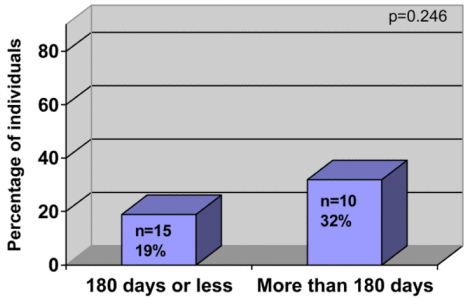

Figure 1c: Proportion with NNRTI mutations.

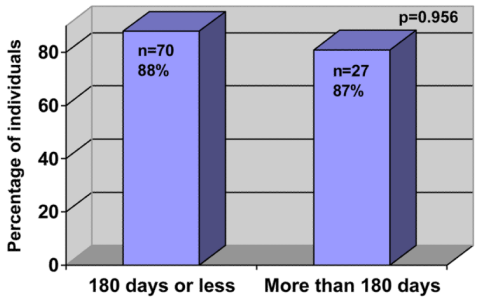

Figure 1.

This illustrates the proportion of individuals with either M184V (figure 1a), TAMs (figure 1b) or NNRTI mutations (figure 1c) by time between initial virological breakthrough (first viral load >1000 copies/ml) and failure (at the time of the second consecutive viral load >1000 copies/ml). Individuals were divided into those whose genotype (taken at the time of failure) was completed on or before 180 days (n=79) from breakthrough or after 180 days (n=31). None of the differences were significant, though a trend to more TAMS with more time on failing therapy was noted.

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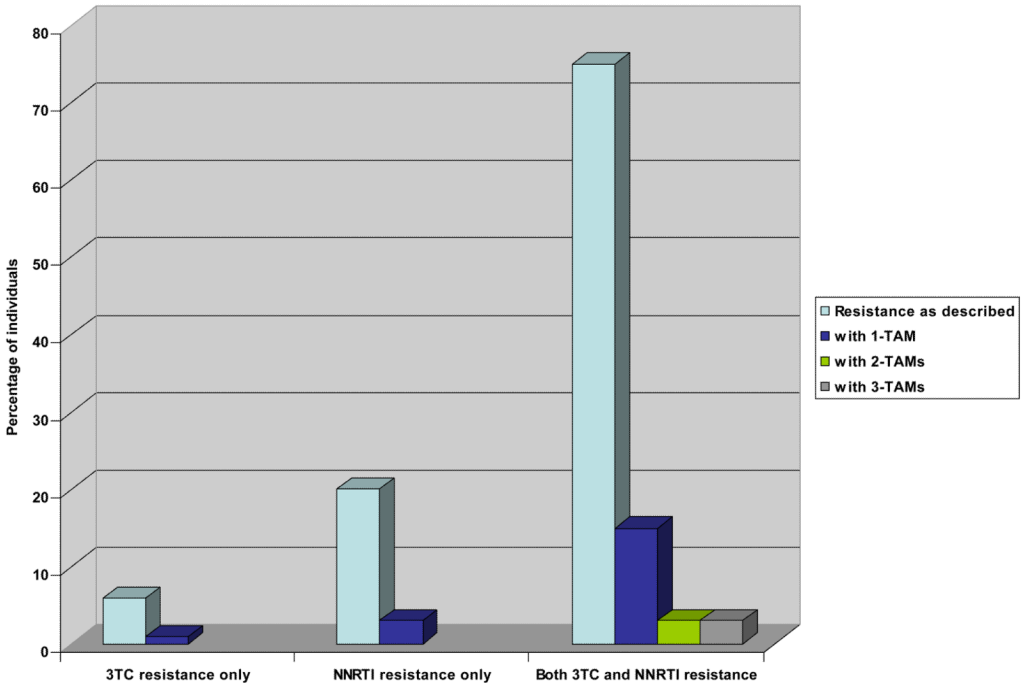

Figure 2.
Association between non-TAM resistance and development of TAMs in the reverse transcriptase gene.

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Table 1

Demographic characteristics and laboratory results for naive and first virological failure groups in a sample from Cape Town, South Africa.

Population	Naïve	Virological failure	P value
Total samples n (%)	120 (100)	110 (100)	
Mean age (years ± IQR)	31 (25–38)	32 (28–35)	p=0.535
Female gender n (%)	75 (63)	77 (70)	p=0.588
Median CD4 count (cells/uL ± IQR)	262 (149–405)	192 (122–283)	p=0.003
Median viral load (log)	4.88 (4.29–5.43)	4.02 (3.61–4.76)	p<0.001
HIV clade C n (%)	117 (98)	56 (97) of 58 available	p=0.966
HIV clade B n (%)	3 (2)	2 (3) of 58 available	
Treatment regimen:			
d4T, 3TC, EFV	-	78 (67.2%)	
d4T, 3TC, NVP	-	18 (15.5%)	
AZT, 3TC, EFV	-	7 (6.0%)	
AZT, 3TC, NVP	-	6 (5.2%)	

[The viral load and CD4 presented for the first time virological failure group are those at the time of second consecutive viral load >1000 copies/ml, a median of 97 days from initial viral load >1000 copies/ml.]

IQR: interquartile range; d4T: stavudine; 3TC: lamivudine; EFV: efavirenz; NVP: nevirapine; AZT: zidovudine

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Table 2

Genotype results in a sample of ART naïve patients in Cape Town, South Africa (n=120). Mutations noted in the IAS-USA listing which were not noted in this group are not listed here.

NRTI mutations			
Ref	**Loci**	**AA**	**n(%)**
K	65	R	1 (0.8)
V	118	I	3 (2.5)

Wild type n(%)	114 (95)

NNRTI mutations			
Ref	**Loci**	**AA**	**n(%)**
Y	181	CY	1 (0.8)
G	190	ACE	1 (0.8)

PI mutations			
Ref	**Loci**	**AA**	**n (%)**
L	10	IVF	6 (5.0)
G	16	E	2 (1.6)
K	20	RT	20 (16.6)
M	36	I	104 (86.7)
M	46	I	1 (0.8)
I	47	IV	1 (0.8)
I	50	V	1 (0.8)
I	54	V	1 (0.8)
L	63	HLPSTV	62 (51.7)
H	69	K	108 (90)
A	71	ATV	3 (2.5)
G	73	S	3 (2.5)
T	74	S	12 (10)
V	77	I	22 (18.3)
LV	82	AF	1 (0.8)
L	89	IM	107 (89.2)

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Table 3

Genotype results in a sample of patients with virological failure to first-line ART in Cape Town, South Africa (n=110). Mutations noted in the IAS-USA listing which were not noted in this group are not listed here.

NRTI mutations			
Ref	**Loci**	**AA**	**n(%)**
M	**41**	**ML**	**1 (0.9)**
K	65	R	10 (9.0)
D	**67**	**N**	**14 (13)**
T	69	DN	2 (1.8)
K	**70**	**KR**	**4 (3.6)**
L	74	L/I/V	1 (0.9)
V	75	IM	3 (2.7)
V	118	I	2 (2.7)
M	184	V	86 (78)
T	**215**	**FSY**	**9 (8.1)**
K	**219**	**EQ**	**5 (4.5)**
K	238	T	2 (1.8)
Wild type n(%)			7 (6.4)

NNRTI mutations			
Ref	**Loci**	**AA**	**n(%)**
A	98	G	4 (3.6)
L	100	I	2 (1.8)
K	101	EP	18 (16)
K	103	N	60 (55)
V	106	M	34 (31)
V	108	IV	13 (12)
E	138	A	2 (1.8)
V	179	DV	6 (5.5)
Y	181	CY	11 (10)
Y	188	HL	9 (8.1)
G	190	ACE	22 (20)
P	225	H	15 (14)
F	227	L	7 (6.4)
M	230	L	8 (7.3)

PI mutations			
Ref	**Loci**	**AA**	**n(%)**
L	10	IVF	7 (6.4)
I	13	V	8 (7.3)
G	16	E	9 (8.1)

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PI mutations			
Ref	**Loci**	**AA**	**n(%)**
K	20	RT	30 (27)
D	30	N	1 (0.9)
L	33	FV	2 (1.8)
M	36	I	95 (86)
L	63	HLPSTV	66 (60)
H	69	K	103 (94)
G	73	S	1 (0.9)
T	74	S	14 (13)
V	77	I	17 (15)
LV	82	AF	1 (0.9)
L	89	IM	90 (82)

[Text in bold indicates thymidine analogue mutations (TAMs).]

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Table 4

Expected resistance patterns according to the Stanford University HIV Resistance Database Genotypic Resistance Interpretation Algorithm in a sample of patients with virological failure to first-line ART in Cape Town, South Africa (n=110).

	Susceptible	Possible low level resistance	Low level resistance	Intermediate resistance	High resistance
Lamivudine/emtricitabine	22 (20%)	-	-	4 (4.0%)	86 (78%)
Abacavir	20 (18%)	55 (50%)	15 (14%)	20 (18%)	-
Zidovudine	98 (89%)	1 (1.0%)	6 (5.5%)	3 (2.7%)	2 (1.8%)
Stavudine	87 (79%)	6 (5.5%)	12 (11%)	5 (4.5%)	-
Didanosine	76 (69%)	9 (8.1%)	9 (8.1%)	14 (13%)	2 (1.8%)
Tenofovir	97 (88%)	1 (1.0%)	4 (3.6%)	8 (7.3%)	-
Efavirenz	10 (9.0%)	2 (1.8%)	-	13 (12%)	85 (77%)
Nevirapine	10 (9.0%)	2 (1.8%)	1 (1.0%)	4 (4.0%)	93 (85%)
Etravirine	10 (9.0%)	15 (14%)	49 (45%)	32 (29%)	4 (4.0%)
Protease Inhibitors	93 (84%)	16 (15%)*	1 (1.0%)**		

* 14 with possible low level resistance to nelfinavir alone; 2 with possible low level resistance to fosamprenavir and tipranavir.

** 1 individual with low level resistance to atazanavir, fosamprenavir, lopinavir and saquinavir and intermediate resistance to indinavir and nelfinavir.