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Original article

Prevalence of HIV type-1 drug-associated mutations in pre-therapy patients in the Free State, South Africa

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Background: We aimed to characterize the molecular epidemiology of HIV type-1 (HIV-1) and the prevalence of drug-associated mutations prior to initiating highly active antiretroviral therapy (HAART) in the Free State province, South Africa. The Free State has a population of 3 million, an antenatal HIV prevalence of approximately 34% and a well established infrastucture for antiretroviral (ARV) provision.

Methods: HIV-1 polymerase genes were sequenced from 425 HAART-naive HIV-1-positive patients at voluntary primary healthcare HIV testing centres, who were subsequently attending district centres for assessment for commencing ARVs. Patients (>18 years) were sampled randomly with no exclusion for gender or clinical criteria. Sequences were analysed according to phylogeny and drug resistance.

Results: Phylogenetic clustering within the cohort was suggestive of multiple introductions of subtype C virus into the region. Drug resistance mutations (according to the International AIDS Society–USA classification) were distributed randomly across the cohort phylogeny with an overall prevalence of 2.3% in the sampled patients. When stratified according to CD4$^+$ T-cell count, the prevalence of resistance was 3.6%, 0.9% and 1.2% for CD4$^+$ T-cell counts <100, 200–350 and >500 cells/μl, respectively, and was most common for non-nucleoside reverse transcriptase inhibitor resistance (3.1% in patients with CD4$^+$ T-cell count <100 cells/μl). We surveyed all drug-selected mutations and found further significant clustering among patients with low CD4$^+$ T-cell counts (P=0.003), suggesting unrecognized exposure to ARVs.

Conclusions: In the Free State population, there was a statistical association between low CD4$^+$ T-cell counts and drug-associated viral polymorphisms. Our data advocate the benefit of detailed history taking from patients starting HAART at low CD4$^+$ T-cell counts with close follow-up of the virological response.

Introduction

Since 2004, the South African government has made antiretroviral (ARV) therapy available to HIV-positive individuals through public sector treatment programmes – an initiative with significant clinical, public health and economic effects [1–4]. A few small- to medium-sized South African cohorts have reported molecular characterization and baseline pre-therapy resistance profiles of HIV infection, predominantly among the KwaZulu-Natal, Gauteng and Limpopo provinces [5–8]. There is currently no similar information available from the Free State Province – the third largest province in South Africa with a population of approximately 3 million and an antenatal clinic HIV prevalence of 33.9% [9]. Under the Free State

Comprehensive Care, Management and Treatment of HIV and AIDS programme, highly active antiretroviral therapy (HAART) has been available since 2004 as a combination of stavudine and lamivudine with either nevirapine or efavirenz, and has significantly reduced the number of HIV-related deaths (hazard ratio 0.14) [10].

Guidelines exist for the surveillance of drug resistance in patients receiving HAART and in drug-naive individuals – the World Health Organization (WHO) and Stanford Drug Resistance Database publish a list of mutations for use in surveillance of transmitted drug resistance mutations (TDRM) and the International AIDS Society (IAS)–USA produce a list for use in the monitoring of treated populations. The WHO, Stanford and IAS–USA committees regularly update the definitions and clinical significance (phenotypical resistance, clinical association and outcome of meta-analyses) of drug resistance mutations and drug-associated polymorphisms [11,12]. Recently, the updated TDRM list from the Stanford database has become unified with the WHO guidelines [13,14]. The mutations in the TDRM surveillance list are selected on the basis that they are non-polymorphic, clinically significant and applicable across all subtypes. The implementation of such a standard should facilitate a specific method to assess changes in drug resistance prevalence in populations over time [14–16].

In patients who report that they are drug-naive, resistance mutations might either reveal transmitted variants or might indicate that patients are not, in fact, drug-naive or are unaware of previous ARV exposure [17]. The extent to which unrecognized access contributes to the level of resistance in patients enrolling onto public sector ARV is unknown in South Africa, and very difficult to measure. Prior to the introduction of the public sector ARV treatment programme, ARV drugs were available for the prevention of mother-to-child transmission (PMTCT) through private clinics and other unregulated routes, although previous studies suggest a low prevalence of baseline drug resistance in South Africa [5–8].

Here, we studied 425 HIV type-1 (HIV-1)-positive patients newly recruited to the public sector ARV treatment programme in the Free State province, South Africa and reporting to be drug-naive. We characterized the molecular epidemiology of HIV-1 infection within this region and measured the prevalence of drug resistance and other polymorphisms associated with drug exposure in these individuals. We found a correlation between low CD4$^+$ T-cell counts and drug-selected polymorphisms, suggesting that many mutations in drug-naive individuals are not transmitted, but are the result of acquired resistance through unrecognised ARV access.

Methods

Patients

We studied 884 adult patients recruited at their first visit to government ARV clinics in the Free State province of South Africa between February and September 2006. All patients had been identified through voluntary screening programmes in the Free State and, following diagnosis with HIV infection at local primary care clinics, were referred to district or regional centres. Here, the patients were counselled by trained HIV nurses and asked directly whether they had previously received PMTCT, mono-, dual- or triple-ARV therapy. Patients who admitted previous drug exposure were excluded from this analysis. For all other patients, an extra 10 ml of blood was taken at the same time as routine bloods for CD4$^+$ T-cell counts and other clinical investigations. The University of the Free State approved the study ethics (ETOVS 10/04 and ETOVS 206/05). The Department of Health of the Free State Province granted approval to conduct the study.

CD4$^+$ T-cell count stratification

The 884 patients were stratified according to CD4$^+$ T-cell count and viral sequences were analysed from 425 patients to form the low (<100 cells/μl; n=195), intermediate (200–349 cells/μl; n=120) and high (>500 cells/μl; n=110) CD4$^+$ T-cell count stratification groups. The mean CD4$^+$ T-cell counts for all patients within the low, intermediate and high groups were 45.4, 270.0 and 651.8 cells/μl, respectively. The mean age of the cohort was 36.1 years (Table 1).

Sequencing

From the 425 patients representing the three CD4$^+$ T-cell count stratifications, 44 were initially sequenced locally in South Africa (ViroSeq Genotyping System; Celera Diagnostics, Alameda, CA, USA) as part of a preliminary analysis (presented previously [18]), and 381 were then sent to the Peter Madawar Building for Pathogen Research (Oxford, UK). There was no difference in the sampling strategy for the two groups. Briefly, RNA was extracted from plasma, amplified by reverse transcriptase (RT)-PCR before sequencing using ABI Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA). The primers used are described elsewhere [19]. Complete protease gene sequences (99 amino acids) and amino acids 1–530 of the RT gene were successfully amplified from 390 and 397 patients, respectively.

Phylogenetic analyses

HIV *pol* sequences were submitted to the BioAfrica database for subtype and locus identification [20,21]. Phylogenetic analysis and maximum likelihood trees were constructed using the General Time Reversible

Table 1. Details of HIV-1-positive patients recruited to three subgroups according to CD4+ T-cell count

Category	Complete cohort	CD4+ T-cell count <100 cells/μl	100–199 cells/μl	200–349 cells/μl	350–499 cells/μl	≥500 cells/μl	*P*-value (χ^2 test for trend)
Patients in cohort, *n* (%)	884	195 (22.1)	212 (24.0)	244 (27.6)	123 (13.9)	110 (12.4)	–
Patients sampled, *n*	425	195	–	120	–	110	–
Patients with both protease and RT gene sequences, *n*	390	192	–	112	–	86	–
Mean age, years (±SD)	36.1 (9.1)	38 (8.9)	–	35.4 (9.0)	–	34.2 (9.2)	–
Mean CD4+ T-cell count, cells/μl (±SD)	271.3 (200.82)	45.36 (28.66)	–	269.96 (40.86)	–	651.80 (178.91)	–
Patients with drug resistance mutations, *n* (%)[a]	9 (2.3)	7 (3.6)	–	1 (0.9)	–	1 (1.2)	0.134 (0.055)
Total drug resistance mutations, *n*	11	9	–	1	–	1	–
Patients with non-accessory drug-associated mutations, *n* (%)[b]	16 (4.1)	13 (6.8)	–	2 (1.8)	–	1 (1.2)	0.015 (0.004)
Total non-accessory drug-associated mutations, *n*	19	16	–	2	–	1	–
Patients with any polymorphism at drug resistance-associated sites, *n* (%)[b]	64 (16.4)	37 (19.3)	–	15 (13.4)	–	12 (14.0)	0.193 (0.099)
Total naturally occurring drug-associated mutations, *n*	73	44	–	15	–	–	–

All patients listed in this table were infected with HIV type-1 (HIV-1) subtype C. All patients were stratified into three discrete groups according to baseline CD4+ T-cell count. Patients not falling into these groups were not analysed. The total number of mutations are also shown because some sequences contained more than one mutation. [a]Defined by the International AIDS Society–USA Drug Resistance Mutation list. [b]Defined by Stanford Drug Resistance Database.

substitution model with optimized proportions of invariable sites and gamma distribution (GTR+G+I) using PAUP* (version 4.0 beta; Sinauer Associates, Inc. Publishers, Sunderland, MO, USA) and PhyML (version 2.4.4) [22]. The *pol* sequences were compared with 986 isolates from the BioAfrica database and the Los Alamos database to determine lineage relationships [21,23]. The Slatkin and Maddison test was used to assess clustering between the Free State Province sequences and other South African sequences, using MacClade (version 4; Sinauer Associates, Inc. Publishers) [24].

Drug resistance analyses

The protease and RT gene sequences were submitted to the Stanford University HIV Drug Resistance Database and mutations were graded as 'accessory', 'potentially low', 'low', 'intermediate' or 'high' resistance. These mutations were then compared with the drug resistance mutation lists of the 2008 IAS–USA and 2009 WHO TDRM surveillance list [11,14]. Each polymorphism was studied further using the online Mutation ARV Evidence Listing programme (MARVEL version 0.8), which summarizes the evidence for each polymorphism according to subtype-specific drug inducibility, viral phenotype and clinical outcome [12].

Statistical analyses

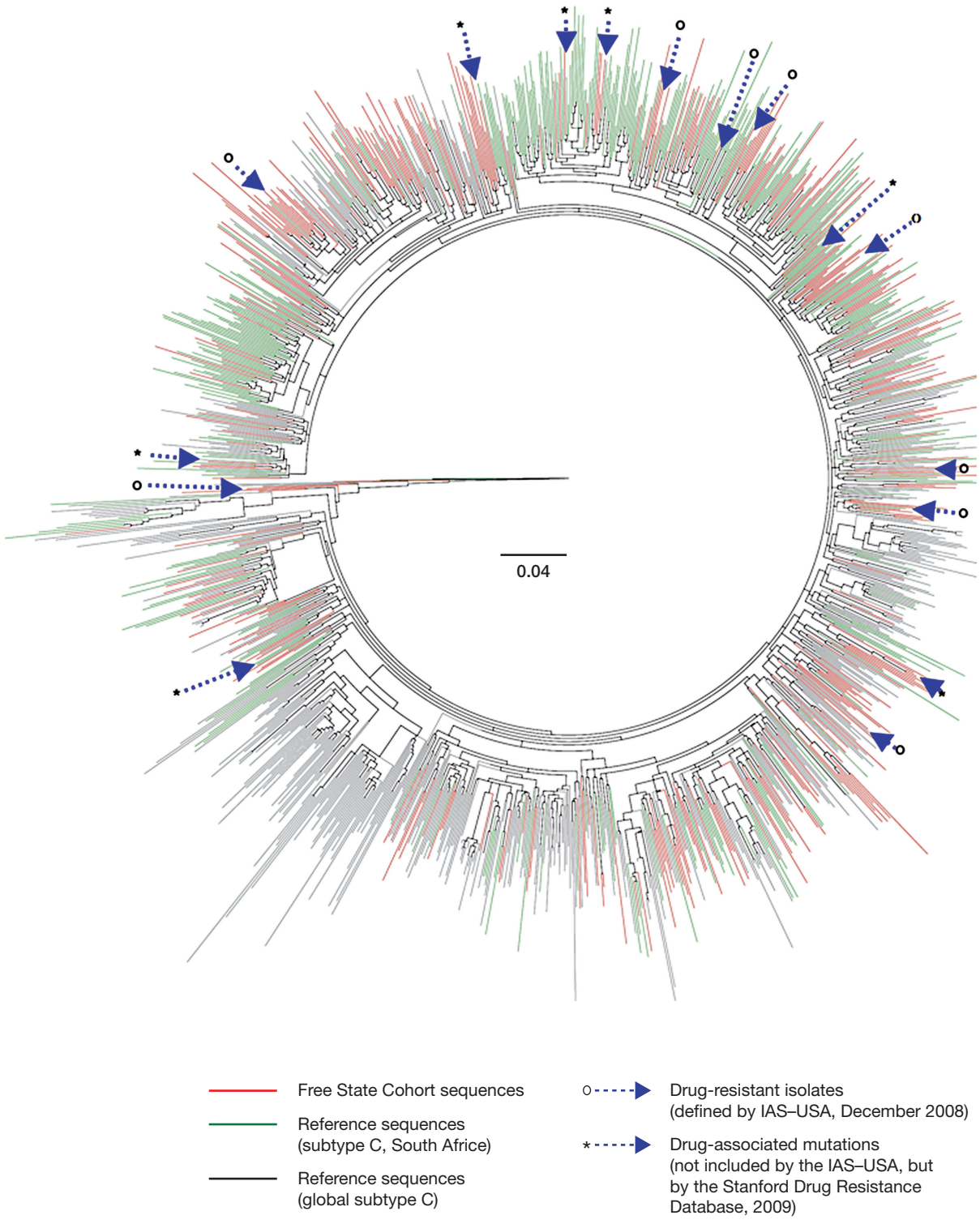
Fisher's exact test (two-tailed) and the χ^2 test for trends were used to compare pre-therapy resistance findings between groups with different CD4+ T-cell counts. The statistical analyses and distributions were carried out using Prism4 for Macintosh (GraphPad Software, La Jolla, CA, USA).

Results

Free State HIV sequences suggest multiple subtype C introductions into the population

In Figure 1, the maximum likelihood tree shows 390 HIV-1 *pol* sequences from the Free State in the context of local and global HIV-1 subtype C viruses from the BioAfrica database. The Free State strains (n=390) are in red, the South African strains (n=428) are in green, and remaining global subtype C strains (n=551) are in black. We found that 70.6% of Free State sequences have a South African reference sequence as the nearest neighbour, but there is also clustering of sequences sampled from the Free State compared with other major South African centres (Slatkins and Maddison test, P<0.001). We found that 52.5% of sequences occurred in 61 clusters of between 2 and 18 patients (mean 3.4 patients), indicating a combination of multiple transmissions between the Free State and the rest of South Africa, together with some small localized epidemics that are limited to the Free State. Sequences that contained any drug-associated polymorphism or a mutation from the IAS–USA surveillance list are indicated and are randomly distributed. The absence

Figure 1. Phylogenetic tree describing HIV-1 molecular epidemiology in the Free State, South Africa in the context of global HIV-1 subtype C reference sequences



HIV type-1 (HIV-1) isolates sampled from Free State patients are shown in the context of published South African and non-South African HIV-1 subtype C reference sequences. Resistant isolates defined by International AIDS Society (IAS)–USA and other drug-associated mutations defined by the Stanford Drug Resistance Database are shown.

of linkage of these variant sequences does not support significant transmission of drug resistance within this cohort (Slatkins and Maddison test, P=0.85).

Low prevalence of pre-therapy drug resistance in the Free State

According to the Stanford Drug Resistance Database list, the prevalence of non-accessory mutations was 4.1%, comprising 16 patients carrying a total of 19 non-accessory mutations (Table 1). The overall prevalence of clinically significant drug resistance mutations (according to the IAS–USA classification) was low (2.3%), comprising 9 patients carrying 11 mutations (Table 1). A total of 64 (16.4%) patients sampled had a mutation at any of the drug resistance amino acid sites, including those that have been found to be naturally occurring polymorphisms (Table 1). Of these, the most common accessory mutations for the protease gene were L10I/V (*n*=7, 1.8%), L11IV (*n*=4, 1.0%) and L89I (*n*=7, 1.8%). For the RT gene, the most common mutations were V118I (*n*=17, 4.4%) and G333E (*n*=4, 1.0%).

The 16 patients with non-accessory mutations – according to the Stanford definition – are detailed in Table 2. Of these, nine had clinically significant mutations according to the IAS–USA list. These were Y181C (*n*=2), K103N (*n*=3), Y188L (*n*=1), V106M (*n*=1), V108I (*n*=1) and K219E (*n*=1) in the RT gene, and M46L (*n*=1) and N88S (*n*=1) in the protease gene. The referral centres and local clinics where these patients had been recruited were either visited or contacted; however, in all but five cases the patients had been lost to follow-up. Of the four who had maintained undetectable viral loads on therapy, none had clinically significant IAS–USA defined mutations at baseline (V179D, M46L, A98G and T69N). The one patient identified with a detectable viral load on ARVs (13,000 RNA copies/ml after 5 months) had V179D at baseline, although no sequence data was available from subsequent samples to determine the development of resistance (Table

Table 2. Patients with drug-associated mutations to antiretroviral drugs during pre-therapy assessment

Patient identification	Gender	Age, years	CD4⁺ T-cell count, cells/μl	PI major mutation (grade of resistance)[a]	NRTI mutation (grade of resistance)[a]	NNRTI mutation (grade of resistance)[a]	Response to therapy
OX2032	F	35	1	–	–	E138K (potentially low) and Y181C (high)	Lost to follow-up
OX428	M	37	2	–	–	A98G (potentially low)	Lost to follow-up
OX927	F	25	3	–	K219E (low)	Y181C (high)	Lost to follow-up
OX195	M	41	5	–	T69N (potentially low)	–	Lost to follow-up
OX693	M	31	14	–	–	V179D (potentially low)	VL=13,000 copies/ml 5 months after starting therapy
OX1	F	42	23	–	–	K103N (high)	Lost to follow-up
OX613	F	24	28	–	–	V179D (potentially low)	VL<25 copies/ml 28 months after starting 3TC/d4T/EFV
OX1082	M	37	30	–	–	V106M and Y188l (both high)	Lost to follow-up
OX2233	F	28	42	–	–	K103N (high)	Lost to follow-up
OX677	F	31	60	M46L (low)	–	–	VL<25 copies/ml 23 months after starting 3TC/d4T/EFV
OX2011	M	37	63	–	–	A98G (potentially low)	VL<25 copies/ml 33 months after starting 3TC/d4T/EFV
OX5	F	56	64	–	T69N (potentially low)	–	VL<25 copies/ml 36 months after starting 3TC/d4T/EFV
OX1006	M	43	64	–	–	V108I (potentially low)	Lost to follow-up
OX2519	F	32	205	–	–	V179D (potentially low)	Lost to follow-up
OX312	F	38	215	–	–	K103N (high)	Lost to follow-up
OX626	F	39	550	N88S (intermediate)	–	–	Lost to follow-up

The table shows observed pre-therapy drug-associated mutations (class of antiretroviral agent, mutation position and grade of resistance), arranged in ascending order of CD4⁺ T-cell count and virological response (if known). [a]Grades of resistance are defined using the Stanford Drug Resistance Database. d4T, stavudine; EFV, efavirenz; F, female; M, male; VL, viral load; 3TC, lamivudine.

2). Of the 16 patients with significant resistance, 6 were male. Of the 10 females, the median age was 34.5 years (range 25–56).

The non-accessory mutations identified in the cohort are explored in more detail in Table 3. For each mutation, the number in each CD4+ T-cell count stratification is shown and compared with the reported prevalence in databases of drug-naive subtype B and C populations and in drug-experienced subtype C populations. To determine the clinical implications of these identified mutations, the Stanford database MARVEL report for each is summarized and the significance of the mutations in the surveillance lists of the WHO (updated 2009) and IAS–USA (updated December 2008) are compared (Table 3). For all the mutations, the prevalence in databases of drug-experienced subtype C cohorts was higher than in drug-naive patients with implications that these mutations might act as markers of drug exposure, even if not conferring clinically relevant resistance.

Clustering of drug-associated mutations among patients with low CD4+ T-cell counts

Drug-associated polymorphisms (based on the Stanford database) were concentrated among patients with low CD4+ T-cell counts – 6.8% of patients with CD4+ T-cell counts <100 cells/μl carried non-accessory mutations compared with 1.8% and 1.2% of patients with intermediate and high CD4+ T-cell counts, respectively (P=0.015; Table 1 and Figure 2). The prevalence of resistance according to the IAS–USA definition was 3.6%, 0.9% and 1.2% for low, intermediate and high CD4+ T-cell count groups, respectively (Table 1). Although not statistically significant, there were more accessory mutations among patients with low CD4+ T-cell counts (19.3%) compared with the intermediate (13.4%) and the high (14.0%) CD4+ T-cell count groups. When using the more relaxed Stanford definition, mutations clustered among patients with low CD4+ T-cell counts for all mutations (Figure 2; P=0.004). Although not statistically significant, when only considering clinically relevant mutations, there was a trend for clustering of resistance among low CD4+ T-cell counts for the IAS–USA (P=0.055) and the WHO TDRM lists (P=0.086; Table 3).

Of the patients with low CD4+ T-cell counts (<100 cells/μl), 5.2% possessed non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations compared with 1.8% of patients with intermediate and 0.0% of patients with high CD4+ T-cell counts (P=0.005; Figure 2 and Table 3). This association remained significant when restricted to NNRTI mutations on the IAS–USA list (P=0.031; Table 3). The distribution of NNRTI mutations within patients with low CD4+ T-cell counts was concentrated further among the individuals with lowest CD4+ T-cell counts (Table 2), who also tended to have higher grade resistant mutations.

Discussion

Here, we present a cohort analysis of the molecular epidemiology of the HIV-1 epidemic in the Free State Province of South Africa, and of the prevalence of circulating pre-therapy drug resistance in this major site for the government ARV treatment programme. There were two key findings. Firstly, there was evidence of multiple introductions of HIV-1 into the Free State, but random distribution of drug resistance-associated polymorphisms. Secondly, the overall prevalence of pre-therapy drug resistance was low, but drug-selected polymorphisms were concentrated among patients with low CD4+ T-cell counts.

The Free State is located in central South Africa and is relatively isolated from other South African centres. Our phylogenetic analysis revealed small but distinct clusters within the Free State, which suggests the mixing of HIV-1 strains from across South Africa, with migration into and through the Free State, possibly a result of the mining industry, transport routes and other economic reasons. However, despite these clusters, viruses with drug-associated mutations are distributed randomly, suggesting that they have evolved recently as a result of individual drug exposure rather than being transmitted within the Free State or introduction into the province, thus resulting in a founder effect.

The prevalence of mutations considered to be clinically important was low (2.3%); however, in a supposedly drug-naive cohort, mutations predominated among patients with low CD4+ T-cell counts. The clustering of mutations with low CD4+ T-cell counts suggests that, rather than being transmitted, these polymorphisms had been selected by drug exposure. Transmitted mutations should be more common in recently infected individuals as they frequently revert to wild type over time in the absence of therapy. Chronically infected patients with low CD4+ T-cell counts would be expected to have fewer mutations if transmission was the only source of drug resistance. Our cohort does not comply with WHO guidelines for the identification of transmitted resistance (that is, patients with recent infection, aged <25 years and no history of pregnancy [15,16,25]), and although transmission of mutations is documented in other cohorts [16,24,26–28], a combination of phylogeny and the association with lower CD4+ T-cell counts in our data suggested that transmission was unlikely to be a significant cause of resistance in the Free State.

The Stanford drug resistance database classifies mutations according to drug inducibility, viral phenotype and clinical outcome [12]. In our cohort, the non-

Table 3. Drug-associated mutations in the Free State Cohort

	PI		NRTI		NNRTI							
Category	M46L	N88S	T69N	K219E	A98G	K103N	V106M	V108I	E138K	179D	Y181C	Y188L
Free State Province cohort												
Patients with CD4⁺ T-cell count <100 cells/μl (*n*=192), *n*[a]	1	0	2	1	2	2	1	1	1	2	2	1
Patients with CD4⁺ T-cell count 200–350 cells/μl (*n*=114), *n*[b]	0	0	0	0	0	1	0	0	0	1	0	0
Patients with CD4⁺ T-cell count >500 cells/μl (*n*=91), *n*[c]	0	1	0	0	0	0	0	0	0	0	0	0
Stanford drug resistance database												
Mutation prevalence in drug-naive subtype B patients, (*n*=7,404 for PR, *n*=5,539 for RT), %	0.3	0	0.4	0	0.2	0.3	0	**0.6**	0.2	**2**	0	0
Mutation prevalence in drug-naive subtype C patients (*n*=2,145 for PR, *n*=1,979 for RT), %	0.1	0	0.1	0	**0.5**	0.3	0	0.3	0.2	**0.5**	0.1	0.1
Other HIV-1 subtypes (drug-naive) with mutation prevalence ≥0.5% (subtypes A, D, F, G, AE and AG)	Subtype G (**0.5**%, *n*=619)	No	Subtype AE (**0.5**%, *n*=762)	No	No	No	No	Subtype AG (**1.2**%, *n*=1,025)	No	Subtype D (**4**%, *n*=320), subtype AE (**1.5**%, *n*=762)	No	No
Mutation prevalence in drug-experienced subtype C patients (*n*=282 for PR, *n*=1063 for RT), %	2.8	3.2	4.2	7	8.2	28	13	4	1.3	4.5	11	4.1
Genotype–treatment correlation?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Genotype–phenotype correlation?	1.8–8.2	0.1–8.9	NA	NA	NA	22–51	11–356	NA	NA	NA	1.3–148	4.3–400
Genotype–virological correlation?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes	NA
Mutation grading (Stanford Drug Resistance Database)	Low	Intermediate	Potentially low	Low	Potentially low	High	High	Potentially low	Low	Potentially low	High	High

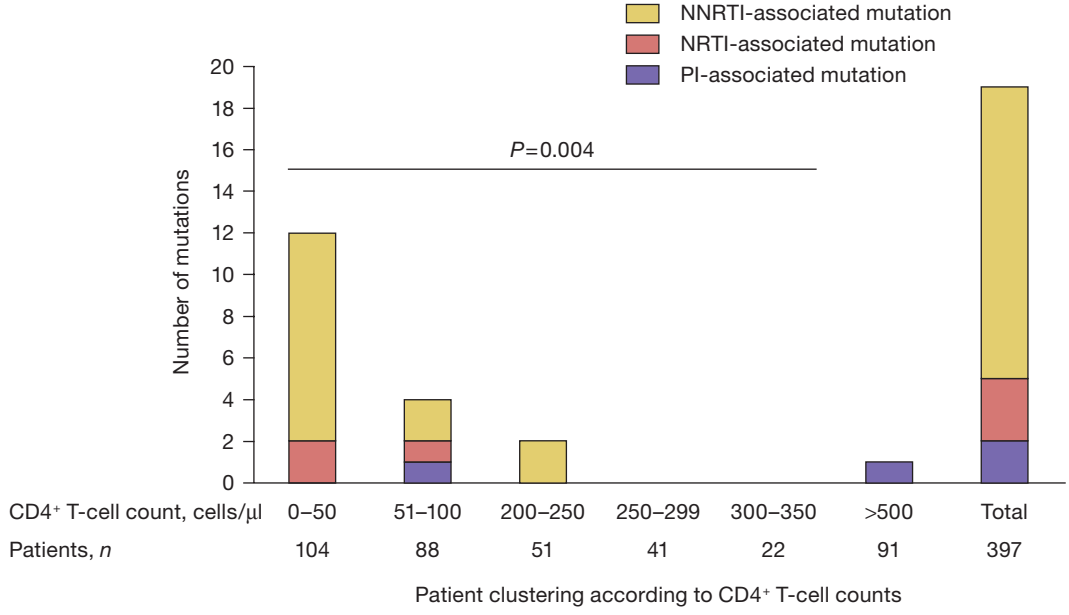
Table 3 shows the non-accessory drug-associated mutations in the Free State cohort and in reference cohorts (values ≥0.5% are in bold). The genotype–treatment correlation row shows whether the mutation is associated with therapy. The genotype–phenotype correlation row shows *in vitro* evidence (fold resistance) for associated phenotypic resistance. The genotype–virological correlation row shows whether mutations have an effect on viral load on therapy. All results are summarized from MARVEL and the Stanford Drug Resistance Database. The significance of each resistance mutation is compared with the drug mutation lists of the International AIDS Society–USA and the World Health Organization. *P*-values were calculated using the χ^2 test for trend, comparing the resistance statistics across the three stratified patient populations. The level of significance for clustering of mutations among low CD4⁺ T-cell counts (χ^2 test for trends) was *P*=0.004 for highly active antiretroviral therapy and *P*=0.005 for non-nucleoside reverse transcriptase inhibitors (NNRTIs). [a]Total mutations =16. [b]Total mutations =2. [c]Total mutations =1. HIV-1, HIV type-1; NA, not applicable; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PR, protease; RT, reverse transcriptase.

accessory mutations not conferring major resistance were T69N, A98G, V179D (all of which were potentially low grade) and E138K (low grade). The WHO TDRM list also excludes these four mutations and one additional mutation (V108I for NNRTI) because they are considered polymorphic, with a pretreatment prevalence of >0.5% in at least one HIV-1 subtype. However, certain mutations excluded by the WHO list are not polymorphic in clade C populations: the prevalence of T69N, V108I and E138K is <0.5% and prevalence of A98G and V179D equals 0.5%. As many of these low-grade resistance mutations are excluded from the IAS–USA and WHO surveillance lists, we have been careful to use the term 'drug-associated mutations' when these mutations were described. We included an analysis with these low grade and potentially low grade mutations, although they are not currently clinically important, because they are selected by therapy and might have a role as surrogate markers of treatment exposure. It is clearly ideal to have a single mutation list to screen all populations; however, the possibility exists that for South Africa, where the dynamics of therapy provision and the more recent introduction of universal access to HAART makes for a unique situation, a subtype C-specific list might become applicable.

Patients were counselled by trained local HIV-1 nurses regarding drug history whether through the prevention of PMTCT, ARV programmes in different provinces, countries and private clinics or other non-governmental sources. A key route of ARV exposure is the nevirapine-only regimen initially used for PMTCT from 2000 [2,5,29]. Those patients with major NNRTI mutations were predominantly women of child-bearing age. Unfortunately, despite contacting or visiting the referring clinics, the demographic details to determine the extent of this effect were not available for this analysis because of loss of follow-up for most of the affected patients. Nevirapine is an inexpensive and effective agent and a key constituent of HAART regimens in resource-limited settings. In our cohort, nevirapine had the highest prevalence of drug-associated mutations. The drug has a long half-life, a simple mutational pathway and is prone to rapid resistance even with the single doses used in PMTCT [5,30,31]. In December 2007, South Africa revised its PMTCT regimen to a combination of zidovudine and nevirapine. Although this measure should improve PMTCT efficacy and reduce the emergence of NNRTI resistance, the logistics of introducing this change might delay its implementation in all areas of South Africa and a proportion of mothers might still receive nevirapine monotherapy.

PMTCT cannot be responsible for all the observed resistance in the cohort – 6 of the 16 patients were men and the age of the females ranged from 25 to 56

Figure 2. Distribution of drug-associated mutations in pre-therapy patients according to CD4⁺ T-cell counts



The plot depicts all pre-therapy patients carrying drug-associated mutations to any of the highly active antiretroviral therapy constituents, including protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), stratified according to CD4⁺ T-cell count. The *P*-value was calculated using the χ^2 test, comparing the mutations across CD4⁺ T-cell counts.

years. These patients predominantly had lower grade mutations, suggestive of exposure but not clinical resistance. It is possible that some individuals had previously received treatment in other clinics, for example, contract workers travelling between provinces. In addition, patients with lower CD4+ T-cell counts are more likely to be symptomatic, to use drugs prescribed to family or friends and might have previously sought medication through other non-government sources [32,33]. At the time of sampling, drugs such as nevirapine, lamivudine, stavudine and didanosine were also available through private practitioners in the Free State, although the duration for which drugs would be prescribed is dependent on the patient's ability to pay [17,34].

In conclusion, this large cohort from South Africa reveals new molecular epidemiological and drug resistance surveillance data. The prevalence of drug-associated mutations among patients is reassuringly low, but the association with low CD4+ T-cell counts is previously undescribed and warrants close monitoring of the virological response when these patients start therapy.

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Disclosure statement

The authors declare no competing interests.

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