**Article:**
(2026) Efficacy and safety of short-cycle, dolutegravir-based antiretroviral therapy in adolescents living with HIV (BREATHER Plus): a multicentre, open-label, randomised, parallel, two-arm, non-inferiority trial. The Lancet HIV. ISSN: 2352-3018

https://doi.org/10.1016/S2352-3018(25)00326-1

# Efficacy and safety of short-cycle, dolutegravir-based antiretroviral therapy in adolescents living with HIV (BREATHER Plus): a multicentre, open-label, randomised, parallel, two-arm, non-inferiority trial

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## Summary

**Background** Short-cycle antiretroviral therapy (ART), comprising 5 days on and 2 days off treatment, offers the potential for ART-free weekends, reduced toxicity, and improved quality of life for adolescents living with HIV, who typically have worse treatment outcomes than other age groups. We aimed to compare the efficacy and safety of short-cycle ART containing tenofovir disoproxil fumarate–lamivudine–dolutegravir with continuous ART in adolescents living with HIV in Africa.

**Methods** We conducted an open-label, randomised, parallel, two-arm, non-inferiority trial (BREATHER Plus) in five clinical research centres across Kenya, South Africa, Uganda, and Zimbabwe. Adolescents (aged ≥12 years to <20 years) with an HIV-1 viral load of less than 50 copies per mL over the previous 12 months and no documented history of treatment failure were eligible for inclusion. Participants were randomly assigned (1:1) by use of permuted blocks to either short-cycle ART or continuous ART, stratified by clinical centre and mode of HIV acquisition (vertical *vs* horizontal or other). Participants received a daily oral fixed-dose combination of tenofovir disoproxil fumarate (300 mg), lamivudine (300 mg), and dolutegravir (50 mg); those assigned to short-cycle ART could choose their two consecutive days off (ie, Friday and Saturday or Saturday and Sunday). The primary outcome was confirmed viral rebound (the first of two consecutive viral load measurements ≥50 copies per mL) by week 96, analysed by intention to treat in all participants with viral load data after baseline assessment by use of adjusted Kaplan–Meier estimated proportions. The non-inferiority margin and confidence level depended on the event rate in the continuous ART group (an 8% margin with 99% CI for a 5% event rate). This trial is registered with ISRCTN (85058577), and follow-up is complete.

**Findings** Between June 29, 2022, and May 10, 2023, 470 adolescents living with HIV were randomly assigned to either short-cycle ART (239 [51%]) or continuous ART (231 [49%]). 263 (56%) participants were female and 207 (44%) were male. Median follow-up was 117 weeks (IQR 108–120). 23 participants in the short-cycle ART group and 11 in the continuous ART group had confirmed viral rebound by 96 weeks, with an estimated probability of confirmed HIV-1 RNA of at least 50 copies per mL of 9·9% (95% CI 6·4–14·3) in the short-cycle ART group versus 4·8% (2·6–7·8) in the continuous ART group. With an estimated difference of 5·1% (99% CI –0·8 to 11·5; bootstrap p=0·034), an 8% higher rate of confirmed virological rebound in the short-cycle ART group was not rejected, and the rate of confirmed virological rebound was significantly higher in the short-cycle ART group than in the continuous ART group. By the end of follow-up, 16 serious adverse events in 15 participants were reported in the short-cycle ART group (including one death unrelated to HIV or ART) and 22 serious adverse events in 16 participants in the continuous ART group.

**Interpretation** BREATHER Plus findings demonstrate that short-cycle ART with tenofovir disoproxil fumarate–lamivudine–dolutegravir should not be recommended for adolescents living with HIV receiving standard-of-care, routine viral load monitoring every 6–12 months.

**Funding** European and Developing Countries Clinical Trials Partnership, and UK Medical Research Council.



## Introduction

Globally in 2023, an estimated 1·5 million adolescents (aged 10–19 years) were living with HIV, with approximately 90% in sub-Saharan Africa.[1,2] Adolescents living with HIV continue to have worse treatment outcomes than younger children and adults with HIV,[3]

*Lancet HIV* 2026

Published Online April 7, 2026 https://doi.org/10.1016/S2352-3018(25)00326-1

See Online/Comment https://doi.org/10.1016/S2352-3018(25)00333-9

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See **Online** for appendix 1

## Research in context

### Evidence before this study

Short-cycle antiretroviral therapy (ART) is a potential strategy to reduce drug exposure and toxicity, while supporting adherence and maintaining virological suppression. This strategy might be of particular importance to adolescents with HIV who face adherence challenges and treatment fatigue, and require ART across their lifespan. We searched PubMed and Embase for articles published between Jan 1, 2000, and June 1, 2025, using the following terms: "HIV", "short cycle", "intermittent", "weekends off", "1 week on", "alternate days", "3-day-per-week", "4-day-per-week", "5-day-per-week", and "6-day-per week". We found mostly small-scale studies showing promising virological and safety outcomes associated with switching from continuous ART to short-cycle ART. Most evidence addressed adults receiving non-integrase strand transfer inhibitor-based ART in high-income settings with frequent real-time HIV viral load monitoring to guide clinical management. Randomised trials of short-cycle ART regimens based on non-nucleoside reverse transcriptase inhibitors (mostly efavirenz) or protease inhibitors have also provided reassuring results for various primary outcomes, including virological suppression, safety, and toxicity. Of particular relevance to BREATHER Plus, the international BREATHER trial, investigating 5 days on and 2 days off efavirenz-based ART in 199 children and young people aged 8–24 years, showed non-inferiority for the primary outcome of confirmed virological rebound by 48 weeks, maintained over median 3·6 years. Besides no safety-related concerns with short-cycle ART, participants reported preferring this approach. Three randomised trials of short-cycle ART included participants receiving integrase strand transfer inhibitor-based ART. The QUATUOR trial's modified intention-to-treat population included 636 adults receiving ART regimens based on non-nucleoside reverse transcriptase inhibitors (296 [47%]), protease inhibitors (36 [6%]), or integrase strand transfer inhibitors (304 [48%]), with 73 (23%) on dolutegravir, 65 (20%) on elvitegravir, and 14 (4%) on raltegravir in the short-cycle ART group. Non-inferiority was shown for the primary endpoint of virological suppression at week 48 with 4 days on and 3 days off ART, with only three treatment failures on short-cycle, integrase strand transfer inhibitor-based ART by 48 weeks and no further failures by 96 weeks. A trial in 60 adults receiving bictegravir-based ART randomly assigned to 5 days on and 2 days off therapy versus daily therapy showed that bictegravir exposure (primary endpoint) and virological suppression over 52 weeks (secondary endpoint) were maintained in the short-cycle ART group, with no safety concerns. Furthermore, a small study in 40 adults comparing daily bictegravir-based ART with this regimen 3 days per week, 2 days per week, or once a week found that dosing could be reduced safely and efficaciously; however, viral rebound was observed over 48 weeks in participants receiving ART 3 days or 1 day per week. Overall, data from previous studies suggest that adults with high treatment adherence might use short-cycle ART safely and effectively, contingent on additional adherence support and viral load monitoring every 12 weeks.

### Added value of this study

To our knowledge, BREATHER Plus is the only trial that has evaluated short-cycle ART with dolutegravir, applying a public health approach to viral load monitoring applicable to low-income and middle-income settings. We show that this regimen cannot be recommended for adolescents living with HIV in this context. Adherence to treatment was high in both trial groups. Reassuringly, most participants with confirmed virological rebound during the study period had re-suppression by the end of the study. Additionally, no ART regimen switches were reported due to treatment failure. However, maintaining an undetectable viral load (<50 copies per mL) is fundamental to the long-term health and wellbeing of adolescents living with HIV and for the prevention of horizontal and vertical HIV transmission.

### Implications of all the available evidence

Our findings indicate vulnerable populations living with HIV, including children, adolescents, and female individuals of childbearing potential, should continue daily ART. Future research should focus on alternative ART simplification strategies and support adherence in regions with limited resources and infrequent viral load monitoring. Long-acting agents provide one such approach; the results of ongoing trials investigating this strategy in adolescents are eagerly awaited.

although the move to dolutegravir suggests an improving landscape for adolescents in this context. Dolutegravir-based antiretroviral therapy (ART) regimens—ie, dolutegravir plus two nucleoside or nucleotide reverse transcriptase inhibitors—have been rolled out globally, with the fixed-dose combination of tenofovir disoproxil fumarate–lamivudine–dolutegravir becoming a near universal regimen for adolescents and adults with HIV.[4]

Short-cycle ART aims to maintain suppression of HIV viral load during planned regular short breaks from ART, with the aim of reducing ART intake, long-term toxicities, and costs, and potentially improving quality of life. Several small single-arm studies[5–11] and randomised controlled trials[12–20] have shown high rates of virological suppression on short-cycle ART strategies of 4 days on and 3 days off, as well as 5 days on and 2 days off. Most studies have investigated the use of efavirenz-based ART, including the BREATHER trial,[17] which showed non-inferior virological suppression on short-cycle ART (5 days on and 2 days off) compared with continuous ART over 144 weeks in 199 young people (aged 8–24 years) enrolled across Africa, Europe, and Thailand. The 2022 ANRS 170 QUATUOR study,[18] a France-wide randomised

controlled trial including 636 participants in the analysis population, showed non-inferiority of short-cycle ART (4 days on and 3 days off) versus daily ART based on virological suppression of less than 50 copies per mL at 48 weeks. Of note, 539 (85%) of these participants were male, median age was 49 years, and 304 (48%) participants were on integrase inhibitor-based regimens. A further small randomised controlled trial involving 60 adults in Taiwan compared 5 days on and 2 days off bictegravir-based ART with continuous ART, providing evidence for adequate bictegravir exposure and high rates of virological suppression with the use of short-cycle ART.[19] Previous short-cycle ART studies have included real-time viral load monitoring every 12 weeks or more frequently to manage participants.

To date, there have been no randomised trials of dolutegravir-based short-cycle ART in adolescents living with HIV and none with a programmatic 6–12-monthly viral load testing approach. We aimed to compare the efficacy and safety of short-cycle (5 days on and 2 days off) ART containing tenofovir disoproxil fumarate–lamivudine–dolutegravir with continuous ART in adolescents living with HIV in Africa with virological suppression over the previous 12 months.[21]

## Methods

### Study design

We conducted a multicentre, open-label, randomised, parallel, two-arm, non-inferiority trial (BREATHER Plus) in five clinical research centres across Kenya, South Africa, Uganda (two centres), and Zimbabwe. The study protocol is available online.[22] The statistical analysis plan is provided in appendix 2.

The study protocol was approved by national and local ethics committees: JCRC Institutional Review Board/Research Ethics Committee (JCRC 2021-19), Uganda National Council of Science and Technology (HS1822ES), and the National Drug Authority (CTC 0201/2021) in Uganda; Pharma Ethics (210624036) and South African Health Products Regulatory Authority (20210615) in South Africa; Moi Teaching and Referral Hospital Institutional Research and Ethics Committee (MTRH-IREC/2021/116) and Kenya Pharmacy and Poisons Board (KPPBECCT/22/05/02) in Kenya; Medicines Control Authority of Zimbabwe (CT228/2021), Medical Research Council of Zimbabwe (MRCZ/A/2644), and Joint Research Ethics Committee (147/2020) in Zimbabwe; and the ethics committee at UCL in the UK (appendix 1 p 5). This trial is registered with ISRCTN (85058577), and follow-up is complete.

The study protocol was amended from version 2.0 to version 3.0 after the trial opened to update information on the risks of neural tube defects associated with dolutegravir use in pregnancy and to reduce the frequency of visits from 8-weekly to 12-weekly after 48 weeks from randomisation. The outline of the statistical analysis plan was updated to be more comprehensive in line with the full statistical analysis plan and other clarifications were made. Youth trial boards in all four countries, comprising young people living with HIV, worked with the trial team throughout. These boards developed participant information, were updated on trial progress, and co-designed material describing the trial results. Additionally, young people were non-voting members on the trial steering committee.

### Participants

Key participant inclusion criteria included adolescents (aged ≥12 years to <20 years) living with HIV who were receiving ART (a dolutegravir–tenofovir-based triple ART for at least 1 month at screening), with virological suppression (HIV-1 viral load <50 copies per mL) over the previous 12 months and no documented history of treatment failure. Previous ART regimen substitutions due to toxicity, simplification, changes in guidelines, or drug availability were not considered to be treatment failure. Main exclusion criteria included pregnancy, breastfeeding, or unwillingness to use highly effective contraception; moderate or high risk of suicidality on the Columbia-Suicide Severity Rating Scale; or ongoing treatment for tuberculosis. A full list of participant inclusion and exclusion criteria has been published previously.[21] Participants aged 18 years and older provided written consent; participants younger than 18 years provided assent, with parents or guardians providing written consent.[21]

Ethnicity and race data were collected at enrolment by investigators according to the following categories: Asian, Black, Hispanic or Latinx, White, and other. Data on participant sex were collected by investigators as sex at birth with two options: male and female. Participant gender identity was not collected as part of this study.

See Online for appendix 2

### Randomisation and masking

Participants were randomly assigned (1:1) to either short-cycle ART or continuous ART, stratified by clinical centre and mode of infection (vertical *vs* horizontal or other). Randomisation was done by use of permuted blocks (sizes 2 and 4) and was undertaken at clinical sites after informed consent was taken by delegated site personnel. The computer-generated randomisation list was prepared by the trial statistician (DF) and incorporated within the database, enabling access only to the next allocation. Although trial statisticians had access to this list, they did not have any day-to-day correspondence with site staff. As an open-label trial, participants, clinical staff, and researchers enrolling, assigning, and prescribing ART to participants according to trial group were unmasked to allocated treatment strategy. Laboratory staff performing viral load measurements were masked to group allocation. Samples were identified by trial number, with no indication of allocated treatment strategy. Only trial statisticians and the independent data monitoring committee reviewed data by treatment group during the trial.

## Procedures

A nested, 4-week randomised pilot safety phase, comprising weekly visits and viral load measurements for 3 weeks (with a fourth viral load measurement if viral load at week 3 was ≥50 copies per mL), was undertaken in 33 participants (16 [48%] in the short-cycle ART group and 17 [51%] in the continuous ART group) to ensure that viral load remained undetectable (<50 copies per mL) after the 2-day break (Saturday and Sunday) in the short-cycle ART group. Participants were recruited to this pilot phase between June 29 and Aug 10, 2022. Trial recruitment continued during the pilot phase. Participants in both treatment groups were seen at screening (when viral load was measured to confirm eligibility), enrolment, week 4 (short-cycle ART group only), and week 8, then every 8 weeks to 48 weeks, and every 12 weeks thereafter. The 8-weekly frequency of visits over 48 weeks was to conduct pregnancy testing because, at that time, there was concern about the increased risk of neural tube defects in pregnancies with dolutegravir,[23,24] which was later refuted. Participants completed a close-out visit at least 96 weeks after enrolment and no earlier than 6 weeks before the last enrolled participant reached 96 weeks. All scheduled visits included a clinical assessment (including systematic ascertainment of adverse events), adherence assessment with a pill count and 7-day recall adherence questionnaire, ART dispensing, and, for adolescent girls after the onset of menarche, a pregnancy test. Bodyweight, height, and waist and hip circumference were measured at baseline; at weeks 8, 24, 48, and 96; and at close-out visit. Haematology, CD4 and CD8 T cells, and glycated haemoglobin ($HbA_{1c}$) tests were performed at baseline, week 48, and week 96, with CD4 and CD8 T cells also measured at week 144. Biochemistry tests, including liver function tests and creatinine measurements to calculate estimated glomerular filtration rate (eGFR) using the Cockcroft–Gault equation, lipid concentrations, and glucose measurements, were performed at baseline and week 96. A mood survey and the Columbia-Suicide Severity Rating Scale were administered at baseline; weeks 8, 24, 48, 72, and 96; and close-out. Acceptability and wellbeing, by use of a modified HIV/AIDS-Targeted Quality of Life questionnaire, and quality of life, by use of the EQ-5D, were assessed at baseline; weeks 24, 48, and 96; and close-out.

Viral loads were measured in real time (with results returned to treating clinicians) every 24–48 weeks from enrolment aligning with national guidelines, and at close-out. In Uganda and Kenya, testing was required at weeks 24, 48, 72, and 96, and every 24 weeks thereafter (until age 20 years in Uganda, when testing frequency reduced to every 48 weeks). In South Africa and Zimbabwe, testing was required at weeks 48 and 96, and every 48 weeks thereafter (with a supplemental test at 24 weeks in Zimbabwe). Participants who had a real-time viral load of at least 50 copies per mL were recalled after at least 7 days for confirmatory testing. Retrospective viral load testing was completed on plasma stored at baseline, every 8 weeks in year 1, every 12 weeks in year 2, and every 24 weeks thereafter, for timepoints where real-time viral loads were not measured (results were not returned to treating clinicians). Real-time and retrospective viral load results informed trial endpoints and were reviewed by the independent data monitoring committee. At the end of the trial, batched genotypic resistance sequencing was performed on stored samples from participants who met the primary endpoint. The earliest sample from the date of meeting the primary endpoint that was above the laboratory-defined resistance testing threshold (viral load range ≥200 to ≥1000 copies per mL by site) was chosen; resistance testing was not performed if no viral loads met this threshold. Drug resistance mutations were classified according to the International Antiviral Society–USA[25] and drug resistance scores were defined according to the Stanford University HIV drug resistance database.[26]

Participants received a daily oral fixed-dose combination of tenofovir disoproxil fumarate (300 mg), lamivudine (300 mg), and dolutegravir (50 mg), provided by national programmes. The protocol allowed for use of emtricitabine (200 mg), instead of lamivudine, and tenofovir alafenamide (25 mg), instead of tenofovir disoproxil fumarate, but only tenofovir disoproxil fumarate–lamivudine–dolutegravir was used. Participants randomly assigned to short-cycle ART could choose their two consecutive days off tenofovir disoproxil fumarate–lamivudine–dolutegravir (ie, Friday and Saturday or Saturday and Sunday). Participants randomly assigned to continuous ART continued daily ART. Participants on short-cycle ART with a confirmed real-time viral load of at least 50 copies per mL, who became pregnant, had incident tuberculosis, or switched off tenofovir disoproxil fumarate–lamivudine–dolutegravir returned to continuous ART. At the end of pregnancy and breastfeeding or on completion of tuberculosis treatment, participants could restart short-cycle ART. Changes to treatment regimen in both treatment groups were at the discretion of the treating clinician. Otherwise, participant management followed local guidelines.

In a Medication Event Monitoring Systems (MEMS Cap) sub-study, participants were randomly selected across sites in Uganda and Kenya to measure adherence to their allocated treatment strategy during weeks 8–32 or weeks 48–72, with a target of 50 participants in each treatment group at each of these timepoints. Selection by the trial statistician for the first period was based on one in two participants who had consented to the MEMS Cap sub-study until site targets were reached. For the second period, all consenting participants who had not previously participated and remained on allocated treatment strategy were selected until site targets were reached. Pill bottle caps with an embedded electronic device recorded each bottle opening as a presumptive dose.

### Outcomes

The primary outcome was confirmed viral rebound, defined as the first of two consecutive viral load measurements of at least 50 copies per mL, by week 96. Secondary efficacy outcomes were confirmed viral load of at least 1000 copies per mL by week 96; viral load of less than 50 copies per mL and no switch to second-line ART for treatment failure at weeks 24, 48, 72, and 96; viral load of at least 50 copies per mL at weeks 48 and 96 by use of a modified US Food and Drug Administration (FDA) snapshot algorithm (appendix 2 p 39); and HIV genotypic drug resistance at confirmed viral rebound.

Safety outcomes were change in lipid concentration, $HbA_{1c}$, phosphate concentration, eGFR, and CD4 and CD8 T-cell counts from baseline to 96 weeks; change in anthropometric measures from baseline to weeks 48 and 96; time to any new or recurrent WHO stage 3–4 event or death; incidence of serious adverse events, grade 3 and worse adverse events, or treatment-modifying adverse events; and the proportion of participants with any change from baseline ART regimen. Patient-reported outcomes, including adherence, acceptability, and neuropsychiatric toxicities, were collected through questionnaires.[22]

### Statistical analysis

The trial was designed with a fixed non-inferiority margin of 10%. A total of 460 participants (230 per group) provided 90% power with a two-sided alpha level of 5% to show non-inferiority of short-cycle ART compared with continuous ART, assuming 11% of participants met the primary endpoint by week 96 in both groups and allowing for a 10% attrition rate. Before the trial opening, a decision was made to use the Smooth Away From Expected (SAFE) non-inferiority frontier[27] to maintain interpretability of results in the case that the control primary endpoint event rate was lower than anticipated. The non-inferiority frontier was clinically prespecified such that, for any observed control event rate of less than 9%, the non-inferiority margin would be less than 10%, with the margin decreasing as the control event risk decreased (appendix 1 pp 6–7). To maintain the nominal type I error rate to less than 0·03 (two-sided alpha level <0·06), a 5% two-sided significance level was planned if the event rate was 9% or higher, and a 1% significance level was planned if the event rate was lower than 9%. These significance levels maintained power of at least 80% for a control event rate between 1% and 15%, holding the sample size and other assumptions fixed. The SAFE frontier was only defined for the primary endpoint analyses.

The primary endpoint was analysed by intention to treat. The analysis population included all participants with viral load data after baseline assessment. The proportion of participants with confirmed viral rebound was estimated by treatment group and clinical centre by use of adjusted Kaplan–Meier methods and censoring at the end of the 96-week window (week 102 minus 1 day), or last viral load of less than 50 copies per mL if not seen at week 102 or beyond. No adjustment was made for mode of infection because 97% of participants had acquired HIV vertically, meaning a high probability of strata with no events. The average cumulative failure function for each treatment group was calculated with standardisation as a weighted average of the corresponding centre-specific estimates.[28] The difference in the probability of confirmed viral rebound between short-cycle ART and continuous ART groups at week 96 was then estimated, with a two-sided 95% CI or 99% CI calculated by use of bias-corrected percentiles of bootstrap estimates (10 000 samples, stratified by centre and treatment group). Short-cycle ART was considered non-inferior to continuous ART if the upper limit of the CI of the difference was less than the selected non-inferiority margin. We then tested for a significant difference in the risk of confirmed viral rebound between short-cycle ART and continuous ART. This difference would correspond to testing the superiority of short-cycle ART over continuous ART if non-inferiority of short-cycle ART was shown, or the superiority of continuous ART over short-cycle ART (equivalent to testing short-cycle ART is significantly worse than continuous ART) if non-inferiority of short-cycle ART was not shown, based on a two-sided 5% significance level. Sensitivity analyses included an equivalent analysis unadjusted for centre, and use of a flexible parametric model allowing a time-varying effect of short-cycle ART.

In the per-protocol analysis, participants were excluded if they did not meet all the eligibility criteria, were randomly assigned in an incorrect stratum, reported taking less than 75% of intended weekend breaks (short-cycle ART group), or reported taking less than 90% of their ART (continuous ART group) up to week 96. Follow-up was censored following a break in any component of ART regimen for more than 7 days, a change to any ART component for any reason, or a change from short-cycle ART to continuous ART for any reason other than meeting the primary endpoint.

The difference between treatment groups in the proportion of participants with a confirmed viral load of at least 1000 copies per mL by week 96 was estimated in the same way as the primary outcome. The proportion of participants with a cross-sectional viral load of less than 50 copies per mL and no switch to second-line ART for treatment failure at each timepoint was compared between both groups by use of an unadjusted test of proportions. The modified FDA snapshot algorithm was used to estimate the proportions of participants with a viral load of at least 50 copies per mL at weeks 48 and 96, which was compared between groups with a Cochran–Mantel–Haenszel test. In an exploratory analysis, we considered time spent above different viral load thresholds from first viral load assessment after baseline to the end of the 96-week window, using last observation carried forward.

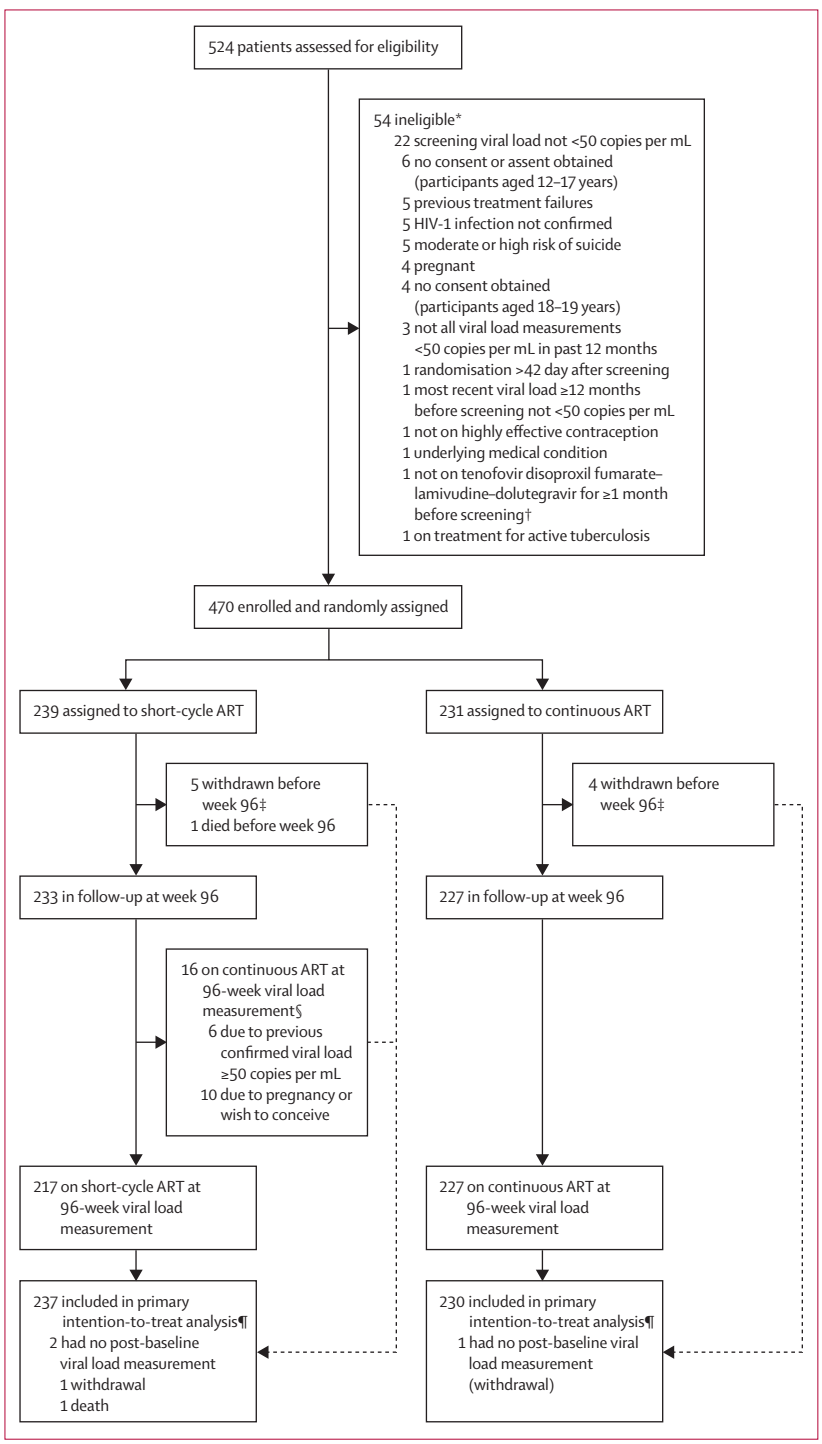


***Figure 1:*** **Trial profile**
ART=antiretroviral therapy. *Reasons for ineligibility were not mutually exclusive. †The protocol allowed for use of emtricitabine instead of lamivudine and tenofovir alafenamide instead of tenofovir disoproxil fumarate. ‡No participants who withdrew had met the primary endpoint. §One further participant in the short-cycle ART group who was withdrawn before week 96 was on continuous ART at withdrawal. ¶Participants with a post-baseline viral load measurement but who withdrew or died before week 96 contributed data up to their last viral load measurement.

We compared adverse events between treatment groups considering time to first event in a Cox regression model. We evaluated change in continuous variables from baseline using linear mixed models, with a random intercept for participants and fixed-effects for baseline measure, treatment group, and visit week, including interaction terms between group and visit week. For participants in the MEMS Cap sub-study, an adherent week was defined as daily openings in the continuous ART group, and as 5 days on (daily openings) from Monday to Friday or from Sunday to Thursday with the corresponding 2 days off (no openings) on Friday and Saturday or on Saturday and Sunday in the short-cycle ART group.

All statistical analyses were performed in StataNow (version 18.5). Further details are provided in the statistical analysis plan (appendix 2).

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

524 adolescents living with HIV were screened for eligibility, of whom 54 (10%) were ineligible (figure 1). Between June 29, 2022, and May 10, 2023, 470 (90%) adolescents were randomly assigned: 239 (51%) to short-cycle ART and 231 (49%) to continuous ART. Three participants did not have a viral load measurement after baseline (one death and one withdrawal in the short-cycle ART group; one withdrawal in the continuous ART group), resulting in 237 participants in the short-cycle ART group and 230 in the continuous ART group included in the primary intention-to-treat analysis. An additional seven participants (four in the short-cycle ART group and three in the continuous ART group) withdrew before the follow-up assessment at 96 weeks. Viral load data at scheduled timepoints during follow-up were almost complete up to and including week 96: 43 (1·8%) of 2390 measurements were missing in the short-cycle ART group and 29 (1·3%) of 2310 measurements were missing in the continuous ART group. Confirmatory viral load measurements were available during follow-up after all initial viral load measurements of at least 50 copies per mL. Median follow-up was 117 weeks (IQR 108–120). Close-out visits were conducted between Jan 29 and May 27, 2025.

Of the 470 randomly assigned adolescents, 212 (45%) were recruited from two Ugandan sites, 142 (30%) from the Zimbabwean site, 84 (18%) from the Kenyan site, and 32 (7%) from the South African site. All participants were Black. 263 (56%) participants were female and 207 (44%) were male (table 1). Median age was 16·5 years (IQR 14·6–18·1), with 140 (30%) participants younger than 15 years. 454 (97%) participants had acquired HIV

vertically and median CD4 T-cell count was 878 cells per µL (IQR 690–1119; table 1). Median duration of exposure to ART before trial enrolment was 11·8 years (IQR 8·6–14·1), including 2·5 years (2·1–3·2; minimum of 1 month) on dolutegravir (table 1; appendix 1 p 8).

23 participants in the short-cycle ART group and 11 in the continuous ART group had confirmed viral rebound by 96 weeks, with an estimated probability of confirmed HIV-1 RNA of at least 50 copies per mL of 9·9% (95% CI 6·4–14·3) in the short-cycle ART group versus 4·8% (2·6–7·8) in the continuous ART group (table 2; figure 2; appendix 1 p 12). The estimated difference in the proportion of participants with confirmed viral rebound between treatment groups (short-cycle ART minus continuous ART) was 5·1% (99% CI –0·8 to 11·5; bootstrap p=0·034). The upper bound of the two-sided 99% confidence limit was above the 8% non-inferiority margin (prespecified for a control event rate of 5%; figure 2; appendix 1 pp 6–7), with evidence at the 5% significance level that participants in the short-cycle ART group were at higher risk of confirmed viral rebound than were participants in the continuous ART group. Results from the sensitivity analyses were similar (figure 2).

In the per-protocol analysis, 20 events were reported in the short-cycle ART group and ten events in the continuous ART group (one event in the continuous ART group dropped due to self-reported adherence, two events in the short-cycle ART group dropped after a break from ART of more than 7 days, and one event in the short-cycle ART group dropped after the participant switched to continuous ART; figure 2). There was no evidence of a difference in the effect of strategy (short-cycle ART *vs* continuous ART) by clinical centre or by age group (appendix 1 pp 13–14). In a post-hoc analysis to 48 weeks, the estimated probability of confirmed viral rebound was 5·1% (95% CI 2·6–8·3) in the short-cycle ART group, compared with 2·2% (0·9–4·3) in the continuous ART group (bootstrap p=0·086; appendix 1 p 15).

Among participants with confirmed viral rebound by 96 weeks, eight participants in the short-cycle ART group and nine in the continuous ART group had both viral load measurements of at least 50 copies per mL performed in real time, with a median duration of 2·1 weeks (range 1·0–7·0) between measurements. Eight participants in the short-cycle ART group and one in the continuous ART group had both viral load measurements of at least 50 copies per mL analysed retrospectively (median 8·7 weeks [range 7·6–12·3] between measurements). The remaining seven participants in the short-cycle ART group and one in the continuous ART group had their initial viral load measurement of at least 50 copies per mL analysed retrospectively and the second measurement performed in real time (median 12·0 weeks [range 8·0–16·3] between measurements).

| | Short-cycle ART (n=239) | Continuous ART (n=231) | Total (n=470) |
|---|---|---|---|
| Age, years | 16·7 (14·9–18·3) | 16·1 (14·3–17·6) | 16·5 (14·6–18·1) |
| Sex | | | |
| Male | 106 (44%) | 101 (44%) | 207 (44%) |
| Female | 133 (56%) | 130 (56%) | 263 (56%) |
| Country | | | |
| Kenya | 43 (18%) | 41 (18%) | 84 (18%) |
| South Africa | 16 (7%) | 16 (7%) | 32 (7%) |
| Uganda | 108 (45%) | 104 (45%) | 212 (45%) |
| Zimbabwe | 72 (30%) | 70 (30%) | 142 (30%) |
| Mode of HIV-1 acquisition | | | |
| Vertical | 229 (96%) | 225 (97%) | 454 (97%) |
| Other* | 7 (3%) | 5 (2%) | 12 (3%) |
| Unknown | 3 (1%) | 1 (0%) | 4 (1%) |
| Total cholesterol, mmol/L | 3·3 (2·9–3·8) | 3·2 (2·9–3·8) | 3·3 (2·9–3·8) |
| Estimated glomerular filtration rate, mL/min | 116 (100–131) | 113 (99–132) | 114 (100–131) |
| CD4 T-cell count, cells per µL | 866 (678–1085) | 909 (693–1163) | 878 (690–1119) |
| CD8 T-cell count, cells per µL | 708 (533–859) | 728 (544–962) | 716 (540–902) |
| Bodyweight, kg | 50·4 (43·8–56·0) | 48·5 (42·0–55·2) | 49·4 (43·0–55·8) |
| Time spent on ART, years | 11·8 (8·3–13·9) | 11·8 (8·7–14·2) | 11·8 (8·6–14·1) |
| Time spent on dolutegravir, years | 2·5 (2·1–3·3) | 2·5 (2·1–3·1) | 2·5 (2·1–3·2) |

Data are median (IQR) or n (%). Two participants were missing baseline CD4 and CD8 T-cell counts. ART=antiretroviral therapy. *Includes sexual contact (four in the short-cycle ART group and three in the continuous ART group), blood product (two in the short-cycle ART group), cross contamination at birth (one in the continuous ART group), suspected intended infection (one in the short-cycle ART group), and suspected sharing of sharps with relative with HIV (one in the continuous ART group). One participant (in the short-cycle ART group) with other mode of acquisition (blood product) was randomly assigned in the vertical stratum in error.

***Table 1:*** **Baseline participant characteristics**

At last viral load measurement, 19 (83%) of 23 participants in the short-cycle ART group with confirmed viral rebound by week 96 had a viral load of less than 50 copies per mL, including ten of 14 who were on continuous ART at last viral load measurement (one of the four without suppression on continuous ART had returned to continuous ART before meeting the primary endpoint) and all nine who had continued on short-cycle ART. In the continuous ART group, eight (73%) of 11 participants with confirmed viral rebound by week 96 had a viral load of less than 50 copies per mL at last viral load measurement (one of three with unsuppressed viral load met the primary endpoint at close-out; appendix 1 pp 16–18). Between week 96 and close-out, an additional two participants in the short-cycle ART group and one in the continuous ART group had confirmed viral rebound (appendix 1 p 19).

By week 96, five (2% proportion estimated by Kaplan–Meier) participants in the short-cycle ART group and five (2%) in the continuous ART group had a confirmed viral load of at least 1000 copies per mL, with an estimated difference in proportion between groups of 0·0% (95% CI –2·6 to 2·7; bootstrap p=0·99; table 2; appendix 1 p 20). No participants switched to second-line ART (table 3; appendix 1 p 46). Based on cross-sectional viral load measurements, the proportions of participants with

| | Short-cycle ART (n=239) | Continuous ART (n=231) | Risk differences (short-term ART *vs* continuous ART) | p value |
|---|---|---|---|---|
| Participants with confirmed HIV-1 RNA ≥50 copies per mL by 96 weeks | 23 | 11 | .. | .. |
| Estimated proportion with confirmed HIV-1 RNA ≥50 copies per mL, % (95% CI) | 9·9% (6·4 to 14·3) | 4·8% (2·6 to 7·8) | 5·1% (99% CI −0·8 to 11·5*) | 0·034 |
| Participants with confirmed HIV-1 RNA ≥1000 copies per mL by 96 weeks | 5 | 5 | .. | .. |
| Estimated proportion with confirmed HIV-1 RNA ≥1000 copies per mL, % (95% CI) | 2·2% (0·8 to 4·3) | 2·2% (0·4 to 4·4) | 0·0% (−2·6 to 2·7) | 0·99 |
| Participants with HIV-1 RNA <50 copies per mL and no switch to second-line ART at week 48 | 223 | 221 | .. | .. |
| Estimated proportion with HIV-1 RNA <50 copies per mL and no switch to second-line ART, % (95% CI) | 94·9% (92·1 to 97·7) | 96·9% (94·7 to 99·2) | −2·0% (−5·6 to 1·6) | 0·27 |
| Participants with HIV-1 RNA <50 copies per mL and no switch to second-line ART at week 96 | 215 | 211 | .. | .. |
| Estimated proportion with HIV-1 RNA <50 copies per mL and no switch to second-line ART, % (95% CI) | 92·3% (88·8 to 95·7) | 93·0% (89·6 to 96·3) | −0·7% (−5·5 to 4·1) | 0·78 |
| Participants with HIV-1 RNA ≥50 copies per mL at 48 weeks using modified FDA snapshot | 7 | 4 | .. | .. |
| Estimated proportion with HIV-1 RNA ≥50 copies per mL, % (95% CI) | 2·9% (1·2 to 5·9) | 1·7% (0·5 to 4·4) | 1·2% (−1·9 to 4·3) | 0·45 |
| Participants with HIV-1 RNA ≥50 copies per mL at 96 weeks using modified FDA snapshot | 14 | 9 | .. | .. |
| Estimated proportion with HIV-1 RNA ≥50 copies per mL, % (95% CI) | 5·9% (3·2 to 9·6) | 3·9% (1·8 to 7·3) | 2·0% (−2·0 to 6·0) | 0·33 |
| Participants with major resistance mutations following confirmed HIV-1 RNA ≥50 copies per mL by week 96† | | | | |
| Integrase strand transfer inhibitors | 0 | 1/10 (10%) | .. | .. |
| Nucleoside or nucleotide analogue reverse transcriptase inhibitors | 0 | 1/11 (9%) | .. | .. |
| Non-nucleoside analogue reverse transcriptase inhibitors | 3/17 (18%) | 2/11 (18%) | .. | .. |
| Protease inhibitors | 0 | 0 | .. | .. |

Data are n or n/N (%), unless otherwise indicated. Proportions for the primary analysis and confirmed HIV-1 RNA ≥1000 copies per mL are based on adjusted Kaplan–Meier estimates; CIs (calculated with bias-corrected percentiles) and p values are based on at least 1000 bootstrap samples, adjusted for site. p values and CIs for HIV-1 RNA <50 copies per mL at each week are based on an unstratified test of proportions at each week. Proportions, as well as CIs and p values for the modified FDA snapshot algorithm, are based on the Cochran–Mantel–Haenszel test stratified by site. Resistance testing was not possible for six participants in the short-cycle ART group with insufficient viraemia at or after confirmed viral rebound: five with all viral load measurements <200 copies per mL and one with all viral load measurements <400 copies per mL. These six participants had HIV-1 re-suppression by close-out visit. ART=antiretroviral therapy. FDA=Food and Drug Administration. *99% CI used for estimated proportion with confirmed HIV-1 RNA ≥50 copies per mL for short-cycle ART versus continuous ART according to the Smooth Away From Expected non-inferiority framework; 95% CIs are presented in all other cases. †The integrase region failed to amplify for one participant in the continuous ART group. No other resistance mutations were detected.

***Table 2:*** **Efficacy outcomes**

suppressed HIV-1 RNA (<50 copies per mL) were similar between treatment groups at weeks 24, 48, 72, and 96 (table 2; appendix 1 pp 22–23). Based on the modified FDA snapshot algorithm and treating switching treatment from short-cycle ART to continuous ART as failure, failure was not significantly higher in the short-cycle ART group than in the continuous ART group at weeks 48 or 96 (table 2; appendix 1 p 24). In an exploratory analysis, participants spent 4·2% of time to week 96 with a viral load of at least 50 copies per mL in the short-cycle ART group, compared with 2·4% of time in the continuous ART group (p=0·011; appendix 1 p 25). Participants spent 2·4% of time to week 96 with a viral load of at least 200 copies per mL in the short-cycle ART group compared with 1·4% of time in the continuous ART group (p=0·083), and 1·5% of time to week 96 with a load of at least 1000 copies per mL in the short-cycle ART group versus 0·8% of time in the continuous ART group (p=0·18; appendix 1 p 25).

Among all 34 participants with viral rebound by 96 weeks in both treatment groups, resistance results were available for 28 (82%) participants: 17 (74%) of 23 participants in the short-cycle ART group and 11 (100%) of 11 in the continuous ART group. One participant in the continuous ART group had major resistance mutations to dolutegravir (G140R; potential low-level resistance to dolutegravir) and to lamivudine (M184I; high-level resistance to lamivudine). Three participants in the short-cycle ART group and two participants in the continuous ART group had major resistance mutations related to non-nucleoside reverse transcriptase inhibitors. No major resistance mutations related to protease inhibitors were identified (table 2; appendix 1 pp 27–28).

By the end of follow-up, there were four new or recurrent WHO stage 3–4 events or deaths (two in each group; table 3). One participant in the short-cycle ART group died 8 days after randomisation, judged unrelated to ART or HIV. There were 16 serious adverse events in 15 participants in the short-cycle ART group, compared with 22 serious adverse events in 16 participants in the continuous ART group (hazard ratio 0·91 [95% CI 0·45–1·85]; p=0·80). 16 serious adverse events (eight in each group) were related to pregnancy (appendix 1 pp 38–39, 43–45). Similar proportions of participants had grade 3 or worse adverse events: 26 events in 23 participants in the short-cycle ART group and 32 events in 23 participants in the continuous ART group (p=0·94; table 3). Five neuropsychiatric toxicity events were reported in five participants, all of whom were receiving continuous ART (appendix 1 p 42).

Among 233 participants in follow-up at week 96 in the short-cycle ART group, 16 (7%) were receiving continuous ART: six had previously reached the primary endpoint

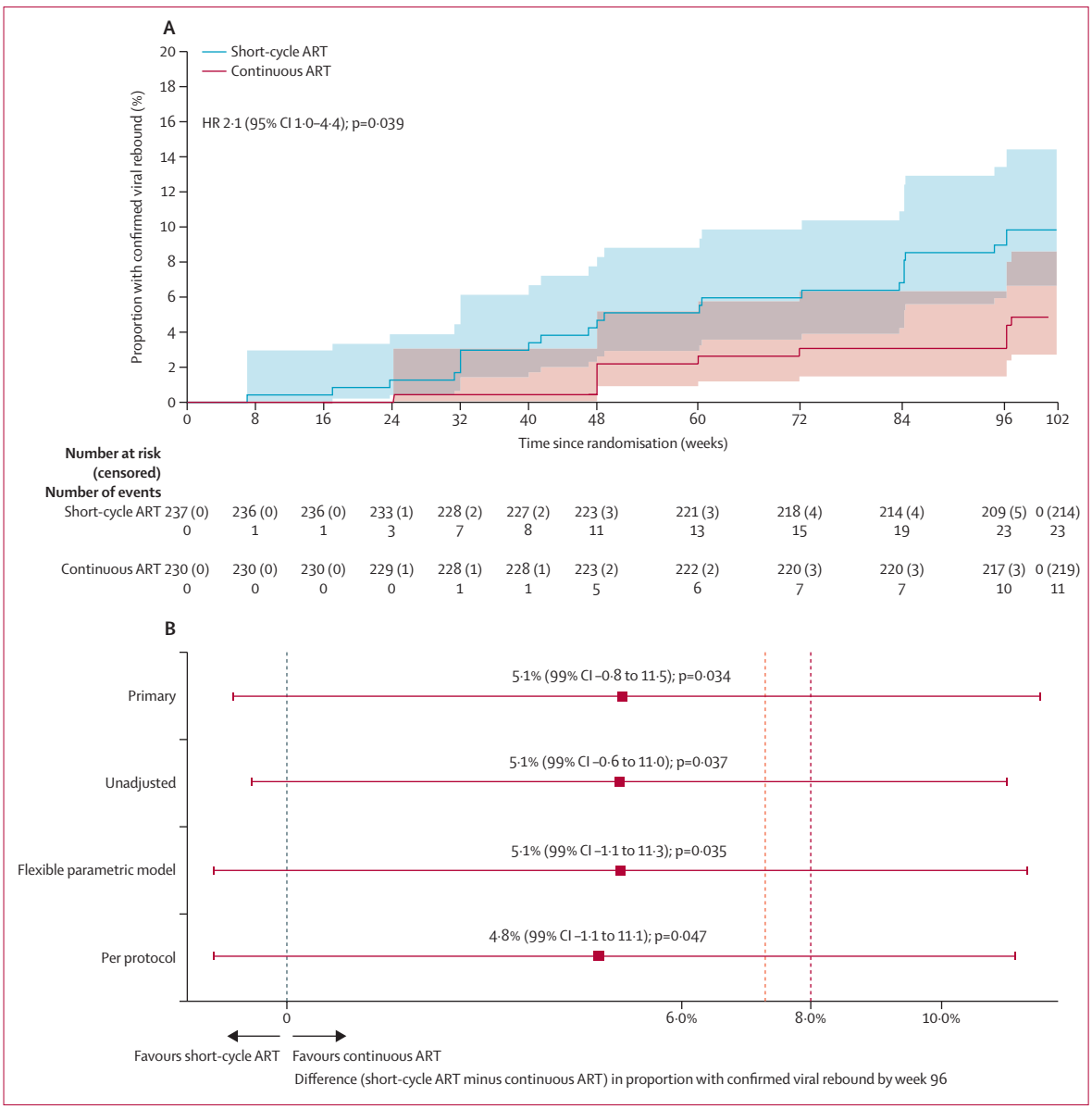


***Figure 2:*** **Proportion of participants with confirmed viral rebound over time and risk difference by week 96**
(A) Kaplan–Meier estimate of the proportion of participants with confirmed viral rebound (first of two consecutive viral load measurements of ≥50 copies per mL) in the short-cycle ART and continuous ART groups. (B) The difference (short-cycle ART minus continuous ART) between trial groups in the proportion of participants with confirmed viral rebound. The flexible parametric model does not assume proportional hazards. The event rate in the continuous ART group corresponded to a Smooth Away From Expected frontier non-inferiority margin of 8·0% in all analyses, excluding the per-protocol analysis in which the event rate corresponded to a margin of 7·3% (pale red line). ART=antiretroviral therapy. HR=hazard ratio.

(based on real-time viral load measurements), five reverted treatment at pregnancy and five reverted treatment due to stopping highly effective contraception. One additional participant in the short-cycle ART group reverted to continuous ART due to participant choice before withdrawing before week 96. A further eight participants in the short-cycle ART group reverted to continuous ART at week 96 (one changed ART, three had confirmed viral rebound at week 96, three reverted treatment at pregnancy, and one due to participant choice) and another five reverted after week 96 (two had confirmed viral rebound, two became pregnant, and one stopped highly effective contraception).

In both treatment groups, mean CD4 and CD8 T-cell count had decreased by a similar extent by week 96 (table 3); only two (1%) participants in the short-cycle ART group and eight (4%) in the continuous ART group had fewer than 350 CD4 T cells per µL at week 96. There was a small significant reduction in the eGFR in the continuous ART group compared with the short-cycle ART group

| | Short-cycle ART (n=239) | Continuous ART (n=231) | Short-cycle ART *vs* continuous ART* | p value |
|---|---|---|---|---|
| Adverse events during follow-up (number of participants) | | | | |
| WHO stage 3–4 events or death | 2 (2) | 2 (2) | 0·98 (0·14 to 6·95) | 0·98 |
| Serious adverse events | 16 (15) | 22 (16) | 0·91 (0·45 to 1·85) | 0·80 |
| Grade ≥3 clinical or laboratory adverse events | 26 (23) | 32 (23) | 0·98 (0·55 to 1·75) | 0·94 |
| ART-modifying adverse events† | 2 (2) | 4 (4) | 0·50 (0·09 to 2·71) | 0·42 |
| Participants with change in baseline ART regimen (%)‡ | 2 (1%) | 2 (1%) | .. | 1·00§ |
| Safety laboratory measures¶ | | | | |
| Estimated mean change from baseline total cholesterol at week 96, mmol/L (95% CI) | 0·08 (0·01 to 0·15) | 0·03 (–0·04 to 0·10) | 0·05 (–0·05 to 0·16) | 0·29 |
| Estimated mean change from baseline eGFR at week 96, mL/min (95% CI) | 0·25 (–2·11 to 2·62) | –3·46 (–5·85 to –1·06) | 3·71 (0·34 to 7·08) | 0·031 |
| Estimated mean change from baseline CD4 T-cell count at week 48, cells per µL (95% CI) | –69·1 (–100·8 to –37·4) | –52·4 (–84·5 to –20·3) | –16·7 (–61·8 to 28·4) | 0·47 |
| Estimated mean change from baseline CD4 T-cell count at week 96, cells per µL (95% CI) | –80·9 (–112·7 to –49·2) | –90·9 (–123·1 to –58·7) | 10·0 (–35·3 to 55·2) | 0·67 |
| Estimated mean change from baseline CD8 T-cell count at week 48, cells per µL (95% CI) | –98·7 (–128·2 to –69·1) | –81·8 (–111·7 to –51·8) | –16·9 (–59·0 to 25·2) | 0·43 |
| Estimated mean change from baseline CD8 T-cell count at week 96, cells per µL (95% CI) | –64·6 (–94·3 to –34·9) | –59·6 (–89·6 to –29·5) | –5·0 (–47·2 to 37·2) | 0·82 |
| Anthropometric measures | | | | |
| Estimated mean change from baseline bodyweight at week 48, kg (95% CI) | 2·62 (2·23 to 3·02) | 2·35 (1·95 to 2·75) | 0·27 (–0·28 to 0·83) | 0·33 |
| Estimated mean change from baseline bodyweight at week 96, kg (95% CI) | 4·33 (3·94 to 4·73) | 4·35 (3·95 to 4·75) | –0·02 (–0·57 to 0·54) | 0·96 |

ART=antiretroviral therapy. eGFR=estimated glomerular filtration rate. HR=hazard ratio. *Short-cycle ART versus continuous ART data represent HRs (95% CIs) for adverse events during follow-up and the differences in change from baseline for safety laboratory and anthropometric measures. HRs are based on time to first event, adjusted for site. All change from baseline estimates are based on a linear random-effects model, adjusted for site and baseline value. †Including chronic kidney disease in one participant in the short-cycle ART group, pulmonary tuberculosis in one participant in the continuous ART group, disseminated tuberculosis in one participant in the continuous ART group, and suicidal ideation in two participants in the continuous ART group. ‡Excluding any modification limited to dose of ART (two participants in the continuous ART group had tuberculosis, resulting in a doubled dose of dolutegravir). §Fisher's exact p value. ¶Estimates are presented for the mean change in laboratory measures from baseline values: total cholesterol 3·36 mmol/L, eGFR 116·8 mL/min, CD4 T-cell count 923 cells per µL, CD8 T-cell count 749 cells per µL, and bodyweight 49·5 kg.

***Table 3:*** **Safety outcomes**

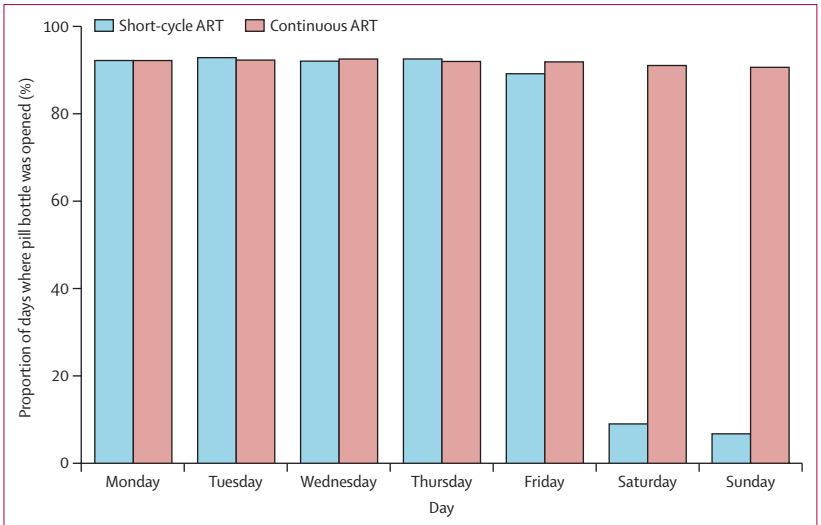


***Figure 3:*** **MEMS Cap sub-study results**
ART=antiretroviral therapy. MEMS Cap=Medication Event Monitoring Systems.

(table 3), but only one participant (short-cycle ART group) switched off tenofovir disoproxil fumarate–lamivudine–dolutegravir for reduced eGFR (at week 109). No significant differences in bodyweight or total cholesterol were reported between treatment groups (table 3). Further results are provided in appendix 1 (pp 47–79).

Self-reported adherence on the 7-day recall questionnaire was high in both groups. At 3085 (96%) of 3210 visits in the short-cycle ART group and at 2797 (96%) of 2907 visits in the continuous ART group, participants reported taking all prescribed ART (according to their allocated strategy) in the previous 7 days. Across the MEMS Cap sub-study, the proportion of days between Monday and Thursday where a pill bottle was opened was similar in both groups: 8348 (92%) of 9039 sub-study days in the short-cycle ART group and 8881 (92%) of 9633 sub-study days in the continuous ART group (figure 3). In the short-cycle ART group, 1602 (71%) of 2264 weeks were considered adherent to short-cycle ART strategy and the remaining 662 (29%) weeks were considered non-adherent: 459 weeks due to greater than two missed tablets and 203 weeks due to either tablets taken on more than 5 days or tablets missed on 2 days other than Friday and Saturday or Saturday and Sunday. In the continuous ART group, 1725 (71%) of 2416 weeks were considered adherent to the continuous ART strategy, with 386 (16%) weeks considered non-adherent due to 1 day off ART and 305 (13%) weeks due to at least 2 days off ART.

All 233 participants in the short-cycle ART group and 227 in the continuous ART group who were in follow-up at week 96 completed the modified HIV/AIDS-Targeted Quality of Life questionnaire. Among these participants, 231 (99%) in the short-cycle ART group and 223 (98%) in the continuous ART group reported that taking HIV medications had not been a burden at any time in the previous 4 weeks; 229 (98%) in the short-cycle ART group and 225 (99%) in the continuous ART group reported that taking these medications had not made it difficult to

live a normal life. Additionally, among the 217 participants in the short-cycle ART group who had not returned to continuous ART at week 96, 199 (92%) reported that short-cycle ART was a lot easier than continuous therapy, 14 (6%) reported it was a little easier, two (1%) reported no difference, and one (<1%) participant reported it was a little more difficult (one response missing). In the EQ-5D questionnaire, only one participant (in the short-cycle ART group) reported any problems performing their usual activities (slight problems) and no participants reported any anxiety or depression, or problems with self-care.

## Discussion

We have evaluated the safety and efficacy of weekends off ART in adolescents on a dolutegravir–tenofovir-based ART regimen over 96 weeks. BREATHER Plus is the first trial to consider short-cycle ART in the context of viral load monitoring every 6–12 months, aligning with standard of care in Africa, and only the second trial to consider short-cycle ART on a three-drug regimen with dolutegravir, which is now the predominant anchor drug globally. We have found that adolescent participants living with HIV receiving short-cycle ART were less likely than those receiving continuous ART to maintain viral suppression below 50 copies per mL over 96 weeks. These results from the BREATHER Plus trial contrast with those of a 2023 meta-analysis that included five cohort studies and two randomised controlled trials, finding high virological efficacy of short-cycle ART at week 48.[29] Additionally, a 2025 meta-analysis of seven randomised controlled trials including 1286 participants (387 on integrase inhibitor-based regimens) showed non-inferiority of short-cycle ART compared with continuous ART at 48 weeks based on a viral load of more than 50 copies per mL and a 4% non-inferiority margin.[30] In the ANRS 170 QUATUOR trial of 636 participants (304 on integrase inhibitor-based ART, 73 on short-cycle ART with dolutegravir, and 76 on continuous ART with dolutegravir), few participants had confirmed virological rebound by 48 weeks (six with short-cycle ART *vs* four with continuous ART), with only one rebound on dolutegravir, in the continuous ART group. The ANRS 170 QUATUOR trial enrolled a long-term adherent population of mostly older men, who were seen at weeks 4 and 12, and every 12 weeks thereafter with real-time viral loads measured at each study visit and results available for clinicians and participants.[18] Such frequent viral load monitoring might reduce the risk of failure by identifying viral blips and providing adherence counselling at blips to help participants quickly reach re-suppression without returning to continuous ART. Two small trials in adults on bictegravir–emtricitabine–tenofovir alafenamide showed promising results;[19,20] however, intracellular concentrations of tenofovir have been shown to be ten times higher with tenofovir alafenamide than with tenofovir disoproxil fumarate[31] and the regimen is not widely available.

Reassuringly, in both treatment groups in the BREATHER Plus trial, most participants (approximately 80%) who had confirmed viral rebound by week 96 had virological suppression at close-out, with no change to ART regimen. Only one participant (in the continuous ART group) had a major resistance mutation to integrase strand transfer inhibitors. However, a persistent viral load of less than 50 copies per mL should be strived for, with less than 200 copies per mL necessary for preventing HIV transmission.[32] An exploratory analysis suggested that participants in the short-cycle ART group were at risk of onward transmission of HIV (viral load ≥200 copies per mL) for a higher proportion of follow-up than were participants receiving continuous ART. It is unclear what effect periods of temporary increases in viral load have on the accumulation of resistance mutations and the size of viral reservoirs, including in sanctuary sites (eg, the brain). Although low, there is a risk that this effect might ultimately lead to treatment failure on tenofovir disoproxil fumarate–lamivudine–dolutegravir.[32] Despite low resistance rates, the identification of resistance to non-nucleoside reverse transcriptase inhibitors at virological rebound might suggest that some participants had treatment failure before enrolment, despite this being an exclusion criterion; however, resistance levels were similar between short-cycle and continuous ART. Resistance testing of baseline samples was not possible because participants had virological suppression and only plasma samples were stored.

Findings from the MEMS Cap sub-study suggest that the total number of prescribed tablets that were taken by participants fell below 95%, with evidence of participants taking more than 2 days off ART in around 20% of monitored weeks in the short-cycle ART group. However, comparison of data from the MEMS Cap sub-study with similar data collected in the BREATHER trial[15] showed similar, if not better, overall adherence in the BREATHER Plus trial (92% of tablets in the short-cycle ART group and 92% in the continuous ART group were taken Monday–Thursday) than in the BREATHER trial (84% and 89%). In BREATHER Plus, a 10% proportion of missed tablets in addition to taking weekends off ART might have contributed to worse virological suppression among participants in the short-cycle ART group than among those in the continuous ART group. Given that BREATHER showed similar virological suppression in both treatment groups,[17] it is possible that differences in the ART regimen (dolutegravir in BREATHER Plus, which has a shorter half-life than efavirenz used in BREATHER[33,34]) and the reduced frequency of viral load monitoring (every 6–12 months in BREATHER Plus *vs* every 12 weeks in BREATHER) contributed to worse outcomes among participants on short-cycle ART in BREATHER Plus.

In the BREATHER Plus trial, confirmed virological rebound among participants in the continuous ART group was low (2% by 48 weeks and 5% by 96 weeks).

However, in the ANRS 170 QUATUOR study, which defined all confirmed viral loads of at least 50 copies per mL by week 48 as virological failure (aligning closely with our primary endpoint), treatment failure rates by 48 weeks were numerically lower in the control group (1% in the modified intention-to-treat population and <1% in the sub-group receiving integrase inhibitor-based ART) than in the continuous ART group in BREATHER Plus.[18]

BREATHER Plus is the first trial to use the SAFE non-inferiority frontier, in which the non-inferiority margin is prespecified dependent on the control event rate.[27] The event rate in the continuous ART group was considerably lower than estimated at the design stage. By considering this scenario before starting recruitment, we maintained the integrity of the design and analysis and modified the non-inferiority margin appropriately to ensure meaningful clinical interpretation of the results.

We recruited participants from four African countries and included adolescents living with HIV spanning the adolescent age range (≥12 years to <20 years). We were unable to recruit the planned numbers of adolescents who had acquired HIV horizontally, likely because participating clinical centres focus on paediatric care. In low-income and middle-income countries (LMICs), there is a growing proportion of adolescents who have acquired HIV horizontally who might face additional challenges with adherence.[35] It is likely that outcomes would be similar or worse among adolescents who have acquired HIV during their teenage years, given that our study population was stable (ie, well known to the clinics where they were enrolled) and on long-term treatment.

The generalisability of our findings to the real world is also limited by the carefully controlled nature of a clinical trial. Visit attendance in BREATHER Plus was more frequent than in routine care, including 8-weekly visits in year 1, and 12-weekly visits from year 2. Participants were contacted following a missed visit and visit attendance was high. Viral loads were measured in real time every 6–12 months in accordance with national guidelines, but more frequently than in many African settings. Adherence counselling was also provided when a measurement first indicated a viral load of at least 50 copies per mL. Given that adolescents typically have lower adherence to ART than adults[3] and in real-world settings, it is likely that adherence to a short-cycle therapy strategy among adolescents living with HIV would be poorer than in a trial, particularly if viral load monitoring was less frequent. This situation could lead to even greater difference between short-cycle therapy and continuous therapy outcomes.

In conclusion, BREATHER Plus shows that short-cycle ART is significantly worse for maintaining virological suppression than continuous ART for adolescents living with HIV in Africa who are receiving tenofovir disoproxil fumarate–lamivudine–dolutegravir and viral load monitoring every 6–12 months. Although short-cycle ART might be desirable in terms of cost reduction and quality of life, the importance of virological suppression for long-term health and the prevention of onward HIV transmission remains fundamental to population health. The impact of these findings on policy in the context of existing reassuring data from smaller trials, mainly conducted in adult populations, requires careful consideration. An evidence-to-decision framework should be applied when reviewing data for guideline development. We urge caution in recommending dosing 4–5 days per week across the general population in LMICs to extend ART supplies.[30] Vulnerable populations living with HIV, including children, adolescents, and female individuals of childbearing potential, should continue daily ART. Although data from other studies suggest that an adult with high adherence to ART might use short-cycle ART safely and effectively, this is contingent on additional adherence support and viral load monitoring every 12 weeks. Given the uncertainty of HIV programme funding, it is unlikely that viral load monitoring even every 6–12 months will remain available in LMICs, where the associated costs remain a considerable barrier and would outweigh any savings in drug costs. Our results emphasise a continued need to assess different strategies to support adherence and to simplify ART safely for adolescents. These strategies could include daily and weekly oral options, dual therapy, and long-acting agents in real-world settings. Results of the LATA trial, conducted by the same consortium, evaluating injectable ART in adolescents living with HIV are eagerly awaited in July, 2026.

#### Contributors

ARK, MB-D, CKit, AS, MA, DF, and SLP designed the study. ARK, MB-D, CKit, AS, MA, GPA, HAM, HM, CKii, RM, RN, RBO, GM, CW, and TA were responsible for study conduct and data collection at clinical research sites. AJ and DF accessed and verified the underlying data. AJ performed the statistical analysis. DF, SLP, AB, and AJ wrote the first draft of the paper. All authors critically reviewed 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

AB reports non-renumerated membership of the Penta-ID scientific steering committee and chairing of Penta–European AIDS Clinical Society guidelines for the treatment of HIV in children in Europe. SLP reports grants from the National Institute for Health and Care Research, Leidos (National Institutes of Health), European and Developing Countries Clinical Trials Partnership (EDCTP)-2, EDCTP-3, Medical Research Council (MRC) UK, Gilead Sciences, Janssen-Cilag, and ViiV Healthcare, paid to UCL. SLP reports being a member of the data and safety monitoring board of the TIPAL trial and chairing the data monitoring committee for the IMPEDE-PKD trial in the UK. AS reports grants from the MRC UK and EDCTP; and an unpaid trustee role at the charity Picturing Health. All other authors declare no competing interests.

#### Data sharing

The BREATHER Plus data are held at the MRC Clinical Trials Unit at UCL, which encourages optimal use of data by use of a controlled access approach to data sharing, incorporating a transparent and robust system to review requests and provide secure data access consistent with the relevant ethics committee approvals. All requests for data are considered and can be initiated by contacting mrcctu.ctuenquiries@ucl.ac.uk.

**Acknowledgments**

This study is part of the EDCTP-2 programme (grant number RIA2017MC-2005-BREATHER Plus), funded by the EU, UK MRC, and Swedish International Development Cooperation Agency. The MRC Clinical Trials Unit at UCL receives core support from the UK MRC (including salary support for SLP and DF from MC_UU_00004/03 and MC_UU_00004/04). We thank the participants of the BREATHER Plus trial and their families. We acknowledge and thank our dear colleague and friend Kusum Nathoo (University of Zimbabwe Clinical Research Centre), who passed away in June, 2025, for her enormous contribution to the trial.

**References**

1 WHO. Treatment and care in children and adolescents. https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/treatment/treatment-and-care-in-children-and-adolescents (accessed Dec 16, 2024).

2 UNAIDS. The urgency of now: AIDS at a crossroads. Joint United Nations Programme on HIV/AIDS, 2024.

3 Auld AF, Agolory SG, Shiraishi RW, et al. Antiretroviral therapy enrollment characteristics and outcomes among HIV-infected adolescents and young adults compared with older adults–seven African countries, 2004-2013. *MMWR Morb Mortal Wkly Rep* 2014; **63:** 1097–103.

4 Clinton Health Access Initiative. 2024 HIV market report. Clinton Health Access Initiative, 2024.

5 Ananworanich J, Hirschel B. Intermittent therapy for the treatment of chronic HIV infection. *AIDS* 2007; **21:** 123–34.

6 Cohen CJ, Colson AE, Sheble-Hall AG, McLaughlin KA, Morse GD. Pilot study of a novel short-cycle antiretroviral treatment interruption strategy: 48-week results of the five-days-on, two-days-off (FOTO) study. *HIV Clin Trials* 2007; **8:** 19–23.

7 Rudy BJ, Sleasman J, Kapogiannis B, et al, and the Adolescent Trials Network for HIV/AIDS Interventions. Short-cycle therapy in adolescents after continuous therapy with established viral suppression: the impact on viral load suppression. *AIDS Res Hum Retroviruses* 2009; **25:** 555–61.

8 Leibowitch J, Mathez D, de Truchis P, Perronne C, Melchior JC. Short cycles of antiretroviral drugs provide intermittent yet effective therapy: a pilot study in 48 patients with chronic HIV infection. *FASEB J* 2010; **24:** 1649–55.

9 Leibowitch J, Mathez D, de Truchis P, et al. Four days a week or less on appropriate anti-HIV drug combinations provided long-term optimal maintenance in 94 patients: the ICCARRE project. *FASEB J* 2015; **29:** 2223–34.

10 de Truchis P, Assoumou L, Landman R, et al, and the ANRS 162-4D Study Group. Four-days-a-week antiretroviral maintenance therapy in virologically controlled HIV-1-infected adults: the ANRS 162-4D trial. *J Antimicrob Chemother* 2018; **73:** 738–47.

11 Sellem B, Abdi B, Lê M, et al. Intermittent bictegravir/emtricitabine/tenofovir alafenamide treatment maintains high level of viral suppression in virally suppressed people living with HIV. *J Pers Med* 2023; **13:** 583.

12 Reynolds SJ, Kityo C, Hallahan CW, et al. A randomized, controlled, trial of short cycle intermittent compared to continuous antiretroviral therapy for the treatment of HIV infection in Uganda. *PLoS One* 2010; **5:** e10307.

13 Rojas J, Blanco JL, Sanchez-Palomino S, et al. A maintenance 3-day-per-week schedule with the single tablet regimen efavirenz/emtricitabine/tenofovir disoproxil fumarate is effective and decreases sub-clinical toxicity. *AIDS* 2018; **32:** 1633–41.

14 Bellagamba R, Giancola ML, Tommasi C, et al, and the ATAD Study Group. Randomized clinical trial on efficacy of fixed-dose efavirenz/tenofovir/emtricitabine on alternate days versus continuous treatment. *AIDS* 2019; **33:** 493–502.

15 Butler K, Inshaw J, Ford D, et al. BREATHER (PENTA 16) short-cycle therapy (SCT) (5 days on/2 days off) in young people with chronic human immunodeficiency virus infection: an open, randomised, parallel-group phase II/III trial. *Health Technol Assess* 2016; **20:** 1–108.

16 Bernays S, Paparini S, Seeley J, Namukwaya Kihika S, Gibb D, Rhodes T. Qualitative study of the BREATHER trial (short cycle antiretroviral therapy): is it acceptable to young people living with HIV? *BMJ Open* 2017; **7:** e012934.

17 Turkova A, Moore CL, Butler K, et al, and the BREATHER (PENTA 16) trial Group. Weekends-off efavirenz-based antiretroviral therapy in HIV-infected children, adolescents and young adults (BREATHER): extended follow-up results of a randomised, open-label, non-inferiority trial. *PLoS One* 2018; **13:** e0196239.

18 Landman R, de Truchis P, Assoumou L, et al, and the ANRS 170 QUATUOR study group. A 4-days-on and 3-days-off maintenance treatment strategy for adults with HIV-1 (ANRS 170 QUATUOR): a randomised, open-label, multicentre, parallel, non-inferiority trial. *Lancet HIV* 2022; **9:** e79–90.

19 Hsin-Yun S, Ya-Ting L, Wen-Chi C, et al. Five-days-on-two-days-off (FOTO) versus daily bictegravir/emtricitabine/tenofovir alafenamide in virologically suppressed people with HIV: a pilot randomized clinical trial. *J Antimicrob Chemother* 2025; **80:** 2179–86.

20 Chivite I, Lazzari ED, Sempere A, et al. Safety, tolerability, and efficacy of a BIC/FTC/TAF dose reduction strategy. Conference on Retroviruses and Opportunistic Infections 2025; March 9–12, 2025 (abstr 659).

21 Katongole F, Arumugam T, Jennings A, et al, and the BREATHER Plus trial team. BREATHER Plus clinical trial design: a randomised non-inferiority trial evaluating the efficacy, safety and acceptability of short cycle (five days on, two days off) dolutegravir/tenofovir-based triple antiretroviral therapy (ART) compared to daily ART in virologically suppressed adolescents living with HIV aged 12 to <20 years in sub-Saharan Africa. *Contemp Clin Trials* 2025; **155:** 107963.

22 MRC Clinical Trials Unit at UCL. BREATHER Plus. https://www.mrcctu.ucl.ac.uk/studies/all-studies/b/breather-plus/ (accessed Feb 23, 2026).

23 Zash R, Makhema J, Shapiro RL. Neural-tube defects with dolutegravir treatment from the time of conception. *N Engl J Med* 2018; **379:** 979–81.

24 Pereira G, Kim A, Jalil E, et al. No occurrences of neural tube defects among 382 women on dolutegravir at pregnancy conception in Brazil. IAS 2019: Conference on HIV Pathogenesis Treatment and Prevention; July 21–24, 2019 (abstr MOAX0104LB).

25 International Antiviral Society–USA. HIV drug resistance mutations. www.iasusa.org/resources/hiv-drug-resistance-mutations/ (accessed June 26, 2025).

26 Stanford University. HIV drug resistance database. https://hivdb.stanford.edu/ (accessed June 26, 2025).

27 Quartagno M, Chan M, Turkova A, Ford D, White IR. The Smooth Away From Expected (SAFE) non-inferiority frontier: theory and implementation with an application to the D3 trial. *Trials* 2023; **24:** 556.

28 Morris TP, Walker AS, Williamson EJ, White IR. Planning a method for covariate adjustment in individually randomised trials: a practical guide. *Trials* 2022; **23:** 328.

29 Parienti J-J, Palich R, Haberer J, et al. Intermittent three-drug regimens (i-3DR) as maintenance ART: a meta-analysis including individual participant data. Abstracts of the 19th European AIDS Conference (#EACS2023); Oct 18–21, 2023 (abstr 419).

30 Hill A, Fairhead C, Manalu S, et al. Could reduced dosing maintain more people on antiretrovirals after the sudden cuts in USAID funding? A crisis response. *AIDS* 2025; **39:** F1–4.

31 Callebaut C, Stepan G, Tian Y, Miller MD. In vitro virology profile of tenofovir alafenamide, a novel oral prodrug of tenofovir with improved antiviral activity compared to that of tenofovir disoproxil fumarate. *Antimicrob Agents Chemother* 2015; **59:** 5909–16.

32 Broyles LN, Luo R, Boeras D, Vojnov L. The risk of sexual transmission of HIV in individuals with low-level HIV viraemia: a systematic review. *Lancet* 2023; **402:** 464–71.

33 Osman N, Mesplède T, Quashie PK, Oliveira M, Zanichelli V, Wainberg MA. Dolutegravir maintains a durable effect against HIV replication in tissue culture even after drug washout. *J Antimicrob Chemother* 2015; **70:** 2810–15.

34 Rotger M, Tegude H, Colombo S, et al. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clin Pharmacol Ther* 2007; **81:** 557–66.

35 Teasdale CA, Brittain K, Zerbe A, et al. Characteristics of adolescents aged 15-19 years living with vertically and horizontally acquired HIV in Nampula, Mozambique. *PLoS One* 2021; **16:** e0250218.