

HIV drug resistance mutations in non-B subtypes after prolonged virological failure on NNRTI-based first-line regimens in sub-Saharan Africa

Cissy Kityo¹

Jennifer Thompson²

Immaculate Nankya¹

Anne Hoppe²

Emmanuel Ndashimye¹

Colin Warambwa³

Ivan Mambule⁴

Joep J. van Oosterhout^{5,6}

Kara Wools-Kaloustian⁷

Silvia Bertagnolio⁸

Philippa J. Easterbrook⁸

Peter Mugenyi¹

A. Sarah Walker² *

Nicholas Paton^{9,2} *

for the Europe Africa Research Network for Evaluation of Second-line Therapy (EARNEST) Trial Team¶

¹Joint Clinical Research Centre (JCRC), Kampala, Uganda; ²MRC Clinical Trials Unit at University College London, United Kingdom; ³University of Zimbabwe Clinical Research Centre, Harare, Zimbabwe; ⁴Infectious Diseases Institute, Kampala, Uganda; ⁵Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine, Blantyre, Malawi; ⁶Dignitas International, Zomba, Malawi; ⁷Moi University School of Medicine, Eldoret, Kenya; ⁸World Health Organisation, Geneva, Switzerland; ⁹Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

* Contributed equally to this article

¶Members of the EARNEST Trial Team are listed in the Acknowledgements

Abstract word count: 204; text word count 2734; 3 tables, 3 figures (plus 2 supplementary tables)

Correspondence to: Professor Nicholas Paton, Yong Loo Lin School of Medicine, National University of Singapore, NUHS Tower Block Level 10, 1E Kent Ridge Road, Singapore 119228. Tel: +65 6772 6988. Email: nick_paton@nuhs.edu.sg

ABSTRACT

Objective: To determine drug resistance mutation (DRM) patterns in a large cohort of patients failing non-nucleoside-reverse-transcriptase-inhibitor (NNRTI)-based first-line antiretroviral therapy (ART) regimens in programmes without routine viral load (VL) monitoring and to examine inter-subtype differences in DRMs.

Design: Sequences from 787 adults/adolescents who failed an NNRTI-based first-line regimen in 13 clinics in Uganda, Kenya, Zimbabwe, Malawi were analysed. Multivariable logistic regression was used to determine the association between specific DRMs and Stanford intermediate/high-level resistance and factors including REGA subtype, first-line ART drugs, CD4 and VL at failure.

Results: The median first-line treatment duration was 4 years (IQR 30-43 months); 42% of participants had VL $\geq 100,000$ c/ml and 63% had CD4 < 100 cells/mm³. Viral subtype distribution was A1 (40%; Uganda, Kenya), C (31%; Zimbabwe, Malawi) and D (25%; Uganda, Kenya) and recombinant/unclassified (5%). In general, DRMs were more common in subtype-C than in subtypes-A and/or -D (NRTI mutations K65R and Q151M; NNRTI mutations E138A, V106M, Y181C, K101E, H221Y). The presence of tenofovir resistance was similar between subtypes ($p(\text{adjusted})=0.32$), but resistance to zidovudine, abacavir, etravirine or rilpivirine was more common in subtype-C than D/A ($p(\text{adjusted}) < 0.02$).

Conclusions: Non-B subtypes differ in DRMs at first-line failure that impact on residual NRTI and NNRTI susceptibility. In particular, higher rates of etravirine and rilpivirine resistance in subtype-C may limit their potential utility in salvage regimens.

INTRODUCTION

Large databases of HIV drug-resistance mutations (DRMs), invaluable for individual patient clinical decision-making, are largely populated with data from subtype-B viruses, dominant in Europe and North America. The prevalence and pattern of DRMs differ between B and non-B subtypes. Studies comparing different non-B subtypes have typically had small numbers of participants failing first-line therapy, and thus limited power to detect subtype differences, given confounding associated with setting-specific factors, such as first-line regimens and monitoring approaches.¹

More than 15 million people receive antiretroviral therapy (ART) in sub-Saharan Africa, mostly delivered using the WHO public health approach that recommends using two nucleoside-reverse-transcriptase-inhibitor (NRTI) drugs combined with a non-NRTI (NNRTI) drug for first-line treatment and with a protease inhibitor for second-line treatment.^{2 3} Regular viral load (VL) monitoring is not widely available and treatment failure is consequently detected late, at which time there is extensive resistance.³ Current WHO guidelines recommend selecting the NRTI drugs for second-line therapy using an algorithm based on first-line NRTI drug exposure.³ An understanding of subtype differences in mutational patterns and residual drug susceptibility might allow more refined NRTI selection predictions in areas where a single subtype predominates and might also clarify the role of second-generation NNRTIs in third-line therapy.

We assessed resistance in a large cohort of sub-Saharan African adults/adolescents with first-line treatment failure to determine the impact of viral subtype on mutational patterns and corresponding drug susceptibility.

METHODS

This study included participants failing NNRTI-based first-line therapy enrolled in the EARNEST trial, a large trial testing several PI-based treatment regimens for second-line therapy, and was performed at 13 sites in 4 sub-Saharan African countries (one trial site excluded from this study, see below).⁴ In the 3 to 5 years preceding this trial, these sites were delivering ART using the public health approach with standardised first-line ART regimens (stavudine- and nevirapine-based regimens predominating in earlier years, with increasing use of tenofovir- and efavirenz-based regimens subsequently). At most sites, first-line failure was detected using clinical monitoring, sometimes supplemented with intermittent CD4 monitoring. In the year prior to enrolment, some sites were performing targeted VL testing in suspected treatment failures, but none had implemented regular routine VL testing.

Trial eligibility required participants to have been taking an NNRTI-based first-line regimen for >12 months and to be currently adherent (no more than 3 ART doses missed in the month prior to screening). Failure of first-line ART was defined by clinical, immunological or virological criteria (modified from WHO 2010 guidelines and confirmed by VL>400 c/ml).⁴ A baseline plasma sample was obtained prior to switch to second-line ART, stored locally and shipped to a central repository at JCRC, Kampala, Uganda within 12 months of collection.

All 37 participants recruited at one site in Zambia (3% of the total sample size) and 41 (3%) participants from the other 13 sites had no baseline samples stored and were excluded. Three participants not taking standard 2NRTI+NNRTI at failure (2 previously NNRTI-exposed, 1 ineligible⁴) were also excluded. Samples from all remaining participants randomised to protease inhibitor (PI)/NRTI (N=398) and PI/raltegravir (N=393) arms were selected, and 15 samples from those randomised to PI-monotherapy who had received only tenofovir and lamivudine/emtricitabine (with NNRTI) in their first-line regimen were added to increase numbers receiving the current WHO-recommended first-line regimen (total 806 baseline samples assayed). Patient demographics, medical and treatment history (including antiretroviral drugs) were obtained from case records and patient self-report. VL and CD4 count were performed at trial screening (<6 weeks before baseline) using standard methods at local sites.

HIV genotyping was performed using an in-house sequencing method encompassing codons 1-300 of reverse transcriptase at a WHO-designated laboratory (JCRC, Kampala, Uganda).⁵ In brief, RNA was extracted using the Qiagen RNA extraction kit and reverse transcribed followed by a nested PCR. The cleaned PCR product was cycle sequenced using the ABI 3730xl. Sequences were edited using the SeqScape version 2.7 and subsequently on Recall as recommended by WHO.⁶ Amino acid sequences were compared to consensus subtype B, DRMs defined using the International AIDS Society-USA list,⁷ and susceptibility predicted using the Stanford algorithm version 7.0 using the full sequence data available.⁸ Subtype was determined by the REGA algorithm version 3.0.⁹ Sequences of viral isolates in this study were submitted to GenBank.

Statistical analysis

Multivariable logistic regression was used to determine associations between specific DRMs and two main factors of interest: subtype and ART exposure at first-line failure (stavudine, tenofovir, zidovudine, or other NRTI for NRTI DRMs; efavirenz or nevirapine for NNRTI DRMs). As well as including these two factors, models adjusted for the following potential confounders: ART drug exposure prior to the regimen they failed on, time on first-line ART, CD4 and log₁₀ VL at failure, and

presence/absence of clinical failure. Exact logistic regression (continuous factors dichotomised at approximate midpoints) was used where logistic regression failed due to small numbers. Similar approaches were used to determine associations between Stanford intermediate/high-level resistance to key drugs and the factors above. Participants with recombinant viruses or where no subtype could be determined were excluded from all models. Models did not adjust for country because national programmes determined the specific NRTIs used in first-line (Table 1); analyses assumed that any relationship between country and DRMs or susceptibility could only be realised through ART received and the other factors above.

All statistical tests presented are two-sided, without adjustment for multiple comparisons. All analyses were done in Stata version 13.1.

RESULTS

Of 806 samples taken between 12 April 2010 and 29 April 2011 and assayed, sequences were obtained in 787 (98%). Forty-two percent of the participants had $VL \geq 100,000$ copies/ml, and 63% had $CD4 < 100$ cells/mm³ (Table 1). The predominant viral subtypes were A1 (40%; Uganda/Kenya), C (31%; Zimbabwe/Malawi) and D (25%; Uganda/Kenya) with 5% recombinants/not predicted by REGA. Subtypes differed significantly in duration of first-line ART and drugs prescribed: more stavudine/nevirapine use and longer duration on first-line ART with subtype-C and more tenofovir use and substitutions in first-line NRTIs (zidovudine/stavudine to tenofovir) with subtype-A/D infection.

The overall prevalence of major DRMs by subtype is shown in Figure 1 and Supplementary Tables 1a/b. One or more major NRTI or NNRTI DRMs were present in 769 (98%); 3 (0.4%) had only NRTI DRMs, 21 (3%) had only NNRTI DRMs and 745 (95%) had both. Only 18 (2%) participants had no NRTI or NNRTI major DRMs.

Overall DRM prevalence (after adjustment) was broadly similar between subtypes but some significant differences were seen (global $p < 0.05$). For NRTI DRMs (Figure 1, supplementary Table 1a), type-2 thymidine analogue (TAM-2) DRMs were more common in A and C than D, K65R and Q151M were more common in C than both A and D (K65R more common in A than D), whereas L210W was less common in C than both A and D. In particular Q151M was seen in 10% of the subtype-C vs $< 1\%$ of subtype-A/D. For NNRTI DRMs (Figure 1, supplementary Table 1b), (i) E138A, V106M, and Y181C were more common in C than both A and D (ii) K101E was significantly more common in C and D than A, (iii) H221Y was more common in A and C than D (and more common in C than D) (iv) V108I was more common in D than A (v) P225H was less common in C than D (the only DRM significantly less common in C). In particular, V106M occurred in 16% subtype-C compared with 1% A/D, whereas P225H occurred in 2% C vs 7% A and 11% D.

Mutation prevalence by drug exposure is shown in Figure 2 and Supplementary Tables 2a/b. After adjustment, participants on zidovudine at failure were more likely to have T215F, T215Y, M41L, K70R, D67N, L210W, type-1 thymidine analogue (TAM-1), TAM-2 and any TAMs; those on tenofovir to have K65R, K70E, Y115F, and M184I; those on efavirenz to have K103N, P225H, Y188L and L100I, and those on nevirapine to have Y181C and G190A. Intermediate/high-level resistance to tenofovir and lamivudine was predicted in 57% and 95% respectively (Figure 3), and to etravirine and rilpivirine in 55% and 65% respectively. The proportion with resistance to all three NRTIs (zidovudine, abacavir, tenofovir) that might be used with lamivudine/emtricitabine in a second-line regimen was 50% (Table 2), and to all NNRTIs (including second-generation) was 55%. Much of the individual drug resistance was due to cross-resistance rather than previous direct exposure to that drug (Figure 4).

After adjustment, subtype-C was associated with greater abacavir and zidovudine resistance compared to subtypes A and D ($p < 0.01$), with prevalence of intermediate/high-level resistance to abacavir and zidovudine of 95% and 86% respectively in subtype-C compared to 84% and 72% respectively in subtype-A, and 81% and 70% respectively in subtype-D. There was no significant impact of subtype on tenofovir resistance (global $p = 0.32$) (Table 3). Subtype-C was associated with greater etravirine and rilpivirine resistance compared to subtypes A and D ($p < 0.003$) with prevalence of intermediate/high-level resistance to etravirine and rilpivirine of 69% and 80% respectively in

subtype-C compared to 49% and 63% respectively in subtype-A, and 55% and 61% respectively in subtype-D (percentages adjusted to average over other model factors).

Those on tenofovir at failure had less zidovudine resistance compared to those on zidovudine/stavudine ($p<0.01$) whereas those on zidovudine appeared to have more tenofovir resistance than those on tenofovir ($p=0.06$); there was no difference in abacavir resistance in those receiving zidovudine vs tenofovir ($p=0.26$). Those with first-line nevirapine exposure had more etravirine and rilpivirine resistance ($p<0.01$). After adjustment, higher VL at failure was strongly associated with greater tenofovir, zidovudine and abacavir resistance ($p<0.01$), and more weakly with greater etravirine resistance ($p=0.04$). Lower CD4 count at failure was independently associated with higher risk of resistance to all drugs ($p<0.01$).

DISCUSSION

We found DRMs conferring resistance to one or more NRTI/NNRTI drugs in 98% of participants failing NNRTI-based first-line ART (resistance to both classes in 95%), similar to smaller studies in Malawi¹⁰, Nigeria¹¹ and the A5230 study.¹² Previous studies have shown that patients monitored without VL accumulate DRMs at a rate of 1 new TAM approximately every 15 months (low-income setting) to 4.3 years (high-income setting), and 1 new NNRTI DRM every 1.6 years (high-income setting) with continued drug exposure following virological failure.¹³⁻¹⁵ The high VL/low CD4 in this cohort suggests that participants had protracted virological failure prior to second-line switch, consistent with the fact that they were managed in programmes using immunological and clinical monitoring (without regular VL testing) to detect treatment failure. The very high level of accumulated DRMs in this cohort is therefore not surprising. Conversely, although regular VL monitoring detects failure earlier and may allow the chance for re-suppression (one third after 3 months' adherence support),¹⁶ resistance often occurs simultaneously with VL rebound so regular VL testing may not always prevent its development. In a research cohort taking WHO-recommended NNRTI-based first-line regimens with VL tested one year after starting ART, 70% of those with detectable VL had one or more DRMs, with 53% and 60% having 184V and NNRTI DRMs respectively.¹⁷ Similarly a large South African programme in which VL was tested 6-monthly found 86% of patients had at least one drug-resistance mutation at second-line switch, likely reflecting delays in acting upon viral load results and illustrating programmatic barriers to reducing resistance development.¹⁸

This study, the largest to date of resistance in patients failing NNRTI-based first-line therapy in sub-Saharan Africa, makes a substantial contribution to the existing literature on differences in mutational patterns in non-B subtypes. In general, resistance-conferring DRMs were more common in subtype-C than in either or both of subtypes-A and -D. K65R was more common in subtype-C than A or D, supporting previous observations in subtype-C compared to subtype-AE and -B in a global study of patients failing stavudine-containing regimens,¹⁹ and in comparison to non-C subtypes in an African cohort study (although this effect was not significant after adjustment for drugs).¹⁷ Differential codon usage has been hypothesised to underlie this difference, and could also contribute to other variations in resistance at failure. Q151M, a rare mutation that confers cross-class NRTI resistance, was present in 11% of subtype-C, but negligible levels in other subtypes (not previously described). Subtype differences in L210W have previously been noted (higher in A than other subtypes in Nigeria).²⁰

We found more inter-subtype differences in NNRTI DRMs, with many being substantially more common in subtype-C than one or both other subtypes. The (almost) exclusive occurrence of V106M in subtype-C has been noted previously.¹⁷ This mutation confers resistance to efavirenz and nevirapine and was found in 31% of those in a small cohort failing first-line in South Africa.²¹ Another substitution at this position, V106I (not seen in our study) is significantly more common in subtype-G.²⁰ Substitutions at position 138 (found in 13% overall) confer resistance to rilpivirine and etravirine and are therefore particularly concerning given that these drugs may be considered for use in third-line therapy.⁷ A Kenyan study reported substitutions at this position in 14% of those failing nevirapine or efavirenz-based first-line, mainly subtype-A;²² and a study in Nigeria, mainly subtype G and CRF02_AG, found these substitution in 9%;¹¹ our finding that these DRMs are significantly more common in subtype-C (18%) than in other non-B subtypes is novel (a previous study of this mutation

in several large databases showed a difference in frequency between subtypes C and B, but not between C and non-B subtypes).²³ The H221Y mutation (conferring rilpivirine resistance⁷ and with Y181C possibly etravirine resistance²⁴) has been noted in patients with non-B subtypes failing first-line nevirapine or efavirenz-based regimens in a predominantly subtype-G and CRF02 Nigerian cohort and subtype-C South African cohort.^{25 26} However, our study is the first to report differences between non-B subtypes for this mutation (more common in subtype-C). To our knowledge, the differences we found in the other NNRTI DRMs, K101E (rilpivirine and etravirine resistance), Y181C (resistance to all NNRTIs), V108I (efavirenz and nevirapine resistance) and P225H (efavirenz resistance) have not been reported previously.

Arising from these DRM differences, viral subtype significantly affected the probability of resistance to drugs that could be used in second or third-line regimens under the public health approach. The much higher zidovudine resistance in subtype-C may be of practical importance given that zidovudine is currently recommended for use in second-line therapy after failure of a tenofovir-based regimen. However, participants were mainly on zidovudine/stavudine first-line and the impact of subtype on zidovudine susceptibility may differ for failure on tenofovir-based first-line regimens (although we adjusted for this). Although we found no significant difference in the proportion with tenofovir resistance between subtypes, only a minority of our participants were taking tenofovir at the time of failure. An analysis of patients failing a tenofovir-based first-line regimen in Western European cohorts showed a higher rate of tenofovir resistance in subtype-C compared to non-C subtypes,²⁷ although subtype does not appear to affect long-term outcomes on tenofovir-based regimens.²⁸ The higher rates of etravirine and rilpivirine resistance seen in subtype-C might also be an important consideration in region-specific programme policy (although overall rates are high regardless of subtype, see below). We confirmed the effects of specific first-line NRTIs on resistance to second-line NRTI drugs, which form the basis for the recommendations in the WHO algorithm. We also confirmed the independent association between first-line nevirapine use and etravirine and rilpivirine resistance.²⁹⁻³¹ Increasing use of efavirenz over nevirapine in first-line therapy may preserve second-generation NNRTIs for potential third-line regimens, but the high rates of resistance seen even in participants failing on efavirenz (40% etravirine, 51% rilpivirine) suggests that these are unlikely to remain sufficiently active to be used in standardised regimens in the public health approach. Furthermore, rates of etravirine and rilpivirine resistance have been shown to increase when tested with deep sequencing.²⁶ Higher VL and lower CD4 counts independently predicted resistance to all potential second-line NRTI drugs, as previously observed.¹² The marginal associations between VL and etravirine and rilpivirine resistance support this arising quickly after virological failure.

Study strengths are the large sample size, the setting within representative sub-Saharan African programmes following the public health approach, and well-defined first-line failure. Limitations are lack of data on CD4/VL monitoring during first-line ART and the few participants using the currently recommended tenofovir-based regimen for first-line. There is potential for residual confounding by setting, since viral subtypes are strongly clustered with countries, and countries used different drug regimens (subtype-C virus predominated in Malawi and Zimbabwe, with more stavudine and less tenofovir or zidovudine use at failure; NNRTI use broadly similar). Also the duration of virological failure on first-line ART is unknown, may be only imperfectly adjusted for by CD4 and VL at failure, and first-line monitoring approaches did differ between countries. Nevertheless, our large sample size allowed us to adjust for different regimens and patient characteristics at failure and identify

some strong independent associations between subtype and specific DRMs. The levels of statistical significance seen likely indicate a true effect rather than an artefact of the multiple comparisons performed. Although we did not adjust for these multiple comparisons, this does not affect the magnitude of observed inter-subtype differences but rather requires caution in the interpretation of borderline differences.

Our study provides important information on the prevalence of DRMs in patients failing NNRTI-based first-line ART in these settings, allowing a more robust comparison between viral subtypes than hitherto possible. We found substantial differences between subtypes, with particular disadvantages for subtype-C, but these are likely to have limited impact on the selection of standardised second-line regimens for use in the public health approach.

Sequences have been deposited with GenBank accession numbers KY061369 - KY062155

ACKNOWLEDGEMENTS

We thank all the participants and staff from all the centres participating in the EARNEST trial.

Members of the EARNEST Trial Team are:

Participating Sites

Uganda

JCRC Kampala (African trial co-ordinating centre; 231): E Agweng, P Awio, G Bakeinyaga, C Isabirye, U Kabuga, S Kasuswa, M Katuramu, C Kityo, F Kiweewa, H Kyomugisha, E Lutalo, P Mugenyi, D Mulima, H Musana, G Musitwa, V Musiime, M Ndigendawan, H Namata, J Nkalubo, P Ocitti Labejja, P Okello, P Olal, G Pimundu, P Segonga, F Ssali, Z Tamale, D Tumukunde, W Namala, R Byaruhanga, J Kayiwa, J Tukamushaba, S Abunyang, D Eram, O Denis, R Lwalanda, L Mugarura, J Namusanje, I Nankya, E Ndashimye, E Nabulime, D Mulima, O Senfuma.

IDI, Kampala (216): G Bihabwa, E Buluma, P Easterbrook, A Elbireer, A Kambugu, D Kamyia, M Katwere, R Kiggundu, C Komujuni, E Laker, E Lubwama, I Mambule, J Matovu, A Nakajubi, J Nakku, R Nalumenya, L Namuyimbwa, F Semitala, B Wandera, J Wanyama

JCRC, Mbarara (97): H Mugerwa, A Lugemwa, E Ninsiima, T Ssenkindu, S Mwebe, L Atwine, H William, C Katemba, S Abunyang, M Acaku, P Ssebutinde, H Kitizo, J Kukundakwe, M Naluguza, K Ssegawa, Namayanja, F Nsibuka, P Tuhirirwe, M Fortunate

JCRC Fort Portal (66): J Acen, J Achidri, A Amone, M. Chamai, J Ditai, M Kemigisa, M Kiconco, C Matama, D Mbanza, F Nambaziira, M Owor Odoi, A Rweyora, G. Tumwebaze

San Raphael of St Francis Hospital, Nsambya (48): H Kalanzi, J Katabaazi, A Kiyangi, M Mbidde, M. Mugenyi, R Mwebaze, P Okong, I Senoga

JCRC Mbale (47): M Abwola, D Baliruno, J Bwomezi, A Kasede, M Mudoola, R Namisi, F Ssennono, S Tuhirwe

JCRC Gulu (43): G Abongomera, G Amone, J Abach, I Aciro, B Arach, P Kidega, J Omongin, E Ocung, W Odong, A Philliam

JCRC Kabale (33): H Alima, B Ahimbisibwe, E Atuhaire, F Atukunda, G Bekusike, A Bulegyeya, D. Kahatano, S Kamukama, J Kyoshabire, A Nassali, A Mbonye, T M Naturinda, Ndrukukire, A Nshabohurira, H. Ntawiha, A Rogers, M Tibyasa;

JCRC Kakira (31): S. Kiirya, D. Atwongyeire, A. Nankya, C. Draleku, D. Nakiboneka, D. Odoch, L. Lakidi, R. Ruganda, R. Abiriga, M. Mulindwa, F. Balmoi, S. Kafuma, E. Moriku

Zimbabwe

University of Zimbabwe Clinical Research Centre, Harare (265): J Hakim, A Reid, E Chidziva, G Musoro, C Warambwa, G Tinago, S Mutsai, M Phiri, S Mudzingwa, T Bafana, V Masore, C Moyo, R Nhema, S Chitongo

Malawi

Department of Medicine, University of Malawi College of Medicine and the Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine (92): Robert Heyderman, Lucky Kabanga, Symon Kaunda, Aubrey Kudzala, Linly Lifa, Jane Mallewa, Mike Moore, Chrissie Mtali, George Musowa, Grace Mwimaniwa, Rosemary Sikwese, Joep van Oosterhout, Milton Ziwoya

Mzuzu Central Hospital, Mzuzu (19): H Chimbaka, B Chitete, S Kamanga, T Kayinga, E Makwakwa, R Mbiya, M Mlenga, T Mphande, C Mtika, G Mushani, O Ndhlovu, M Ngonga, I Nkhana, R Nyirenda

Kenya

Moi Teaching and Referral Hospital (52): P Cheruiyot, C Kwobah, W Lokitala Ekiru, M Mokaya, A Mudogo, A Nzioka, A Siika, M Tanui, S Wachira, K Wools-Kaloustian

Zambia

University Teaching Hospital (37): P Alipalli, E Chikatula, J Kipaila, I Kunda, S Lakhi, J Malama, W Mufwambi, L Mulenga, P Mwaba, E Mwamba, A Mweemba, M Namfukwe

The Aids Support Organisation (TASO), Uganda: E Kerukadho, B Ngwatu, J Birungi

MRC Clinical Trials Unit: N Paton, J Boles, A Burke, L Castle, S Ghuman, L Kendall, A Hoppe, S Tebbs, M Thomason, J Thompson, S Walker, J Whittle, H Wilkes, N Young

Monitors: C Kapuya, F Kyomuhendo, D Kyakundi, N Mkandawire, S Mulambo, S Senyonjo

Clinical Expert Review Committee: B Angus, A Arenas-Pinto, A Palfreeman, F Post, D Ishola

European Collaborators:

J Arribas (Hospital La Paz, Madrid, Spain), R Colebunders (Institute of Tropical Medicine, Antwerp, Belgium), M Floridia (ISS, Italy), M Giuliano (ISS, Italy), P Mallon (University College Dublin, Ireland), P Walsh (University College Dublin, Ireland), M De Rosa (CINECA, Italy), E Rinaldi (CINECA, Italy)

Trial Steering Committee: I Weller (Chair), C Gilks, J Hakim, A Kangewende, S Lakhi, E Luyirika, F Miiro, P Mwamba, P Mugenyi, S Ojoo, N Paton, S Phiri, J van Oosterhout, A Siika, S Walker, A Wapakabulo,

Data Monitoring Committee: T Peto (Chair), N French, J Matenga

Pharmaceutical companies: G Cloherty, J van Wyk, M Norton, S Lehrman, P Lamba, K Malik, J Rooney, W Snowden, J Villacian

Funding and in-kind support:

The EARNEST trial was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP, Grant Code: IP.2007.33011.003) with contributions from the Medical Research Council, UK; Instituto de Salud Carlos III, Spain (Grant A107/90015); Irish Aid, Ireland; Swedish International Development Cooperation Agency (SIDA), Sweden; Instituto Superiore di Sanita (ISS), Italy; The World Health Organisation; and Merck, USA. Substantive in-kind contributions were made by the Medical Research Council Clinical Trials Unit, UK, CINECA, Bologna, Italy, Janssen Diagnostics, Beerse, Belgium; GSK/ViiV Healthcare Ltd., UK; Abbott Laboratories, USA. Trial medication was donated by AbbVie, Merck, Pfizer, GSK and Gilead. The Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine receives core funding from the Wellcome Trust UK.

REFERENCES

1. Bhargava M, Cajas JM, Wainberg MA, Klein MB, Pant Pai N. Do HIV-1 non-B subtypes differentially impact resistance mutations and clinical disease progression in treated populations? Evidence from a systematic review. *Journal of the International AIDS Society* 2014; **17**: 18944.
2. Gilks CF, Crowley S, Ekpini R, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. *Lancet* 2006; **368**(9534): 505-10.
3. World Health Organisation. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. . Geneva: World Health Organisation 2016.
4. Paton NI, Kityo C, Hoppe A, et al. Assessment of second-line antiretroviral regimens for HIV therapy in Africa. *N Engl J Med* 2014; **371**(3): 234-47.
5. Richard N, Juntilla M, Abraha A, et al. High prevalence of antiretroviral resistance in treated Ugandans infected with non-subtype B human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* 2004; **20**(4): 355-64.
6. Woods CK, Brumme CJ, Liu TF, et al. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. *Journal of clinical microbiology* 2012; **50**(6): 1936-42.
7. Wensing AM, Calvez V, Gunthard HF, et al. 2014 update of the drug resistance mutations in HIV-1. *Top Antivir Med* 2014; **22**(3): 642-50.
8. Stanford HIV Drug Resistance Database. 2014. <http://hivdb.stanford.edu/>.
9. de Oliveira T, Deforche K, Cassol S, et al. An automated genotyping system for analysis of HIV-1 and other microbial sequences. *Bioinformatics* 2005; **21**(19): 3797-800.
10. Hosseinipour MC, van Oosterhout JJ, Weigel R, et al. 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**(9): 1127-34.
11. Etiebet MA, Shepherd J, Nowak RG, et al. Tenofovir-based regimens associated with less drug resistance in HIV-1-infected Nigerians failing first-line antiretroviral therapy. *AIDS* 2013; **27**(4): 553-61.
12. Wallis CL, Aga E, Ribaud H, et al. Drug susceptibility and resistance mutations after first-line failure in resource limited settings. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **59**(5): 706-15.
13. Sigaloff KC, Ramatsebe T, Viana R, de Wit TF, Wallis CL, Stevens WS. Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa. *AIDS Res Hum Retroviruses* 2012; **28**(2): 171-5.
14. Cozzi-Lepri A, Paredes, Phillips AN, et al. The rate of accumulation of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance in patients kept on a virologically failing regimen containing an NNRTI*. *HIV medicine* 2012; **13**(1): 62-72.
15. Cozzi-Lepri A, Phillips AN, Martinez-Picado J, et al. Rate of accumulation of thymidine analogue mutations in patients continuing to receive virologically failing regimens containing zidovudine or stavudine: implications for antiretroviral therapy programs in resource-limited settings. *J Infect Dis* 2009; **200**(5): 687-97.
16. Hoffmann CJ, Charalambous S, Sim J, et al. Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009; **49**(12): 1928-35.
17. Hamers RL, Sigaloff KC, Wensing AM, et al. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2012; **54**(11): 1660-9.
18. Manasa J, Lessells RJ, Skingsley A, et al. High-levels of acquired drug resistance in adult patients failing first-line antiretroviral therapy in a rural HIV treatment programme in KwaZulu-Natal, South Africa. *PLoS One* 2013; **8**(8): e72152.

19. Tang MW, Rhee SY, Bertagnolio S, et al. Nucleoside reverse transcriptase inhibitor resistance mutations associated with first-line stavudine-containing antiretroviral therapy: programmatic implications for countries phasing out stavudine. *J Infect Dis* 2013; **207 Suppl 2**: S70-7.
20. Chaplin B, Eisen G, Idoko J, et al. Impact of HIV type 1 subtype on drug resistance mutations in Nigerian patients failing first-line therapy. *AIDS Res Hum Retroviruses* 2011; **27**(1): 71-80.
21. Orrell C, Walensky RP, Losina E, Pitt J, Freedberg KA, Wood R. HIV type-1 clade C resistance genotypes in treatment-naïve patients and after first virological failure in a large community antiretroviral therapy programme. *Antiviral therapy* 2009; **14**(4): 523-31.
22. Crawford KW, Njeru D, Maswai J, et al. Occurrence of etravirine/rilpivirine-specific resistance mutations selected by efavirenz and nevirapine in Kenyan patients with non-B HIV-1 subtypes failing antiretroviral therapy. *Aids* 2014; **28**(3): 442-5.
23. Sluis-Cremer N, Jordan MR, Huber K, et al. E138A in HIV-1 reverse transcriptase is more common in subtype C than B: implications for rilpivirine use in resource-limited settings. *Antiviral research* 2014; **107**: 31-4.
24. Maiga AI, Descamps D, Morand-Joubert L, et al. Resistance-associated mutations to etravirine (TMC-125) in antiretroviral-naïve patients infected with non-B HIV-1 subtypes. *Antimicrob Agents Chemother* 2010; **54**(2): 728-33.
25. Taiwo B, Chaplin B, Penugonda S, et al. Suboptimal etravirine activity is common during failure of nevirapine-based combination antiretroviral therapy in a cohort infected with non-B subtype HIV-1. *Curr HIV Res* 2010; **8**(3): 194-8.
26. Casadella M, Noguera-Julian M, Sunpath H, et al. Treatment options after virological failure of first-line tenofovir-based regimens in South Africa: an analysis by deep sequencing. *AIDS* 2016; **30**(7): 1137-40.
27. TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. *Lancet Infect Dis* 2016; **16**: 565-75.
28. White E, Smit E, Churchill D, et al. No Evidence That HIV-1 Subtype C Infection Compromises the Efficacy of Tenofovir-Containing Regimens: Cohort Study in the United Kingdom. *J Infect Dis* 2016.
29. Anta L, Llibre JM, Poveda E, et al. Rilpivirine resistance mutations in HIV patients failing non-nucleoside reverse transcriptase inhibitor-based therapies. *AIDS* 2013; **27**(1): 81-5.
30. van Zyl GU, van der Merwe L, Claassen M, Zeier M, Preiser W. Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa. *J Med Virol* 2011; **83**(10): 1764-9.
31. Kiartiburanakul S, Wiboonchutikul S, Sukasem C, Chantratita W, Sungkanuparph S. Using of nevirapine is associated with intermediate and reduced response to etravirine among HIV-infected patients who experienced virologic failure in a resource-limited setting. *J Clin Virol* 2010; **47**(4): 330-4.

Table 1: Characteristics of study population at first line failure

Characteristic at first line failure	Overall N=787 n (%)	Subtype-A N=311 40% n (%)	Subtype-C N=244 31% n (%)	Subtype-D N=194 25% n (%)	Recombinant* N=38 5% n (%)	P value
Country n(%)						
Kenya	33 (4%)	20 (6%)	3 (1%)	7 (4%)	3 (8%)	<0.001
Malawi	69 (9%)	0	68 (28%)	0	1 (3%)	
Uganda	516 (66%)	289 (93%)	12(5%)	186 (96%)	29 (76%)	
Zimbabwe	169 (21%)	2 (1%)	161 (66%)	1 (1%)	5 (13%)	
Sex n(%)						
Female	485 (62%)	193 (62%)	141 (58%)	126 (65%)	25 (66%)	0.44
Male	302 (38%)	118 (38%)	103 (42%)	70 (36%)	13 (34%)	
Age median(IQR)	37 (30-43)	36 (30-42)	39 (33-45)	35 (30-42)	34 (29-43)	<0.001
Years on first-line therapy median (IQR)	4.0 (2.8-5.4)	3.8 (2.7-5.3)	4.3 (3.1-5.5)	3.9 (2.8-5.4)	3.8 (2.8-5.2)	0.02
2 or more CD4s on first-line therapy**	549 (70%)	284 (91%)	71 (29%)	163 (84%)	31 (82%)	<0.001
1 or more VLs on first-line therapy**	160 (20%)	75 (24%)	26 (11%)	51 (26%)	8 (21%)	<0.001
Drug exposure in first line therapy n(%)						
Number of regimens						
1	398 (51%)	151 (49%)	141 (58%)	88 (45%)	18 (47%)	0.09
2	324 (41%)	129 (41%)	89 (36%)	91 (47%)	15 (39%)	
3+	65 (8%)	31 (10%)	14 (6%)	15 (8%)	5 (13%)	
NNRTI						
Number exposed to						
1	660 (84%)	270 (87%)	194 (80%)	166 (86%)	30 (79%)	0.09
2	127 (16%)	41 (13%)	50 (20%)	28 (14%)	8 (21%)	
NNRTI at failure						
Efavirenz	193 (25%)	84 (27%)	45 (18%)	54 (28%)	10 (26%)	0.05
Nevirapine	594 (75%)	227 (73%)	199 (82%)	140 (72%)	28 (74%)	
NRTI						
Number exposed to						
2	467 (59%)	173 (56%)	174 (70%)	100 (52%)	24 (63%)	0.001
3	293 (37%)	125 (40%)	69 (28%)	86 (44%)	13 (34%)	
4/5	27 (3%)	13 (4%)	5 (2%)	8 (4%)	1 (3%)	
NRTI at failure						
Tenofovir	96(12%)	56 (18%)	3 (1%)	31 (16%)	6 (16%)	<0.001
Stavudine	200 (25%)	21 (7%)	162 (66%)	8 (4%)	9 (24%)	
Zidovudine	486 (62%)	233 (75%)	76 (31%)	154 (79%)	23 (61%)	
None of the above †	5 (1%)	1 (0%)	3 (1%)	1 (1%)	0	
CD4 (cells/mm ³)						
median (IQR)	67 (27-136)	69 (26-151)	66 (25-116)	71 (30-140)	54 (25-129)	0.47
<100 n(%)	495 (63%)	191 (61%)	161 (66%)	119 (61%)	24 (63%)	

Viral load (copies/ml)						
median (IQR)	74,500 (25,400- 194,130)	74,100 (23,906- 183,935)	77,679 (27,591- 250,864)	59,760 (24,135 - 163,417)	148,500 (43,542- 240,725)	0.33
≥100,000 n(%)	334 (42%)	131 (42%)	109 (45%)	72 (37%)	22 (58%)	

* called by REGA as a recombinant or not called by REGA.

** excluding values at ART initiation and within the 90 days preceding switch to second-line (since some additional testing was done as part of trial recruitment initiatives)

† 1 didanosine, 4 abacavir

Table 2: Resistance to potential future NRTI and NNRTI drug options

Drug	Intermediate/ high resistance (N=787) n(%)	High resistance (N=787) n(%)
NRTI		
ABC TDF ZDV	392 (50%)	191 (24%)
ABC ZDV (not TDF)	168 (21%)	177 (22%)
ABC TDF (not ZDV)	54 (7%)	51 (6%)
ABC (not TDF ZDV)	32 (4%)	18 (2%)
ZDV (not TDF ABC)	0	111 (14%)
TDF (not ZDV ABC)	0	2
None of ABC TDF ZDV	141 (18%)	237 (30%)
NNRTI		
EFV NVP ETR RPV	432 (55%)	83 (11%)
EFV NVP RPV (not ETR)	79 (10%)	98 (12%)
NVP ETR RPV (not RPV)	0	19 (2%)
EFV NVP (not ETR RPV)	252 (32%)	373 (47%)
NVP RPV (not EFV ETR)	2	6 (1%)
NVP (not EFV ETR RPV)	1	184 (23%)
None of EFV NVP ETR RPV	21 (3%)	24 (3%)

ABC=abacavir, TDF=tenofovir, ZDV=zidovudine,
 EFV=efavirenz, NPV=nevirapine, ETR=etravirine, RPV=rilpivirine

Table 3: Factors predicting intermediate/ high level resistance to drugs for potential use in a second-line regimen

	Abacavir resistance		Tenofovir resistance		Zidovudine resistance		Etravirine resistance		Rilpivirine resistance	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
NRTI at failure		<0.001		<0.001		<0.001				
Tenofovir vs Stavudine	10.77 (3.51, 33.05)	<0.001	1.76 (0.82, 3.78)	0.15	0.31 (0.13, 0.75)	0.009				
Zidovudine vs Stavudine	6.24 (3.00, 13.01)	<0.001	3.13 (1.76, 5.59)	<0.001	6.27 (3.24, 12.13)	<0.001				
Zidovudine vs Tenofovir	0.58 (0.23, 1.48)	0.26	1.78 (0.98, 3.24)	0.06	20.21 (9.98, 40.96)	<0.001				
NNRTI at failure										
Nevirapine vs Efavirenz							3.39 (2.11, 5.47)	<0.001	2.88 (1.84, 4.50)	<0.001
NRTIs prior to failure										
None (failed on initial NRTIs)	1		1		1					
Stavudine only	1.78 (0.99, 3.22)	0.04	1.31 (0.86, 2.01)	0.14	1.51 (0.90, 2.55)	<0.001				
Zidovudine only	7.82 (0.94, 65.01)		2.58 (0.93, 7.14)		3.80 (1.34, 10.80)					
Stavudine and Zidovudine	4.21 (0.40, 44.19)		2.11 (0.69, 6.51)		8.59 (2.70, 27.28)					
NNRTIs prior to failure (vs failed on first NNRTI)										
Efavirenz							0.51 (0.26, 1.00)	0.004	0.39 (0.20, 0.76)	0.005
Nevirapine							2.47 (1.28, 4.76)		1.76 (0.92, 3.37)	
Subtype		0.002		0.32		0.02		<0.001		<0.001
C vs A	3.31 (1.59, 6.88)	0.001	1.31 (0.78, 2.20)	0.30	2.34 (1.24, 4.42)	0.009	2.37 (1.61, 3.47)	<0.001	2.28 (1.51, 3.46)	<0.001
C vs D	4.08 (1.85, 9.03)	0.001	1.53 (0.88, 2.67)	0.13	2.54 (1.28, 5.05)	0.008	1.88 (1.23, 2.87)	0.003	2.45 (1.56, 3.85)	<0.001
D vs A	0.81 (0.48, 1.36)	0.43	0.85 (0.57, 1.27)	0.44	0.92 (0.58, 1.46)	0.73	1.26 (0.87, 1.83)	0.23	0.93 (0.64, 1.36)	0.72
Log ₁₀ viral load at failure	1.58 (1.15, 2.16)	0.003	1.95 (1.50, 2.53)	<0.001	1.53 (1.15, 2.02)	0.003	1.29 (1.01, 1.64)	0.04	1.21 (0.94, 1.56)	0.15
CD4 at failure (per 100 cell increase)	0.59 (0.48, 0.72)	<0.001	0.48 (0.39, 0.59)	<0.001	0.66 (0.55, 0.80)	<0.001	0.79 (0.67, 0.93)	0.004	0.75 (0.64, 0.89)	0.001
Clinical failure	0.79 (0.43, 1.46)	0.45	0.99 (0.61, 1.61)	0.96	0.75 (0.44, 1.29)	0.31	1.24 (0.76, 2.00)	0.39	1.32 (0.78, 2.25)	0.30
Years on ART	1.13 (0.99, 1.28)	0.07	1.03 (0.95, 1.13)	0.47	1.19 (1.06, 1.33)	0.003	0.99 (0.92, 1.07)	0.85	0.99 (0.91, 1.07)	0.74
Fit statistic										
Area under the ROC curve	76% (71%, 80%)		75% (71%, 78%)		78% (74%, 82%)		69% (65%, 73%)		70% (66%, 74%)	

Figure 1: Prevalence of major IAS-USA drug resistance mutations by HIV-1 subtype

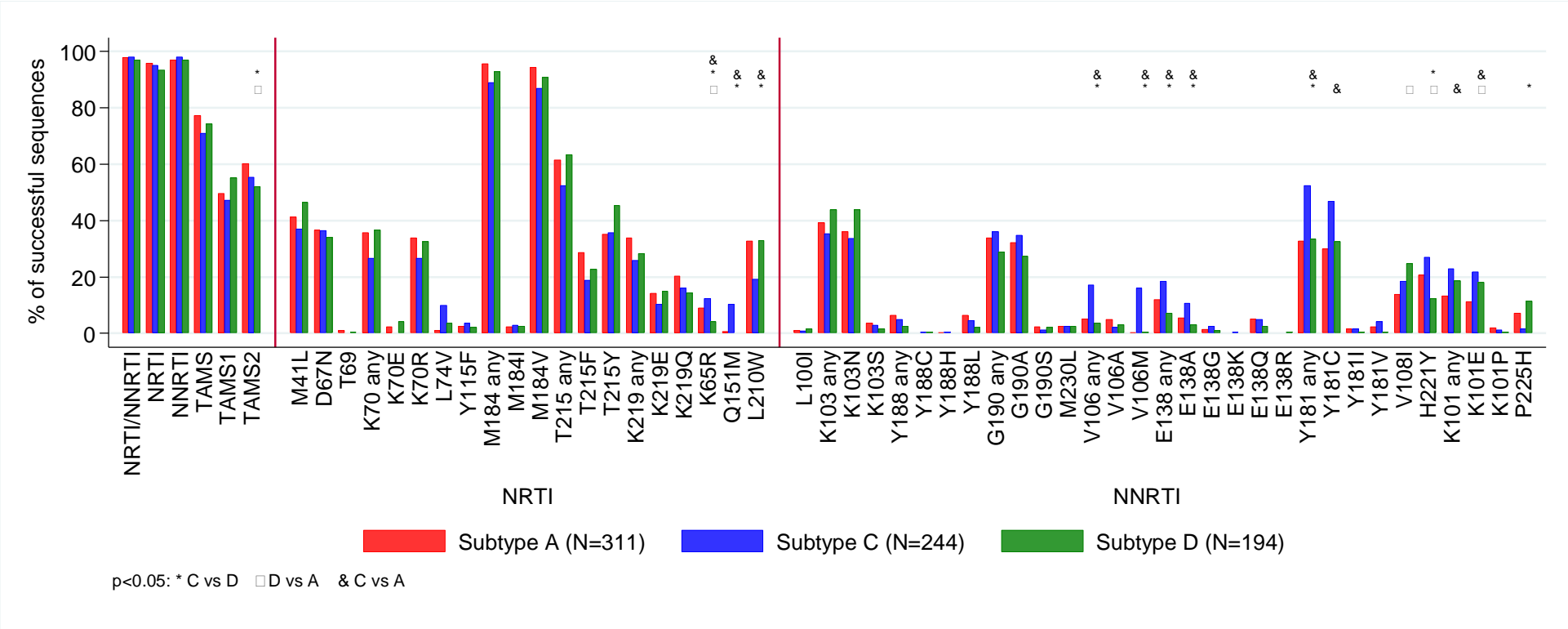


Figure 2 Prevalence of major IAS-USA drug resistance mutations by drug exposure at first line failure

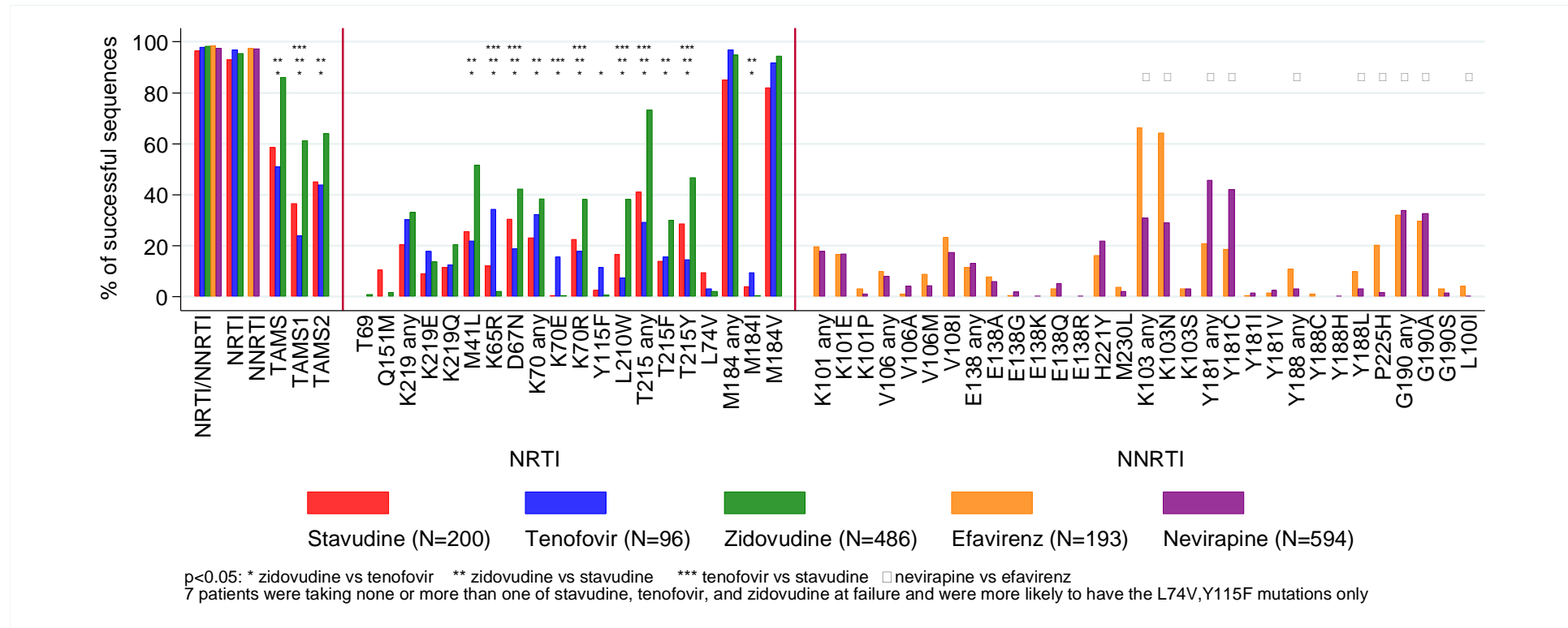


Figure 3: Overall resistance to NRTI and NNRTI drugs

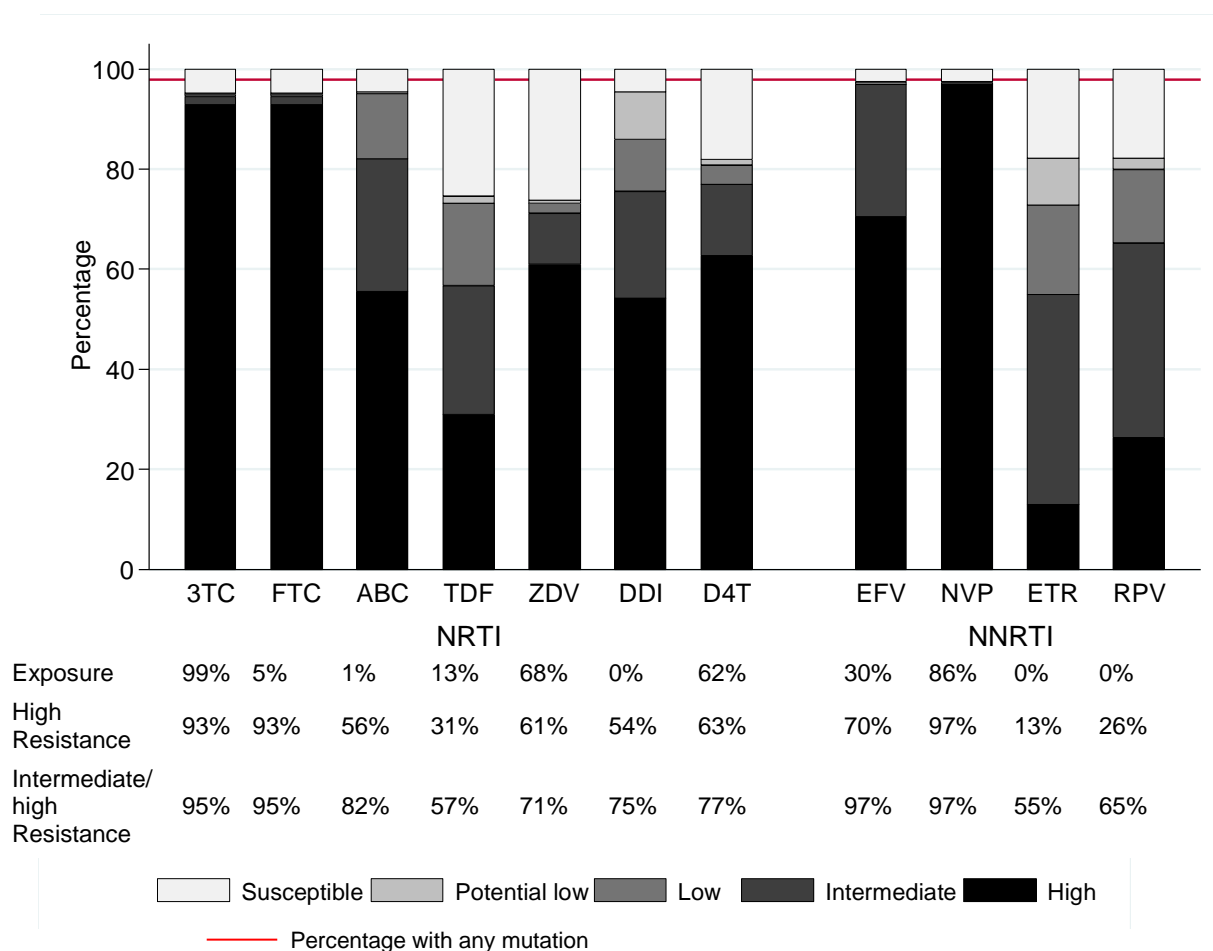


Figure 4a: Intermediate/high level resistance according to exposure or cross-resistance

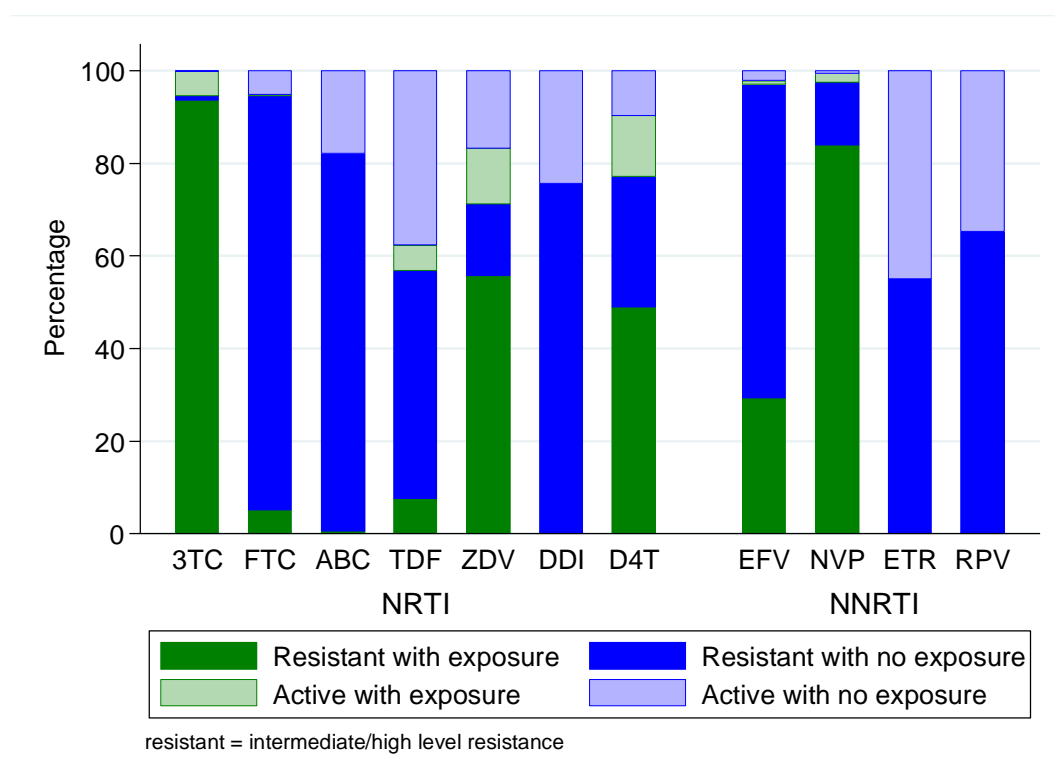


Figure 4b: High level resistance according to exposure or cross-resistance

