



Efficacy and safety of dolutegravir or darunavir in combination with lamivudine plus either zidovudine or tenofovir for second-line treatment of HIV infection (NADIA): week 96 results from a prospective, multicentre, open-label, factorial, randomised, non-inferiority trial

Nicholas I Paton, Joseph Msaazi, Cissy Kityo, Stephen Walimbwa, Anne Hoppe, Apolo Balyegisawa, Jesca Asienzo, Arvind Kaimal, Grace Mirembe, Abbas Lugemwa, Gilbert Ategeka, Margaret Borok, Henry Mugerwa, Abraham Siika, Eva Laker A Odongpiny, Barbara Castelnovo, Agnes Kiragga, Andrew Kambugu, for the NADIA Trial Team*



Summary

Background WHO guidelines recommend dolutegravir plus two nucleoside reverse transcriptase inhibitors (NRTIs) for second-line HIV therapy, with NRTI switching from first-line tenofovir to zidovudine. We aimed to examine whether dolutegravir is non-inferior to darunavir, the best-in-class protease inhibitor drug, and whether maintaining tenofovir in second-line therapy is non-inferior to switching to zidovudine.

Methods In this prospective, multicentre, open-label, factorial, randomised, non-inferiority trial (NADIA), participants with confirmed HIV first-line treatment failure (HIV-1 RNA ≥ 1000 copies per mL) were recruited at seven clinical sites in Kenya, Uganda, and Zimbabwe. Following a 2×2 factorial design and stratified by site and screening HIV-1 RNA concentration, participants were randomly assigned (1:1:1:1) to receive a 96-week regimen containing either dolutegravir (50 mg once daily) or ritonavir-boosted darunavir (800 mg of darunavir plus 100 mg of ritonavir once daily) in combination with either tenofovir (300 mg once daily) plus lamivudine (300 mg once daily) or zidovudine (300 mg twice daily) plus lamivudine (150 mg twice daily). The NRTI drugs allocated by randomisation were administered orally in fixed-dose combination pills; other drugs were administered orally as separate pills. The previously reported primary outcome was the proportion of participants with a plasma HIV-1 RNA concentration of less than 400 copies per mL at 48 weeks. Here, we report the main secondary outcome: the proportion of participants with a plasma HIV-1 RNA concentration of less than 400 copies per mL at 96 weeks (non-inferiority margin 12%). We analysed this outcome and safety outcomes in the intention-to-treat population, which excluded only those who were randomly assigned in error and withdrawn before receiving trial drugs. This study was registered at ClinicalTrials.gov, NCT03988452, and is complete.

Findings Between July 30 and Dec 18, 2019, we screened 783 patients and enrolled 465. One participant was randomly assigned in error and immediately withdrawn. The remaining 464 participants were randomly assigned to receive either dolutegravir ($n=235$) or ritonavir-boosted darunavir ($n=229$) and to receive lamivudine plus either tenofovir ($n=233$) or zidovudine ($n=231$). At week 96, 211 (90%) of 235 participants in the dolutegravir group and 199 (87%) of 229 participants in the darunavir group had HIV-1 RNA less than 400 copies per mL (percentage point difference 2.9, 95% CI -3.0 to 8.7), indicating non-inferiority. Nine (4%) participants (all in the dolutegravir group) developed dolutegravir resistance; no participants developed darunavir resistance ($p=0.0023$). In the other randomised comparison, 214 (92%) of 233 patients in the tenofovir group and 196 (85%) of 231 patients in the zidovudine group had HIV-1 RNA less than 400 copies per mL (percentage point difference 7.0, 95% CI 1.2 to 12.8), showing non-inferiority and indicating the superiority of tenofovir ($p=0.019$). The proportions of participants with any grade 3–4 adverse event were similar between the dolutegravir (26 [11%]) and darunavir (28 [12%]) groups and between the tenofovir (22 [9%]) and zidovudine (32 [14%]) groups. There were no deaths related to study medication.

Interpretation Dolutegravir-based and darunavir-based regimens maintain good viral suppression during 96 weeks; dolutegravir is non-inferior to darunavir but is at greater risk of resistance in second-line therapy. Tenofovir should be continued in second-line therapy, rather than being switched to zidovudine.

Funding Janssen.

Copyright © 2022 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

Lancet HIV 2022; 6: e381–93

Published Online

April 20, 2022

[https://doi.org/10.1016/S2352-3018\(22\)00092-3](https://doi.org/10.1016/S2352-3018(22)00092-3)

See [Comment](#) page e368

*A complete list of members of the NADIA Trial Team can be found in appendix 1

Infectious Diseases
Translational Research
Programme and Department
of Medicine, Yong Loo Lin
School of Medicine, National
University of Singapore,
Singapore (Prof N I Paton MD);
Department of Clinical
Research, London School of
Hygiene and Tropical
Medicine, London, UK
(Prof N I Paton); Infectious
Diseases Institute, Makerere
University, Kampala, Uganda
(J Msaazi MSc,
S Walimbwa MD, A Hoppe PhD,
A Balyegisawa MD,
J Asienzo BPLM, A Kaimal MD,
E L A Odongpiny MSc,
B Castelnovo PhD,
A Kiragga PhD,
A Kambugu MMed); Joint
Clinical Research Centre,
Kampala, Uganda (C Kityo PhD,
H Mugerwa MD); Makerere
University Walter Reed
Project, Kampala, Uganda
(G Mirembe MMed); Joint
Clinical Research Centre,
Mbarara, Uganda
(A Lugemwa MD); Joint Clinical
Research Centre, Fort Portal,
Uganda (G Ategeka MD);
University of Zimbabwe
Clinical Research Centre,
Harare, Zimbabwe
(Prof M Borok MBChB);
Department of Medicine,
Moi University School of
Medicine, Eldoret, Kenya
(Prof A Siika MMed)

Correspondence to:
 Prof Nicholas I Paton, Infectious
 Diseases Translational Research
 Programme and Department of
 Medicine, Yong Loo Lin School
 of Medicine, National University
 of Singapore, Singapore 119228
nick_paton@nus.edu.sg

See Online for appendix 1

Research in context

Evidence before this study

We searched PubMed without language restrictions for articles published between database inception and Dec 31, 2021, that described randomised controlled trials (RCTs) comparing dolutegravir with a protease inhibitor, or comparing zidovudine with tenofovir, in patients with HIV treatment failure on a first-line regimen based on a non-nucleoside reverse transcriptase inhibitor (NNRTI). We used the search terms “dolutegravir OR darunavir OR protease inhibitor OR tenofovir OR zidovudine” and “trial OR randomised” and “second-line OR first-line failure”. From the 330 articles retrieved, we identified three that reported relevant RCTs. First, the DAWNING trial compared dolutegravir with ritonavir-boosted lopinavir in adults with first-line treatment failure on an NNRTI-based regimen; nucleoside reverse transcriptase inhibitors (NRTIs) were selected by the clinician following resistance testing and viral load was monitored every 4–12 weeks. The trial showed the superiority of dolutegravir versus ritonavir-boosted lopinavir for the primary outcome of viral load suppression to less than 50 copies per mL at 48 weeks. Second, the ODYSSEY trial compared dolutegravir-based regimens with standard-of-care regimens for first-line and second-line HIV therapy in children and adolescents; NRTIs were selected by clinicians on the basis of resistance testing or treatment history, and viral load was monitored every 6–12 months. In the subset of participants receiving second-line therapy, fewer participants in the dolutegravir group than in the protease inhibitor group (mostly taking ritonavir-boosted lopinavir) had treatment failure at 96 weeks. Finally, the third report was on 48-week outcomes from NADIA (this trial), which showed that dolutegravir was non-inferior to ritonavir-boosted darunavir and that tenofovir was non-inferior to zidovudine for the primary outcome of viral load suppression to less than 400 copies per mL at 48 weeks.

Added value of this study

The 96-week findings from the NADIA trial provide important new data that inform the public health approach to HIV treatment and regimens for second-line therapy. The trial shows the non-inferiority of dolutegravir to darunavir, but not superiority, and confirms the high efficacy and durability of responses to each, including when the drugs are combined with NRTIs that have no predicted activity. This trial has provided the best estimate to date of the risk and profile of emergent dolutegravir resistance when this drug is used with NRTIs to which there is pre-existing resistance. To our knowledge, this RCT is the first to compare, head-to-head, dolutegravir with ritonavir-boosted darunavir (the best tolerated and most

potent drug in the protease inhibitor class) in second-line therapy and is the only one to have shown the non-inferiority of a protease inhibitor to dolutegravir in this setting. The evidence that the genetic barrier to resistance is higher with darunavir than with dolutegravir when combined with NRTIs to which participants have pre-existing drug resistance is also a new finding. NADIA is also the only RCT to have tested the longstanding WHO recommendation to switch from tenofovir to zidovudine at transition to second-line therapy. After 96 weeks' follow-up, maintaining tenofovir in the second-line regimen was superior to switching to zidovudine in achieving viral suppression. Maintaining tenofovir versus switching to zidovudine also reduced the chance of viral rebound, increased CD4 cell count, and possibly reduced the risk of high-level dolutegravir resistance.

Implications of all the available evidence

The 96-week results of the NADIA trial, taken together with results of two other trials, now provide sufficient evidence to support WHO's existing recommendation to use dolutegravir with two NRTIs in second-line therapy in the public health approach, and show that this combination will produce durable viral suppression, even when substantial NRTI resistance is present and when delivered with sparse viral load monitoring, which is relevant to the setting of the public health approach. These findings also support the global, programme-wide switch of stable patients from NNRTI-based to dolutegravir-based regimens, indicating that viral suppression is likely to be achieved even if patients have occult NRTI resistance. However, there is a clear risk of dolutegravir resistance when used in second-line therapy that has not been observed in first-line therapy. Although the overall rate is modest in clinical trials, resistance might increase in treatment programme settings and mitigation strategies should be considered (including optimising the choice of NRTIs). The finding in NADIA that darunavir achieves high rates of viral suppression that are non-inferior to those of dolutegravir contrasts with the results of trials comparing dolutegravir with other protease inhibitors (lopinavir and atazanavir) in second-line therapy, which found the protease inhibitor regimen to be inferior to dolutegravir. Darunavir therefore merits an expanded role in the public health approach as a preferred protease inhibitor; its high genetic barrier to resistance also confers a possible advantage over dolutegravir. WHO's longstanding recommendation to switch from tenofovir to zidovudine for second-line therapy in the public health approach should be revised to recommend maintaining tenofovir (and lamivudine) when introducing a new third drug (either dolutegravir or darunavir).

Introduction

WHO recommends a public health approach to HIV treatment involving the use of a small number of standardised regimens and simplified monitoring.¹ Current treatment guidelines recommend dolutegravir,

an integrase strand transfer inhibitor, combined with two drugs from the nucleoside reverse transcriptase inhibitor (NRTI) drug class, as the preferred second-line therapy in patients who develop treatment failure on initial therapy with a non-dolutegravir-based regimen.²

However, patients switching to second-line therapy often have HIV that has acquired resistance mutations to NRTIs used in the first-line regimen. Before this trial, the efficacy of dolutegravir as second-line therapy had only been tested with NRTIs selected by resistance testing to ensure that at least one NRTI was fully active, and the performance of dolutegravir with NRTIs that lack predicted activity on resistance testing was unknown.^{3,4} Furthermore, dolutegravir had only been compared with lopinavir, an older drug in the protease inhibitor drug class that, although recommended in WHO treatment guidelines, requires twice daily dosing and has common gastrointestinal side-effects.³⁻⁵

Instead of resistance testing to guide the choice of NRTIs in a second-line regimen, WHO recommends an empirical switch from tenofovir, the more commonly used NRTI in first-line therapy, to zidovudine, an alternative NRTI, with lamivudine continued throughout.² However, before now, this recommendation to switch to zidovudine, present in WHO treatment guidelines since 2010, had never been tested in a randomised controlled trial.

The Nucleosides and Darunavir/Dolutegravir In Africa (NADIA) trial is designed to address two questions. First, whether dolutegravir is non-inferior to darunavir, the best-in-class protease inhibitor drug,^{6,7} in a population of patients with high levels of background NRTI resistance switching to second-line therapy. Second, whether maintaining tenofovir in second-line therapy is non-inferior to switching to zidovudine. The trial was conducted with conditions of simplified monitoring and care recommended by WHO's public health approach.^{1,4} The primary outcome results, reported after 48-week follow-up, showed non-inferiority of dolutegravir and non-inferiority of maintaining tenofovir.⁸ Here, we report results of the 96 week follow-up that provide further important information relevant to antiretroviral treatment policies and clinical practice in the public health approach and beyond.

Methods

Study design and participants

In this prospective, multicentre, open-label, 2×2 factorial, randomised, non-inferiority trial (NADIA), participants were recruited via referral clinics and followed up at seven clinical sites in three countries (Kenya, Uganda, and Zimbabwe); the trial was coordinated from Uganda. Results after the first 48 weeks of follow-up have been previously reported.⁸ Patients were required to be at least 12 years old; to have taken a regimen with tenofovir plus lamivudine or emtricitabine plus a non-nucleoside reverse transcriptase inhibitor (NNRTI) for at least 6 months continuously before screening; to have missed no more than 3 days of treatment in the month before screening; and to have HIV-1 RNA of 1000 copies per mL or more (HIV first-line treatment failure) at screening and on a second sample obtained before or after the screening sample. The main exclusion criteria were

previous use of protease or integrase inhibitor drugs, current pregnancy or breastfeeding, severe hepatic impairment, or an estimated glomerular filtration rate of less than 50 mL/min per 1.73 m².⁹ Further inclusion and exclusion criteria can be found in appendix 1 (pp 2–3). The trial received approval from all local ethics committees and national regulatory agencies. All patients provided written informed consent.

Randomisation and masking

Following a 2×2 factorial design and stratified by site and screening HIV-1 RNA concentration (<100 000 copies per mL vs ≥100 000 copies per mL), participants were randomly assigned (1:1:1:1) to receive a regimen containing either dolutegravir or ritonavir-boosted darunavir in combination with either tenofovir plus lamivudine or zidovudine plus lamivudine. Randomisation used a secure web-based system preprogrammed with a computer-generated, sequentially numbered randomisation list using random permuted blocks (block size 4 or 8), and was maintained by a data management group that was independent of the trial management team. The trial management team did not have access to aggregate unmasked data except for serious adverse event and pregnancy reports; the trial statistician had access to unmasked data through formal request to the data management group when required for study analyses. Randomisation was done by the study coordinator at each site, who could access the next number on the system but not the whole list. Treatment allocation was not masked to site staff or participants.

Procedures

For 96 weeks, participants received a regimen containing either 50 mg of dolutegravir once daily or ritonavir-boosted darunavir (800 mg of darunavir plus 100 mg of ritonavir) once daily given in combination with either 300 mg of tenofovir plus 300 mg of lamivudine once daily or 300 mg of zidovudine plus 150 mg of lamivudine twice daily. The NRTI drugs allocated by randomisation were administered orally in a fixed-dose combination pill; other drugs were administered orally as separate pills. Participants assigned to received zidovudine who had hepatitis B virus coinfection added tenofovir. The protocol allowed NRTI substitution for toxicity and switching dolutegravir to darunavir for pregnancy. Patients with tuberculosis took 50 mg of dolutegravir twice daily with standard tuberculosis treatment or ritonavir-boosted darunavir with rifabutin-based tuberculosis treatment.

Visits were scheduled at baseline and weeks 4, 8, and 12, and then every 12 weeks thereafter until week 96 and were mostly nurse-led. Adherence was assessed at each visit by standard questions and overall adherence calculated as the proportion of visits attended at which the participant missed at least one dose of study medication in the previous 4 weeks. Peripheral neuropathy screens, waist circumference measurements, and assessments for

fat changes (patient self-report and clinician confirmation) were done at baseline, week 48, and week 96. Complete blood count, alanine aminotransferase, and creatinine were measured at screening and weeks 12, 48, and 96, and urine dipstick tests for protein were done at baseline, week 48, and week 96. CD4 cell counts were measured at screening and weeks 24, 48, and 96.

Real-time, open (unmasked to site clinician) HIV-1 RNA measurement was done at screening, weeks 24, 48, and 96; measurement was also done at week 72 for participants who did not meet stability criteria at week 48. Stability criteria were HIV-1 RNA concentration of less than 1000 copies per mL on the test done at week 48 and on the previous test, good understanding of the importance of continued adherence, no pregnancy, and no major current illness.^{2,10} Testing was done with the assay in routine use at each site laboratory. Participants with HIV-1 RNA concentrations of 1000 copies per mL or more received intensive adherence counselling and the test was repeated after 12 weeks (window 10–16 weeks) in accordance with WHO recommendations (window 2–12 weeks at week 96).¹⁰ If participants had confirmed HIV-1 RNA concentrations of 1000 copies per mL or more on the repeat test, they were evaluated for switch to third-line treatment. If adherence remained suboptimal, a further period of adherence counselling and a repeat HIV-1 RNA test were allowed, at clinicians' discretion, before considering a treatment switch.

Retrospective, closed HIV-1 RNA measurement was done in batches on plasma samples stored at weeks 12 and 72 (in participants who met stability criteria at week 48 and who were therefore not eligible for open testing). Tests were done in the site laboratory by use of the same routine assay, but results were blinded, seen only by the independent data monitoring committee, and returned to the site clinician after the participant completed trial follow-up.

Genotypic resistance testing was done in a WHO-accredited central laboratory (Joint Clinical Research Centre, Kampala, Uganda). Drug susceptibility prediction used the Stanford algorithm. Resistance testing was done in real time (reverse transcriptase in all, and protease, integrase, or both, according to drug exposure) only for participants with confirmed viral load rebounds of HIV-1 RNA of 1000 copies per mL or more (detected on open viral load testing), with results returned to the site clinician.

Supplementary genotypic resistance testing was done retrospectively in batches on stored plasma samples from all participants at baseline (reverse transcriptase in all, and protease, integrase, or both, according to mutations detected at rebound) and from all participants with confirmed viral load rebound of 400 copies per mL or more during the trial (whether detected on open or closed viral load tests) or a single result of 400 copies per mL or more at week 96 (reverse transcriptase in all, and protease, integrase, or both, according to drug

exposure). Results were returned to site clinicians after participants completed trial follow-up.

Outcomes

The primary outcome for both factorial comparisons in this trial was proportion of patients with plasma viral loads less than 400 copies per mL at week 48, and has been reported.⁸ The main secondary outcome for the analysis reported here is the proportion of patients with plasma HIV-1 RNA loads of less than 400 copies per mL at week 96 (at the end of the planned full duration of trial follow-up), determined in the same way as week 48. Further secondary outcomes assessed at week 96 were the proportion of patients with plasma viral loads less than 1000 copies per mL; confirmed viral rebound to 1000 copies per mL or more (two consecutive viral loads of 1000 copies per mL or more); confirmed viral load rebound (≥ 1000 copies per mL) with at least one major resistance mutation to dolutegravir or darunavir; and the change in CD4 cell count from baseline. Prespecified other outcomes at the week 96 visit that we present here are the proportion of patients with a plasma viral load of less than 50 copies per mL; confirmed viral load rebound to 400 copies per mL or more; change in bodyweight, waist circumference, and body-mass index from baseline; incident obesity (body-mass index >30 kg/m²); facial lipoatrophy; fat accumulation; and symptomatic peripheral neuropathy. Prespecified other outcomes that are not presented here (and will be reported in future publications) are viral load rebound to 1000 copies per mL or more with at least one major resistance mutation to tenofovir or zidovudine; quality of life; and resource use and costs. The main safety assessments were proportions of patients that had at least one grade 3 or 4 clinical adverse event or at least one serious adverse event by week 96. Clinical adverse events were defined and graded by use of standard criteria.¹¹ More information on outcomes can be found in the statistical analysis plan (appendix 2).

Statistical analysis

Sample size justification was based on the assumption that 82% in each randomised group would have viral load of less than 400 copies per mL (based on previous data).¹² With a non-inferiority margin of 12% (selected on the basis of clinical consensus and the range used in previous second-line treatment trials)^{3,12–15} and a 2.5% one-sided significance level, we calculated that 440 patients (220 per randomised group) would provide 90% power to show non-inferiority.

The proportion of participants with viral suppression (<400 copies per mL at week 96) was calculated by use of a modified US Food and Drug Administration (FDA) snapshot algorithm¹⁶ in the intention-to-treat population, which excluded only those randomly assigned in error and withdrawn before receiving trial drugs. Non-inferiority of dolutegravir versus darunavir could be concluded if the lower limit of the two-sided 95% CI

See Online for appendix 2

For the Stanford algorithm see <http://hivdb.stanford.edu/>

(unadjusted; binomial methods) of the difference in the proportion with viral loads less than 400 copies per mL in the dolutegravir minus the darunavir group was higher than -12%. Four prespecified sensitivity analyses of the main secondary outcome at week 96 were done: one used a per-protocol population (appendix 1 p 3); one used a binomial linear regression model adjusted for the other factorial comparison, site, baseline viral load, baseline CD4 cell count, and sex; one used imputation for missing data; and one used complete case analysis. We also did a post-hoc sensitivity analysis of the main secondary outcome at week 96 in which we excluded patients with hepatitis B virus coinfection.

Superiority testing was planned only if non-inferiority was shown in both the primary intention-to-treat analysis and the per-protocol analysis. Non-inferiority of dolutegravir versus darunavir was prespecified as the main hypothesis to be tested; a test for interaction was done to identify whether the comparison of zidovudine with tenofovir could be analysed independently of the main comparison. No adjustment for multiplicity was required for these analyses of the main secondary outcome, given the prespecified hierarchical testing approaches.

We did analyses of viral suppression to less than 400 copies per mL at week 96 in prespecified subgroups of other drugs in the regimen (group assigned by the other factorial randomisation), sex, baseline viral load (test done at screening), baseline CD4 cell count (test done at screening), number of predicted-active NRTIs in the prescribed regimen, presence of key NRTI mutations (Lys65Arg or Lys65Asn and Met184Val or Met184Ile), and the presence of an intermediate-to-high level of tenofovir or zidovudine resistance at baseline. With the same subgroups, we did post-hoc analyses of viral suppression to less than 1000 copies per mL and less than 50 copies per mL at week 96.

We also fitted a multivariable logistic regression model to explore the association between viral suppression to less than 400 copies per mL and randomised drugs, baseline resistance mutations, adherence, and other factors (baseline viral load, viral subtype, baseline CD4 cell count, sex, and age; details in appendix 1 [pp 3–4]).

All efficacy and safety analyses used the intention-to-treat population, with the exception of the per-protocol sensitivity analysis of the main secondary outcome. For analyses of viral suppression, missing data were assigned values by the FDA snapshot algorithm, or by imputation in one sensitivity analysis of the main secondary outcome. All other missing outcome values were handled by complete case analyses.

Outcomes other than viral suppression that are expressed as proportions were analysed by use of risk difference and the χ^2 test or Fisher's exact test. Continuous outcomes were analysed with a two-sample Student's *t* test. There was no correction for multiplicity, so results are reported as point estimates and 95% CIs. All analyses used

Stata, version 16. The trial was monitored by an independent data monitoring committee, and an independent trial steering committee provided trial oversight. The trial was registered with ClinicalTrials.gov, NCT03988452.

Role of the funding source

The funder of the study reviewed and commented on the initial concept sheet but had no role in the development of the final protocol, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit the paper for publication.

Results

Between July 30 and Dec 18, 2019, we formally screened 783 patients for eligibility and enrolled 465 (figure 1). Of these, one participant was randomly assigned in error and immediately withdrawn. The remaining 464 participants were randomly assigned to receive either dolutegravir (n=235) or darunavir (with ritonavir; n=229) and to receive either tenofovir with lamivudine (n=233) or zidovudine with lamivudine (n=231). Of these 464, eight (2%) died and three (1%) withdrew or were lost to follow-up before week 96 (figure 1).

Baseline characteristics were similar between the randomised groups (appendix 1 p 5).^{*} Overall, 282 (61%) of 464 participants were female and 182 (39%) were male, 238 (51%) had CD4 cell counts of less than 200 cells per μ L, and 128 (28%) had HIV-1 RNA of at least 100 000 copies per mL. A viral sequence was obtained from a stored baseline plasma sample in 453 (98%) of 464 participants; mutations classified as conferring intermediate-to-high level resistance were detected in 265 (58%) for tenofovir, 83 (18%) for zidovudine, and 416 (92%) for lamivudine (appendix 1 p 5).

Participants received their strictly assigned or substituted regimen (substituted for reasons permitted in the protocol) for a mean of 95% (SD 18) and 4% (18) of the follow-up time, respectively (appendix 1 p 6). Overall, 4542 (98%) of 4640 scheduled study visits to week 96 were attended and participants reported complete adherence at 3720 (82%) visits, with no difference between the randomised groups (appendix 1 p 7). Additional open HIV-1 RNA measurement at week 72 was done in 53 (11%) participants who did not meet week 48 stability criteria.

At week 96, 211 (90%) of 235 participants in the dolutegravir group and 199 (87%) of 229 participants in the darunavir group had viral suppression to less than 400 copies per mL (percentage point difference 2.9, 95% CI -3.0 to 8.7) in the intention-to-treat population, thus meeting the prespecified non-inferiority criterion (table 1; appendix 1 p 13). The non-inferiority criterion was also met in the per-protocol population that excluded 35 patients who died, were lost to follow-up, or interrupted or switched treatment for reasons not permitted by the protocol (table 1). As non-inferiority was confirmed in both intention-to-treat and per-protocol populations, we tested for the superiority of dolutegravir

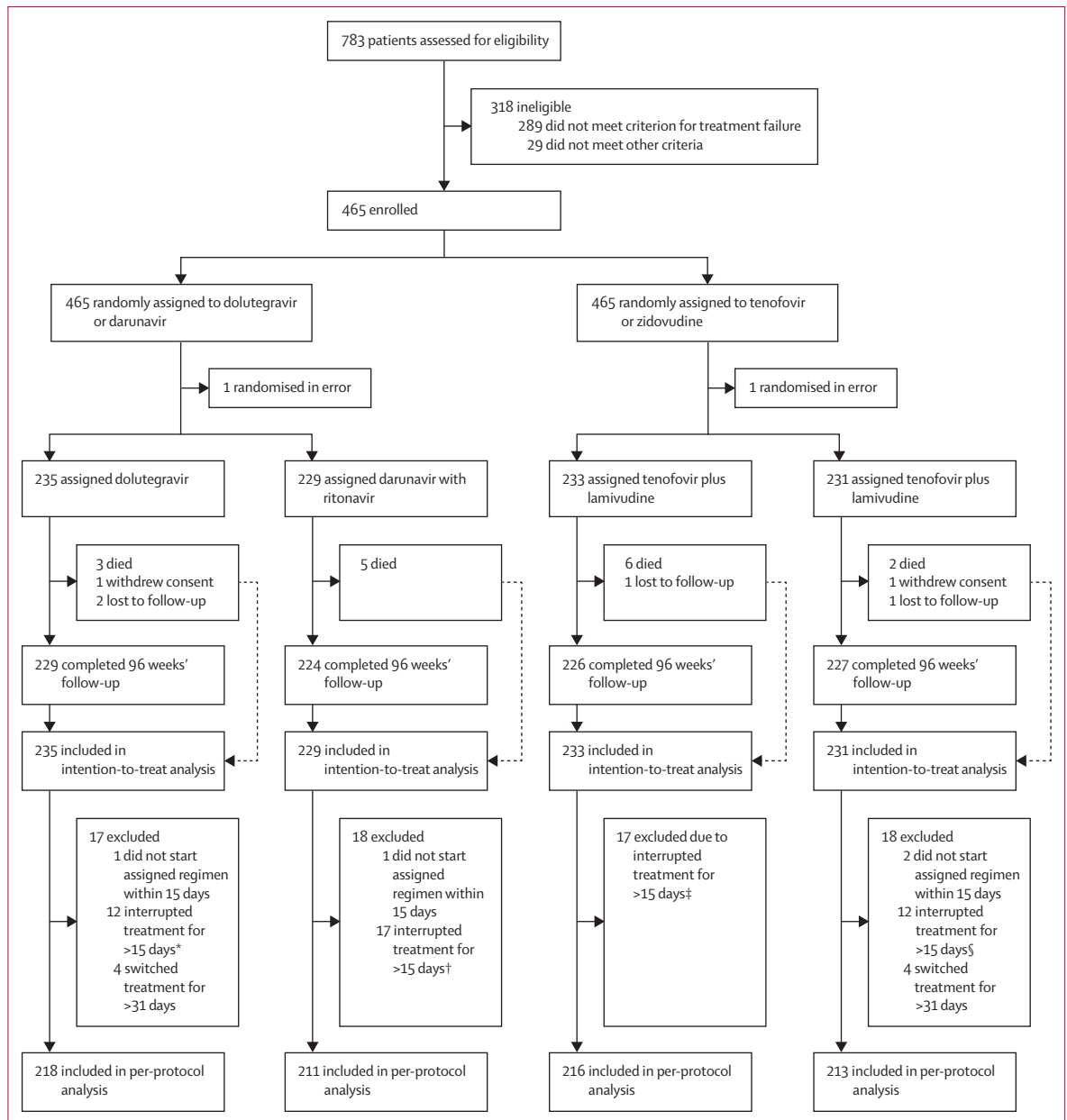


Figure 1: Trial profile to week 96

*Includes the six who either died, were lost to follow-up, or withdrew. †Includes the five who died. ‡Includes the seven who died or were lost to follow-up. §Includes the four who either died, were lost to follow-up, or withdrew.

versus darunavir but found no evidence for such ($p=0.33$; table 1). Results were consistent in other prespecified sensitivity analyses (table 1); for the other HIV-1 RNA thresholds (<1000 copies per mL and <50 copies per mL; table 1); and in a post-hoc sensitivity analysis in which we excluded 22 patients with hepatitis B virus coinfection (appendix 1 p 8).

The finding of similar rates of suppression to less than 400 copies per mL with dolutegravir versus darunavir was consistent across prespecified subgroups (figure 2A). In the subgroup with no predicted-active NRTIs, the

proportion of participants with concentrations of HIV-1 RNA of less than 400 copies per mL exceeded 90% in both groups (figure 2A). Overall findings were similar in additional, post-hoc, subgroup analyses at other viral load thresholds (appendix 1 pp 15, 17).

Confirmed viral rebound to 1000 copies per mL or more occurred in 20 (9%) participants in the dolutegravir group and in 26 (11%) participants in the darunavir group ($p=0.31$); the proportions with confirmed viral rebound to 400 copies per mL or more were also similar between the two groups ($p=0.27$; table 1). Seven participants with

	Dolutegravir (n=235)	Darunavir (n=229)	Difference in percentage points (95% CI)	p value*	Tenofovir (n=233)	Zidovudine (n=231)	Difference in percentage points (95% CI)	p value*
Main secondary outcome: HIV-1 RNA suppression to <400 copies per mL in the intention-to-treat population†								
<400 copies per mL	211 (90%)	199 (87%)	2.9 (-3.0 to 8.7)	0.33	214 (92%)	196 (85%)	7.0 (1.2 to 12.8)	0.019
≥400 copies per mL‡	20 (9%)	25 (11%)	13 (6%)	32 (14%)
No virological data	4 (2%)	5 (2%)	6 (3%)	3 (1%)
Withdraw due to adverse events or death	3 (1%)	5 (2%)	6 (3%)	2 (1%)
Withdraw for other reasons	1 (<1%)	0	0	1 (<1%)
Sensitivity analyses: HIV-1 RNA suppression to <400 copies per mL								
<400 copies per mL in the adjusted analysis§	90%	87%	3.5 (-2.8 to 9.8)	0.28	92%	84%	7.9 (1.9 to 14.0)	0.0096
<400 copies per mL in the per-protocol analysis¶	201/218 (92%)	192/211 (91%)	1.2 (-4.0 to 6.5)	0.65	206/216 (95%)	187/213 (88%)	7.6 (2.4 to 12.8)	0.0047
<400 copies per mL using missing data imputation	93%	88%	5.1 (-0.2 to 10.3)	0.059	95%	87%	7.5 (2.3 to 12.7)	0.0049
<400 copies per mL in the complete case analysis	214/229 (93%)	198/224 (88%)	5.1 (-0.2 to 10.3)	0.061	213/225 (95%)	199/228 (87%)	7.4 (2.2 to 12.6)	0.0062
Secondary and other efficacy outcomes								
HIV-1 RNA <1000 copies per mL	213 (91%)	203 (89%)	2.0 (-3.6 to 7.5)	0.48	216 (93%)	200 (87%)	6.1 (0.6 to 11.6)	0.030
HIV-1 RNA <50 copies per mL	189 (80%)	172 (75%)	5.3 (-2.2 to 12.9)	0.17	188 (81%)	173 (75%)	5.8 (-1.8 to 13.3)	0.13
Confirmed viral rebound to ≥1000 copies per mL	20 (9%)	26 (11%)	-2.8 (-8.3 to 2.6)	0.31	13 (6%)	33 (14%)	-8.7 (-14.1 to -3.3)	0.0017
Confirmed viral rebound to ≥400 copies per mL	24 (10%)	31 (14%)	-3.3 (-9.2 to 2.6)	0.27	15 (6%)	40 (17%)	-10.9 (-16.7 to -5.1)	0.0003
Confirmed viral rebound to ≥1000 copies per mL with at least one major resistance mutation to dolutegravir or darunavir**	7 (3%)	0	3.0 (0.8-5.3)	0.0075	2 (1%)	5 (2%)	-1.3 (-3.6 to 0.9)	0.24
At least one dolutegravir or darunavir major resistance mutation††	9 (4%)	0	4.0 (1.4-6.5)	0.0023	3 (1%)	6 (3%)	-1.4 (3.9 to 1.2)	0.30
Change from baseline in CD4 cell count, cells per µL‡‡	221 (197)	218 (246)	3 (-38 to 44)	0.89	250 (204)	191 (235)	59 (18 to 100)	0.0046

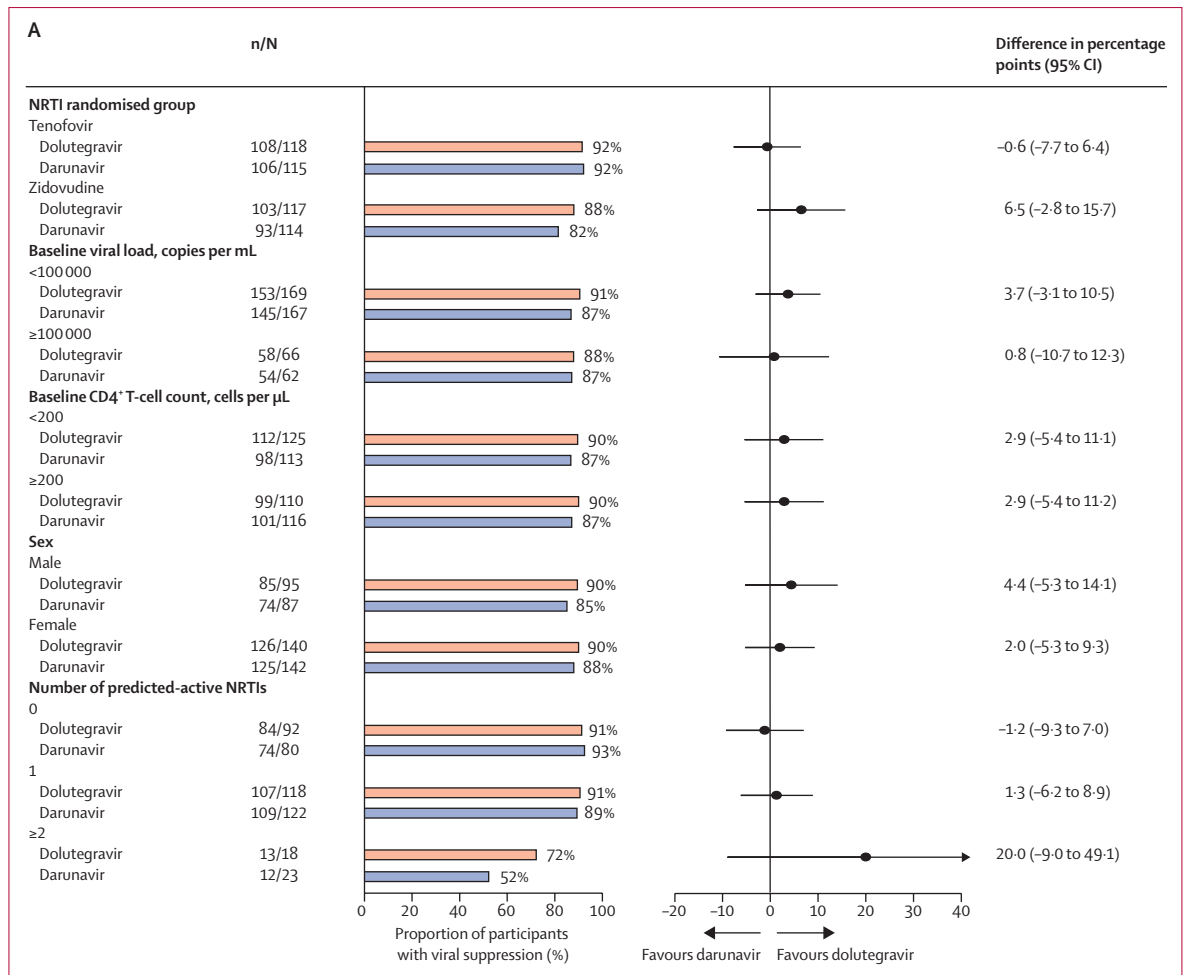
Data are n (%), n/N (%), or mean (SD), unless otherwise specified. Percentages might not total 100% because of rounding. All analyses of viral load suppression (according to a threshold) were done with the use of the US Food and Drug Administration snapshot algorithm and involved the intention-to-treat population, except where stated for sensitivity analyses. Analyses of viral load rebound and changes in CD4 cell count were done by use of complete case analyses. The widths of the CIs have not been adjusted for multiple comparisons; therefore, the intervals cannot be used to infer treatment effects with respect to the outcomes other than the main secondary outcome. *p values are for superiority (not non-inferiority). †p=0.23 for the interaction between the two factorial comparisons. ‡Of the 45 patients with a viral load of 400 copies per mL or more, categorisation was based on a measured value in the week 96 window in 40 participants and on the value before withdrawal or treatment switch in one and four participants, respectively. §Estimates were from a binomial linear regression model, with adjustment for the other factorial comparison, site, baseline viral load, CD4 cell count, and sex. ¶The per-protocol population excludes 35 patients who died, were lost to follow-up, or interrupted or switched treatment for reasons not permitted by the protocol. ||One additional patient (assigned to receive dolutegravir plus tenofovir) had a viral load of at least 1000 copies per mL at week 48 that could not be confirmed (the patient had not taken any antiretroviral drugs for >3 months and withdrew from the trial after the visit). **Based on available viral sequences for 43 of 46 patients with confirmed viral rebound to 1000 copies per mL or more by week 96. ††Sequences from 49 of 55 with rebound to 400 copies per mL or more by week 96; nine participants had at least one major viral mutation associated with dolutegravir resistance: Thr66Thr/Ala, Gly118Arg, Glu138Lys, Gly149Gly/Ala, and Gly163Gly/Arg (n=1; high resistance level; zidovudine group); Thr66Thr/Ala/Ile/Val, Thr97Ala, Gly118Arg, and Glu138Lys (n=1; high resistance level; zidovudine group); Glu138Lys, Gly140Ala, and Glu148Arg (n=1; high resistance level; zidovudine group); Thr66Ile, Gly118Arg, Glu138Lys, and Gly149Gly/Ala (n=1; high resistance level; zidovudine group); Thr66Ala, Gly118Arg, and Glu138Lys (n=1; high resistance level; zidovudine group); Arg263Arg/Lys (n=1; intermediate resistance level; zidovudine group); and Met50Ile and Arg263Lys or Arg263Arg/Lys (n=3; intermediate resistance level; tenofovir group); no patients had viral mutations associated with darunavir resistance. ‡‡CD4 cell count data were available for 453 patients; missing values were due to death (eight patients [three who received dolutegravir, five who received tenofovir, and two who received zidovudine]), loss to follow-up (two patients [two who received dolutegravir, one who received tenofovir, and one who received zidovudine]), or withdrawal (one who received tenofovir).

Table 1: Main efficacy outcomes at week 96

confirmed viral rebound to at least 1000 copies per mL and a further two participants with confirmed viral rebound to at least 400 copies per mL, equalling a total of nine (4%) participants in the dolutegravir group, had major dolutegravir resistance mutations, but no participants had dolutegravir or darunavir major resistance mutations in the darunavir group (p=0.0023; table 1). Of the nine participants who had major dolutegravir resistance mutations, seven had confirmed viral rebound (≥400 copies per mL) at or before week 48, of whom five reported missed medication doses at multiple visits at or before week 48. Five participants had high-level resistance to dolutegravir (associated with Gly118Arg mutation in four and Glu148Arg mutation in one, with accessory mutations) and four participants (Arg263Lys mutation in all) had intermediate-level resistance to

dolutegravir. No patient developed a major resistance mutation to darunavir. The increase in CD4 cell count from baseline was similar between the dolutegravir and darunavir groups (table 1).

There was no interaction between the zidovudine versus tenofovir and darunavir versus dolutegravir randomisation factors for the main secondary outcome (p=0.23), so results for the zidovudine and tenofovir comparison are also presented by randomised group (table 1). At week 96, 214 (92%) of 233 participants in the tenofovir group and 196 (85%) of 231 participants in the zidovudine group had HIV-1 RNA concentrations of less than 400 copies per mL (percentage point difference 7.0, 95% CI 1.2–12.8) in the intention-to-treat population, meeting the non-inferiority criterion (table 1; appendix 1 p 14). The non-inferiority criterion was also met in the



(Figure 2 continues on next page)

per-protocol population and so we tested for, and found evidence of, the superiority of tenofovir versus zidovudine in the intention-to-treat population ($p=0.019$). The direction and magnitude of the risk difference between tenofovir and zidovudine was similar in other prespecified sensitivity analyses (table 1); for other viral load thresholds (HIV-1 RNA <1000 copies per mL and <50 copies per mL; table 1); and in a post-hoc sensitivity analysis in which we excluded those with hepatitis B virus coinfection (table 1; appendix 1 p 8).

Rates of viral suppression to less than 400 copies per mL were higher in the tenofovir group than in the zidovudine group across many of the prespecified subgroup analyses (figure 2B). By contrast, there was no subgroup in which zidovudine resulted in substantially better viral suppression (<400 copies per mL) than tenofovir, even in those participants with the Lys65Arg or Lys65Asn mutations or an intermediate-to-high level of tenofovir resistance at baseline (figure 2B). Overall findings were similar in additional, post-hoc, subgroup analyses at other viral load thresholds (appendix 1 p 16, 18).

Confirmed viral rebound to at least 1000 copies per mL occurred in 13 (6%) participants in the tenofovir group and in 33 (14%) participants in the zidovudine group (percentage point difference -8.7 , 95% CI -14.1 to -3.3 ; $p=0.0017$; table 1). The proportion with confirmed viral rebound to at least 400 copies per mL was also lower in the tenofovir group than in the zidovudine group ($p=0.0003$; table 1). Of the nine participants with dolutegravir major resistance mutations, three (two with confirmed viral rebound to ≥ 1000 copies per mL) were in the tenofovir group and six (five with confirmed viral rebound to ≥ 1000 copies per mL) were in the zidovudine group (table 1). The mutations and resistance level clustered by NRTI group, with all three participants with major resistance mutations in the tenofovir group having the intermediate-level resistance profile and five of the six participants in the zidovudine group having the high-level resistance profile (table 1). The increase in CD4 cell count from baseline was greater in the tenofovir group than in the zidovudine group (table 1).

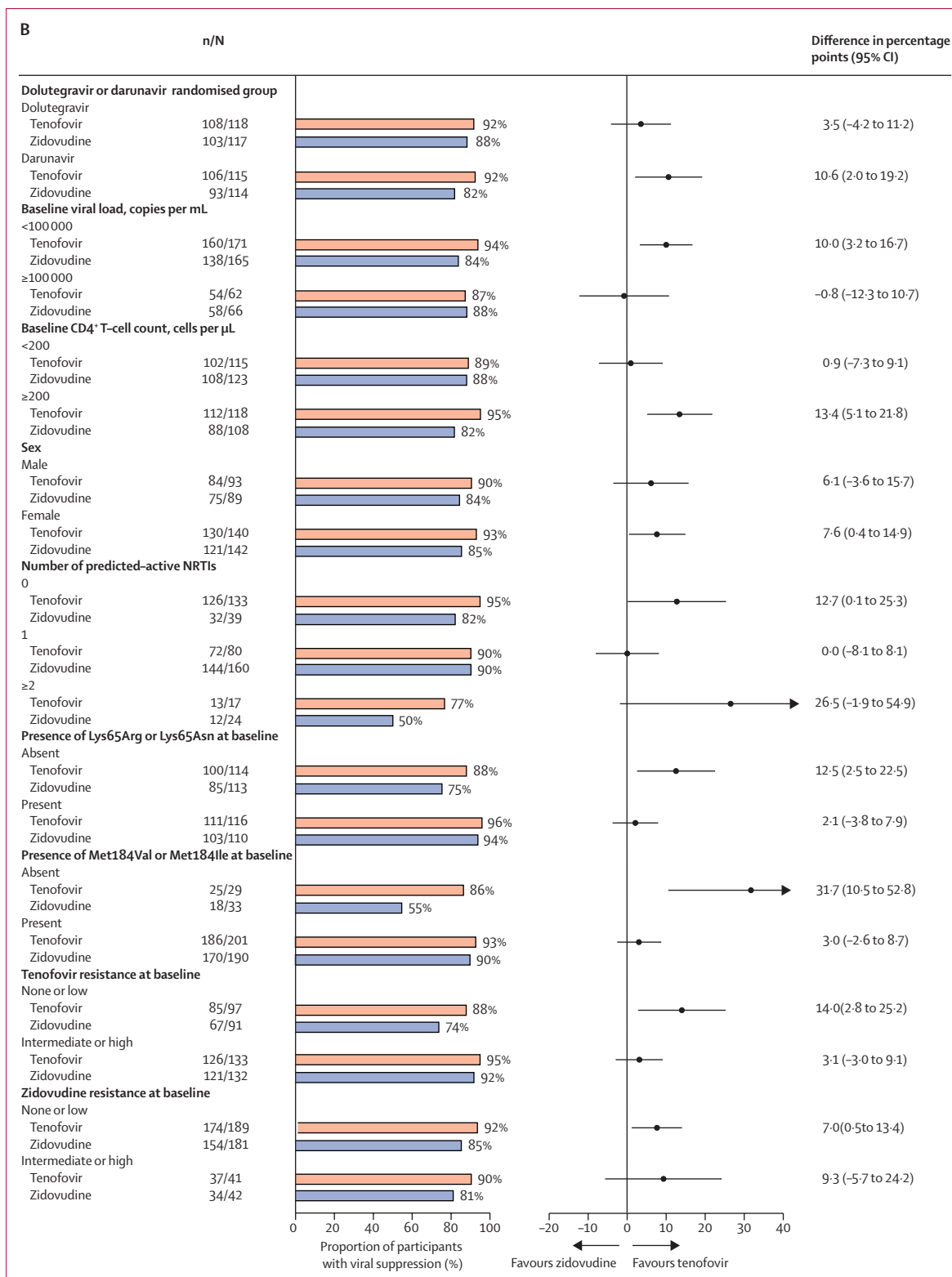


Figure 2: Subgroup analyses of viral suppression to less than 400 copies per mL at week 96 in the intention-to-treat population (A) The dolutegravir and darunavir groups. (B) The tenofovir and zidovudine groups. Viral suppression is based on the US Food and Drug Administration snapshot outcome and includes all cases with baseline data available for subgroup classification. The left side of the figure shows the subgroups and the proportion of participants with viral suppression to less than 400 copies per mL at week 96. The right side of the figure shows the point estimate of the (unadjusted) difference in proportions between the treatment groups (dolutegravir minus darunavir or tenofovir minus zidovudine) and the 95% CI within a specific stratum. The widths of the CIs have not been adjusted for multiplicity and cannot be used to infer treatment effects. NRTI=nucleoside reverse transcriptase inhibitor.

	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)*	p value
Dolutegravir or darunavir randomised treatment group				
Darunavir	1 (ref)	..	1 (ref)	..
Dolutegravir	1.31 (0.70-2.43)	0.40	0.99 (0.49-2.01)	0.99
NRTI randomised treatment group				
Zidovudine	1 (ref)	..	1 (ref)	..
Tenofovir	2.76 (1.41-5.42)	0.0031	2.92 (1.39-6.13)	0.0046
Adherence (visits with missed antiretroviral therapy)				
0	1 (ref)	..	1 (ref)	..
1	0.97 (0.28-3.41)	0.96	0.88 (0.24-3.26)	0.84
2	0.22 (0.08-0.61)	0.0032	0.29 (0.10-0.85)	0.024
≥3	0.17 (0.07-0.41)	<0.0001	0.23 (0.09-0.58)	0.0021
HIV-1 RNA concentration at baseline				
<100 000 copies per mL	1 (ref)
≥100 000 copies per mL	1.06 (0.53-2.13)	0.87
Viral subtype				
A	1 (ref)
C	1.17 (0.45-3.01)	0.75
D	0.78 (0.35-1.73)	0.54
Other	0.58 (0.24-1.40)	0.23
CD4 T-cell count at baseline				
<200 cells per µL	1 (ref)
≥200 cells per µL	0.76 (0.41-1.41)	0.38
Sex				
Male	1 (ref)
Female	1.11 (0.60-2.08)	0.73
Age				
<35 years	1 (ref)
35-49 years	0.89 (0.47-1.68)	0.72
≥50 years	1.23 (0.35-4.35)	0.74
Presence of Lys65Arg or Lys65Asn at baseline				
No	1 (ref)	..	1 (ref)	..
Yes	7.52 (3.11-18.16)	<0.0001	5.93 (2.33-15.06)	0.0002
Presence of Met184Val or Met184Ile at baseline				
No	1 (ref)	..	1 (ref)	..
Yes	5.03 (2.54-9.93)	<0.0001	4.99 (2.23-11.18)	<0.0001
Presence of thymidine analogue mutations† at baseline				
No	1 (ref)	..	1 (ref)	..
Yes	0.88 (0.44-1.74)	0.71	0.89 (0.40-1.97)	0.78

The model was based on the 444 (96%) of 464 participants that had values for all variables in the model. Separate models were also fitted that included a term for the interaction between the presence of Lys65Arg/Asn at baseline and allocation to tenofovir (interaction term p=0.68; stratum-specific OR for tenofovir use with Lys65Arg/Asn absent 3.13 [95% CI 1.38-7.06]; p=0.0061; stratum-specific OR for tenofovir use with Lys65Arg/Asn present 2.07 [0.36-12.04]; p=0.42) or a term for the interaction between the presence of Met184Val/Ile at baseline and allocation to tenofovir (interaction term p=0.060; stratum-specific OR for tenofovir use with Met184Val/Ile absent 9.87 [95% CI 2.14-45.56]; p=0.0033; stratum-specific OR for tenofovir use with Met184Val/Ile present 1.83 [0.78-4.27]; p=0.17), adjusting for factors that were in the main model. NRTI=nucleoside reverse transcriptase inhibitor. OR=odds ratio. *Adjusted for all factors in main model (dolutegravir or darunavir randomised treatment group, NRTI randomised treatment group, adherence, presence of Lys65Arg/Asn at baseline, presence of Met184Val/Ile at baseline, and presence of thymidine analogue mutations at baseline). †One or more of Met41Leu, Asp67Asn, Lys70Arg, Leu210Trp, Thr215Tyr, Thr215Phe, Lys219Gln, and Lys219Glu.

Table 2: Multivariable logistic regression model of HIV-1 RNA suppression to less than 400 copies per mL at week 96

Multivariable logistic regression modelling found that the allocation to tenofovir versus zidovudine, the presence versus the absence of the Lys65Arg or Lys65Asn

mutations at baseline, and the presence versus the absence of the Met184Val or Met184Ile mutations at baseline were each independently associated with higher odds for HIV-1 RNA suppression to less than 400 copies per mL at week 96 (table 2). Worse adherence was independently associated with lower odds for HIV-1 RNA suppression to less than 400 copies per mL at week 96 (table 2). Models that included additional terms for the interactions between allocation to tenofovir and the presence of Lys65Arg or Lys65Asn mutations, or allocation to tenofovir and the presence of Met184Val or Met184Ile mutations, found higher odds for viral load suppression to less than 400 copies per mL by tenofovir compared with zidovudine in all strata, and the advantage of tenofovir was greater in the strata in which resistance mutations were absent (table 2).

Change in bodyweight, body-mass index, waist circumference, incident obesity (30 patients; 29 women and one man), incident facial lipoatrophy, incident fat accumulation, and incident symptomatic peripheral neuropathy were similar between randomised groups (appendix 1 p 12). Treatment was well tolerated overall, with similar proportions of patients with at least one grade 3-4 adverse event or at least one serious adverse event between the randomised groups (table 3; appendix 1 pp 9-11). The study drug was discontinued due to adverse events in five patients (zidovudine in three patients, tenofovir in one patient, and dolutegravir in one patient; table 3).

Discussion

The results of the 96-week follow-up of the NADIA trial provide important new information relevant for clinical practice and policy in the public health approach to HIV treatment and beyond. The regimen of dolutegravir plus two NRTIs was non-inferior to the regimen of ritonavir-boosted darunavir plus two NRTIs for the outcome of viral suppression to less than 400 copies per mL in second-line therapy, confirming the published week 48 findings.⁸ However, these longer-term follow-up data provide essential reassurance that even when dolutegravir is combined with NRTIs to which the virus has acquired resistance, and where treatment is delivered under conditions typical of the public health approach (including sparse [usually annual] viral load monitoring), viral suppression can be sustained in a high proportion of patients, compatible with targets considered necessary for achieving overall reduction in incident HIV infections at a population level.¹⁷ Similarly high rates of viral suppression have been shown in other studies of second-line treatment with dolutegravir plus NRTIs, with varied approaches to NRTI selection and the frequency of viral load monitoring.^{3,5,18} Our findings also increase confidence that switching all patients on non-NRTI-based regimens to dolutegravir, now widely implemented in the public health approach, is likely to maintain virological suppression even if done without previous

testing of viral load to identify those with occult treatment failure and possible NRTI resistance.

These positive findings for viral suppression with dolutegravir are tempered by our observation that nine participants developed dolutegravir resistance by week 96. This result is striking because dolutegravir resistance is usually very rare; isolated cases have been observed in other second-line trials^{3,5} and in trials of dolutegravir monotherapy,¹⁹ but resistance has been negligible when dolutegravir is given with two NRTIs in treatment-naive populations, including in trials done in sub-Saharan Africa.^{20–22} The rate of dolutegravir resistance we observed (4% in 96 weeks) is similar to that of efavirenz resistance (3–4%) in first-line trials or lopinavir resistance (2%) in second-line trials during 96 weeks, which were done in similar settings.^{12,21,22} Although this trial was implemented with treatment monitoring and delivery relevant to the public health approach, the risk of resistance might be higher in field settings that are unable to meet this standard. The widespread switch from NNRTI-based to dolutegravir-based first-line regimens, although likely to be successful in maintaining viral suppression in most patients, will expand the population of patients taking dolutegravir plus NRTIs that have pre-existing resistance and could increase this problem. However, our results also suggest that the risk and consequences of dolutegravir resistance could be manageable. First, most who developed dolutegravir resistance mutations had viral load rebound by or at week 48, which, in many cases, was associated with self-reported non-adherence at multiple clinic visits, indicating a potential opportunity for additional intervention. On the basis of this result, it seems prudent to regard patients with past treatment failure and pre-existing NRTI resistance (known or suspected) who switch to dolutegravir as a separate, higher-risk group who can be targeted for prompt and more intensive intervention at the first sign of non-adherence or finding of a detectable viral load result on an early viral load test. An earlier repeat viral load test might also be considered after implementing intensive adherence counselling. Second, although knowledge is sparse concerning the clinical consequences of dolutegravir resistance mutations when combined with pre-existing resistance to NRTIs, some dolutegravir mutations, such as that resulting in Arg263Lys, seen in all three incidences of dolutegravir resistance in the tenofovir group, confer relatively modest levels of resistance in vitro and impair viral replication capacity.²⁰ Spontaneous re-suppression can occur with improved adherence, even after the appearance of resistance. Experience to date is insufficient to estimate a probability for this occurrence, but our finding of key NRTI resistance mutations having little impact on viral suppression is a salutary warning of the need to collect substantial data on the clinical impact of mutations before reaching a definitive interpretation. Third, combining dolutegravir with tenofovir rather than zidovudine is likely to decrease the risk of rebound and

	Dolutegravir (n=235)	Darunavir (n=229)	Tenofovir (n=233)	Zidovudine (n=231)
Any grade 3 or 4 event	26 (11%)	28 (12%)	22 (9%)	32 (14%)
Grade 3–4 event related to a study drug*	3 (1%)	3 (1%)	1 (<1%)	5 (2%)
Event (any grade) leading to discontinuation of study drug or drugs†	4 (2%)	1 (<1%)	2 (1%)	3 (1%)
Any serious adverse event	18 (8%)	16 (7%)	17 (7%)	17 (7%)
Serious adverse event (death)‡	3 (1%)	5 (2%)	6 (3%)	2 (1%)
WHO stage 4 event§	2 (1%)	4 (2%)	4 (2%)	2 (1%)
Haemoglobin <9 g/dL	6 (3%)	7 (3%)	6 (3%)	7 (3%)
eGFR <60 mL/min per 1.73 m ² ¶	1 (<1%)	3 (1%)	3 (1%)	1 (<1%)

Data are n (%). eGFR=estimated glomerular filtration rate. *Grade 3 or 4 events considered related to a study drug or drugs were diabetic ketoacidosis (n=1; considered related to dolutegravir), severe drug-induced nausea (n=1; considered related to zidovudine, darunavir, ritonavir, or a combination thereof), neutropenia (n=1; neutrophil count 0.61×10^9 neutrophils per L; considered related to zidovudine), and anaemia (n=3; haemoglobin of 4.3 g/dL, 4.8 g/dL, and 5.0 g/dL, respectively; considered related to zidovudine). †Zidovudine was discontinued in three patients (anaemia); tenofovir was discontinued in one patient (progression of renal parenchymal disease); and dolutegravir was discontinued in one patient (diabetic ketoacidosis). ‡Causes of death were cryptococcal meningitis (n=1), decompensated liver disease (n=1), advanced HIV infection (n=1), and unknown (n=5). One additional death occurred after week 96 and is not included here (Hodgkin lymphoma, with onset at week 84 and death at week 105; the patient was randomly assigned to the dolutegravir and zidovudine group). The case of cryptococcal meningitis was possibly unmasked by treatment-related immune reconstitution, but there were no deaths directly related to study medications. §WHO stage 4 events in the six patients were cryptococcal meningitis (n=3), extrapulmonary tuberculosis (n=1), cryptococcal meningitis and extrapulmonary tuberculosis (n=1), and oesophageal candidiasis (n=1). ¶Minimum eGFR values were 53 mL/min per 1.73 m², 55 mL/min per 1.73 m², 58 mL/min per 1.73 m², and 59 mL/min per 1.73 m².

Table 3: Adverse events that occurred from baseline to week 96 in the intention-to-treat population

possibly avoid the development of high-level dolutegravir resistance. Finally, in the event of treatment failure with dolutegravir resistance, NADIA has shown that darunavir plus two NRTIs is likely to represent a viable treatment option.

Our results suggest that darunavir merits an expanded role in the public health approach. The finding that the proportion with viral suppression was non-inferior between the darunavir and dolutegravir groups contrasts with other second-line trials that have found that other protease inhibitors (lopinavir and atazanavir, designated as preferred drugs in the public health approach) produce inferior results to dolutegravir.^{3,5} Taken together, the evidence would support regarding darunavir as a preferred protease inhibitor in this setting. The absence of resistance is a further advantage for darunavir and is consistent across multiple studies of once-daily darunavir, including a second-line trial in Africa that found no darunavir resistance among patients with treatment failure,^{15,23} and across other studies challenging darunavir in dual or monotherapy regimens.^{24–26} Both darunavir and dolutegravir are second-generation drugs considered to have the highest genetic barrier to resistance within their respective drug classes.^{7,20} However, based on the head-to-head comparison in this trial, the genetic barrier appears to be more robust with darunavir when protection from NRTIs in the regimen is more limited. Pragmatic considerations, and, principally, the cost and availability of a fixed-dose combination with NRTIs, favour the use of dolutegravir over darunavir for patients with previous treatment failure and NRTI resistance, but our results

suggest that the regimens are probably interchangeable and could be used in either sequence.

Our finding of superiority of maintaining tenofovir versus switching to zidovudine for the outcome of viral suppression at 96 weeks is more remarkable and likely to have a greater impact on guidelines, policy, and clinical practice than the previous report of non-inferiority at 48 weeks.⁸ Further exploration in subgroup analyses and logistic regression models yielded a plausible explanation, in keeping with *in vitro* and clinical trial data.^{27,28} The overall superiority of tenofovir versus zidovudine appears to be driven most strongly by the relatively low rate of viral suppression with zidovudine (both absolute and relative to tenofovir) in patients without the Lys65Arg or Lys65Asn mutations, the Met184Val or Met184Ile mutations, or both, at baseline, which is consistent with a trial showing clear superiority of tenofovir over zidovudine in treatment-naïve patients.²⁷ In the presence of Lys65Arg/Asn or Met184Val/Ile, the proportion of participants with viral suppression on zidovudine improved markedly, in keeping with the known *in-vitro* effects of these mutations, greater in combination than individually, to enhance susceptibility to zidovudine.²⁸ Although Lys65Arg/Asn decreases susceptibility to tenofovir *in vitro*, Met184Val/Ile enhances susceptibility *in vitro* and might abrogate the effects arising from Lys65Arg/Asn,²⁸ which is consistent with our finding of a high proportion of patients on tenofovir attaining viral suppression in the presence of Lys65Arg/Asn (accompanied by Met184Val/Ile in almost all cases), suggesting that the net clinical impact of these mutations is minimal. Lamivudine, administered to all groups, might also contribute to the overall efficacy of the regimens with either tenofovir or zidovudine, despite high-level resistance to lamivudine conferred by Met184Val/Ile, by maintaining these mutations that affect viral replication capacity.²³

Current WHO treatment guidelines for the public health approach to HIV treatment continue to recommend, as they have since 2010, a switch from tenofovir to zidovudine at transition to second-line therapy.² Our findings of superior viral suppression, less frequent viral rebound, greater CD4 cell count increase, and possible reduced risk of developing high-level dolutegravir drug resistance with maintaining tenofovir versus switching to zidovudine indicate that this recommendation should be revised to one of maintaining tenofovir. We did not observe any substantive difference in toxicity or tolerability between tenofovir and zidovudine that would alter this conclusion. Although the magnitude of tenofovir benefit differed according to the presence of resistance mutations, this finding does not justify a more complex algorithm involving resistance testing because tenofovir offers non-inferior or greater efficacy to zidovudine, irrespective of the mutation profile. For patients who develop treatment failure on a first-line regimen with zidovudine, now taken by a minority of patients in HIV treatment programmes, switching to tenofovir, as recommended by current guidelines, is likely to be appropriate, although we did not

directly test this. Previous studies have identified paradoxical relationships between NRTI drug resistance and outcomes from second-line therapy (more resistance being associated with better outcomes) that are likely, at least in part, to have arisen from the effects we have elucidated here;^{29,30} these findings prompted the decision to randomise the NRTIs in this trial. The clarity that this trial has provided for disentangling treatment and mutation effects illustrates the importance of testing public health recommendations in rigorous randomised controlled trials designed to be relevant to the settings in which treatment is delivered, even if such studies must be done after the interventions have been implemented in treatment programmes (as was the case for both of the interventions tested in this trial).

The main strength of this trial is that treatment was administered and monitored in conditions broadly generalisable to the public health approach. This trial was conducted at seven sites in three countries in sub-Saharan Africa; the sites were heterogeneous and it is likely that the findings can be generalised to other resource-limited settings, although overall outcomes might be worse in programme settings with less rigorous implementation and oversight than is possible in a trial. The similarity of findings across various subgroups, including viral subtypes, also supports the generalisability of our findings. The main limitation of our study is the use of open-label treatment, but blinding with placebos and administration of a twice-daily regimen for all participants would have seriously limited the relevance of our trial to treatment programme settings. The risk of bias arising from use of open-label treatment is small given the excellent participant retention in the trial, high participant adherence to the randomly assigned treatment regimens, and objective laboratory-measured outcomes.

In summary, NADIA has shown that dolutegravir plus two NRTIs produced durable viral suppression at 96 weeks in conditions representative of the public health approach, including among patients with pre-existing NRTI resistance. The rate of dolutegravir resistance, although relatively modest, is of concern, and its potential impact on clinical outcomes, as well as any possible mitigation strategies, should be assessed. Darunavir also produced durable viral suppression without risk of resistance and merits consideration for an expanded role in the public health approach. Maintaining tenofovir was superior to switching to zidovudine for the outcome of viral suppression and can be adopted as the standard for second-line therapy, without consideration of NRTI resistance mutations accumulated during previous treatment failure. The findings from NADIA might also have applicability beyond the public health approach to settings of individualised therapy, highlighting the importance of a nuanced approach to interpreting the impact of NRTI resistance mutations and the possibility of differentiating second-line regimens not only on their likelihood of achieving viral suppression, but also by the

risk of developing resistance with loss of future treatment options.

Contributors

NIP, CK, and AKam designed the study. NIP, JM, SW, AH, AB, AKir, and AKam coordinated the study. CK, AKai, GM, AL, GA, MB, HM, AS, and BC enrolled patients into the study. JA and ELAO oversaw the procurement and distribution of study drugs. JM did the statistical analysis. All authors interpreted the data. NIP and JM accessed and verified the data and wrote the first version of the manuscript. All authors provided input into the manuscript and approved the final version of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

NIP reports grants paid to his institution and personal fees from Janssen. CK, AL, and HM report grants and the donation of drugs and trial supplies to their institutions from Janssen. AKam reports grants paid to his institution from Janssen. All other authors declare no competing interests.

Data sharing

Anonymised individual participant data and study documents can be requested from the corresponding author (nick_paton@nus.edu.sg) and will be made available, subject to the approval of the Trial Steering Committee, from 6 months until 2 years after the publication of this Article. The statistical analysis plan can be found in appendix 2.

Acknowledgments

This study was funded by Janssen. We thank the trial participants for their commitment to the trial and adherence to study visits, assessments, and treatment.

References

- 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**: 505–10.
- WHO. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. Geneva: World Health Organization, 2021.
- Aboud M, Kaplan R, Lombaard J, et al. Dolutegravir versus ritonavir-boosted lopinavir both with dual nucleoside reverse transcriptase inhibitor therapy in adults with HIV-1 infection in whom first-line therapy has failed (DAWNING): an open-label, non-inferiority, phase 3b trial. *Lancet Infect Dis* 2019; **19**: 253–64.
- WHO. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV. Geneva: World Health Organization, 2018.
- Turkova A, White E, Mujuru HA, et al. Dolutegravir as first- or second-line treatment for HIV-1 infection in children. *N Engl J Med* 2021; **385**: 2531–43.
- Santos JR, Cozzi-Lepri A, Phillips A, et al. Long-term effectiveness of recommended boosted protease inhibitor-based antiretroviral therapy in Europe. *HIV Med* 2018; **19**: 324–38.
- Lefebvre E, Schiffer CA. Resilience to resistance of HIV-1 protease inhibitors: profile of darunavir. *AIDS Rev* 2008; **10**: 131–42.
- Paton NI, Musaaizy J, Kityo C, et al. Dolutegravir or darunavir in combination with zidovudine or tenofovir to treat HIV. *N Engl J Med* 2021; **385**: 330–41.
- Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604–12.
- WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva: World Health Organization, 2016.
- National Institutes of Health, Division of AIDS. Division of AIDS table for grading the severity of adverse events (clarification 9). Bethesda, MD: National Institutes of Health, Division of AIDS, 2004.
- 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**: 234–47.
- SECOND-LINE Study Group. Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study. *Lancet* 2013; **381**: 2091–99.
- La Rosa AM, Harrison LJ, Taiwo B, et al. Raltegravir in second-line antiretroviral therapy in resource-limited settings (SELECT): a randomised, phase 3, non-inferiority study. *Lancet HIV* 2016; **3**: e247–58.
- Ciaffi L, Koulla-Shiro S, Sawadogo A, et al. Efficacy and safety of three second-line antiretroviral regimens in HIV-infected patients in Africa. *AIDS* 2015; **29**: 1473–81.
- US Food and Drug Administration. Human immunodeficiency virus-1 infection: developing antiretroviral drugs for treatment. Rockville, MD: US Food and Drug Administration, 2015.
- Joint United Nations Programme on HIV/AIDS. 2017 global AIDS update—ending AIDS: progress towards the 90–90–90 targets. July 20, 2017. https://www.unaids.org/en/resources/documents/2017/20170720_Global_AIDS_update_2017 (accessed Jan 15, 2022).
- Keene CM, Griesel R, Zhao Y, et al. Virologic efficacy of tenofovir, lamivudine and dolutegravir as second-line antiretroviral therapy in adults failing a tenofovir-based first-line regimen. *AIDS* 2021; **35**: 1423–32.
- Wijting I, Rokx C, Boucher C, et al. Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised non-inferiority trial. *Lancet HIV* 2017; **4**: e547–54.
- Rhee SY, Grant PM, Tzou PL, et al. A systematic review of the genetic mechanisms of dolutegravir resistance. *J Antimicrob Chemother* 2019; **74**: 3135–49.
- Calmy A, Tovar Sanchez T, Kouanfack C, et al. Dolutegravir-based and low-dose efavirenz-based regimen for the initial treatment of HIV-1 infection (NAMSAL): week 96 results from a two-group, multicentre, randomised, open label, phase 3 non-inferiority trial in Cameroon. *Lancet HIV* 2020; **7**: e677–87.
- Venter WDF, Sokhela S, Simmons B, et al. Dolutegravir with emtricitabine and tenofovir alafenamide or tenofovir disoproxil fumarate versus efavirenz, emtricitabine, and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection (ADVANCE): week 96 results from a randomised, phase 3, non-inferiority trial. *Lancet HIV* 2020; **7**: e666–76.
- Ciaffi L, Koulla-Shiro S, Sawadogo AB, et al. Boosted protease inhibitor monotherapy versus boosted protease inhibitor plus lamivudine dual therapy as second-line maintenance treatment for HIV-1-infected patients in sub-Saharan Africa (ANRS12 286/MOBIDIP): a multicentre, randomised, parallel, open-label, superiority trial. *Lancet HIV* 2017; **4**: e384–92.
- Lathouwers E, Wong EY, Luo D, Seyedkazemi S, De Meyer S, Brown K. HIV-1 resistance rarely observed in patients using darunavir once-daily regimens across clinical studies. *HIV Clin Trials* 2017; **18**: 196–204.
- Pulido F, Ribera E, Lagarde M, et al. Dual therapy with darunavir and ritonavir plus lamivudine vs triple therapy with darunavir and ritonavir plus tenofovir disoproxil fumarate and emtricitabine or abacavir and lamivudine for maintenance of human immunodeficiency virus type 1 viral suppression: randomized, open-label, noninferiority DUAL-GESIDA 8014-RIS-EST45 trial. *Clin Infect Dis* 2017; **65**: 2112–18.
- Paton NI, Stöhr W, Arenas-Pinto A, et al. Protease inhibitor monotherapy for long-term management of HIV infection: a randomised, controlled, open-label, non-inferiority trial. *Lancet HIV* 2015; **2**: e417–26.
- Pozniak AL, Gallant JE, DeJesus E, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naïve patients: virologic, immunologic, and morphologic changes—a 96-week analysis. *J Acquir Immune Defic Syndr* 2006; **43**: 535–40.
- Ly JK, Margot NA, MacArthur HL, Hung M, Miller MD, White KL. The balance between NRTI discrimination and excision drives the susceptibility of HIV-1 RT mutants K65R, M184V and K65R+M184V. *Antivir Chem Chemother* 2007; **18**: 307–16.
- Paton NI, Kityo C, Thompson J, et al. Nucleoside reverse-transcriptase inhibitor cross-resistance and outcomes from second-line antiretroviral therapy in the public health approach: an observational analysis within the randomised, open-label, EARNEST trial. *Lancet HIV* 2017; **4**: e341–48.
- Boyd MA, Moore CL, Molina J-M, et al. Baseline HIV-1 resistance, virological outcomes, and emergent resistance in the SECOND-LINE trial: an exploratory analysis. *Lancet HIV* 2015; **2**: e42–51.