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

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Little is known about the impact of pretreatment drug resistance (PDR) on the efficacy of second generation integrase inhibitors. We sequenced pretreatment plasma specimens from the ADVANCE trial (NCT03122262). Our primary outcome was 96-week virologic success, defined as a sustained viral load <1000 copies/mL from 12 weeks onwards, <200 copies/mL from 24 weeks onwards, and <50 copies/mL after 48 weeks. Here we report how this outcome was impacted by PDR, defined by the World Health Organization (WHO) mutation list. Of 1053 trial participants, 874 (83%) have successful sequencing, including 289 (33%) randomized to EFV-based therapy and 585 (67%) randomized to DTG-based therapy. Fourteen percent (122/874) have ≥1 WHO-defined mutation, of which 98% (120/122) are NNRTI mutations. Rates of virologic suppression are lower in the total cohort among those with PDR 65% (73/112) compared to those without PDR (85% [605/713], P < 0.001), and for those on EFV-based treatment (60% [12/20] vs 86% [214/248], P = 0.002) and for those on DTG-based treatment (61/92 [66%] vs 84% [391/465] P < 0.001, P for interaction by regimen 0.49). Results are similar in multivariable models adjusted for clinical characteristics and adherence. NNRTI resistance prior to treatment is associated with long-term failure of integrase inhibitor-containing first-line regimens, and portends high rates of first-line failure in sub Saharan Africa.

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he increasing prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance in those initiating or re-initiating antiretroviral therapy (ART), along with the advantageous safety, potency, and cost-effectiveness characteristics of dolutegravir (DTG), prompted the World Health Organization (WHO) to recommend DTG-based ART as a preferred first-line regimen. However, recent concerns about DTG have emerged. For example, early data suggested a slightly increased risk of neural tube defects following DTG exposure in pregnancy, although more recent data have been reassuring.

Secondly, greater weight gain was observed in patients treated with DTG compared to efavirenz (EFV) in two clinical trials in sub-Saharan Africa, as well as in cohort studies from the North America and Europe, leading to concerns about long-term effects of obesity with lifelong ART.

Whilst cost-effectiveness analyses continue to support the use of DTG as first-line therapy despite these issues, 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. Pretreatment 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.

By contrast, the ANRS 12249 Treatment as Prevention Trial 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. A study in Kenya similarly suggested isolated K103N might have limited impact on EFV-based ART.

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-naive individuals and as a switch regimen in the absence of significant background resistance, there is relatively little 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. As such, additional studies are needed to better elucidate the impact of pretreatment NNRTI drug resistance on virologic outcomes with both EFV-based and DTG-based used first-line regimens in the region.

Here we report results of next-generation sequencing of stored plasma specimens from participants in the ADVANCE clinical trial to determine the contributions of NNRTI pretreatment drug resistance (PDR) on 96-week virologic outcomes for individuals initiating EFV- and DTG-based ART. We hypothesize that NNRTI PDR significantly affects efficacy of EFV-containing regimens but has a negligible effect on outcomes for those initiating DTG-based therapy.

### Results

#### Study population

A total of 1053 individuals were enrolled in the ADVANCE trial. Of these, 991 (94%) consented for specimen storage and had pretreatment plasma available for testing, and 874 (83%) had successful sequencing of a pretreatment plasma specimen (Fig. 1). We found no differences in clinical or demographic characteristics between those who successfully underwent sequencing and those who did not (Supplementary information).

Of participants included in PDR analyses, 289 (33%) were randomized to an EFV-based regimen and 585 (67%) were randomized to a DTG-based regimen. At the time of data extraction, all had completed observation up to 96 weeks. A total of 48 and 82 individuals were excluded from our 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 in our primary analytic sample (Table 1). However, individuals starting DTG-based regimens had a higher prevalence of PDR than those initiating EFV-based regimens (16.5 vs 7.4%, P < 0.001).

#### Pretreatment drug resistance

Approximately 14% (122/874) 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 harboring WHO-defined PDR having at least one NNRTI mutation. The most common single mutation was K103N, present in 9% (81/874).

Only 20 (2%) individuals had a 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] vs 86% [214/248], P = 0.002) and DTG-based ART (61/92 [66%] vs 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, as well as additive effects of PDR and adherence in both the EFV and DTG arms (Table 3 and Fig. 4). The effect size and confidence interval estimated would mean that an unmeasured confounder would require an odds ratio of 2.7 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. 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 assessed for persistent virologic failure with two consecutive viral loads >200 copies/mL, 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 (Supplementary information). Among 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 (75%) had M184V.
In contrast to the effect seen with long-term virologic outcomes, PDR only had an effect on initial virologic response for individuals on EFV-based ART, but not those on DTG-based ART. The change in log_{10} viral load from enrollment to week 12 was greater for those without PDR in the EFV arm (1.89 vs 2.61 log_{10} copies/mL, \( P < 0.001 \)), but not in the DTG arms (2.76 vs 2.68 log_{10} copies/mL, \( P = 0.43, P = 0.001 \) for interaction between arms, Table 2, Supplementary information). In survival analyses, individuals in the EFV arm with PDR experienced a longer time to suppression than whose without PDR (\( P = 0.04 \) by log-rank testing), whereas those with and without PDR had similar time to initial suppression in the DTG arms (\( P = 0.54 \) by log-rank testing, Supplementary information). In adjusted Cox proportional hazards models, the effect of PDR remained significant only for
mutations detected at >20% of the viral quasispecies. ADVANCE trial, using the WHO Surveillance Drug Mutations list for 95% confidence intervals around the proportion estimates.

| Table 1 Cohort characteristics for participants who completed pretreatment HIV drug resistance testing and included in our primary analysis of virologic failure, divided by regimen. |
|---------------------------------------------------------------|
|                                         | Efavirenz arm | Dolutegravir arms | P valuea |
|                                         | (n = 269)     | (n = 557)         |          |
| Female sex (n, %)                             | 153 (56.9%)   | 341 (61.2%)       | 0.23     |
| Age (median, IQR)                             | 32 (27–37)    | 32 (27–38)        | 0.83     |
| Married or partner (n, %)                     | 60 (22.3%)    | 108 (19.4%)       | 0.34     |
| Tertiary education (n, %)                      | 18 (6.7%)     | 51 (9.2%)         | 0.22     |
| Employed (n, %)                               | 170 (63.7%)   | 349 (63.8%)       | 0.97     |
| Pretreatment CD4 count (n, %)                  |               |                   |          |
| ≤200 cells/µL                                | 80 (29.7%)    | 179 (32.1%)       |          |
| 201–350 cells/µL                             | 81 (30.1%)    | 166 (29.8%)       |          |
| 351–500 cells/µL                             | 58 (21.6%)    | 99 (17.8%)        |          |
| >500 cells/µL                                | 50 (18.6%)    | 118 (20.3%)       |          |
| Pretreatment viral load (n, %)                |               |                   |          |
| <10,000 copies/mL                             | 113 (42.0%)   | 264 (47.4%)       |          |
| 10,000–100,000 copies/mL                     | 67 (24.9%)    | 122 (21.9%)       |          |
| >100,000 copies/mL                           | 113 (42.0%)   | 252 (45.2%)       |          |
| Low self-reported adherenceb (n, %)           |               |                   |          |
| <90%                                         | 12 (4.5%)     | 33 (6.3%)         | 0.45     |
| 90–95%                                       | 23 (8.6%)     | 58 (10.5%)        |          |
| 95–100%                                      | 233 (87.0%)   | 463 (83.6%)       |          |
| Presence of any WHO-defined pretreatment drug resistance | 20 (7.4%)     | 92 (16.5%)        | <0.001 |

*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 nonparametric tests to compare median age.

bLow adherence defined as self-report of less than perfect adherence in the 4 days prior to any study visits during the observation period.

Pill count adherence (n, %) ≥

| Mutation frequency |
|--------------------|
| Total cohort       | Efavirenz arm (n = 269) | Dolutegravir arm (n = 557) |
|-------------------|-------------------------|---------------------------|
| Mutation with mutation | 0.00 | 0.00 | 0.20 |
| Proportion with mutation | 0.05 | 0.05 | 0.15 |
| Any WHO NRTI* or NNRTI* mutation | 0.00 | 0.00 | 0.20 |
| Any major NRTI mutation | 0.00 | 0.00 | 0.20 |
| Any major NNRTI mutation | 0.00 | 0.00 | 0.20 |
| K65R               | 0.00 | 0.00 | 0.20 |
| M184V              | 0.00 | 0.00 | 0.20 |
| M184I + K65R       | 0.00 | 0.00 | 0.20 |
| K65R + K103N       | 0.00 | 0.00 | 0.20 |

The observed effect we identified may be those on EFV-based ART (AHR 0.58, 95%CI 0.35, 0.96), but not for those DTG-based ART (AHR 1.01, 95%CI 0.80, 1.27).

We considered the impact of isolated K103N (as majority virus population, >20%) on virologic response to EFV and DTG (Supplementary Information). 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 information). We found similar effects of PDR on virologic success in analyses stratified by EFV vs DTG-based treatment (Supplementary information). In a subset of 38 individuals who had sequencing data available both prior to enrollment and at the time of failure, we found that new NRTI and NNRTI mutations developed among those in the EFV-based ART arm in 31% (5/16) and 40% (4/10) of individuals respectively, but that new resistance in the reverse transcriptase gene was rare among those in the DTG-based ART arms (6% [1/17] new NRTI mutations and 0% [0/11] new NNRTI mutations, Supplementary information). Finally, we found no difference in the effect of PDR on any outcomes in sub-analyses restricted to sequences with at least 1000× average depth coverage (Supplementary information).

Discussion

We report a strong association between drug resistance before treatment initiation, primarily to the NNRTI class, and virologic failure for people initiating first-line ART in the ADVANCE clinical trial. The effect was seen among individuals in the EFV arm and 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 focused on persistent virologic failure (two or more consecutive visits with a high viral load), the effect of PDR on DTG persisted, but to a lower degree. In contrast to the effects seen for long-term outcomes, we did not find that PDR had an impact on time to initial suppression or change in quantified viral load from enrollment to 12 weeks, suggesting that NRTI PDR affects longer-term maintenance of suppression for DTG-based ART or via a non-virally mediated behavioral mechanism. Nonetheless, the finding that NNRTI resistance appears to ultimately predict treatment failure among individuals initiating DTG-based ART in LMIC was unexpected, and to our knowledge not previously reported in the literature.

A virologic mechanism to explain our findings has not been established. NNRTI mutations are not known to affect susceptibility of DTG.
Table 2 Virologic success in the ADVANCE Trial by the presence of WHO-de
defined pretreatment drug resistance.

| Pretreatment Drug Resistance | Total cohort | Efavirenz arm | Dolutegravir arms | Interaction P value |
|------------------------------|-------------|--------------|------------------|--------------------|
| PDR                          |             |              |                  |                    |
| No PDR                       | 73/12 (62%) | 12/20 (60%)  | 5/24 (21%)      | -0.001             |
| PDR                          | 69/11 (65%) | 17/27 (63%)  | 9/21 (43%)      | -0.001             |
| PDR                          | 69/11 (65%) | 17/27 (63%)  | 9/21 (43%)      | -0.001             |
| PDR                          |             |              |                  |                    |
| PDR                          | 7/12 (58%)  | 25/49 (51%)  | 6/16 (38%)      | -0.004             |
| PDR                          | 6/11 (55%)  | 25/49 (51%)  | 6/16 (38%)      | -0.004             |
| PDR                          | 11/24 (46%) | 25/49 (51%)  | 6/16 (38%)      | -0.004             |
| PDR                          |             |              |                  |                    |
| PDR                          | 10/12 (83%) | 12/20 (60%)  | 5/24 (21%)      | -0.001             |
| PDR                          | 10/11 (91%) | 12/20 (60%)  | 5/24 (21%)      | -0.001             |
| PDR                          | 10/11 (91%) | 12/20 (60%)  | 5/24 (21%)      | -0.001             |
| PDR                          |             |              |                  |                    |
| PDR                          | 7/12 (58%)  | 25/49 (51%)  | 6/16 (38%)      | -0.004             |
| PDR                          | 6/11 (55%)  | 25/49 (51%)  | 6/16 (38%)      | -0.004             |
| PDR                          | 11/24 (46%) | 25/49 (51%)  | 6/16 (38%)      | -0.004             |
| PDR                          |             |              |                  |                    |
| Mean change in log10 viral load from baseline to 12 weeks (SD) | 2.63 (0.95) | 2.63 (0.95) | 2.63 (0.95) | 2.63 (0.95) |

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

**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 continue with virologic suppression are considered as achieving virologic success.

**Secondary outcome**: Virologic success in our secondary outcome was defined as the absence of two consecutive visits with a viral load > 100 copies/mL from 12 weeks, >200 copies/mL from 24 weeks, and >500 copies/mL from 48 weeks onwards. Individuals who are censored after 48 weeks continue with virologic suppression are considered as achieving virologic success.

**48-week Snapshot**: This outcome refers to Food and Drug Administration-defined Snapshot outcomes for HIV therapeutic trials.

PDR: presence of WHO-defined pretreatment drug resistance.
Fig. 3 Virologic success in the ADVANCE Trial divided by the presence or absence of WHO-defined pretreatment 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). Error bars indicate 95% confidence intervals around the proportion estimates. P values represent results of two-sided two-proportion Z tests.

fitness deficit. Making this distinction about the clinical implications of drug resistance for clinical and public health purposes could be crucial. Fifth, 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. Finally, these results signal the importance of future work to determine optimal treatment recommendations for individuals failing DTG-based ART, for which minimal data are currently available. Without such data, treatment programs should be advised to maintain virologic monitoring programs, adherence monitoring and support programs for those with failure, and regimen change guidance for individuals with intolerance or persistent virologic failure, even when documented drug resistance is absent. More novel strategies, such as real-time adherence and resistance monitoring, and long-acting injectable formulations of ART for those with adherence challenges should also be explored as these options become more widely available.

NAMSAL is the only other randomized controlled trial that has compared DTG vs 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. DTG was non-inferior to EFV in that study, but baseline VL > 100,000 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. In NAMSAL, investigators reported no impact of baseline NNRTI resistance on outcomes, although 6/16 failures in the EFV arm 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 appear 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 very 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 naive and who responded poorly to first-line ART. While the proportion is low, this finding is concerning from the point of view of the large-scale EFV to DTG-transition in sub-Saharan Africa, during which multi-class drug resistance is likely to be more prevalent.

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. However, many studies, and particularly those considering newer ART regimens, have failed to demonstrate a role for these low-level mutant viruses in determining clinical outcomes. We also found no association between PDR and outcome when considering individuals with mutations in between 2 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 the 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...
randomization was minimal. Our estimates could be susceptible to unmeasured or residual confounding, particularly due to the effects of prior ART exposure and/or imperfect adherence not captured by self-report or pharmacy pill counts. Notably, our estimates of the effect of PDR on virologic outcomes remained large and significant after adjustment for confounders, including adherence, meaning an unmeasured confounder would have to have a strong association (OR of 2.7 or greater) with both PDR and virologic success to reduce the effect of pretreatment drug resistance. Notably, known predictors of treatment success, such as adherence and pretreatment viral load, each predicted virologic success, which enhances the internal validity of our estimates. We also were unable to sequence 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. Finally, our sequencing did not consider as achieving virologic success.

In summary, our study suggests that the presence of PDR to NNRTIs is negatively associated with long-term virologic 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 pretreatment integrase mutations to outcomes, elucidate the impact of prior exposure to ART on treatment outcomes, and

### Table 3 Logistic regression models for 96-week virologic success in the ADVANCE Trial (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).

| Covariable | Univariable models | Baseline viral load-adjusted multivariable model | Fully adjusted multivariable model |
|------------|--------------------|-----------------------------------------------|-----------------------------------|
|            | Odds ratio (95%CI) | P valuea                                | Adjusted odds ratio (95%CI)       | P valuea                                |
|            |                    |                                | Adjusted odds ratio (95%CI)       |                                |
| 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                      | 1.77 (1.17, 2.67)                 | 0.01                                |
| Pretreatment CD4 count |                  |                            |                                   |                                    |
| <200 cells/μL | REF                |                            | REF                               |                                    |
| 201–350 cells/μL | 1.31 (0.83, 2.07)  | 0.25                       | 1.27 (0.76, 2.13)                 | 0.37                                |
| 351–500 cells/μL | 1.12 (0.67, 1.87)  | 0.66                       | 0.98 (0.54, 1.77)                 | 0.95                                |
| >500 cells/μL | 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 (n, %) | 0.36 (0.25, 0.52) | <0.001                     | 0.41 (0.27, 0.63)                 | <0.001                               |
| Pill count adherence (n, %) |                  |                            |                                   |                                    |
| <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 |                    |                                |                                   |                                    |
| 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                     | 0.34 (0.22, 0.53)                 | <0.001                               |

*P values represent results of two-sided tests of significance for coefficients in multivariable logistic regression models.

**Low adherence defined as self-report of less than perfect adherence in the 4 days prior to any study visits during the observation period.

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

Fig. 4 96-week treatment outcomes among participants in the ADVANCE Trial divided by treatment arm, presence or absence of WHO-defined pretreatment drug resistance, and achievement of greater than vs less than 95% adherence based on pharmacy pill count. Error bars represent 95% confidence intervals of the proportion estimates.
whether treatment failure observed on DTG-based ART is associated with emergence of integrase inhibitor mutations.

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 (NC/TGW 02222). Individuals were recruited from 11 public HIV clinics in Johannesburg, South Africa. All study visits and data collection procedures were performed at one of two research clinics in Johannesburg operated by the study staff. Consenting participants were randomized in a 1:1:1 ratio to (i) tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), EFV; (ii) TDF, FTC, DTG, or (iii) tenofovir alafenamide (TAF), FTC, and DTG53,54. 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 6 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 pretreatment viral load and CD4 T-cell count, demographics, demographic data. During observation, participants were scheduled for visits at week 4, 12, then every 12 weeks thereafter. Data for this analysis is 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. The first 30 days prior to each visit, study pharmacists and study nurses recorded dispensed pills and performed a pill count of remaining pills at each follow-up visit. Pretreatment plasma specimens were shipped to KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP) for extraction (Chemagic 360; Perkin Elmer, Germany). HIV-1 genotyping and nucleotide sequencing. We performed a genotypic resistance analysis (using BASTAR 5.2.1, Aachen, Germany) and performed a genotypic resistance analysis using the Geno2Pheno tool (https://www.genotypetools.org) to predict protease inhibitor resistance.

Statistical analysis. We first described and graphically depicted the analytic sample to determine which study participants were included and excluded from this analysis. We assessed for selection bias in this sub-analysis, we compared characteristics between individuals who had 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 observed in drug resistance testing.

We first described the presence of at least one of the WHO list of virology drug mutations detected in at least 20% of the viral population53,54. Our primary outcome of interest was 96-week virologic success, which we defined as sustained a viral load <1000 copies/mL from 12 weeks through 96 weeks, <200 copies/mL from 24 weeks through 96 weeks, and <50 copies/mL from 48 weeks through 96 weeks. Individuals censored with virologic suppression at 48 weeks or after are considered to have achieved virologic success.

We conducted an analysis (but are included in the 48- and 96-week Food and Drug Administration regulatory database and, for administrative reasons, we were able to compare sequences from this study with the deidentified database and code for all analyses upon request on the corresponding author.

Data availability

Full data are available from Professor Ravi Gupta (rkg20@cam.ac.uk) or Professor Francois Venter (fventer@ezintsha.org). Sequences generated in this study are available from SRA: https://www.ncbi.nlm.nih.gov/sra under accession number: PRJNA669549.

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W.D.F.V., M.J.S., R.K.G., T.d.O., R.L. Validation: Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs: M.J.S., B.S., T.d.O., R.L., B.C., J.G., G.A., S.A.K. Visualization: Preparation, creation and/or presentation of the published work, specifically visualization/data presentation: M.J.S. Writing—original draft preparation: Creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation): M.J.S. Writing—review and editing: Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision—including pre- or post-publication stages: M.J.S., M.A.M., B.S., T.d.O., R.L., J.G., S.A.K., B.C., G.A., C.M.S., W.D.F.V., A.H., R.K.G.

Competing interests
R.K.G. has received ad hoc consulting fees from Gilead, ViiV and UMOVIS Lab. W.D.F.V. received drug donations from ViiV Healthcare and Gilead Sciences for investigator-led clinical studies, including ADVANCE. In addition, he receives honoraria for talks and board membership for: Gilead, ViiV, Mylan, Merck, Adcock-Ingram, Aspen, Abbott, Roche, and G.A. M.A.M. 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. No other authors have any conflict of interest.

Additional information
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