Journal of
AIDS & Clinical Research
ISSN: 2155-6113

El-Khatib et al. J AIDS Clinic Res 2011, 2:2
DOI: 10.4172/2155-6113.1000117

Research Article Open Access

Drug Resistance Patterns and Virus Re-Suppression among HIV-1 Subtype C Infected Patients Receiving Non-Nucleoside Reverse Transcriptase Inhibitors in South Africa

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Abstract

Background: Emergence of HIV-1 drug resistance is at times an inevitable and anticipated consequence of antiretroviral therapy (ART) failure. We examined drug resistance patterns and virus re-suppression among subtype C-infected South African patients receiving first-line ART.

Methods: Treatment records of 431 patients on NNRTI-containing regimens for a median of 45 months were analyzed. Patients with viral load (VL) >400 copies/mL were followed and drug resistance mutations (DRM) were re-assessed. Associations between clinical/demographic measures and drug resistance/virologic outcomes were examined using Fisher exact and ordinal and logistic regression.

Results: Ten percent of patients (43/431) were viremic at enrollment (98%) sequences were obtained from 38/43. Of those, 82% had 1-7 DRM. In bivariate analysis remote exposure to single-dose nevirapine or prior ART; higher CD4 counts; lower VL; and >6 months of virologic failure were significantly associated with number of DRM. Of 25 viremic patients followed for a median of 8 months on a continued first-line regimen, 12 (48%) re-suppressed, six with K103N and three with M184V. Thirteen (52%) had continued virologic failure which was significantly associated with detectable VL >6 months prior to enrollment and number of DRM.

Conclusion: Among these HIV-1 subtype C-infected patients, DRM numbers and patterns were associated with prior exposure to sub-optimal ART, adherence and duration of virologic failure. Viral re-suppression in the presence of K103N and M184V challenges assumptions about drug resistance. In resource-limited settings, where genotyping and alternative drug options are unavailable, continuing first-line treatment, reinforcing adherence and regular virologic monitoring may be effective even after virologic failure.

Keywords: HIV-1; Subtype C; Drug resistance; Mutations; NNRTI; First-line ART; South Africa; ART experienced

Background

More than 1 million HIV-1 subtype C infected patients in South Africa are receiving antiretroviral therapy (ART) [43]. In 2004 a national treatment program was initiated, including a first-line regimen containing a non-nucleoside reverse transcriptase inhibitor (NNRTI), either efavirenz or nevirapine, in combination with NRTI, stavudine or zidovudine, and lamivudine [27] The virologic outcomes of first-line regimens among subtype C-infected people in South Africa are comparable to those among subtype B infected patients in Switzerland, where approximately 10% of patients experience virologic failure after 12 months and up to 25% experience virologic failure by two years on ART [20].

Risk factors contributing to virologic failure and drug resistance in sub-Saharan Africa include incomplete adherence [1,11,30,36], treatment interruptions [39,41], low CD4 cell counts [14,15,21,41] low body weight before ART initiation [21] and prior exposure to single dose nevirapine (sdNVP) for prevention of mother-to-child transmission (pMTCT) and/or dual nucleoside treatment [6,22,25]. The majority (>80%) of viremic patients harbor drug resistance mutations (DRM) [2,11,14,28,33] and maintaining a failing ART regimen can lead to accumulation of DRM [14,21,32] and increased ART cross-resistance [5,18].

Five recent Southern African studies among NNRTI recipients identified treatment failure by virologic or immunologic criteria [11,14,28,33,42]. The prevalence of DRM ranged from 62% to 95% [14,15,28,33,42]. In the first year of treatment Marconi et al. [28] in KwaZulu-Natal (n=115) and Orrell et al. [33] in Cape Town (n=110), identified DRM among 83% and >87% respectively. In longer term studies, Hoffmann et al. [3,14] in South Africa (n=68) and Hosseinipour et al. [15] in Malawi (n=94) reported DRM after a median >36 months among 62%, and 95% respectively. Wallis et al. [42] in Johannesburg

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Received January 12, 2011; **Accepted** February 15, 2011; **Published** February 18, 2011

Citation: El-Khatib Z, DeLong AK, Katzenstein D, Ekstrom AM, Ledwaba J, et al. (2011) Drug Resistance Patterns and Virus Re-Suppression among HIV-1 Subtype C Infected Patients Receiving Non-Nucleoside Reverse Transcriptase Inhibitors in South Africa. J AIDS Clinic Res 2:117. doi:10.4172/2155-6113.1000117

reported DRM among 84% of 226 viremic patients, however duration of treatment was not reported.

Previously [11], we surveyed viremia and drug resistance prevalence among 998 patients in Soweto, South Africa and found that 94/883 (11%) receiving first-line regimens for a median of 42 months were viremic and 78/94 (83%) had drug resistance. Here, we obtained retrospective data on 431 of these patients, enrolled at a single clinic, and examined factors associated with the evolution and patterns of DRM. Additionally, we followed 25 of these viremic patients to explore the implications of DRM on continued NNRTI-based treatment.

Methods

Study sites and patients

The study was done at the Perinatal HIV Research Unit (PHRU), a non-governmental organization (NGO) research clinic in Chris Hani Baragwanath Hospital, Soweto, outside Johannesburg, South Africa(11, 29). At the time of the study, the clinic staff consisted of five medical doctors, two nursing assistants and two counselors managing around 1500 ART recipients, with 50 daily visits [11].

Consenting patients were enrolled from March through September 2008 if they were ≥18 years old and >12 months on a first-line regimen [11]. At study enrollment, viral load (VL), CD4 cell count and HIV-1 reverse transcriptase (RT) genotype were assessed and basic demographic information obtained. Virologic failure was defined as VL>400 copies/mL at study enrollment [11]. Persistent virologic failure and re-suppression were defined as VL>400 copies/mL and return to ≤ 400 copies/mL, respectively, at follow-up (Figure 1).

Medical records were reviewed retrospectively to extract information on potential risk factors that may be associated with DRM or re-suppression, including age, gender, year of HIV diagnosis, ART regimens and dates, history of sdNVP or other ART exposure, tuberculosis (TB) treatment, pre-ART initiation VL and CD4 cell counts, WHO stage prior to ART initiation and any treatment interruptions in the last six months prior to study enrollment. Virologic failure prior to study enrollment, was defined as VL>400 copies/mL at either of the prior two visits in the last 12 months. Poor adherence was considered to be returning more than seven days late for the drug refill appointment pre-study enrollment. An instrument was designed and tested to extract information from medical records using Epi Data [9,10]. The study and consent forms were approved by the University of the Witwatersrand Human Research Ethics Committee in South Africa and the Regional Medical Ethics Board in Stockholm, Sweden.

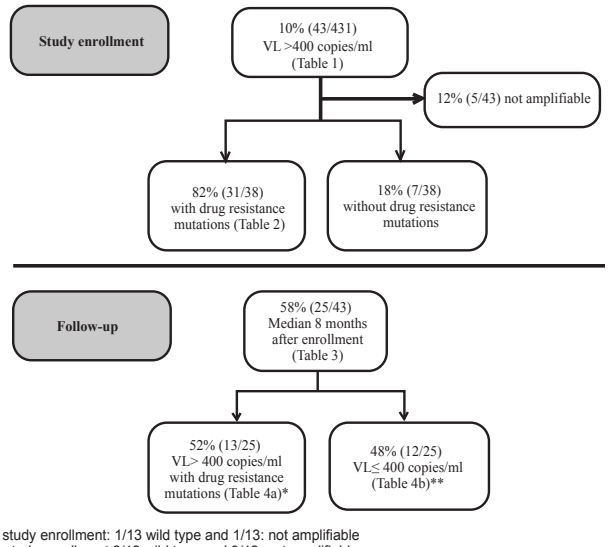

Figure 1: Study design

Laboratory assessments

CD4 cell counts were performed by FACSCountTM (Becton Dickinson BioSciences, Immunocytochemistry Systems, San Jose, California, USA) and VL was measured using the Roche Amplicor, version 1.5 (Roche Molecular Diagnostics, Basel, Switzerland) with a lower limit of detection of 400 copies/mL. Both assays were performed at the National Institute for Communicable Diseases (NICD), Johannesburg. VL data extracted from medical records that were done as part of routine clinical care used the Versant HIV-1 RNA 3.0 (Siemens Deerfield, IL, USA) bDNA technology. For HIV-1 drug resistance testing, an in-house genotyping assay was performed at the NICD [35].

Drug resistance mutations and susceptibility scoring

Mutations were identified by the Stanford HIVdb genotypic resistance algorithm [13] and coded as major DRM as defined by the International AIDS Society (IAS) December 2009 list [16]. Subtype was established using the Rega subtyping tool v.2.0, which incorporates rigorous phylogenetic analyses [7]. Sequence quality was confirmed prior to analysis by[18] inspecting sequences for possible frame shifts, high numbers of ambiguous nucleic and/or amino acids, extreme levels of pair-wise genetic distances, and unique amino acids or stop codons. To predict phenotypic drug resistance the Stanford HIV database (HIVdb) scoring system was applied [13] and a resistance score calculated as (i) susceptible (0-9) to potentially low-level (10-14); (ii) low (15-29); (iii) intermediate (30-59); and (iv) high level of resistance (score ≥ 60).

Data analysis and statistics

Risk factors were examined for associations with two drug resistance outcomes: (i) number of all-class DRM at enrollment; and (ii) continued viremia versus re-suppression at follow-up. Associations between viral re-suppression and presence of any DRM, number of NRTI and NNRTI DRM and the total number of DRM at enrollment were examined. Due to the small sample size, bivariate analysis was performed without adjusting for confounding variables and the results must be interpreted with this in mind.

Ordinal logistic regression was used to examine the association between risk factors and the number of DRM at enrollment expressed as odds ratios (OR), and 95% confidence intervals (95% CI). Unlike Poisson regression, ordinal logistic regression can be fitted to zero-inflated data and does not assume that the events (i.e. accumulation of DRM) are independent and occur at a constant rate. Each model was checked to ensure the assumption of proportional odds between successive DRM categories was met. To examine risk factors associated with persistent virologic failure at follow-up, Fisher exact tests were used for categorical risk factors (OR, 95% CI) and Wilcoxon rank sum tests for continuous risk factors (difference in median, 95% CI). Analysis was performed using Stata/SE College Station, Texas (version 10.1) (38) and R (version 2.11.1) (40). P-values less than 0.05 were considered statistically significant.

Results

Patient characteristics

(Table 1) displays characteristics of the 431 patients who had been on ART for at least 12 months where 75% were females, 96% were born in South Africa, 90% had above primary school education and the median age at study enrollment was 38 years. Ninety-one percent were receiving efavirenz-based therapy and 9% a nevirapine-based therapy.

Before ART initiation, the median VL and CD4 were 71,995 (range 1,078 to >500,000) copies/mL and 93 (range 1 to 444) cells/mm3 respectively. At study enrollment, patients had received ART for a median of 45 months (range 13 to 152) and the CD4 cell count increased to 419 (range 16 to 1,270).

Forty-three patients (10%) had VL>400 copies/mL (median 6,510; range 407 to >500,000) at enrollment, almost all (98%) previously suppressed on ART. Median time on ART was similar among patients with VL < and >400 copies/mL (p=0.86). However at study enrollment, those with VL>400 copies/mL had a significantly lower CD4 cell count compared to patients with VL<400 copies/mL (p<0.01).

Among 40/43 patients for whom there was a record of previous ART exposure: 8/31 (26%) females had received sdNVP prior to initiating ART and 5/40 (13%) patients had been exposed to other ART before initiating the current ART regimen. Compared to unexposed individuals, there was a borderline association between viremia at study enrollment and exposure to sdNVP (p=0.05).

Drug resistance mutations at study enrollment

Thirty-eight of 43 samples were successfully genotyped; 31/38 (82%) had at least one DRM and 24 (63%) had ≥3 DRM (Table 2). Of the genotyped samples 5/38 (13%) had K103N alone; 2/38 (5%) had M184V and K103N; and 10/38 (26%) had three mutations with M184V/I, K103N and an additional NNRTI mutation. Finally 14/38 (37%) had ≥4 mutations: 13/14 (93%) M184V/I and 8/14 (57%) K103N, all with ≥2 NNRTI and most with one or more thymidine analogue mutations (TAM) or other NRTI mutations. Overall, 8/38 (21%) had one or more TAMs, three had A62V or V75I and only one patient had K65R.

Several risk factors were significantly associated with increased numbers of DRM (Table 3). Patients with prior exposure to either sdNVP or other ART had more mutations than those not previously exposed. The ordinal regression OR was 3.8 (95%CI 1.1 to 15.2; p=0.03), i.e. it was 3.8 times more likely for patients with prior ART exposure to have ≥1 vs. 0, ≥2 vs. ≤1, ≥3 vs. ≤2 DRM and so on. Number of DRM was positively associated with being female (OR 5.6; 95%CI 1.3 to 24.5; p=0.02), having a higher CD4 cell count (OR 1.7 per 100 CD4 cells; 95%CI 1.1 to 2.7; p=0.02) and having detectable VL at one of two earlier scheduled visits (OR 8.4; 95%CI 1.9 to 42.4; p<0.01). The association with gender was mainly explained by prior exposure to pMTCT, mainly sdNVP, among the women. Only one male had any prior exposure to ART. The number of DRM was negatively associated with coming late for the drug refill visit in the last month (OR 0.1; 95%CI 0 to 0.5; p=0.01) and with VL such that for participants with 1-log unit higher VL the odds of having a higher number of DRM was 0.5 (95%CI 0.2 to 1.0; p=0.04). Finally the median VL of the seven patients with no DRM was 83,000 copies/mL compared with a median VL of 6,510 copies/mL among those with at least one DRM, providing evidence for existing but incomplete drug pressure amongst those with DRM.

Risk factors for persistent virologic failure and drug resistance mutations at follow-up

Follow-up data and samples were available for 25/43 (58%) of the viremic patients, after a median of 8 (range 4 to 10) months (Figure 1). Persistent virologic failure at follow-up, in 13/25 (52%) patients, was associated with a detectable VL in the two visits prior to study enrollment (p<0.01) and the number of DRM at study enrollment (OR 2.36; 95%CI 1.11 to 5.02; p=0.04), particularly NRTI mutations (OR 3.68; 95%CI 1.11 to 12.17; p=0.05) (Table 3). All 13 patients had genotypic resistance with six additional DRM acquired at follow-up, leading to high level predicted resistance to efavirenz and/or nevirapine (100%) and lamivudine (100%) and intermediate to low predicted resistance to etravirine in 7/13 (54%) (Table 4a). Although viremic, patient number 35 did not have any DRM at study enrollment, but with continued treatment and presumably better adherence, three DRM were selected at follow-up with a persistent, albeit lower, VL for nine months. At study enrollment nine of these patients had failed first-line regimens with full predicted susceptibility to NRTIs.

Twelve of the 25 (48%) patients re-suppressed at follow-up, after a median of eight additional months on treatment with the same NNRTI (mostly efavirenz)-based regimen. Comparison of clinical and laboratory characteristics of these 12 patients with the 13 who had persistent virologic failure showed no significant differences in sex, median CD4 and VL prior to ART initiation. At study enrollment 3/12 (25%) re-suppressed patients could not be amplified and three (25%) had no DRM. However, the remaining six patients who were re-suppressed had NNRTI DRM, three had K103N, one had K103N and M184V and one had K103N, V106M and M184V (Table 4b). The sixth patient had three NNRTI and 3 NRTI mutations. Thus, six patients with high level NNRTI resistance and three patients with high level NNRTI and lamivudine resistance achieved re-suppression while continuing the same first-line regimen.

Discussion

HIV-1 drug resistance is a potential cause and is often a consequence of virologic failure. In this study, we examined drug resistance in HIV-1 subtype C infected South African patients failing first-line regimens after a minimum of 12 months on ART. Of 43 long-term ART recipients with viremia, most had multiple DRM. Among 25 of these patients followed on continued NNRTI-based ART, 12 achieved virologic suppression and 13 had persistent VL>400 copies/mL. We examined the characteristics and patterns of DRM, the estimated drug resistance and implications for further therapies among these ART experienced, subtype C infected patients.

In examination of co-occurrence of DRM, 82% of 38 available RT sequences demonstrated a wide spectrum (range 1 to 7) of co-occurring, DRM. The majority (63%) had ≥3 DRM, mostly including K103N accompanied by M184V/I, at times with complex mixtures of additional NRTI and NNRTI mutations. Patients with prior ART drug exposure and those with detectable VL and higher CD4 cell counts prior to or at study enrollment, respectively, tended to have a higher number of DRM. These results are likely to reflect the selection of drug resistance due to lapses in adherence, reduced drug exposure and inadequate drug pressure. Conversely, patients who were late to pharmacy or who had higher VL at study enrollment tended to have fewer DRM, perhaps reflecting a very low adherence.

Drug resistance mutations among subtype C NNRTI recipients have been identified after virologic or immunologic failure based on

	All patients N=431	Suppressed at study enrollment N=388	Viremic at study enrollment N=43	p value
Female	325 (75%)	291 (75%)	34 (79%)	0.56
Age, median in years (range)	38 (21, 64)	38 (21, 64)	36 (25, 60)	0.35
Born in South Africa	414 (96%)	373 (96%)	41 (95%)	0.80
Education				
Never been to school	7 (2%)	6 (2%)	1 (2%)	0.64
Primary school	42 (10%)	39 (10%)	3 (7%)	
Secondary school	357 (83%)	319 (82%)	38 (88%)	
Tertiary school	25 (6%)	24 (6%)	1 (2%)	
In any form of a relationshipa				
Yes	250 (58%)	227 (59%)	23 (54%)	
No	181 (42%)	161 (41%)	20 (46%)	0.53
Months on ART, at study enrollment (range)	45 (13, 152)	45 (13, 152)	45 (13, 55)	0.86
ART regimen				
Efavirenz containingb	393 (91%)	357 (92%)	36 (94%)	
Stavudine containingc	254 (59%)	225 (58%)	29 (67%)	0.35
VL prior to starting ART				
Median copies/mL (range)d	70 870 (1 078, >500 000)	67 315 (2 102, >500 000)	86 718 (1 078, >500 000)	0.48
CD4 prior to starting ART				
Median cells/mm3 (range)	93 (1, 444)	95 (1, 760)	107 (6, 314)	0.34
Mean	96	104	109	0.74
CD4 at study enrollment				
Median cells/mm3 (range)	419 (16, 1 270)	437 (119, 1 270)	276 (16, 642)	<0.01
Prior exposure to ART				
No	335 (81%)	308 (82%)	27 (68%)	
PMTCT only	45 (11%)	37 (10%)	8 (20%)	0.05f
Othere	35 (8%)	30 (8%)	5 (13%)	0.21
Exposure to sdNVP or other ART vs. no exposuree	80 (19%)	67 (18%)	13 (32%)	0.03f
WHO stage prior to starting ART				
I	61 (21%)	55 (20%)	6 (25%)	
II	86 (29%)	82 (31%)	4 (17%)	0.32f
III	137 (47%)	123 (46%)	14 (58%)	1.00f
IV	9 (3%)	9 (3%)	0 (0%)	---

ART, antiretroviral therapy; VL, virus load; WHO, world health organization, PMTCT, prevention mother-to-child-transmission by administrating a single dose nevirapine; N, number

[a]Yes: cohabitation, married, sexual relationship, No: Single/divorced/separated/widow
[b]The other drug option was nevirapine
[c]The other drug option was zidovudine
[d]VL done using Versant HIV-1 RNA 3.0 (Siemens Deerfield, IL, USA) bDNA technology
[e]Exposure to other antiretroviral drugs prior to ART initiation
[f]2-sided Fisher exact test

Table 1: Demographic, clinical and laboratory characteristics of patients

Number of drug resistance mutations (n pts)	Months on ART	Sex	VL at study enrollment (copies/mL)	CD4 at study enrollment (cells/mm3)	NNRTI	NRTI
≥5 (n=8 pts)						
1*	48	F	3 010	524	K101E+V108I+Y181C	D67N+K70R+M184V+K219E
2**	49	F	2 680	213	K101H+K103N+V106M+G190A+F227L	D67N+M184V
3*,^	41	F	4 310	354	K101E+V106M+G190A	D67N+M184V
4	44	F	75 000	89	K103N+P225H	D67N+K70R+M184V+T215F+K219E
5*	22	F	1 850	342	K103N+V108I	A62V+V75I+M184V
6*	45	F	773	384	V106M+G190A	M41L+D67N+K70R+M184V
7*	44	F	2 530	199	V106M+Y188LH	D67N+M184V+ K219E
8	45	F	18 200	496	V106M+Y188L	M41L+D67N+K70R+ M184V+T215Y
4 (n=6 pts)						
9	15	F	16 900	160	K101E+V108I+G190A	M184V
10	45	F	11 000	464	K101H+K103N+G190A	M184V
11	46	F	3 000	354	K103N+P225H	V75I+M184V

12*	37	M	49 900	16	K103N+Y181C+P225H	M184I
13^	47	F	54 700	419	K103N+G190A+P225H	M184V
14	47	F	9 670	377	K103N+V106A+G190A	K65R
3 (n=10 pts)						
15*	48	F	18 300	331	K101E+Y188L	M184I
16	13	F	6 890	214	K101E+V106M	M184V
17	41	F	5 340	319	K103N	D67N+M184V
18*	46	F	407	269	K103N+P225H	M184V
19	47	F	6 510	190	K103N+V108I	M184V
20*	47	F	1 330	277	K103N+V108I	M184V
21	47	M	31 000	99	K103N+V108I	M184V
22**	47	F	1 280	642	K103N+V106M	M184V
23*	40	F	955	276	V106M	A62V+M184V
24	84	F	43 800	174	V106M+Y188C	M184V
2 (n=2 pts)						
25	43	M	430	151	K103N	M184V
26**,^	47	F	1 370	320	K103N	M184V
1 (n=5 pts)						
27**	44	M	178 000	47	K103N	---
28**	46	F	56 500	187	K103N	---
29**	45	F	42 800	100	K103N	---
30*	45	M	1 130	439	K103N	---
31^	46	F	43 900	193	K103N	---
32	46	F	552	369	No major mutations	No major mutations
33**	41	F	882 000	90	No major mutations	No major mutations
34**	47	F	1 450	192	No major mutations	No major mutations
35*,^	43	M	83 000	103	No major mutations	No major mutations
36^	47	F	77 500	232	No major mutations	No major mutations
37**,^	43	M	312 000	203	No major mutations	No major mutations
38^	50	M	493 000	157	No major mutations	No major mutations
Not amplifiable (n=5 pts)						
39	44	F	463	357	---	---
40*,^	43	F	4 660	554	---	---
41**,^	41	M	1 060	430	---	---
42**	13	F	557	445	---	---
43**	55	F	66 800	379	---	---

Pts, patients; F, female; M, male; ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI, VL: virus load; *Persistent virologic failure at follow-up; ** Re-suppressed at follow-up; ^ Came late >7 days for the last drug-refill visit

Table 2: Patterns of reverse transcriptase drug resistance mutations

different clinical guidelines. Here, the frequency of DRM upon virologic failure using a threshold of >400 copies/mL was similar to thresholds of VL >1,000 or >5,000 copies/mL [14,28,33,42] or immunologic failure criteria (8, 15). We compared the patterns of DRM found here to 418 published sequences from adult patients failing first-line regimens in subtype C studies, mostly from Southern Africa and India (8, 15, 19, 28, 35, 37, 42), accessed, in July 2010 at the Stanford HIV Sequence Database (13). Patterns of DRM, including the overall frequency of any DRM (82% in the current study vs. 83% among published sequences), ≥1 NRTI resistance mutations (11% vs 9%), the prevalence of K103N (55% vs 42%), M184V/I (66% vs. 74%), and K65R (3% vs. 6%) were not significantly different. However, the data presented here compared to the published sequences demonstrated significant differences in the frequency of ≥1 NNRTI mutation (58% vs 40%, p=0.03) and a lower rate of TAM (21% vs 37%, p=0.05). These modest differences may be ascribed to differences in clinical management strategies, specific drug combinations or duration of virologic failure. Among eight patients with TAMs, the most common pathway (seen in 6/8 patients) was TAM-2 related (D67N, K70R, K219Q/R/E),; and the rest (2/8) were mixed with the TAM-1 pathway (M41L, L210W, T215Y) extending similar prior Southern African observations [28,31] .

Although K103N and other NNRTI resistance mutations confer high level NNRTI resistance [12,13] , six patients in our study who harbored such mutations, re-suppressed VL after 4 to 10 months with no change in their first-line regimen. Three of these patients had high level resistance to two drug classes, with the addition of the M184V mutation, conferring resistance to lamivudine. These findings extend observations by Hoffmann and colleagues who reported 11 males with either NNRTI and NRTI mutations, who re-suppressed with continued first-line regimens, raising questions about potential cautious re-use or continuation of those medications in certain circumstances [14] . It is plausible that improved adherence was a factor in this observed resuppression, though this could not be confirmed in this patient population. The observation that successful re-suppression was strongly associated with recent failure and a low number of DRM seems logical, but should be confirmed prospectively in larger studies.

Thirteen patients found to be viremic both at enrollment and at

	Number of DRM at study enrollment (n=43)		Persistent virologic failure (n=13) vs. re-suppression (n=12) at follow-up	
Risk factor	**Odds ratio (95% CI)[a]**	**p-value**	**Effect (difference in median, 95% CI 95% CI)[b]**	**p-value**
Female vs. Male	5.6 (1.3, 24.5)	**0.02**	1.1 (0.1, 10.5)	1.00
Age (years)	1.0 (0.9, 1.0)	0.17	1.4 (-6.0, 9.0)	0.62
Current TB therapy (yes/no)[c]	-	-	0.9 (0, 78.4)	1.00
Tested HIV positive after 2003 (yes/no)	0.4 (0.1, 1.8)	0.23	0.7 (0.1, 5.5)	1.00
Number of months on ART	1.0 (0.9, 1.0)	0.16	2.1 (-1.4, 6.4)	0.17
ARV exposure prior to ART initiation[d]		-		0.78
none			reference	
pMTCT	---	---	0.3 (0, 4.3)	0.59
Other ARV	---	---	0.8 (0, 70.5)	1.00
Exposure to sdNVP or other ART vs. no exposure[d]	3.8 (1.1, 14.2)	**0.03**	0.42 (0, 3.8)	0.64
CD4 at study enrollment per 100 cells/mm^3	1.7 (1.1, 2.7)	**0.02**	-0.7 (-1.9, 1.0)	0.41
CD4 pre-ART initiation per 100 cells/mm^3	0.8 (0.3, 2.1)	0.64	0 (-0.6, 0.5)	0.98
Log_{10} (VL) at study enrollment[e]	0.5 (0.2, 1.0)	**0.04**	0.4 (-0.2, 1.6)	0.27
Log_{10} (VL) pre-ART initiation[e]	1.3 (0.5, 3.4)	0.60	-0.4 (-1.0, 0.2)	0.22
VL >400 copies/mL at least once in the two tests prior to study enrollment (yes/no)[e]	8.4 (1.9, 42.4)	**<0.01**	infinity (2.4, infinity)	**<0.01**
Late to the drug refill visit at least 7 days in previous month (yes/no)	0.1 (0, 0.5)	**0.01**	0.9 (0.1, 8.6)	1.00
History of treatment interruption in the last 6 months (yes/no)[c]	---	---	infinity (0.2, infinity)	0.48
Taking d4T vs. AZT	0.6 (0.2, 1.9)	0.36	0.3 (0, 2.8)	0.38
Taking EFV vs. NVP[c]	---	---	1.1 (0.1, 17.8)	1.00
≥1 DRM (yes/no)			5.1 (0.3, 313.7)	0.27
Number of NRTI DRM mutations			3.7 (1.1, 12.2)	**0.05**
Number of NNRTI DRM mutations			2.9 (0.9, 9.0)	0.08
Total number of DRM mutations			2.4 (1.1, 5.0)	**0.04**

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; VL, virus load; TB, tuberculosis; DRM, drug resistance mutations; d4T, stavudine; AZT, zidovudine; EFV, efavirenz; NVP, nevirapine; ARV, antiretroviral drugs

[a]For each 1-unit difference in the covariate (e.g. going from no to yes) the odds ratio expresses (for each possible value of the # DRM (0-7)) the ratio of the odds of having at least that number of DRM versus fewer than that number of DRM.

[b]Effects presented as the odds ratio for categorical risk factors and the difference in medians for continuous risk factors (difference in median, 95% CI).

[c]The ordinal logistic regression model could not be fitted because there was too little variability in the risk factor or the assumption of proportional odds ratios was not met (i.e. only 4 were on TB therapy, 4 had taken other ARV prior to starting ART, 3 had treatment interruption, and the assumption was not met for EFV vs. NVP.

[d]One female was exposed to both of sdNVP and other ARV, pre-ART initiation, and she had VL <400 copies/mL at study enrollment.

[e]VL done using Versant HIV-1 RNA 3.0 (Siemens Deerfield, IL, USA) bDNA technology

Table 3: Risk factors associated with the number of DRM at study enrollment and persistent virologic failure versus re-suppression at follow-up

follow-up, had a longer duration of virologic failure on treatment and a higher number of DRM. These findings substantiate the observation that resistance evolves as a function of continued, albeit suboptimal, drug pressure due to reduced adherence, treatment interruptions or both. Despite the relatively short time between sequences - 8 months, and a median of 3 DRM per patient, mutations accumulated with a rise in high level predicted 2-class resistance. This is consistent with observations in HIV-1 subtype B [5,18]. Some of the accumulated DRM were associated with etravirine resistance, conferring intermediate resistance to this drug after first-line regimen failure, suggesting the need for further studies of the use of this NNRTI in subsequent regimens [23].

Among women, we found a borderline association between exposure to sdNVP and virologic failure, albeit years afterwards and with a period of suppression, consistent with the results of a recent study from the Western Cape, South Africa [6] and another report of a significant association between detection of minority NNRTI mutations and treatment failure, even after 18 months had elapsed since sdNVP [4] . These findings are not entirely in line with other reports

	Study enrollment					Follow-up					
				Predicted phenotypic resistance						Predicted phenotypic resistance	
Patient	**VL (ART used)***	**NNRTI mutations**	**NRTI mutations**	**NNRTI**	**NRTI**	**Months after enrollment**	**VL (ART used)***	**NNRTI mutations**	**NRTI mutations**	**NNRTI**	**NRTI**
1	3,010 (1b)	K101E+ V108I+ Y181C	D67N+ K70R+ M184V + K219E	H: NVP I: EFV; ETR	H: 3TC I: AZT L: d4T; ABC PL: ddI S: TDF	7	3,680 (1b)	K101E+ Y181C	D67N+ K70R+ M184V+ K219E	H: NVP I: EFV ETR	H: 3TC I: AZT L: d4T; ABC PL: ddI S: TDF
3	4,310 (1a)	K101E+ V106M+ G190A	D67N+ M184V	H: EFV; NVP I: ETR	H: 3TC L: ABC PL: ddI S: d4T; AZT TDF	11	3,790 (1a)	K101E+ V106M+ G190A	D67N+ **K70R**+ M184V+ **K219Q**	H: EFV; NVP I: ETR	H: 3TC I: AZT L: d4T; ABC PL: ddI S: TDF
5	1,850 (1a)	K103N+ V108I	A62V+ V75I+ M184V	H: EFV; NVP S: ETR	H: 3TC L: ddI; ABC S: d4T; AZT TDF	6	6,150 (1a)	K103N+ V108I	A62V+ V75I+ M184V	H: EFV; NVP PL: ETR	H: 3TC L: ddI; ABC S: d4T; TDF AZT
6	773 (1a)	V106M+ G190A+ *A98G*	M41L D67N+ K70R+ M184V	H: EFV; NVP L: ETR	H: 3TC I: d4T, ABC AZT L: TDF ddI	9	4,530 (1a)	*A98G*+ V106M+ G190A	D67N+ K70R+ M184V+ **K219Q**	H: EFV; NVP L: ETR	H: 3TC I: AZT L: d4T ABC PL: ddI S: TDF
7	2,530 (1a)	V106M+ Y188LH	D67N+ M184V + K219E	H: EFV; NVP L: ETR	H: 3TC L: d4T; ABC AZT PL: ddI S: TDF	12	25,000 (1a)	***V90I***+ V106M+ Y188L	M184V	H: EFV; NVP L: ETR	H: 3TC PL: ABC S: d4T; TDF ddI; AZT
12	49,900 (1a)	K103N+ Y181C+ P225H	M184I	H: EFV; NVP I: ETR	H: 3TC PL: ABC S: d4T; TDF AZT	6	8,540 (1a)	***V90I***+ K103N+ Y181C+ P225H	M184V	H: EFV; NVP I: ETR	H: 3TC PL: ABC S: d4T; TDF AZT; ddI
15	18,300 (1a)	K101E+ Y188L	M184I	H: EFV; NVP L: ETR	H: 3TC PL: ABC S: d4T; TDF ddI; AZT	9	3,710 (1a)	K101E+ Y188L	M184V	H: EFV; NVP L: ETR	H: 3TC PL: ABC S: d4T; TDF ddI; AZT
18	407 (1a)	K103N+ P225H	M184V	H: EFV; NVP L: ETR	H: 3TC PL: ABC S: d4T; TDF DDI; AZT	10	937 (1b)	K103N	M184V	H: EFV, NVP S: ETR	H: 3TC PL: ABC S: d4T; TDF ddI; AZT
20	1,330 (1a)	K103N+ V108I	M184V	H: EFV; NVP S: ETR	H: 3TC PL: ABC S: d4T; DDI TDF; AZT	10	3,010 (1a)	K103N+ V108I	M184V	H: EFV; NVP S: ETR	H: 3TC PL: ABC S: d4T; TDF ddI; AZT
23	955 (1b)	V106M	A62V+ M184V	H: EFV; NVP PL: ETR	H: 3TC PL: ABC S: d4T; TDF DDI; AZT	11	2,540 (1b)	V106M+ **E138A**	A62V+ M184V	H: EFV; NVP L: ETR	H: 3TC PL: ABC S: d4T; TDF ddI; AZT
30	1,130 (1a)	K103N	---	H: EFV; NVP S: ETR	S: all drugs	9	15,700 (1a)	K103N	V75I	H: EFV; NVP S: ETR	PL: d4T; ddI S: 3TC; TDF ABC; AZT
35	83,000 (1a)	Wild type	Wild type	S: all drugs	S: all drugs	9	2,670 (1a)	V106M	K70R+ M184V	H: EFV; NVP PL: ETR	H: 3TC PL: ABC; AZT S: d4T; TDF; ddI
40	4 660	Not amplifiable	Not amplifiable	---	---	9	31,300	K103N+ P225H	V75I+ M184V	H: EFV; NVP L: ETR	H: 3TC L: ABC; DDI S: d4T; AZT; TDF

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; VL, virus load (copies/mL); ART, antiretroviral therapy; EFV, Efavirenz; NVP, Nevirapine; ETR, Etravirine; 3TC, Lamivudine; d4T, Stavudine; TDF, Tenofovir; ABC, Abacavir; AZT, Zidovudine; DDI, Didanosine; S, Susceptible; PL, potentially low-level of resistance; L, low-level resistance; I, intermediate level of resistance; H, High level of resistance; Line 1a, EFV + 3TC + d4T; Line 1b, NVP + 3TC + d4T

Underlined mutations = lost mutations; Bolded mutations=gained mutations and Italic mutations=Minor mutations, for Etravirine, according to the IAS USA-2009 list.

Background colors for predicted phenotypic resistance were: White if susceptible or potentially-low; Light gray if low or intermediate and dark gray if highly resistant.

Table 4a: Genotypic and predicted phenotypic drug resistance among patients with persistent virologic failure at follow-up

	Routine clinical VL testing		Study enrollment				Routine clinical VL testing		Study follow-up	
					Predicted phenotypic resistance					
Patient	N months before enrollment	VL (ART used)*	VL (copies/mL)	Mutations	NNRTI	NRTI	N months after study enrollment	VL (copies/mL)	N months of last VL test after study enrollment	VL (copies/mL)
2	6	535 (1a)	2 680	D67N+ K101H+ V106M+ M184V+ G190A+ F227L	H: EFV; NVP	H: 3TC S: d4T	6	982	10	≤400
22	2	42,187 (1a)	1 280	K103N+ V106M+ M184V	H: EFV; NVP	H: 3TC S: d4T	6	64,312	9	≤400
26	7	≤400 (1a)	1 370	K103N+ M184V	H: EFV; NVP	H: 3TC S: d4T	6	≤400	9	≤400
27	Treatment was interrupted for 10 months	NA (1a)	178 000	K103N	H: EFV; NVP	S: 3TC; d4T	NA	NA	5	≤400
28	6	191 (1a)	56 500	K103N	H: EFV; NVP	S: 3TC; d4T	4	≤400	7	≤400
29	3	19,653 (1a)	42 800	K103N	H: EFV; NVP	S: 3TC; d4T	3	≤400	10	≤400
33	7	≤400 (1b)	882 000	Wild type	S: EFV; NVP	S: 3TC; d4T	NA	NA	6	≤400
34	2	20,758 (1b)	1 450	Wild type	S: EFV; NVP	S: 3TC; d4T	NA	NA	4	≤400
37	7	≤400 (1a)	312 000	Wild type	S: EFV; NVP	S: 3TC; d4T	3	≤400	8	≤400
41	6	≤400 (1a)	1 060	Not amplifiable	---	---	6	≤400	9	≤400
42	4	≤400 (1a)	557	Not amplifiable	---	--	1	≤400	8	≤400
43	4	≤400 (1a)	66 800	Not amplifiable	---	--	3	≤400	7	≤400

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; VL, virus load (copies/mL); TB, tuberculosis; DRM, drug resistance mutations; Line 1a, Efavirenz (EFV) + Lamivudine (3TC) + Stavudine (d4T); Line 1b, Nevirapine (NVP) + 3TC + d4T; Colors for predicted phenotypic resistance were: White if susceptible (S) or potentially-low (PL); Light gray if low (L) or intermediate (I) and dark gray if highly (H) resistant.

Table 4b: Characteristics of patients with virologic failure at study enrollment and re-suppression at follow-up

from sub-Saharan Africa and Asia that suggest that in the short-term, administration of ART >12 months after sdNVP may not jeopardize the efficacy of NNRTI based ART [17,26].

Where genotyping is routinely available, the interpretation of DRM following the failure of first-line regimens drives decisions about clinical management, adherence counseling, switching to second-line regimens and the need for new ART combinations. Among these HIV-1 subtype C infected patients, a proportion of those failing first-line regimens responded to continuing their current regimen despite predicted high-level resistance. In resource-limited settings, where genotyping is not available, the practice of reinforcing adherence, continuing a first-line regimen and repeating a VL test after 6 months may be justified. However, it will be important to monitor such patients closely to determine if they ultimately fail due to the presence of archived resistance mutations. In such cases, switching to a second-line regimen, as mandated by treatment national guidelines, should not be deferred.

There are several limitations to this study. First, the number of patients with drug resistance, longitudinal sequences and re-suppression was relatively small and longer term CD4 cell counts and VLs from 25 failing patients were not available. Second, a small proportion of patients' samples, particularly among those who resuppressed, could not be successfully amplified and sequenced. Third, genotyping at onset of ART was not available. However, the prevalence of transmitted ART resistance, in South Africa, is still low (<5%) [24,34] . Finally, confirmation of VL was not immediately available, and clinical practice included continued first line-treatment for 4-6 months between detection of virologic failure, reinforcing adherence and re-testing VL. The fact that patients were not switched to second-line regimens, despite repeated virologic failure, reflects the current limited treatment options in this setting.

In summary, this study extends observations of the range of DRM patterns among HIV-1 subtype C patients in South Africa receiving long-term first-line regimens. Surprisingly, a number of patients with DRM that predict high-level resistance, K103N with or without M184V, were successfully re-suppressed on the same first-line regimen. Reinforcing adherence without changing treatment among

patients with first-line virologic failure could spare the expense and toxicity of second-line regimens in resource-limited settings with high HIV burdens. While additional studies are needed to confirm these observations and examine their longevity, detection of viremia on treatment should prompt repeat testing, adherence counseling and, if viremia is persistent, provision of second-line therapy.

Acknowledgments

We would like to thank patients for agreeing to participate, Ms. Albertina Dambuza and Belinda Dambuza for assisting in reviewing medical records; Sisters Noreen Boikanyo, Rebecca Phofa, Catherine Lephoto, Agnes Ramashiga and Fikile Mbatha for study logistics; Ms Sarah Cohen, Mary Phoswa and Maphuti Madiga for processing blood specimens; Ms Ewalde Cutler and Professor Adrian Puren for the VL and CD4 cell counts; Drs. Penny Moore for technical assistance and Robert Shafer for constructive critiques.

This study was funded by: the Swedish International Development Cooperation Agency to Z.EK and NICD; Sven Gard's Fund for Virology Research, The Royal Swedish Academy of Sciences and a Karolinska Institutet Faculty Funds (KID) to Z.EK; African Programme for Training in HIV/TB Research Fogarty International Center/NIH/2U2RTW006878 and from the Freeman-Spogli Institute at Stanford University; D.K, A.DL and R.K are funded by an NIH/RO1 grant/AI66922. A.DL is also funded by the National Institute Of Allergy And Infectious Diseases/ P30AI042853.

Gen Bank accession number: HQ12279-12308;HQ12354-12365.

Competing Interests: The authors have declared that no competing interests exist

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