Published in final edited form as:
Antivir Ther. 2012 ; 17(2): 313–320. doi:10.3851/IMP1985.

Low rates of nucleoside reverse transcriptase inhibitor (NRTI) resistance detected in a well monitored cohort in South Africa accessing antiretroviral therapy

Carole L Wallis[1,*], **Maria A Papathanasopolous**[1], **Matthew Fox**[1,2,5], **Francesca Conradie**[1], **Prudence Ive**[1], **Catherine Orrell**[3], **Jennifer Zeinecker**[3], **Ian Sanne**[1], **Robin Wood**[3], **James McIntyre**[4], and **Wendy Stevens**[1] **on behalf of the CIPRA-SA project 1 study team**

[1]University of the Witwatersrand, Johannesburg, South Africa

[2]Center for Global Health and Development, Boston University, Boston, USA

[3]University of Cape Town, Cape Town, South Africa

[4]Anova Health, Johannesburg, South Africa

[5]Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA

Abstract

Background—Emergence of complex HIV-1 drug resistance mutations has been linked to the duration of time on a failing antiretroviral (ARV) drug regimen. This study reports on resistance profiles in a closely monitored subtype C infected cohort.

Methods—A total of 812 participants were enrolled into the CIPRA-SA 'safeguard the household' study, viral loads (VLs) were performed 12 weekly for 96 weeks. Virological failure was defined as either <1.5 log drop in VL at week 12 or 2 consecutive VL measurements >1000 RNA copies/ml after week 24. Regimens prescribed were in-line with the South African roll-out program (d4T, 3TC, EFV or NVP). Viral RNA was extracted from patients with virological failure, and *pol* RT-PCR and sequence analysis were performed to determine drug resistance mutations.

Results—Eighty three participants experienced virological failure on the first-line regimen during the study period, of which 61 (73%) had HIV-1 drug resistance mutations. The M184V mutation was the most frequent (n=46; 65%), followed by K103N (46%) and Y181C (21%). TAMS were infrequent (1%) and Q151M was not observed.

Conclusion—Drug resistance profiles were less complex than has been previously reported in South Africa using the same ARV drug regimens. This data suggests that frequent viral load monitoring limits the level and complexity of resistance observed in HIV-1 subtype C, preserving susceptibility to second-line options.

Keywords

HIV-1 drug resistance; subtype C; first-line failure; South Africa

Corresponding Author and reprints request: Dr Carole Wallis, 3B22, 3rd Floor Wits Medical School, Department of Molecular Medicine & Hematology, University of the Witwatersrand, 7 York Road, Parktown, 2193, South Africa, cwallis@mweb.co.za, Telephone: +27-11-489-8567, Fax: +27-11-484-5812.

Meetings Presented At: This work was presented as a poster at San Francisco, USA at the 17th Conference of Retroviruses and Opportunistic Infections, 2010.

Potential conflicts of interest: No conflict of interest for any authors

INTRODUCTION

Access to antiretroviral therapy (ART) in sub-Saharan Africa has increased rapidly over the last decade, and now efforts must be put into maintaining individual patients on lifelong ART. The long-term challenges of providing ART include managing toxicities associated with extended antiretroviral (ARV) use and the development of ARV drug resistance, both of which limit future drug options available to the patient. The current ARV drug resistance patterns that have been documented in resource limited settings (RLS) show high levels of complex nucleoside reverse transcriptase inhibitor (NRTI) resistance profiles, which is likely to impair future NRTI usage [1–5]. Furthermore, the presence of non-NRTI (NNRTI) mutations with delayed detection of ART failure, could compromise the use of second-generation NNRTIs such as Etravirine.

In most RLS, immunological and virological monitoring is conducted infrequently due to cost, limited infrastructure and shortage of technical skills. In such settings, where ART failure is generally assessed through clinical staging and/or CD4 counts, usually without viral load testing, a complex pattern of resistance has been observed. A recent study from Malawi has shown that 95% of patients failing first-line regimen (stavudine [d4T], lamivudine [3TC], nevirapine [NVP]) had drug resistance [1, 2]. A large proportion of these patients harboured mutations associated with cross-resistance to most NRTIs (K65R [19%], Q151M [19%] and/or thymidine analogue mutations (TAMs) [56%]), limiting the potential future use of fully susceptible NRTIs [1]. High levels of TAMs have been observed in Tanzania (28%) [6], Botswana (59%) [7], and South Africa (11–32%) [3–5] and in Uganda (74%) [8]. Furthermore, K65R has been observed in a high frequency, which is linked to nucleotide changes in HIV-1 subtype C [4, 5, 9–11]. The high level of NRTI resistance, due to mutations K65R, Q151M and TAMs would weaken second-line regimens, containing a boosted PI as the only active ARV. This is known to lower the barrier for selection of PI resistance [12].

In South Africa, where viral load testing is widely available as part of public-sector-ART, prior to April 2010, switching to second-line therapy was only considered under South African guidelines when two consecutive viral load measurements greater than 5000 HIV RNA copies/ml were detected [13]. In this setting, consistent resistance data from ART centres reflect a maximum of 39% complex drug resistance patterns (defined as the presence of either K65R and/or Q151M and/or 2 or more thymidine analogue mutations) [3–5].

Routine resistance monitoring is currently not performed within the South African national ARV treatment program and it remains difficult in many clinics to analyse clinical and laboratory data due the absence of linked laboratory and clinical electronic medical records. The CIPRA-SA ‘Safeguard the household’ study was a randomised controlled trial of ARV monitoring strategies in South Africa, with the primary objective of evaluating the care given by nurses versus doctors [14]. Because the first- and second-line regimens used in the study were those of the national ARV roll-out programme up until April 2010 [13], laboratory data from this study allow for a unique opportunity to examine the resistance patterns within the South African ARV roll-out programme in a well monitored cohort. We set out to describe the resistance patterns emerging in HIV-1 Subtype C infected patients in South Africa receiving ART in order to guide future government programs.

METHODS

Study Participants

Eight hundred and twelve HIV-1 positive participants were enrolled into the CIPRA-SA study over a period of 2 years (Feb 2005–Jan 2007) from either Soweto in Gauteng Province

or Masiphumelele in the Western Cape. Participants were ≥ 18 years of age, had a CD4+ T-cell count <350 cells/mm^3, had no active opportunistic infections at time of enrolment and were ART naive (excluding previous single dose NVP exposure). The participants were randomized into two arms (primary health care sister versus doctor managed arm), initiated on first-line ART, monitored every three months (viral load, CD4, hepatic and renal function) and followed for a minimum of 96 weeks [14]. Adherence data was collected at every scheduled visit from week 4 until study completion using clinic-based pill count.

ARV Treatment Regimens

All subjects in the CIPRA-SA cohort were given a first-line ART regimen containing d4T and 3TC, and the majority was given EFV as the third drug. However, if female subjects were of child-bearing age and unwilling to use two forms of contraception, NVP was prescribed instead. Lopinavir boosted with ritonavir (LVP/r) or nelfinavir (NLF) could be prescribed for women pregnant at treatment initiation. One drug substitution was permitted if drug toxicity above grade 3 was observed.

Study Design

We conducted a study of resistance patterns among participants in the CIPRA-SA cohort who failed first-line treatment at two time-points, at enrolment and at time of virological failure. HIV-1 drug resistance testing was conducted on all participants determined to have viral treatment failure, women that had previous sdNVP exposure to prevent PMTCT were also included. Treatment failure was defined as either a) failure to suppress, defined as failure to achieve a 1.5 log drop in HIV viral load by 12 week on ART; or b) virological rebound defined as two consecutive HIV viral load measurements of >1000 RNA copies/mL after week 24 on ART recorded more than four weeks apart.

The study was approved by the Human Research and Ethics Committee of the University of the Witwatersrand, Johannesburg South Africa and the University of Cape Town Research Ethics Committee, Cape Town South Africa and approval was given by the Boston University IRB for analysis of anonymized data.

Population Genotype Analysis

Population based genotyping was performed using an in-house drug resistance assay[15]. Viral RNA was extracted from 200ul of plasma samples using the automated Roche MagNa Pure LC analyzer and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany). A 1.7 kb amplicon was generated by RT-initiated polymerase chain reaction (PCR) encompassing the entire PR and partial RT coding regions using primers designed from the consensus HIV-1 subtype C sequence available on the Los Alamos Database (www.hiv.lanl.gov/). The amplicon was sequenced using five primers that ensure bidirectional coverage from codons 1-99 of PR and codons 1-230 of RT. Sequencing was performed with an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, USA).

Data Analysis

Sequences were assembled, manually edited using Sequencher version 4.5 software (Genecodes, Ann Arbor, MI), and submitted to the ViroScore database, which uses the IAS-USA mutation list[16] to identify known HIV-1 drug resistance mutations associated with decreased activity of the PR and RT inhibitors. The frequency for each of these mutations was analyzed by ART regimen and is presented with 95% confidence intervals. Sequences generated were subtyped using the published REGA subtyping tool version 2 [17, 18].

Statistical analysis was performed using SAS version 9.2 (Cary, North Carolina). We compared baseline characteristic between those who failed and those who did not using

simple proportions (with 95% confidence intervals where appropriate) for categorical variables and medians and inter-quartile ranges for continuous variables. We compared baseline characteristics using t-tests for continuous variables and chi-squared tests for categorical variables. The difference in frequency of mutations was summarized using simple proportions and comparisons between EFV and NVP exposure were compared using a chi-squared or Fisher's exact tests. A P-value of <0.05 was considered significant.

RESULTS

Table 1 shows demographic and clinical characteristics of the cohort at enrolment. At enrolment, 517 of the 812 participants (64%) had CD4+ T-cell counts <200 cells/mm^3. The median baseline CD4+ T-cell was 164 cells/mm^3 and median log viral load was 5.1 copies/ml. Thirty five percent of participants enrolled in the study had a CDC stage C classification, defined by CD4+ T-cell count and symptomatic conditions attributed to HIV-1 infection. Seventy-one percent (573/812) of the cohort was female and 99% were of African descent. A total of 83 participants experienced virological failure on first-line ART (10.2%; 95% CI: 8.3%–12.5%), the majority of whom were initiated on d4T-3TC-EFV (n=49; 59%) or d4T-3TC-NVP (n=22; 27%). Twelve subjects (pregnant at enrolment) were on a PI-based regimen at time of failure (n=3; d4T-3TC-NLF and n=9; d4T-3TC-LPV/r). The median viral load at failure was 3.9 log copies (IQR: 3.5–4.8) with a median time to virological failure of 60 weeks.

Baseline characteristics showed that subjects who failed ART had lower baseline CD4+ T-cell counts (median 167 vs. 147 respectively, p=0.045) than those who did not fail. Women who failed were more often exposed to single dose nevirapine (sdNVP) compared to those with ART success, but this difference was not significant (39% vs. 28%, p=0.079). Failing patients were more likely to be on a NVP-based regimen than those who did not fail (59% vs. 75%, p=0.001). Age, gender, treatment arm, study site and baseline viral load were not related to virological failure.

Resistance

Of the 83 participants experiencing virological failure, sequencing data was available for all samples at both study entry and virological failure. At virological failure known HIV-1 drug resistance mutations were observed in 73% (n=61), and 27% (n=22) had wild-type virus. The M184V mutation was the most frequent (n=47; 57%), followed by K103N (46%) and Y181C (21%). TAMS were infrequent (1%) and Q151M was not observed (Figure 1). Thirteen of the 83 (16%) participants failed to suppress viral load and 70 (84%) experienced viral rebound.

Of the thirteen participants who failed to suppress viral load, none had mutations associated with resistance at study enrolment, whereas at failure four (31%; 95% CI 11%–59%) had mutations. The most frequent mutation was K103N (n=3; 23%) followed by V106A/M (n=2; 15%). The Y181C and M184V mutations occurred in one subject each.

Of the 70 subjects who experienced viral rebound, baseline sequencing revealed that 5 (7%) were found to have resistance at study entry and 57 (81%) had resistance at failure. At time of failure the following NRTI mutations were observed: A62V (n=1; 1%), K65R (n=2; 3%), D67G/N (n=2; 3%), T69L (n=1; 1%), K70R (n=1; 1%), V75I (n=1; 1%), M184V (n=46; 66%) and K219K (n=1; 1%). The M184V mutation was the most prevalent mutation occurring in 46 subjects experiencing viral rebound (66%; 95% CI: 54%–76%) (Figure 2) followed by the K65R mutation. Only one subject had TAMs and Q151M was not observed. Nineteen percent (n=13) of the participants had no mutations associated with resistance (Figure 2).

Of the five with resistance at enrollment, no NRTI resistance was observed, two had resistance to NNRTIs (K103N n=1; V106M, K103N n=1), two had protease resistance (M46I n=1, M46L n=1) and one had both protease and NNRTI resistance (M46V, K101E, G190A). All three participants with NNRTI mutations were female, but only one reported previous sdNVP exposure to prevent two separate cases of mother-to-child-transmission (1 and 36 months prior to study entry). Only one of these subjects with baseline resistance (M46I) would have been fully susceptible to the regimen they were prescribed (d4T, 3TC, EFV). The subjects that were not completely susceptible to their regimens suppressed for an average of eleven months, whereas the subject that was fully susceptible suppressed for 30 months.

Resistance by Regimen

Of the 83 subjects experiencing virological failure, different mutation patterns were observed in the 71 participants accessing either an EFV (n=49) or NVP-containing (n=22) regimen (Figure 3). Of the subjects failing an EFV or NVP regimen 27% (n=13) and 18% (n=4), respectively, had no NNRTI mutation present at virological failure. The Y181C and V106A mutations only occurred in participants accessing a failing NVP-containing regimen (41% and 9%, respectively). Both the K103N and V106M mutations occurred more frequently in EFV- than NVP-exposed subjects, but differences were not significant (51% vs. 32%, p=0.0064 and 16% vs. 9%, p=0.1345, respectively). EFV selected for a wider range of mutations in the RT region compared to NVP (Figure 3).

Of the 12 subjects failing a PI-containing regimen 58% (n=7) had no resistance at virological failure. Nine of the 12 were accessing a LPV/r and three a NLF-based regimens. Protease resistance was only observed in one subject accessing LPV/r; however the M46L mutation was present at enrollment. Of the remaining nine participants failing a LPV/r regimen, M184V was observed in one subject and K103N, V106A and Y188C in another female subject (with no previous sdNVP exposure reported). All three subjects failing the NLF-based regimen had the M184V mutation and the two with prior sdNVP exposure also had NNRTIs mutations (K103N, V106M n=1; K103N, Y188H n=1).

DISCUSSION

This is the first study describing the HIV-1 drug resistance mutation patterns in HIV-1 subtype C infected individuals in a well monitored cohort, using ARV drug regimens that mirrored that of the South African national roll-out program [13]. Of the 83 subjects defined as meeting the virological failure criteria of the study, 73% (n=61) had an NRTI and/or NNRTI resistance mutation. The most frequently observed mutations were M184V (57%), K103N (46%) and Y181C (21%). The mutations K65R and TAMs were observed infrequently, and the Q151M was not present at all, thereby preserving the use of AZT, ddI or TNF used in second-line regimens.

The mutation patterns were less complex than those reported in published data from the region [1, 3–5], K65R (3%) was considerably lower than that observed in first-line failures in previously published data from Malawi [1] and South Africa [5], which showed M184V present in up to 81% and K65R present in up to 19%. Furthermore, only one participant in this study harbored TAMs, in complete contrast to all other published studies from the region which report levels of 23% to 56% [1, 3–5] and the Q151M mutation was not observed. The differences in resistance levels observed could be attributed to several different factors. Firstly, stringent monitoring and switch criteria (switch based on viral load >1000 RNA copies/ml versus a higher threshold of >5000 RNA copies/ml, or switch is based on clinical/immunological criteria) could prevent prolonged ART failure. A comparison of viral loads at failure between this study and those in the region [3–5],

indicated that the median viral load of this cohort (3.95 log copies/ml) was half a log lower compared to the other studies (4.29, 4.88 and 4.43 log copies/ml, respectively). Secondly, the increased frequency of CD4 and viral load monitoring, namely, 3-monthly compared to 6-monthly in the South African national program until April 2010, could decrease ART failure. Both of these factors are in line with studies from developed countries which have linked duration of treatment failure to frequency and complexity of mutation profiles [19, 20]. A

The second most frequent NRTI mutation observed was K65R (3%), which is uncommon in HIV-1 subtype B infected patients receiving d4T [21]. This finding is similar to those in the region [1, 4, 5] and *in vitro* cell culture studies [9, 22, 23]. The increased frequency of K65R has been linked to nucleotide changes in the sequence prior to codon 65 in HIV subtype C[10, 11]. The K65R mutation results in broad cross-resistance to NRTIs [16] and has consequences for the subsequent use of most NRTIs, especially tenofovir (TDF) in second-line regimens possibly making it better suited in first-line regimens. Furthermore, the presence of K65R may have implications for TDF usage in pre-exposure prophylaxis (PREP) and further investigation is required into transmission and fitness of viruses with K65R.

NVP and EFV were used by participants in this study and it was observed that EFV selected for a wider range of resistance mutations in the RT area investigated, than NVP, though again, the numbers were small. The K103N (41%) mutation was the most frequent NNRTI mutation observed. NVP uniquely selected for Y181C (41%) and the V106A (9%) and both the K103N and V106M mutations were more frequent in participants accessing EFV. These NNRTI mutation patterns are the same as those observed in the South African public sector programme [5] with K103N being the most prevalent. The difference in mutations selected by EFV and NVP could have an impact on the usage of second generation NNRTIs, like etravirine (ETR) in future second- or third-line regimens. For example, the use of NVP can lead to emergence of Y181C which results in a significant reduction in the susceptibility to ETR, whereas the emergence of K103N from the use of EFV does not affect drug susceptibility to second generation ETR.

Twenty seven percent of subjects had no HIV drug resistance mutations present. This could be a result of poor adherence, which was not addressed in this paper, especially in the group of participants that did not achieve a 1.5 log drop in viremia by week 12. This finding substantiates the use of drug resistance testing after first-line failure to decrease the amount of patients that are switched unnecessarily to more expensive second-line regimens. Instead of switching these patients without mutations to the second-line regimen, they should undergo intensive adherence counseling.

In conclusion, this study has shown that the complexity of drug resistance patterns in RLS can be greatly reduced when both strict and frequent virological monitoring is used to detect ART failure. This underscores the importance of using routine viral load testing and strict switching criteria to reduce the duration on a failing regimen and limit the development of complex resistance patterns. This strategy will preserve future treatment options for either second- or third-line ARV treatment regimens. Furthermore, the use of HIV-1 drug resistance testing after first-line failure will reduce the number of unnecessary switches to more expensive second-line regimens.

Acknowledgments

We would like to acknowledge the work of the CIPRA-SA Study Team (Sharlaa Badal-Faesen, Mildred Botile, Nastassja Choonilal, Jennipher Gelant, Janet Grab, Veronica Graham, Najma Hafejee, Lynda Hamber, Sindesh Harduth, Johean Hendricks, Colleen Herman, Mellissa Hero, Richard Kaplan, Nicola Killa, Daniella Klemp, Faisel

Laher, Thandi Mabiletsa, Zanele Madlala, Ntombekaya Mafukuzela, Bontle Mahlatsi, Helgard Marias, Nomakhaya Mfundisi, Buang Motloba, Cindy Moyo, Mcebisi Mtshizana, Lundi Ncana, Kevin Newell, Sean Palmer, Deborah Pearce, Mary-Ann Potts, Daphne Radebe, Anne Reyneke, Anna Segeneco, Jennifer Sekgale, Jan Steyn, Pinky Thebe, Handre Truter, Diederik van Niekerk, Frieda Verheye-Dua, Karlien Voges, Helen Woolgar), the Endpoint Review Committee (Gary Maartens, David Spencer; Charles van der Horst), Contract Laboratory Services (Charlotte Ingram, Debbie Glencross) and especially the contribution of our patients. **Support for the Study:** The CIPRA-SA project is an NIH funded study (CIPRA grant: U19 AI53217-01, Principal Investigator Dr J McIntyre).

References

1. Hosseinipour MC, van Oosterhout JJ, Weigel R, Phiri S, Kamwendo D, Parkin N, Fiscus SA, Nelson JA, Eron JJ, Kumwenda J. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. AIDS. 2009; 23:1127–1134. [PubMed: 19417582]
2. Hosseinipour MC, Kumwenda JJ, Weigel R, Brown LB, Mzinganjira D, Mhango B, Eron JJ, Phiri S, van Oosterhout JJ. Second-line treatment in the Malawi antiretroviral programme: high early mortality, but good outcomes in survivors, despite extensive drug resistance at baseline. HIV Med. 2010; 11:510–518. [PubMed: 20345885]
3. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, Carpenter S, Giddy J, Ross D, Holst H, Losina E, Walker BD, Kuritzkes DR. Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. Clin Infect Dis. 2008; 46:1589–1597. [PubMed: 18419495]
4. Orrell C, Walensky RP, Losina E, Pitt J, Freedberg KA, Wood R. HIV type-1 clade C resistance genotypes in treatment-naive patients and after first virological failure in a large community antiretroviral therapy programme. Antivir Ther. 2009; 14:523–531. [PubMed: 19578237]
5. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa. J Acquir Immune Defic Syndr. 2010; 53:480–484. [PubMed: 19801944]
6. Johannessen A, Naman E, Kivuyo SL, Kasubi MJ, Holberg-Petersen M, Matee MI, Gundersen SG, Bruun JN. Virological efficacy and emergence of drug resistance in adults on antiretroviral treatment in rural Tanzania. BMC Infect Dis. 2009; 9:108. [PubMed: 19583845]
7. Doualla-Bell F, Gaolathe T, Avalos A, Cloutier S, Ndwapi N, Holcroft C, Moffat H, Dickinson D, Essex M, Wainberg MA, Mine M. Five-year follow up of genotypic resistance patterns in HIV-1 subtype C infected patients in Botswana after failure of thymidine analogue-based regimens. J Int AIDS Soc. 2009; 12:25. [PubMed: 19852859]
8. Lyagoba F, Dunn DT, Pillay D, Kityo C, Robertson V, Tugume S, Hakim J, Munderi P, Chirara M, Ndembi N, Goodall RL, Yirrell DL, Burke A, Gilks CF, Kaleebu P. Evolution of drug resistance during 48 weeks of zidovudine/lamivudine/tenofovir in the absence of real-time viral load monitoring. J Acquir Immune Defic Syndr. 2010; 55:277–283. [PubMed: 20686411]
9. Doualla-Bell F, Avalos A, Brenner B, Gaolathe T, Mine M, Gaseitsiwe S, Oliveira M, Moisi D, Ndwapi N, Moffat H, Essex M, Wainberg MA. High prevalence of the K65R mutation in human immunodeficiency virus type 1 subtype C isolates from infected patients in Botswana treated with didanosine-based regimens. Antimicrob Agents Chemother. 2006; 50:4182–4185. [PubMed: 17015626]
10. Coutsinos D, Invernizzi CF, Xu H, Brenner BG, Wainberg MA. Factors affecting template usage in the development of K65R resistance in subtype C variants of HIV type-1. Antivir Chem Chemother. 2010; 20:117–131. [PubMed: 20054099]
11. Coutsinos D, Invernizzi CF, Xu H, Moisi D, Oliveira M, Brenner BG, Wainberg MA. Template usage is responsible for the preferential acquisition of the K65R reverse transcriptase mutation in subtype C variants of human immunodeficiency virus type 1. J Virol. 2009; 83:2029–2033. [PubMed: 19073730]
12. Delaugerre C, Flandre P, Chaix ML, Ghosn J, Raffi F, Dellamonica P, Jaeger H, Shurmann D, Cohen-Codar I, Van PN, Norton M, Taburet AM, Delfraissy JF, Rouzioux C. Protease inhibitor resistance analysis in the MONARK trial comparing first-line lopinavir-ritonavir monotherapy to lopinavir-ritonavir plus zidovudine and lamivudine triple therapy. Antimicrob Agents Chemother. 2009; 53:2934–2939. [PubMed: 19451297]

13. Department of Health. [Accessed 29 October 2010] National Antiretroviral Treatment Guidelines. 2004. http://southafrica.usembassy.gov/media/2004-doh-art-guidelines.pdf

14. Sanne I, Orrell C, Fox MP, Conradie F, Ive P, Zeinecker J, Cornell M, Heiberg C, Ingram C, Panchia R, Rassool M, Gonin R, Stevens W, Truter H, Dehlinger M, van der Horst C, McIntyre J, Wood R. Nurse versus doctor management of HIV-infected patients receiving antiretroviral therapy (CIPRA-SA): a randomised non-inferiority trial. Lancet. 2010; 376:33–40. [PubMed: 20557927]

15. Wallis CL, Papathanasopoulos MA, Lakhi S, Karita E, Kamali A, Kaleebu P, Sanders E, Anzala O, Bekker LG, Stevens G, de Wit TF, Stevens W. Affordable in-house antiretroviral drug resistance assay with good performance in non-subtype B HIV-1. J Virol Methods. 2009; 163:505–508. [PubMed: 19917318]

16. Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, Pillay D, Schapiro JM, Richman DD. Update of the Drug Resistance Mutations in HIV-1: Spring 2008. Top HIV Med. 2008; 16:62–68. [PubMed: 18441382]

17. Alcantara LC, Cassol S, Libin P, Deforche K, Pybus OG, Van Ranst M, Galvao-Castro B, Vandamme AM, de Oliveira T. A standardized framework for accurate, high-throughput genotyping of recombinant and non-recombinant viral sequences. Nucleic Acids Res. 2009; 37:W634–642. [PubMed: 19483099]

18. de Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, Snoeck J, van Rensburg EJ, Wensing AM, van de Vijver DA, Boucher CA, Camacho R, Vandamme AM. An automated genotyping system for analysis of HIV-1 and other microbial sequences. Bioinformatics. 2005; 21:3797–3800. [PubMed: 16076886]

19. Hatano H, Hunt P, Weidler J, Coakley E, Hoh R, Liegler T, Martin JN, Deeks SG. Rate of viral evolution and risk of losing future drug options in heavily pretreated, HIV-infected patients who continue to receive a stable, partially suppressive treatment regimen. Clin Infect Dis. 2006; 43:1329–1336. [PubMed: 17051502]

20. Cozzi-Lepri A, Phillips AN, Ruiz L, Clotet B, Loveday C, Kjaer J, Mens H, Clumeck N, Viksna L, Antunes F, Machala L, Lundgren JD. Evolution of drug resistance in HIV-infected patients remaining on a virologically failing combination antiretroviral therapy regimen. AIDS. 2007; 21:721–732. [PubMed: 17413693]

21. Margot NA, Lu B, Cheng A, Miller MD. Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903. HIV Med. 2006; 7:442–450. [PubMed: 16925730]

22. Brenner BG, Oliveira M, Doualla-Bell F, Moisi DD, Ntemgwa M, Frankel F, Essex M, Wainberg MA. HIV-1 subtype C viruses rapidly develop K65R resistance to tenofovir in cell culture. AIDS. 2006; 20:F9–13. [PubMed: 16816549]

23. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Chantratitra W. Tenofovir resistance among HIV-infected patients failing a fixed-dose combination of stavudine, lamivudine, and nevirapine in a resource-limited setting. AIDS Patient Care STDS. 2007; 21:711–714. [PubMed: 17949267]

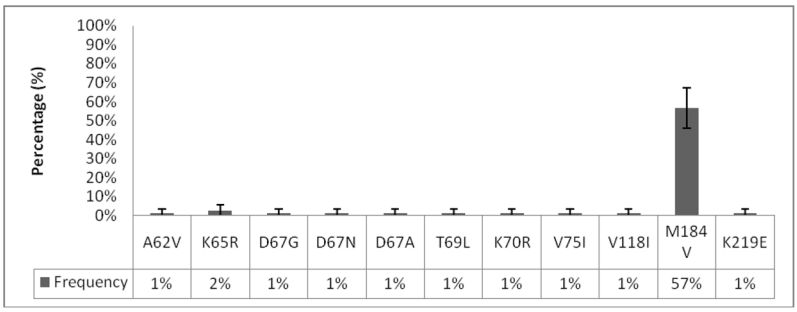


Figure 1. Frequency of the HIV-1 antiretroviral drug resistance mutations associated with NRTIs[a] resistance in the 83CIPRA-SA[b] participants experiencing viral failure onARV therapy in South Africa

[a]NRTI = nucleoside reverse transcriptase inhibitor

[b]CIPRA-SA is the Comprehensive International Program of Research on AIDS-South Africa.

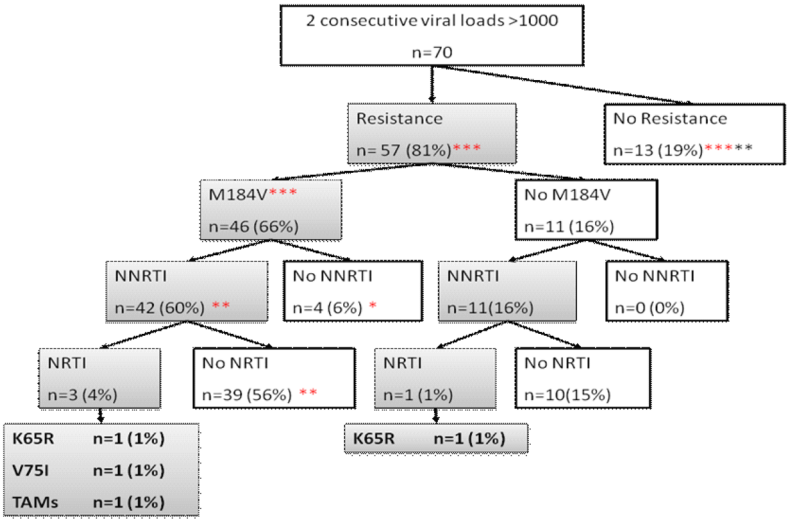
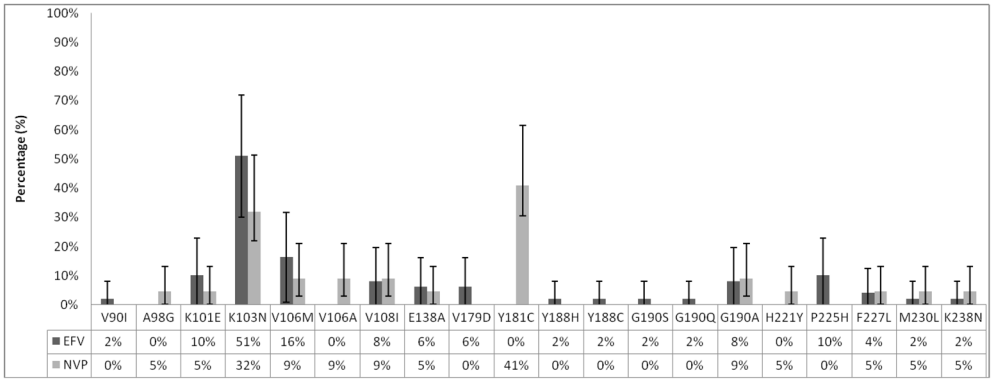


Figure 2. HIV-1 antiretroviral resistance patterns in 70 participants experiencing viral rebound on first-line therapy in the CIPRA-SA[b] study in South Africa

[a] Participants on a PIs-based regimen are represented by black asterisks, if they were also exposed to sdNVP to prevent mother-to-child-transmission the asterisks is red.

[b] CIPRA-SA is the Comprehensive International Program of Research on AIDS-South Africa.



	V90I	A98G	K101E	K103N	V106M	V106A	V108I	E138A	V179D	Y181C	Y188H	Y188C	G190S	G190Q	G190A	H221Y	P225H	F227L	M230L	K238N
EFV	2%	0%	10%	51%	16%	0%	8%	6%	6%	0%	2%	2%	2%	2%	8%	0%	10%	4%	2%	2%
NVP	0%	5%	5%	32%	9%	9%	9%	5%	0%	41%	0%	0%	0%	0%	9%	5%	0%	5%	5%	5%

Figure 3. Distribution of NNRTIs[a] mutations in patients failing Efavirenz (n=49)or Nevirapine(n=22) -containing first-line antiretroviral regimens in the CIPRA-SA[b] study in South Africa

The error bars represent the confidence intervals.

[a] NNRTI = non-nucleoside reverse transcriptase inhibitor.

[b] CIPRA-SA is the Comprehensive International Program of Research on AIDS-South Africa.

Table 1

Baseline characteristics at enrolment of the 812 Participants and 83 Virologic Treatment Failures in the CIPRA-SA[a] Study in South Africa

Variable	No Virologic Failure (n=729)	Virologic Failure (n=83)	Total (n=812)	p-values[c]
Female	511 (70.1%)	62 (74.7%)	573 (70.6%)	0.3833
Age				
in years [median (IQR[b])]	32.3 (28.0–37.2)	31.9 (28.0–36.2)	32.3 (28.0–37.1)	0.6046
Nurse managed care	360 (49.4%)	44 (53.0%)	404 (49.8%)	0.5309
First-line ART regimen[b]				0.0012
D4T-3TC-EFV	548 (75.2%)	49 (59.0%)	597 (73.5%)	
D4T-3TC-NVP	131 (18.0%)	22 (26.5%)	153 (18.8%)	
D4T-3TC-LPVr	46 (6.3%)	9 (10.9%)	55 (6.8%)	
D4T-3TC-NLF	4 (0.5%)	3 (3.6%)	7 (0.9%)	
Study Site				0.0590
Soweto	395 (54.2%)	54 (65.1%)	449 (55.3%)	
Masiphumelele	334 (45.8%)	29 (34.9%)	363 (44.7%)	
Single dose NVP[b] **exposed**[d]	143 (28.0%)	24 (38.7%)	167 (29.1%)	0.0793
CDC Stage				0.4549
A	265 (36.4%)	36 (43.4%)	301 (37.1%)	
B	208 (28.5%)	21 (25.3%)	229 (28.2%)	
C	256 (35.1%)	26 (31.3%)	282 (34.7%)	
CD4+ T-cell count				
(cells/mm^3) [Median, (IQR[b])]	167 (109–234)	147 (106–198)	164 (109 – 229)	0.0453
CD4 count				
<200	454 (62.3%)	63 (75.9%)	517 (63.7%)	0.0145
≥200	275 (37.7%)	20 (24.1%)	295 (36.3%)	
BMI*				
[median (IQR[b])]	23.5 (20.7–27.3)	23.5 (21.1–26.7)	23.5 (20.8–27.2)	0.4601
Baseline HIV-1 RNA log$_{10}$				
copies/ml [Mean, (IQR[b])]	5.1 (4.6 – 5.6)	5.2 (4.8 – 5.5)	5.1 (4.6–5.6)	0.2320

[a] CIPRA-SA is the Comprehensive International Program of Research on AIDS-South Africa.

[b] IQR – interquartile range, NVP = nevirapine, D4T = Stavudine, 3TC = Lamivudine, EFV = Efavirenz, LPVr = Lopinavir-ritonavir, NLF = Nelfinavir, BMI = body mass index, ART = antiretroviral therapy

[c] p-values from chi-squared test for categorical variables and t-tests for continuous variables

[d] denominator is only female