**BRIEF REPORT**

**An apparent paradox: resistance mutations in HIV-1 DNA predict improved virological responses to antiretroviral therapy**

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**Abstract**

**Objective:** In sub-Saharan Africa, detecting resistance-associated mutations (RAMs) at failure of first-line ART with 2 NRTIs plus an NNRTI predicts improved virological responses to second-line therapy with 2 NRTIs plus a ritonavir-boosted PI (PI/r). This indicates residual NRTI activity in the presence of RAMs, although additional factors may contribute to the effect. This study investigated the influence of pre-existing RAMs on the outcomes of maintenance monotherapy with ritonavir-boosted darunavir within a randomised trial in Cameroon.

**Methods:** RAMs were detected in HIV-1 DNA using PBMC collected at initiation of darunavir/ritonavir monotherapy. Adherence was assessed by pill count and visual analogue scale (VAS). Predictors of virological failure (confirmed or last available viral load >400 copies/mL) were explored by logistic regression analysis.

**Results:** After NNRTI-based therapy, participants (n=81) had received PI/r-based therapy for a median of 3.2 years and had a confirmed viral load <60 copies/mL and a median CD4 count of 466 cells/mm3. NRTI and NNRTI RAMs were detected in 39/60 (65.0%) and 41/60 (68.3%) HIV-1 DNA sequences, respectively. Over 48 weeks of monotherapy, 16/81 (19.8%) patients experienced virological failure. After adjusting for age, HIV-1 DNA load, adherence by VAS, and RAM status, virological failure was less likely with higher VAS-measured adherence (adjusted odds ratio 0.04, 95% CI 0.01-0.37; p=0.004) and detectable HIV-1 DNA RAMs (adjusted odds ratio 0.15, 95% CI 0.03-0.82; p=0.028).

**Conclusions:** Pre-existingNRTI and NNRTI RAMs are associated with improved virological responses to NRTI-sparing ART in sub-Saharan Africa, indicating a predictive effect that is independent of residual NRTI activity.

**Keywords:** Resistance, adherence, Africa, monotherapy, NRTI, HIV-1 DNA

**Introduction**

The WHO recommends standardised regimens for first-line and second-line ART in sub-Saharan Africa, comprising 2 NRTIs plus an NNRTI, a PI/r, or more recently an integrase inhibitor.1 Pooled estimates indicate adherence rates of 67-77% in the region,2,3 and rates of virological suppression of 65% and 62% after 24 months of NNRTI-based first-line ART4 and PI/r-based second-line5 ART, respectively. Sparse virological monitoring and delayed introduction of second-line ART in viraemic patients favour the emergence of drug resistance, and RAMs affecting both the NRTIs and the NNRTIs are common at failure of first-line NNRTI-based ART.6-10 The significance of NRTI RAMs in terms of predicting reduced responses to second-line NRTI-containing ART has been called into question.5,11 In a meta-analysis of pooled data from sub-Saharan Africa, we observed that detection of RAMs at failure of NNRTI-based ART, most commonly M184V and thymidine analogue mutations (TAMs), predicted higher (rather than lower) odds of virological suppression after starting 2NRTIs + PI/r.5 Thus, it has been inferred that resistance testing underestimates the continued activity of NRTIs such as lamivudine and tenofovir in the presence of M184V and TAMs. Here we report the same predictive effect in patients receiving NRTI-sparing ART.

**Methods**

***Study population***

MANET (Monotherapy in Africa, New Evaluations of Treatment) was a randomised, open-label trial based at Hôpital Central Yaoundé (NCT02155101). Between August 2014 and July 2015, 120 virologically suppressed patients on 2NRTIs + PI/r either switched to darunavir/ritonavir monotherapy (800/100mg once daily) for 48 weeks (n=81) or continued 2NRTIs + PI/r for 24 weeks (n=39). Eligibility criteria comprised receiving 2NRTIs + PI/r for ≥12 weeks, CD4 count >100 cells/mm3, viral load <60 copies/mL in 2 measurements 4-12 weeks apart (median 7 weeks), and hepatitis B surface antigen (HBsAg)-negative. This analysis focused on patients receiving monotherapy.

**Ethics**

Approval was granted by the University of Liverpool Ethics Committee (RETH000605) and the Cameroon National Ethics Committee (2013/07/347). Participants gave written informed consent.

***Adherence***

Participants collected a prescription from pharmacy every 4 weeks and attended study visits at weeks 4, 12, 24, 36, and 48. At each pharmacy visit, the number of left-over pills was counted to infer the percentage of pills taken. At each study visit, adherence was self-reported via a visual analogue scale (VAS) graded from 0% (complete non-adherence) to 100% (complete adherence) in 10% increments.12

***Laboratory testing***

Safety parameters, CD4 cell counts, and viral load (Biocentric, Bandol, France; lower limit of quantification 60 copies/mL) were measured at the Centre Pasteur of Cameroon in Yaoundé. In the United Kingdom, total HIV-1 DNA was quantified in PBMC as described.12 RAMs were detected by Sanger sequencing of protease (aa 1-99) and reverse transcriptase (aa 1-335) as described,13 and defined by the Stanford HIV drug resistance algorithm (v8.4). Darunavir RAMs comprised V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V.

***Analyses***

The characteristics of patients with or without HIV-1 DNA sequencing results were compared using Wilcoxon rank-sum test, *t*-test, or Fisher’s exact test. Pill counts and VAS percentages were averaged in each patient over the entire study period, and compared by Spearman’s rank correlation. Patients with virological rebound were recalled for adherence counselling, followed by repeat testing 4 weeks later. Mean adherence levels in the period before versus the period after the first counselling session were compared by *t*-test. Treatment failure comprised virological failure (either confirmed or last available viral load >400 copies/mL) or discontinuation of monotherapy prior to week 48 due to a significant adverse event, death, or loss to follow-up. Predictors of virological failure were explored by logistic regression analysis; all available variables were considered for inclusion and those with p≤0.2 in the univariate analysis were retained in the multivariable model.

**Results**

***HIV-1 DNA RAMs at study entry***

The population is described in Table 1. Of 76 patients with PBMC samples, 60 (78.9%) yielded a HIV-1 DNA sequence. Relative to patients with sequencing results, those without had a lower HIV-1 DNA load (p=0.0004) and a higher nadir CD4 cell count (p=0.05) (Supplementary Table 1). NRTI and NNRTI RAMs occurred in 39/60 (65.0%) and 41/60 (68.3%) samples, respectively (Table 1); 37/60 (61.6%) samples had dual-class mutations.

***Outcomes of DRV/r monotherapy***

Between week 12 and week 48 (median week 24), 16/81 (19.8%) patients experienced virological failure. A further 6/81 (7.4%) prematurely discontinued monotherapy owing to significant adverse events (n=5) or loss to follow-up (n=1) (Supplementary Table 2). A further 12/81 (14.8%) patients did not meet the definition of virological failure but experienced 1 viraemic episode (median 2.2log10 copies/mL; range 1.9-2.9), with 9/12 experiencing a single episode. Between week 12 and week 48 (median week 36), 21/28 viraemic patients (median viral load 3.0 log10 copies/mL; range 2.0-4.1) underwent resistance testing. Largely reflecting the profiles of HIV-1 DNA, NRTI and NNRTI RAMs occurred in 7/21 (33.3%) and 8/21 (38.1%) samples, respectively; 6/21 (28.6%) samples had dual-class mutations. No sample had darunavir RAMs.

***Adherence***

Mean adherence was 97.3% (±2.9) by pill count and 97.3% (±3.5) by VAS overall, and 97.3% (±2.5) and 94.9% (±5.2) respectively in the virological failure population. The two adherence measures were weakly correlated (Spearman’s r=0.26; p=0.021) (Supplementary Figure 1). Among viraemic patients, mean adherence levels in the period preceding versus that following adherence counselling were 97.6% versus 97.4% (p=0.677) by pill count and 96.5% versus 95.7% (p=0.264) by VAS.

***Predictors of virological failure***

By univariate analysis, the odds of virological failure were lower in older patients and those with a lower HIV-1 DNA load, higher adherence by VAS, and either detected HIV-1 DNA RAMs or unknown HIV-1 DNA RAM status (due to missing data) (Table 2). After adjustment, higher VAS-measured adherence and detection of HIV-1 DNA RAMs remained independently predictive of reduced odds of virological failure.

**Discussion**

Among patients who switched from suppressive second-line ART with 2NRTIs + PI/r to darunavir/ritonavir monotherapy, 19.8% experienced virological failure over 48 weeks. Detection of HIV-1 DNA RAMs at study entry and higher VAS-measured adherence during the study predicted reduced odds of virological failure in this population.

Previous randomised studies from Western Europe investigated darunavir/ritonavir monotherapy in patients without a history of treatment failure, and reported frequent viraemia but a low risk of darunavir RAMs.14 A study from Burkina Faso, Cameroon, and Senegal evaluated patients who after failure of first-line ART with 2NRTIs + NNRTI had achieved virological suppression on 2NRTIs + PI/r,15 thus resembling the population of this study. Of 133 patients randomised to either darunavir/ritonavir (n=56) or lopinavir/ritonavir monotherapy, 21% experienced virological failure (viral load >500 copies/mL) over 48 weeks. Similarly high rates of virological failure were observed in this study. Together, the data indicate that darunavir/ritonavir monotherapy is not indicated for patients in sub-Saharan Africa due to the high risk of viraemia.16 Adding a further dimension, we previously observed a risk of both incident and reactivated hepatitis B virus (HBV) infection after discontinuation of NRTIs that may exert prophylactic activity in a high HBV endemicity setting such as Cameroon.17

VAS-measured adherence was an independent predictor of virological outcomes, and performed better than directly measured pill counts. This is promising given that VAS is an easy tool to implement in resource-limited settings. Although there exists conflicting evidence about the relative performance of self-reported versus directly assessed adherence measures in sub-Saharan Africa,18,19 in the study from Burkina Faso, Cameroon, and Senegal, pill counts were also less likely to detect adherence problems relative to the use of questionnaires.15 It is possible that the personal interaction required to administer a VAS (or a questionnaire) may bring benefit when assessing adherence in these settings.

The study provides novel insights about the role of RAMs as predictors of virological outcomes in treatment-experienced populations in sub-Saharan Africa. At study entry, the mutational patterns in HIV-1 DNA were overall reflective of prolonged viraemia on first-line NNRTI-based ART. Mutations selected by lamivudine (M184V) and zidovudine or stavudine (TAMs) were common, as were the nevirapine and efavirenz RAMs K103N and Y181C. Further emergence of NRTI RAMs may have occurred during undocumented periods of viraemia on 2 NRTIs + PI/r. Our previous pooled analysis indicated that detecting RAMs at failure of first-line ART with 2NRTIs + NNRTI predicted improved responses after starting second-line ART with 2NRTIs + PI/r.5 Here, detecting NRTI and NNRTI RAMs in HIV-1 DNA of virologically suppressed patients receiving 2NRTIs + PI/r predicted a reduced risk of virological failure after switching to darunavir/ritonavir monotherapy. The effect persisted after considering other proposed predictors of responses to PI/r monotherapy, including adherence, HIV-1 DNA load, and nadir CD4 cell counts.20 Whereas NRTIs are expected to retain partial antiviral activity despite the presence of RAMs, the findings imply a predictive role for pre-existing NRTI RAMs that is partially independent of residual NRTI activity. RAMs may be a proxy for unknown co-variables. It may also be proposed that patients who develop resistance while receiving ART have relatively higher levels of adherence (hence higher drug selective pressure) than patients who experience failure without resistance, and maintain higher adherence levels during subsequent treatment lines. Thus, RAMs may act as an additional, sensitive indicator of overall compliance with treatment in these populations.

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**Transparency declaration**

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*Authors’ contribution*

AMG: Study design, data collection and analysis, preparation of the manuscript; AA: sequencing and data analysis; OMF: study coordination, samples and data acquisition; LB: statistical analysis; VFD, SM, and CK: clinical supervision and data acquisition; JF: laboratory data acquisition; JT: laboratory supervision. All authors contributed to the preparation of the manuscript.

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**Table 1**. Characteristics of the study population at study entry (n=81)

|  |  |  |
| --- | --- | --- |
| Characteristic | |  |
| Female, n (%) | | 61 (75.3) |
| Age, median years (IQR) | | 45 (38, 51) |
| Time since HIV diagnosis, median years (IQR) | | 8.8 (5.9, 11.1) |
| CD4 count, median cells/mm3 (IQR) | | 466 (341, 615) |
| Nadir CD4 count, median cells/mm3 (IQR) | | 90 (37, 167) |
| History of AIDS, n (%) | | 12 (14.8) |
| HIV-1 DNA, median log10 copies/106 PBMC (IQR) | | 2.9 (2.5, 3.3) |
| Antiretroviral drugs  ever experienced, n (%) | Lamivudine | 81 (100) |
| Tenofovir | 73 (90.1) |
| Zidovudine | 72 (88.9) |
| Stavudine | 41 (50.6) |
| Didanosine | 13 (16.0) |
| Abacavir | 9 (11.1) |
| Nevirapine | 50 (61.7) |
| Efavirenz | 43 (53.1) |
| Lopinavir/ritonavir | 79 (97.5) |
| Indinavir/ritonavir | 9 (11.1) |
| Atazanavir/ritonavir | 5 (6.2) |
| Duration of exposure,  median years (IQR) | Total ART | 7.6 (5.3, 9.8) |
| Tenofovir | 2.9 (1.3, 4.6) |
| Zidovudine | 2.4 (1.5, 4.0) |
| Stavudine | 2.6 (1.3, 4.0) |
| NNRTI | 3.1 (1.6, 5.5) |
| PI/r | 3.2 (1.3, 5.8) |
| ART regimen  at study entry, n (%) | Tenofovir/lamivudine + lopinavir/ritonavir | 69 (85.2) |
| Tenofovir/lamivudine + atazanavir/ritonavir | 2 (2.5) |
| Tenofovir + abacavir + lopinavir/ritonavir | 1 (1.2) |
| Abacavir + didanosine + lopinavir/ritonavir | 5 (6.2) |
| Zidovudine/lamivudine + lopinavir/ritonavir | 4 (4.9) |
| GSS of ART regimen  at study entry, n (%)a | 3 | 22 (27.2) |
| 2 | 22 (27.2) |
| 1.5 | 1 (1.2) |
| 1.25 | 10 (12.3) |
| 1 | 5 (6.2) |
|  | Not availableb | 21 (25.9%) |
| HIV-1 subtype, n (%)c | CRF02\_AG | 35 (43.2) |
| A1 | 10 (12.3) |
| G | 5 (6.2) |
| Other | 10 (12.3) |
|  | Not availableb | 21 (25.9%) |
| RAMs in HIV-1 DNA,  n (%)d | Any | 44 (54.3) |
| None | 16 (19.8) |
| Not available | 21 (25.9%) |
| Any NRTI | 39 (48.1) |
| 1 TAM | 13 (16.0) |
| 2 TAMs | 5 (6.2) |
| ≥3 TAMs | 11 (13.6) |
| TAM-1 profile | 10 (12.3) |
| TAM-2 profile | 15 (18.5) |
| Mixed/other TAM profile | 4 (4.9) |
| K65R | 2 (2.5) |
| L74V/I | 5 (6.2) |
| M184I/V | 35 (43.2) |
| Q151M/L | 3 (3.7) |
| T69ins | 1 (1.2) |
| T69D/N | 1 (1.2) |
| Any NNRTI | 41 (50.6) |
| A98G | 9 (11.1) |
| L100I | 1 (1.2) |
| K101E/H/P/T | 6 (7.4) |
| K103N | 20 (24.7) |
| V106A | 3 (3.7) |
| V108I | 6 (7.4) |
| E138G/K | 3 (3.7) |
| Y181C | 14 (17.3) |
| Y188L/C/F/H/L | 4 (4.9) |
| G190A | 9 (11.1) |
| H221Y | 5 (6.2) |
| P225H | 2 (2.5) |
| F227L | 3 (3.7) |
| M230I/L | 4 (4.9) |
| K238T | 4 (4.9) |
| Y318F | 1 (1.2) |

aGSS were determined from HIV-1 DNA sequencing results using the Stanford HIV drug resistance algorithm (v8.4) and assigning to each drug in the regimen 0 for high-level resistance, 0.25 for intermediate resistance, 0.5 for low-level resistance, and 1 for potential low-level resistance/full susceptibility. bFive patients did not have a PBMC sample for sequencing; 16 samples did not yield a HIV-1 DNA sequence in ≥2 attempts and no further attempts were possible due to limited sample volume. cHIV-1 subtypes were determined phylogenetically from HIV-1 DNA sequences; other subtypes comprised CRF11\_cpx (n=3), and subtypes D, H, F1, CRF01\_AE, CRF37\_cpx, CRF06\_cpx, and CRF18\_cpx (one of each). dNRTI and NNRTi RAMs of 60 patients with HIV-1 DNA sequencing results are detailed; 3 patients had D30N in protease but no record of exposure to a PI/r other than lopinavir/ritonavir and including nelfinavir; 11 RT sequences showed stop codons (positions 24, 42, 48, 71, 88, 120, 153, 212, 219, 229, 239) suggestive of defective proviruses; among 29 patients with ≥1 TAM, the median number of TAMs was 2 (range 1-5); TAM-1 profile comprised 1 of [M41L, L210W, T215Y]; TAM-2 profile comprised 1 of [D67N/G, K70R, T215F (or T215I/V occurring with other TAM-2 RAMs), K219 Q/E/R]; mixed/other TAM profile comprised mixtures of TAM-1 and TAM-2 RAMs or occurrence of T215S as the sole TAM.

Abbreviations: IQR= interquartile range; PI/r= ritonavir-boosted PI; GSS= genotypic susceptibility score; RAMs= resistance associated mutations; TAM= thymidine analogue mutation; RT= reverse transcriptase.

**Table 2.** Factors associated with virological failure (confirmed or last available viral load >400 copies/mL)

Abbreviations: OR= odds ratio; AOR= adjusted odds ratio; PI/r= ritonavir-boosted PI; RAM= Resistance associated mutation; VAS= Visual analogue scale.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Univariate analysis | | Multivariable analysis | |
| Characteristic |  | OR (95% CI) | *P* value | AOR (95% CI) | *P* value |
| Gender | female versus male | 0.66 (0.20-2.20) | 0.500 |  |  |
| Age | per 5 year older | 0.75 (0.54-1.04) | 0.087 | 0.94 (0.87-1.01) | 0.119 |
| Time since HIV diagnosis | per year longer | 0.92 (0.78-1.09) | 0.348 |  |  |
| CD4 count | per 50 cells/mm3 higher | 1.04 (0.94-1.14) | 0.496 |  |  |
| Nadir CD4 count | per 50 cells/mm3 higher | 0.92 (0.64-1.31) | 0.867 |  |  |
| History of AIDS | yes versus no | 1.44 (0.34-6.06) | 0.622 |  |  |
| HIV-1 DNA | per log10 copies/106 PBMC higher | 1.80 (0.72-4.50) | 0.211 | 2.80 (0.80-9.99) | 0.109 |
| ART duration | per year longer | 0.91 (0.76-1.09) | 0.325 |  |  |
| PI/r duration | per year longer | 0.96 (0.79-1.16) | 0.667 |  |  |
| 1 RAM in HIV-1 DNA | yes versus no | 0.31 (0.09-1.09) | 0.069 | 0.15 (0.03-0.82) | 0.028 |
|  | unknown versus no | 0.17 (0.03-0.94) | 0.042 | 0.29 (0.04-2.06) | 0.218 |
| Pill count | Per 10% increment | 0.55 (0.15-2.03) | 0.367 |  |  |
| VAS | Per 10% increment | 0.21 (0.06-0.69) | 0.011 | 0.04 (0.01-0.37) | 0.004 |