Reduced efficacy of HIV-1 integrase inhibitors in patients with drug resistance mutations in reverse transcriptase

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Abstract

Background: Little is known about the impact of pre-treatment drug resistance (PDR) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) on the efficacy of second generation integrase inhibitors, now the standard of care drug class for HIV-1 treatment globally.

Methods: We conducted next-generation sequencing on stored plasma specimens from the ADVANCE trial collected prior to treatment initiation. Our primary outcome was 96-week virologic success, defined as achievement of a viral load < 1000 copies/mL from 12 weeks, < 200 copies/mL from 24 weeks, and < 50 copies/mL from 48 through 96 weeks. We estimated the impact of PDR, defined by the presence of drug resistance on the World Health Organization (WHO) mutation list, on virologic outcomes in the entire cohort, and stratified by EFV-based versus DTG-based regimens. In sensitivity analyses, we allowed virologic failure with re-suppression, assessed FDA 48 and 96-week Snapshot outcomes, and considered minority resistance mutations (5–20% frequency).

Results: Of 1,053 trial participants, 873 (83%) had plasma available and successful sequencing completed. Of these, 288 (33%) were randomized to an EFV-based regimen and 585 (67%) were randomized to a DTG-based regimen. Fourteen percent (122/873) had at least one WHO-defined mutation, of which over 98% (120/122) had NNRTI mutations. NRTI mutations were rare (20/873, 2%). Rates of virologic suppression were significantly lower in those with PDR 65% (73/112) compared to those without PDR (85% [605/713], $P < 0.001$). This phenomenon was consistent for both EFV-based (60% [12/20] versus 86% [214/248], $P = 0.002$) and DTG-based ART (61/92 [66%] versus 84% [391/465] $P < 0.001$, P for interaction by regimen 0.49). In multivariable models adjusted for clinical characteristics and treatment adherence, PDR strongly predicted failure [adjusted OR 0.38 (0.23–0.61), P < 0.001]. Although suppression rates were greater when allowing for non-consecutive visits with failure, PDR significantly predicted greater risk of failure for both regimens in all outcome definitions. We found no effect of mutations at frequencies 5–20% on any of our outcomes.

Interpretation: NNRTI resistance prior to treatment initiation is associated with failure of integrase inhibitor-containing first-line regimens. These results portend high rates of first-line treatment failure in sub Saharan Africa, where circulating NNRTI resistance is common.

Introduction

The increasing prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance in those initiating or re-initiating antiretroviral therapy (ART),[1] along with the advantageous safety, potency, and cost-effectiveness characteristics of dolutegravir (DTG),[2] prompted the World Health Organization (WHO) to recommend DTG-based ART as a preferred first-line regimen.[3] However, recent concerns about DTG have emerged. For example early data suggested a slight increased risk of neural tube defects following DTG exposure in pregnancy, although more recent data has been reassuring.[4] Secondly, greater weight gain was observed in patients treated with DTG compared to efavirenz (EFV) in two clinical trials in sub-Saharan Africa, and elsewhere, leading to concerns about long-term effects of obesity with lifelong ART.[5–9]

Whilst cost-effectiveness analyses continue to support the use of DTG as first-line therapy despite these issues,[10] the WHO and others are revisiting targeted use of efavirenz (EFV). Concerns remain about the use of EFV with widespread NNRTI resistance, which exceeds 10–15% in much of sub-Saharan Africa.[11] Pre-treatment NNRTI resistance has been associated with a 2–3 fold greater risk of virologic failure (VF) for people initiating NNRTI-based regimens, both with older combinations such as nevirapine (NVP) and with EFV.[12–14] By contrast, the ANRS 12249 Treatment as Prevention Trial[15] reported that the most common NNRTI mutation, K103N, when detected alone, was not associated with increased risk of VF on an NNRTI-based single tablet regimen containing tenofovir, emtricitabine and efavirenz.[16] A study in Kenya similarly suggested isolated K103N might have limited impact on EFV-based ART.[17]

These conflicting data have generated controversy in the field on optimal first-line regimens to balance safety, tolerability, cost, and the impact of circulating NNRTI drug resistance on virologic outcomes. Although clinical trial data in the United States suggest that DTG performs exceptionally well in ART-naïve individuals and as a switch regimen in the absence of significant background resistance,[18–20] there are relatively few data available on the efficacy of DTG in the context of high circulating NNRTI resistance. In the DAWNING trial, in which individuals failing NNRTIs were randomized to DTG or lopinavir/ritonavir, and over 90% had some evidence of NNRTI resistance, approximately 84% of individuals in the DTG arm achieved virologic suppression at 48 weeks. Notably the proportion of people suppressed at 48-weeks on DTG arm was lower than in most prior clinical trials, albeit of first-line therapy.[21] As such, additional studies are needed to better elucidate the impact of pre-treatment NNRTI drug resistance on virologic outcomes with both EFV-based and DTG-based used first-line regimens in the region.

We conducted next generation sequencing of stored plasma specimens from participants in the ADVANCE clinical trial to determine the contributions of NNRTI pre-treatment drug resistance (PDR) on 96-week virologic outcomes for individuals initiating EFV and DTG-based ART. We hypothesized that NNRTI PDR would significantly affect efficacy of EFV-containing regimens but would have a negligible effect on outcomes for those initiating DTG-based therapy.

Methods

Study design

The ADVANCE trial is an open-label, non-inferiority, phase three clinical trial comparing three regimens for the initial treatment of HIV. Individuals were recruited from an urban centre in Johannesburg, South Africa, and randomized in a 1:1:1 ratio to (i) tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), EFV; (ii) TDF, FTC, DTG, or (iii) tenofovir alafenamide fumarate (TAF), FTC, and DTG. The study design and results of 48-week outcomes by treatment regimen have been reported previously.[22] In brief, the study enrolled non-pregnant individuals over 12 years old without chronic kidney disease. Individuals were excluded if they had more than 30 days of prior ART use, any ART use in the past six months, were pregnant, or were actively undergoing therapy for tuberculosis.

Study visits and measures

Study participants were seen for screening and randomization visits, which included collection of blood for pre-treatment viral load and CD4 T-cell count measurements. Data on demographics, employment, marital status, and education attainment were collected. During observation, participants were scheduled for visits at week four, 12, then every 12 weeks thereafter. Data for this analysis are limited to 96 weeks of observation. At each follow-up visit, plasma was collected for viral load estimation. Participants were asked about self-reported adherence over the past four days prior to each visit. Finally, study pharmacists recorded dispensed pills and performed a pill count of remaining pills at each follow-up visit.

Pre-treatment plasma specimens were shipped to KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP) for extraction (Chemagic 360; Perkin Elmer, Germany), HIV-1 pol gene amplification (ThermoFisher HIV-1 genotype amplification module; Life Technologies, CA, USA), and next generation sequencing (Illumina MiSeq; Ilumina, CA, USA) as previously described[23].

Statistical analysis

We first described and graphically depicted the analytic sample to determine which study participants were included and excluded from this analysis. To assess for selection bias in this sub-analysis, we compared characteristics between individuals who had sequencing results available for this analysis with those who did not due to lack of available plasma specimens or failed sequencing. We then summarized clinical and demographic features of the analytic sample in total,

and divided into those initiating EFV and DTG-based regimens. We then described the frequency and proportion of WHO-assigned PDR mutations overall, by drug class, and by treatment regimen.

Our primary exposure of interest was PDR, which we defined as the presence of at least one of the WHO list of surveillance drug mutations detected in at least 20% of the viral population.[24] Our primary outcome of interest was 96-week virologic success, which we defined as achievement of a viral load < 1000 copies/mL from 12 weeks onwards, < 200 copies/mL from 24 weeks onwards, and < 50 copies/mL from 48 weeks onwards through 96 weeks. Individuals censored with virologic suppression at 48-weeks or after are considered to have achieved virologic success. Individuals who did not complete 12 weeks of observation are not included in this analysis (but are included in the 48 and 96-week Food and Drug Administration [FDA] Snapshot sensitivity outcomes as failures, as described below). We derived this definition to reflect treatment response in individuals who attain and maintain virologic suppression over the course of study observation. We estimated the proportion of participants who achieved virologic suppression by the presence or absence of PDR for the total cohort, and by EFV versus DTG treatment regimens.

We fitted logistic regression models with virologic success as the outcome of interest to estimate the contributions of both PDR and regimen to 96-week virologic success, with and without a regimen by PDR product interaction term to assess whether the effect of PDR differed by EFV versus DTG use. We then fitted multivariable logistic regression models with virologic success as the outcome of interest, including the following potential confounding variables, which have been shown to determine virologic success in prior work[25,26]: sex, age, partnership status (defined as married or with a primary partner versus not), educational attainment (dichotomized as tertiary education or less), active employment status, pre-treatment CD4 T-cell count (categorized as $\leq$200 cells/uL, 201–350 cells/uL, 351–500 cells/uL, and >500 cells/uL), pre-treatment viral load (categorized as <10,000 copies/mL, 10,000-100,000 copies/mL, >100,000 copies/mL), pill count-based adherence (calculated as the number of pills taken since the prior visit divided by the expected number of pills taken, capped at 100% at each visit, averaged over the course of the 96-week observation period, and categorized as 95–100%, 90–95%, and <90% average adherence), and self-reported adherence (dichotomized as perfect adherence in the past four days versus any treatment interruptions in the past four days).

In sensitivity analyses, we varied our definition of virologic success. To assess for the impact of NNRTI PDR on more persistent virologic failure, for a secondary outcome we defined success in individuals without two consecutive visits up to 96-weeks with a viral load > 200 copies/mL. In this definition, individuals censored after a single viral load > 200 copies/mL are considered failures, whereas those who discontinue with virologic suppression are considered as achieving virologic success. This outcome is meant to allow for virologic blips or episodic failure followed by re-suppression. We also conducted analyses using the FDA-defined 48-week and 96-week Snapshot to define virologic success. In these analyses, individuals who dropped out prior to the 48 and 96-week windows are considered as failures, irrespective of the reason. Finally, we considered three stratified analyses in which we 1) restricted the definition of PDR to individuals with only the K103N mutation, 2) restricted the definition of PDR to individuals who had WHO-defined PDR mutations at variant frequencies of 2–20%, and 3) assessed finding stratified by those in the EFV- or DTG-based arms. Finally, we estimate an E-value to determine the magnitude of the effect size an unmeasured confounder who need to have to reduce the association between PDR and virologic success to null.[27] Data analysis was conducted in Stata (Version 15, Statacorp, College Station, Texas, USA), coded by two separate investigators (MJS and BS) and compared for reproducibility.

Ethical considerations

The study was approved by the institutional review board at the University of the Witwatersrand. All study participants gave written informed consent to participate.

Results

Study population

A total of 1,053 individuals were enrolled in the ADVANCE trial. Of these 989 (94%) consented for specimen storage and had pre-treatment plasma available for testing, and 873 (83%) had successful sequencing of a pre-treatment plasma specimen (Fig. 1). We found no differences in clinical or demographic characteristics between those who successfully underwent sequencing and those who did not (Supplemental Table 1). The analytic sample was thus comprised of 873 individuals, 288 (33%) of whom were randomized to an EFV-based regimen and 585 (67%) of whom were randomized to a DTG-based regimen. At the time of data extraction, all 873 had completed observation up to 96 weeks. A total of 48 and 91 individuals were excluded from the primary and secondary analyses, respectively, for not remaining in the study to 12 or 24 weeks. There were no differences by treatment regimen in clinical or demographic factors (Table 1). However, individuals starting DTG-based regimens had a higher prevalence of PDR than those initiating EFV-based regimens (16.8 versus 8.0%, $P < 0.001$).

Table 1
Cohort characteristics for participants who completed pre-treatment HIV drug resistance testing in the ADVANCE clinical trial, divided by regimen

	Efavirenz arm (n = 288)	Dolutegravir arms (n = 585)	*P*-value[a]
Female sex (n, %)	163 (56.6%)	357 (61.0%)	0.21
Age (median, IQR)	31 (27–37)	32 (27–37)	0.85
Married or Partner (n, %)	64 (22.2%)	114 (19.5%)	0.35
Tertiary education (n, %)	21 (7.3%)	56 (9.7%)	0.36
Employed (n, %)	175 (61.2%)	363 (63.1%)	0.65
Pretreatment CD4 count (n, %)			0.71
$\leq$200 cells/uL	85 (29.5%)	186 (31.8%)	
201–350 cells/uL	87 (30.2%)	175 (29.9%)	
351–500 cells/uL	61 (21.2%)	106 (18.1%)	
>500 cells/uL	55 (19.1%)	118 (20.2%)	
Pretreatment viral load (n, %)			0.36
<10,000 copies/mL	98 (34.0%)	185 (31.6%)	
10,000-100,000 copies/mL	121 (41.0%)	276 (47.2%)	
>100,000 copies/mL	69 (24.0%)	124 (21.2%)	
Low self-reported adherence[b] (n, %)	112 (38.9%)	254 (43.4%)	0.24
Pill count adherence (n, %)[c]			0.26
<90%	12 (4.3%)	36 (6.3%)	
90–95%	23 (8.2%)	60 (10.5%)	
95–100%	244 (87.5%)	476 (83.2%)	
Presence of Any WHO-defined pretreatment drug resistance	24 (8.3%)	98 (16.8%)	< 0.001

[a] *P*-values represent statistical tests comparing those included and excluded from the analytic dataset, using chi-squared testing to compare categorical variables and Mann-Whitney non-parametric tests to compare median age.

[b] Low adherence defined as self-report of less than perfect adherence in the four days prior to any study visits during the observation period

[c] Pill count was calculated at each visit by study pharmacists, capped at 100%, then averaged across the 96-week observation period

Pre-treatment drug resistance

Approximately 14% (122/873) of individuals had at least one WHO-defined PDR mutation at variant frequencies of 20% or greater (Fig. 2). The majority of PDR was accounted for by mutations conferring resistance to NNRTIs, with over 98%

(120/122) of those harbouring WHO-defined PDR having at least one NNRTI mutation. The most common single mutation was K103N, present in 9% (81/873). Only 20 (2%) of individuals had an nucleoside reverse transcriptase inhibitor (NRTI) mutation, with M184V being the most common, present in 12 (1%) individuals, followed by K65R, which was present in 8 (1%) individuals. The combination of at least one NRTI mutation and one NNRTI mutation was identified in 18 (2%) participants.

Virologic Suppression Rates

After excluding 48 individuals who were censored before 12 weeks, virologic success over 96-weeks of observation, as defined by our primary outcome, was achieved in approximately 83% of study participants (678/825, Table 2). In the overall cohort, rates of virologic suppression were significantly lower in those with PDR 65% (73/112) compared to those without PDR (85% [605/713], $P < 0.001$). This pattern was true for participants initiating EFV-based ART (60% [12/20] versus 86% [214/248], $P = 0.002$) and DTG-based ART (61/92 [66%] versus 84% [391/465], P-value < 0.001, Fig. 3). In multivariable regression models, PDR remained a strong predictor of virologic success (AOR 0.38, 95%CI 0.21 ,0.61) after adjustment for demographic and clinical factors, and both self-reported and pill count-based adherence (Table 3). The effect size and confidence interval estimated, would mean that an unmeasured confounder would require an odds ratio of 2.9 or greater with both PDR and virologic suppression (conditional on other confounders, including self-reported adherence) to reduce the effect seen between PDR and virologic success to the null.[27] Viral suppression was also lower in those with higher baseline viral loads and in those with lower self-reported adherence. The effect of PDR did not differ by treatment arm (P-value for interaction term by regimen = 0.42). Rates of virologic success were higher for both regimens for those with and without PDR in our secondary outcome, which allowed for resuppression after an episode of virologic failure, although the effect of PDR persisted (85% [73/86] vs 94% 428/453], $P = 0.001$ for DTG-based ART; 68% [13/19] vs 93% [217/233], $P < 0.001$ for EFV-based ART, Table 2). The effect of PDR on treatment outcomes persisted as well as for both the FDA 48-week and 96-week Snapshot Analyses, including in multivariable analyses (Supplemental Table 2).

Table 2
Virologic success in the ADVANCE Trial by the presence of WHO-defined pretreatment drug resistance

	Total Cohort			Efavirenz arm			Dolutegravir arms			Interaction *P*-value[a]
	PDR	No PDR	P-value	PDR	No PDR	P-value	PDR	No PDR	P-value	
Primary Outcome[b]	73/112 (65%)	605/713 (85%)	< 0.001	12/20 (60%)	214/248 (86%)	0.002	61/92 (66%)	391/465 (84%)	< 0.001	0.42
Secondary Outcome[c]	86/105 (82%)	645/686 (94%)	< 0.001	13/19 (68%)	217/233 (93%)	< 0.001	73/86 (85%)	428/453 (94%)	0.001	0.28
48-week Snapshot[d]	84/122 (69%)	631/751 (84%)	< 0.001	11/24 (46%)	213/264 (81%)	< 0.001	73/98 (74%)	418/487 (86%)	0.005	0.09
96-week Snapshot[d]	71/122 (58%)	598/751 (80%)	< 0.001	11/24 (46%)	200/264 (76%)	0.001	60/98 (61%)	398/487 (82%)	< 0.001	0.59

[a] *P*-value indicates results of interaction terms comparing virologic suppression rates in the efavirenz versus dolutegravir arms by presence of WHO-defined pretreatment drug resistance

[b] Primary outcome: Virologic success in our primary outcome was defined as achievement of a sustained viral load < 1000 copies/mL from 12 weeks, < 200 copies/mL from 24 weeks, and < 50 copies/mL from 48 weeks onwards. Individuals who are censored after 48-weeks with virologic suppression are considered as achieving virologic success.

[c] Secondary outcome: Virologic success in our secondary outcome was defined as the absence of two consecutive visits with a viral load > 200 copies/mL. Individuals who are censored with a single viral load > 200 copies/mL are considered failures, whereas those who discontinue with virologic suppression are considered as achieving virologic success.

[d] 48 and 96-week Snapshot outcome refer to Food and Drug Administration-defined Snapshot outcomes for HIV therapeutic trials

PDR: presence of WHO-defined pretreatment drug resistance

Table 3

Logistic regression models for 96-week virologic success in the ADVANCE Trial[a]

	Univariable Models		Multivariable Model	
Covariable	**Odds Ratio (95%CI)**	**P-value**	**Adjusted Odds Ratio (95%CI)**	**P-value**
Female Sex	0.90 (0.62, 1.29)	0.59	0.82 (0.54, 1.25)	0.35
Age (each year)	1.05 (1.02, 1.07)	< 0.001	1.02 (0.99, 1.05)	0.14
Married or Partner	1.38 (0.86, 2.23)	0.18	0.95 (0.56, 1.60)	0.84
Tertiary education	0.83 (0.45, 1.54)	0.66	0.81 (0.41, 1.57)	0.53
Employed	2.07 (1.43, 2.98)	< 0.001	1.79 (1.17, 2.67)	0.007
Pretreatment CD4 count				
$\leq$200 cells/uL	REF		REF	
201–350 cells/uL	1.31 (0.83, 2.07)	0.25	1.27 (0.76, 2.13)	0.37
351–500 cells/uL	1.12 (0.67, 1.87)	0.66	0.98 (0.54, 1.77)	0.95
>500 cells/uL	1.13 (0.68, 1.88)	0.63	0.99 (0.54, 1.83)	0.97
Pre-treatment viral load				
<10,000 copies/mL	REF		REF	
10,000-100,000 copies/mL	0.59 (0.37, 0.92)	0.02	0.52 (0.31, 0.88)	0.01
>100,000 copies/mL	0.49 (0.29, 0.82)	0.006	0.39 (0.21, 0.72)	0.003
Low self-reported adherence[b] (n, %)	0.36 (0.25, 0.52)	< 0.001	0.41 (0.27, 0.63)	< 0.001
Pill count adherence (n, %)[c]				
<90%	REF		REF	
90–95%	2.99 (1.39, 6.43)	0.005	2.71 (1.15, 6.38)	0.02
95–100%	6.16 (3.31, 11.46)	< 0.001	3.51 (1.70, 7.24)	0.001
Regimen				

[a]Virologic success in our primary outcome was defined as achievement of a sustained viral load < 1000 copies/mL from 12 weeks, < 200 copies/mL from 24 weeks, and < 50 copies/mL from 48 weeks onwards. Individuals who are censored after 48-weeks with virologic suppression are considered as achieving virologic success.

[b]Low adherence defined as self-report of less than perfect adherence in the four days prior to any study visits during the observation period

[c]Pill count was calculated at each visit by study pharmacists, capped at 100%, then averaged across the 96-week observation period<

	Univariable Models		Multivariable Model	
Efavirenz-based regimen	REF		REF	
Dolutegravir-based regimen	0.80 (0.54, 1.18)	0.26	1.02 (0.67, 1.57)	0.92
Presence of WHO-defined pretreatment drug resistance	**0.33 (0.22, 0.52)**	**< 0.001**	**0.38 (0.23, 0.61)**	**< 0.001**
[a]Virologic success in our primary outcome was defined as achievement of a sustained viral load < 1000 copies/mL from 12 weeks, < 200 copies/mL from 24 weeks, and < 50 copies/mL from 48 weeks onwards. Individuals who are censored after 48-weeks with virologic suppression are considered as achieving virologic success.				
[b]Low adherence defined as self-report of less than perfect adherence in the four days prior to any study visits during the observation period				
[c]Pill count was calculated at each visit by study pharmacists, capped at 100%, then averaged across the 96-week observation period<				

Amongst those with the TDF-associated resistance mutation K65R at baseline (n = 8), two were in the EFV arm (both failures) and of the six in the DTG arm, 2/6 (33%) achieved 96-week virologic suppression as defined by the primary outcome measure. Participants with K65R all had NNRTI mutations and 6/8 had M184V.

We considered the impact of isolated K103N (as majority virus population, > 20%) on virologic response to EFV and DTG (Supplementary Table 3). Rates of virologic suppression were similar in participants taking EFV-based ART with and without the K103N mutation, although the number of individuals with K103N was small in this arm (n = 8). Isolated K103N was associated with lower virologic success for individuals on DTG-based ART, with the exception of our secondary outcome, for which the effect size was similar but the effect was not statistically significant.

We next examined the impact of minority variant PDR in 2–20% of viral quasispecies on outcome of first-line ART. Individuals with mutations in minority populations had similar virologic outcomes as those without PDR overall, and for both those taking DTG- or EFV-based ART (Supplementary Table 4). Finally,, we found persistent effects of PDR on virologic success in analyses stratified by EFV versus DTG-based treatment (Supplemental Table 5).

Discussion

We report a strong and pervasive association between NNRTI resistance before treatment initiation and virologic failure for people initiating first-line ART with both DTG and EFV-based ART in the ADVANCE clinical trial. The effect was stronger among individuals in the EFV arm, but also highly significant in the DTG arms, and persisted after adjusting for self-reported and pill count-based adherence and baseline viral load. When we considered a secondary outcome, which allowed for re-suppression after an episode of VF, the effect of PDR on DTG persisted, but to a lower degree. The finding that NNRTI resistance appears to predict failure among individuals initiating DTG-based ART in LMIC was unexpected, and to our knowledge not previously reported in the literature.

Although NNRTI mutations are not known to affect susceptibility of DTG, the observed effect we identified may be due to higher replication or fitness of NNRTI mutant viruses in the context of drug pressure from integrase inhibitors.[28] Although we found relatively little minority resistance and no effect of minority resistance on outcomes, existence of NNRTI resistance could be a surrogate marker of archived NRTI resistance.[29] Integrase resistance mutations were not assessed in this study, but are generally believed to be rare (< 1%) in this region.[30,31] Alternatively, the lack of suppression may be due to a behavioural component – pre-existing EFV mutations may be a surrogate of prior default among participants not disclosing previous ART exposure. Our multivariable logistic regression models included a measure of self-reported

adherence and pill count-based adherence, both of which were highly predictive of virologic outcomes, and addition of which to our model did not meaningfully alter the effect size of PDR on virologic success. However, both self-reported and pill count-based adherence are imperfect measures, and can have a relatively low sensitivity to detect poor adherence, so residual confounding might be present.[32–34] The South African public program has used EFV in first line therapy since its inception in 2004, and the programme has over 5 million on treatment. As such, the numbers who have defaulted and are re-initiating therapy are likely to be significant, and it is impossible to identify this within clinical trials using existing South African data systems. Of note, a number of studies have reported denial of ART use among individuals determined to be taking therapy based on drug level testing.[35–37]

Whether the mechanism of effect is due to poor adherence or virologic mechanisms, our finding that NNRTI resistance, present in 10–20% of individuals initiating DTG in the region, is associated with a reduction in efficacy of DTG-based ART is has multiple public health implications. First, ensuring adequate virologic monitoring occurs with DTG-based regimens will remain a priority. Second, treatment programs will require ongoing attention to second and third-line options, particularly if DTG failure or intolerance becomes more common than previously expected, and NNRTI-based regimens become more commonly used again. Third, integrase resistance testing, which is rarely done outside of research studies in resource-limited settings should become a consideration for referral laboratories in countries where DTG becomes the treatment of choice. Finally, our findings might signal a warning for national programs in the midst of large-scale switching from EFV-based to DTG-based ART, and support increased vigilance for the presence of treatment failure at the time of switch. Future work should explore the efficacy and feasibility of innovative means of mitigating the effect of drug resistance on treatment outcomes, such as targeted point-of-care resistance testing to identify individuals at greater risk of VF, or longer acting regimens to reduce imperfect adherence in those most susceptible to it.[38–40]

NAMSAL is the only other randomized controlled trial which has compared DTG versus EFV-based first-line ART in sub Saharan Africa. That study, conducted in Cameroon, compared low-dose 400 mg EFV to DTG as third agent at 48 weeks.[6] DTG was non-inferior to EFV in that study, but baseline VL > 100,00 copies/ml predicted failure in both arms. NAMSAL reported a much lower prevalence of NNRTI resistance (6%) than we did (14%), which is consistent with other data in the region.[41] In NAMSAL, investigators reported no impact of baseline NNRTI resistance on outcomes, although 6/16 failures on EFV had pre-existing NNRTI resistance. In that study, none of the three failures in the DTG arm at 48 weeks had baseline resistance to NNRTIs, the 6% of those that did appeared to suppress during the study. By contrast, in our study, isolated K103N in the DTG arm was associated with lower virologic success in the primary analysis, albeit at 96 weeks. As in prior studies, we identified a small number of individuals with resistance to both the NRTI and NNRTI drug classes, including K65R, M184V who we believe were unlikely to be treatment naïve and who responded poorly to first-line ART. Whilst the proportion is low, this finding is concerning from the point of view of the largescale EFV to DTG-transition in sub-Saharan Africa, during which multi-class drug resistance is likely to be more prevalent.[42–46]

Next generation sequencing is becoming more widely used in research studies to measure the prevalence and impact of drug resistance in LMIC, and has the added advantage of being able to detect resistant viruses at low frequencies.[47–49] However, many studies have failed to demonstrate a role for these low-level mutant viruses in determining clinical outcomes.[50] We also found no association between PDR and outcome when considering individuals with mutations in between 5 and 20% of viral quasispecies, which supports current practice to use major resistance mutation frequencies for determination of clinically significant drug resistance.

Our study should be generalized in light of its conduct in South Africa, and the presence of NNRTI resistance-conferring mutations as the large majority of the PDR detected. As this is the first study to show an impact of PDR on the efficacy of first-line DTG, it requires corroboration from future studies of similar cohorts. The presence of a higher prevalence of PDR in the DTG arm suggests that there might have been imbalance between groups, which is most likely due to chance, because study arm was determined by computer randomization. Nonetheless, we have low suspicion for selective

dropout in the study because interest in DTG among patients and within society at the time of randomisation was minimal. Our estimates could be susceptible to unmeasured or residual confounding, particularly due to effects of adherence not captured by self-report. Notably, our estimates of the effect of PDR on virologic outcomes remained large and strongly significant after adjustment for confounders, including adherence, meaning an unmeasured confounder would have to have a strong association (OR of 2.8 or greater) with both PDR and virologic success to reduce the effect of pre-treatment drug resistance to null.[27] Moreover, known predictors of treatment success, such as adherence and pre-treatment viral load, each predicted virologic success, which enhances the internal validity of our estimates. We also were unable to sequence approximately 15% of the study cohort due to unavailable specimens or failed sequencing. Despite that, our sample size remained large enough to detect relatively small changes in outcomes, and we detected no differences in characteristics between those who were and were not included in this sub-study, which reduces the risk of selection bias.

In summary, our study suggests that the presence of PDR to NNRTIs is negatively associated with outcome of both EFV- and DTG-based first-line ART in South Africa. In the context of highly prevalent PDR NNRTI resistance, our findings, if corroborated, have implications for first-line ART selection and treatment monitoring guidelines in the region. Future work should validate our findings, assess the contribution of pre-treatment integrase mutations to outcomes, elucidate the impact of prior exposure to ART on treatment outcomes, and whether treatment failure observed on DTG-based ART is associated with emergence of integrase inhibitor mutations.

Declarations

Competing interests

No competing interests were disclosed.

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Conflicts of Interest

RKG has received ad hoc consulting fees from Gilead, ViiV and UMOVIS Lab

WDFV received drug donations from ViiV Healthcare and Gilead Sciences for investigator-led clinical studies, including ADVANCE. In addition, he receive honoraria for talks and board membership for: Gilead, ViiV, Mylan, Merck, Adcock-

Ingram, Aspen, Abbott, Roche, J&J.

MM received drug donations from ViiV Healthcare and Gilead Sciences for investigator-led clinical studies, including ADVANCE. In addition, she received honoraria for talks and board membership for: Gilead, ViiV, Mylan, Aspen, AbbVie, Johnson & Johnson, Sanofi, Pfizer and Southern African HIV Clinicians Society. She also received meeting/conference sponsorship from Johnson and Johnson, BD, Gilead, Merck, Cipla, Mylan and Canopy Growth.

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Figures

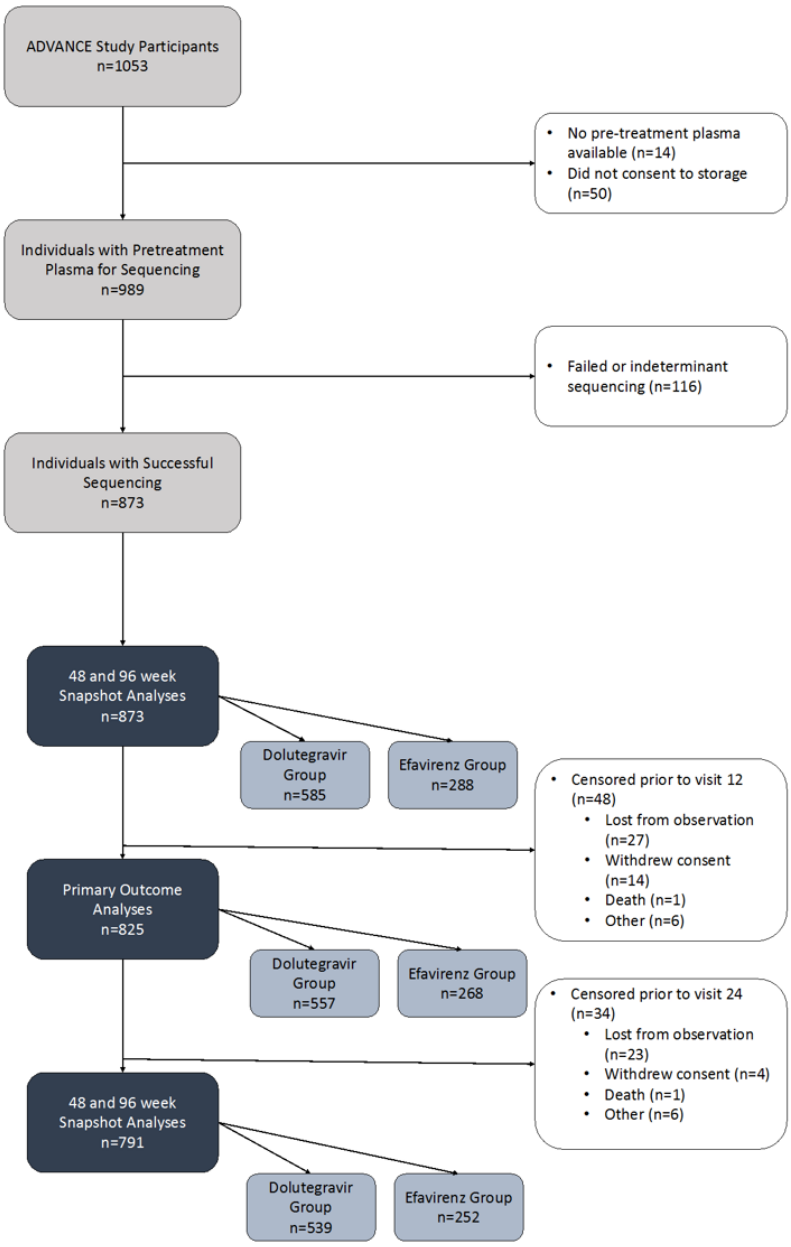

Figure 1

Study schema

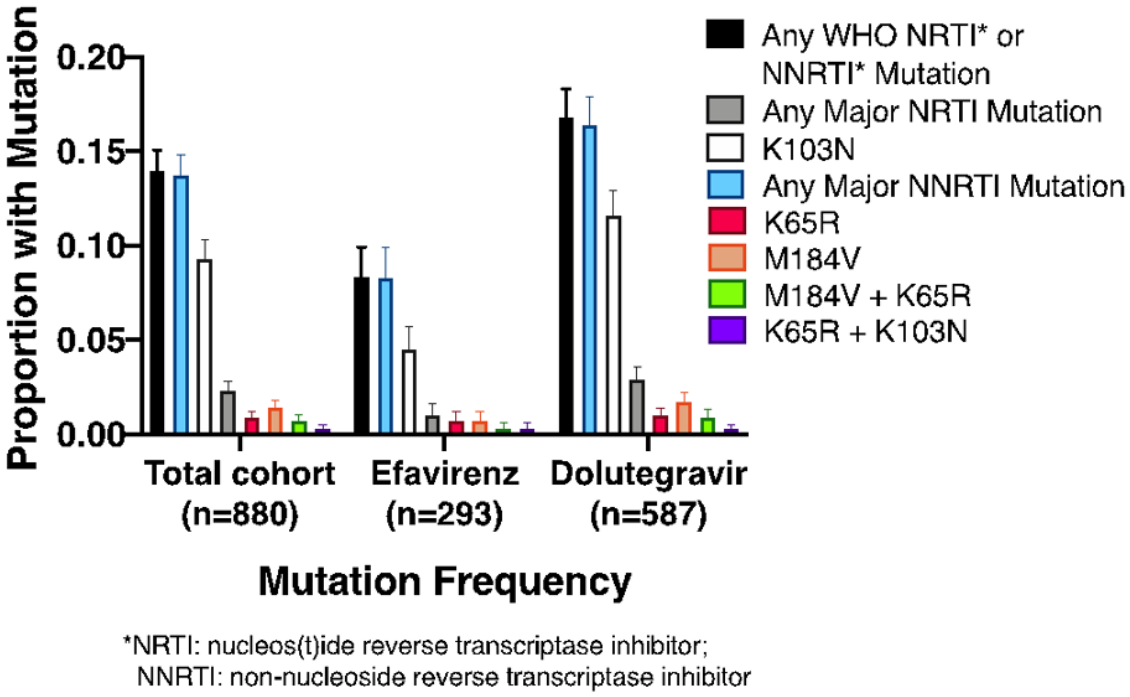

Figure 2

Distribution of WHO-defined pre-treatment drug resistance in the ADVANCE trial, using the WHO Surveillance Drug Mutations list for mutations detected at >20% of the viral quasispecies.

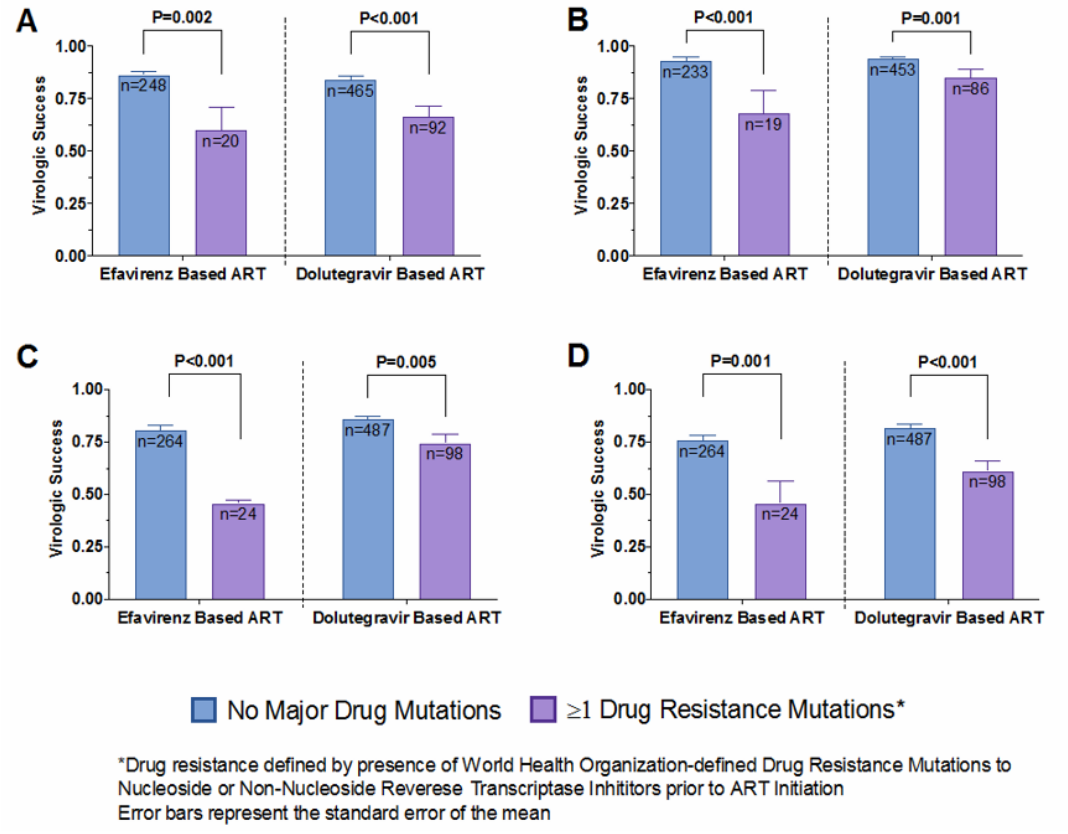

Figure 3

Virologic success in the ADVANCE trial divided by the presence or absence of WHO-defined pre-treatment major drug mutations and by use of efavirenz- or dolutegravir-based regimen. Results are for virologic success defined by our primary outcome (A), secondary outcome (B), FDA 48-week Snapshot (C), and FDA 96-week Snapshot (D).

Supplementary Files

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