Short-cycle therapy (weekends-off) on dolutegravir-based antiretroviral therapy in adolescents living with HIV (BREATHER Plus): a randomised, open-label, 96-week non-inferiority trial

Authors

Adeodata R. Kekitiinwa (1), Angus Jennings (2), Mutsa Bwakura Dangarembizi (3), Cissy Kityo (4), Abraham Siika (5), Moherndran Archary (6,9), Alexandra Green (2), Lungile Jafta (7), George Patrick Akabwai (1), Hilda Angela Mujuru (3), Henry Mugerwa (4), Cecilia Kiilu (5), Rosie Mngqibisa (6), Janet Seeley (8,9,10), Rashidah Nazzinda (4), Naomi Apoto (1), Simon Walker (11), David Burger (12), Carlo Giaquinto (7), Resty Babirye Okello (1); Godfrey Musoro (3), Charity Wambui (5), Tiyara Arumugam (6), Magda Conway (7), Nadine Van Looy (2), Annabelle South (2), Alasdair Bamford (2), Margaret J Thomason (2), Deborah Ford* (2), Sarah L Pett* (2), on behalf of the BREATHER Plus trial team**

*equal contribution

**names listed in Appendix 0

1. Baylor College of Medicine Children's Foundation, Kampala, Uganda
2. Medical Research Council Clinical Trials Unit at University College London (UCL), London, United Kingdom
3. University of Zimbabwe Clinical Research Centre, Harare, Zimbabwe
4. Joint Clinical Research Centre, Kampala, Uganda
5. Moi University Clinical Research Centre, Eldoret, Kenya
6. Department of Paediatrics and Children Health, Victoria Mxenge Hospital, Enhancing Care Foundation, Durban, South Africa
7. Fondazione Penta ETS, Padova, Italy
8. Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe, Uganda
9. Africa Health Research Institute, Nelson R. Mandela School of Medicine, 3rd FlooK-RITH Tower Building, Durban, KwaZulu-Natal, South Africa
10. Department of Global Health and Development, London School of Hygiene & Tropical Medicine, London, UK.
11. Centre for Health Economics, University of York, United Kingdom
12. Department of Pharmacy, Pharmacology & Toxicology, Radboud University Medical Center Nijmegen, Nijmegen, The Netherlands

Length: Abstract: 300 words

Text: 4491 words; max 4500

3 tables

3 figures

Correspondence to: D Ford, MRC Clinical Trials Unit at UCL, 90 High Holborn, London; email deborah.ford@ucl.ac.uk

SUMMARY

Background

Adolescents living with HIV (ALHIV) have poorer treatment outcomes compared to other age-groups. Short-cycle therapy (SCT, 5-days-on, 2-days-off) offers potential for drug-free weekends, less toxicity and better Quality-of-Life. We evaluated efficacy and safety of SCT using tenofovir disoproxil fumarate/lamivudine/dolutegravir (TLD) in ALHIV in Africa.

Methods

BREATHER Plus (ISRCTN #:85058577), an open-label, randomised (1:1) non-inferiority trial, compared SCT with continuous therapy (CT). Participants were ALHIV aged 12-<20 years in 5 clinical centres in Kenya, South Africa, Uganda and Zimbabwe, with HIV-1 viral load (VL) <50 copies/mL for >12 months, and no documented prior treatment failure. The primary outcome was the intent-to-treat adjusted Kaplan-Meier estimated proportion with confirmed VL ≥50 copies/mL by week 96. The non-inferiority margin and confidence level depended on the CT event rate (8% margin, 99% CI for 5% event rate). Participants were managed using real-time routine 6-12 monthly VLs; additional 8-12 weekly VLs were measured retrospectively.

Findings

BREATHER Plus enrolled 470 (239 SCT, 231 CT) ALHIV (56% female; median (IQR) age 16.5 (14.6–18.1) years, prior ART duration 11.8 years (8.6-14.1)) between 29-June-2022 and 10-May-2023. Median (IQR) follow-up was 117 weeks (108-120). Twenty-three participants in the SCT arm had confirmed VL ≥50 copies/mL by 96-weeks (Kaplan-Meier estimated proportion 10%) versus 11 (5%) in CT, with estimated difference (SCT-CT) of 5% (99%CI: -0.8,11.5); an 8% higher rate of confirmed virological rebound in SCT was not rejected. At the 5% significance level, SCT was inferior to CT (p=0.034). By end of follow-up, there were 16 SAEs (15 participants) in SCT arm (including 1 death unrelated to HIV or ART) versus 22 (16) in CT.

Interpretation

BREATHER Plus findings demonstrate that SCT tenofovir disoproxil fumarate/lamivudine/dolutegravir cannot be recommended for ALHIV, using standard-of-care routine 6-12 monthly VL monitoring.

Funding: European and Developing Countries Clinical Trials Partnership (EDCTP2), UK Medical Research Council

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 may be of particular importance to adolescents with HIV who face adherence challenges, treatment fatigue, and require ART across their lifespan. We searched Pubmed and Embase for articles published between Jan 1, 2000 and Jun 1, 2025, using the terms “HIV”, "short cycle", "intermittent", "weekends off”, "1 week on", "alternate days", "3-day-per-week", “4-day-per-week”, “5-day-per-week”, “6-day-per week”. We found mostly small-scale studies demonstrating promising virological and safety outcomes switching from continuous therapy to short-cycle therapy. Evidence came from mostly adults receiving non-integrase inhibitor (INSTI)-based ART in high-income settings with frequent real-time HIV viral load monitoring to guide clinical management. Following early evidence that a one-week-on, one-week-off strategy resulted in unacceptable failure rates, studies of short cycle therapy with less time off ART demonstrated encouraging results.

Single arm studies: A single-arm pilot study (5-days-on, 2-days-off) for adults with protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART found durable virological suppression, especially with efavirenz. A subsequent small (n=32) proof-of-principle study in children and adolescents with HIV receiving PI-based therapy (4-days-on, 3-days-off) found high rates of virological rebound. Further small single-arm adult studies employing a sequential reduction in days on therapy, showed that four and five days a week with mainly efavirenz or PI-based ART maintained virological suppression. An additional single-arm study provided further evidence of durable virological suppression with 4-days-on, 3-days-off efavirenz or PI-based ART. Similarly high and durable rates of virological suppression have been reported in 85 adults receiving bictegravir-based ART 4 or 5-days-a-week.

Randomised studies of NNRTI or PI-based regimens: A randomised trial including adults found 5-days-on, 2-days-off efavirenz or PI-based ART to be non-inferior to continuous therapy for a combined virological and clinical primary outcome. No virological failures occurred in a small randomised trial of a Monday/Wednesday/Friday dosing of efavirenz-based ART in adults, with additional evidence of reduced treatment-related toxicity. Additional randomised trial efficacy and safety data were provided by a study including adults receiving continuous efavirenz-based therapy compared to alternate-day therapy, with high rates of virological suppression maintained in both arms. The BREATHER trial, an international phase II/III trial investigating 5-days-on, 2-days off

efavirenz-based ART in 199 children and young people aged 8-24 years, demonstrated non-inferiority for a primary outcome of confirmed virological rebound by 48 weeks. There were no safety-related concerns with short-cycle therapy and participants preferred it. Non-inferiority was maintained over a median of 3.6 years.

Randomised studies of integrase inhibitor-based ART: Two randomised trials of short cycle therapy included participants receiving INSTI-based ART. The QUATUOR trial, included 647 adults receiving NNRTI (47%), PI (6%) or INSTI (48%) based ART (including 73 on dolutegravir, 65 on elvitegravir and 14 on raltegravir in the short-cycle arm). Non-inferiority was demonstrated for the primary endpoint of virological suppression at week 48 with 4-days-on, three-days-off ART, with only three failures on INSTI-based short-cycle therapy by 48 weeks and no further failures by 96 weeks. Reassuring safety data, improved daily-life satisfaction and reduced cost were also reported for the short-cycle strategy. Most recently, a trial included 60 adults receiving bictegravir-based ART randomized to daily versus five-days-on, two-days-off therapy. The primary endpoint was plasma bictegravir C_{trough} above the in vitro protein-adjusted 95% effective concentration at weeks 4, 28 and 52 with secondary endpoint including proportion of participants with viral load of >50 copies/ml at weeks 4, 28, 40 and 52. Bictegravir exposure and virological suppression were maintained in the short-cycle therapy group and there were no safety concerns.

Despite the above evidence, short-cycle ART is not widely recommended. Further investigation of efficacy, safety and acceptability of short-cycle strategy for adolescents living with HIV in Africa with real-world frequency of viral load monitoring was therefore justified. Furthermore, additional data for dolutegravir-based therapy, which is now universally recommended, are essential to support any recommendations for this approach.

Added value of this study

The BREATHER Plus trial demonstrated that short-cycle therapy with dolutegravir-based ART for adolescents in the context of 6-12 monthly viral load monitoring was inferior in terms of virological suppression. Maintaining an undetectable viral load is fundamental to long-term health and well-being of adolescents for multiple reasons, including sustained CD4+ T-cell count recovery, reduction of immune activation and associated comorbidities, as well as prevention of horizontal and vertical HIV transmission. It is also associated with a reduced risk of genotypic resistance and reduced HIV reservoir size, which is a key element of possible cure strategies.

Implications of all the available evidence

BREATHER Plus is the only trial that has evaluated short-cycle therapy with dolutegravir, using a public health approach to viral load monitoring applicable to low-and-middle-income settings and has shown that this cannot be recommended for adolescents in this context. Viral load testing frequency is likely to fall further with funding cuts, and visit frequency and adherence counselling intensity in the trial are not applicable in routine care. Future research should focus on alternative ART simplification strategies and support adherence in regions with limited resources and reduced viral load monitoring. Long-acting agents provide one such approach and the results of ongoing trials are eagerly awaited.

INTRODUCTION

Globally in 2023, an estimated 1.5 million adolescents (10-19 years) were living with HIV (ALHIV), with approximately 90% in sub-Saharan Africa[1,2]. ALHIV continue to have poorer treatment outcomes than younger children and adults with HIV, although the move to dolutegravir suggests an improving landscape for ALHIV[3-7]. Dolutegravir-based antiretroviral therapy (ART) regimens (dolutegravir+ 2 nucleos(t)ide reverse transcriptase inhibitor (NRTI)) have been rolled out globally and the fixed-dose combination of tenofovir disoproxil fumarate/lamivudine/dolutegravir (TLD) has become a near universal regimen for adolescents and adults.[8]

Short cycle therapy (SCT) aims to maintain suppression of HIV viral load (VL) 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[9-15] and randomised controlled trials (RCTs)[16-23] have demonstrated high rates of virological suppression on SCT strategies of 4-days-on/3-days-off and 5-days-on/2-days-off. Most used efavirenz-based antiretroviral therapy (ART), including the BREATHER trial (n=199)[21] which showed non-inferior virological suppression on SCT (5--days-on/2-days-off) versus continuous therapy (CT) over 144 weeks in young people (aged 8-24 years) enrolled in Africa, Europe and Thailand. More recently, the ANRS-170 QUATUOR study (n=636)[22], a France-wide RCT (85% male, median age 49 years; 48% on integrase-inhibitor (INSTI) based regimens (50% dolutegravir)), demonstrated non-inferiority of SCT (4-days-on, 3-days-off) versus daily ART based on virologic suppression <50copies/mL at 48 weeks. A further small RCT including 60 adults in Taiwan, comparing continuous bictegravir-based ART with 5-days-on-2-days off (FOTO) provided evidence for adequate bictegravir exposure and high rates of virological suppression using SCT[23]. Previous SCT studies have included frequent real-time VL monitoring (12-weekly or more often) to manage participants.

There have been no randomised trials of dolutegravir-based SCT in ALHIV and none with a programmatic 6-12 monthly VL testing approach. The objective of the BREATHER Plus trial was to evaluate whether SCT while receiving TLD with standard-of-care VL monitoring in ALHIV is non-inferior to CT in maintaining virological suppression, while improving Quality-of-Life[24].

METHODS

Study design

BREATHER Plus was an open-label randomised, parallel-2 arm non-inferiority trial evaluating SCT (five days on ART, two days off) versus CT on TLD in ALHIV who had been virologically suppressed on ART for more than one year. Recruitment was between 29-June-2022 and 10-May-2023 in five clinical research centres in Kenya, South Africa, Uganda (two centres) and Zimbabwe. Close-out visits were scheduled at least 96 weeks after enrolment and between 29-January-2025 and 22-April-2025. The trial protocol was approved by national and local ethics committees and the ethics committee at University College London, London, UK (Appendix 1).

Participants

Participants were ALHIV aged ≥12 to <20 years, who were receiving ART (a dolutegravir/tenofovir triple ART for at least one month at screening) with virological suppression (<50 copies/mL) for 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 treatment failure. Main exclusion criteria included pregnancy or breastfeeding or unwillingness to use highly effective contraception, moderate or high risk of suicidality on the Columbia-Suicide Severity Rating Scale (CSSRS), or ongoing treatment for tuberculosis. Participants aged ≥18 years provided written consent; younger participants provided assent, with parents/guardians providing written consent[24].

Randomisation and masking

This was an open-label strategy trial. Randomisation was 1:1 to SCT versus CT using permuted blocks and stratified by clinical centre and mode of infection (vertical or horizontal/other). The computer-generated randomisation list was prepared by the Trial Statistician and incorporated within the database, enabling access only to the next allocation. Randomisation at clinical sites was undertaken after informed consent was taken by delegated site personnel. VLs were measured by laboratory staff blind to trial arm. Only the Trial Statisticians and the Independent Data Monitoring Committee (IDMC) reviewed data by randomised arm during the trial.

Procedures

An nested four-week randomised pilot safety phase (weekly visits and VLs for three weeks, with a fourth VL if the week-3 VL was ≥50 copies/mL) was undertaken in 33 participants (16 SCT) to ensure those in the SCT arm maintained undetectable VL (<50 copies/mL) after the two-day break

(Saturday/Sunday). Trial recruitment continued during the pilot phase. Participants were seen at screening (when VL was measured to confirm eligibility), enrolment, week 4 (SCT arm only), week 8 and then 8-weekly for 48 weeks (frequency of visits was to conduct pregnancy testing, because at that time, there was concern about the increased risk of neural tube defects in pregnancies with dolutegravir[25-27], later refuted) and 12-weekly thereafter. All scheduled visits included a clinical assessment, adherence assessment with pill-count and 7-day recall adherence questionnaire, ART dispensing, and, for girls post menarche, a pregnancy test. Weight, height, and waist and hip circumference were measured at baseline, weeks 8, 24, 48, 96 and close-out. Haematology, CD4+ and CD8+ T-cells and glycosylated haemoglobin (HbA1c) tests were performed at baseline, 48 and 96 weeks, with CD4+ and CD8+ T-cells also measured at week 144. Biochemistry tests including estimated glomerular filtration rate (eGFR, calculated using the Cockcroft-Gault equation), lipids and glucose measurements were performed at baseline and 96 weeks. A mood survey and the Columbia Suicide Severity Rating Scale (C-SSRS) were administered at baseline, weeks 8, 24, 48, 72, 96 and close-out. Acceptability and wellbeing and Quality-of-Life were assessed at baseline, weeks 24, 48, 96 and close-out.

VLs were measured in real-time (with results returned to treating clinicians) 24-48 weekly 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 24-weekly thereafter (until age 20 in Uganda, when testing frequency reduced to 48-weekly). In South Africa and Zimbabwe, testing was required at weeks 48 and 96 and 48-weekly thereafter (with a supplemental 24-week test in Zimbabwe). Participants who had a real time VL ≥50 copies/mL were recalled after ≥7 days for confirmatory testing. Retrospective VL testing was completed on plasma stored at baseline, 8-weekly in year 1, 12-weekly in year 2 and 24-weekly thereafter, for timepoints where real-time VLs were not done (results were not returned to treating clinicians). Real-time and retrospective VL results informed trial endpoints and were reviewed by the IDMC. 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 (ranging from VL ≥200 to ≥1000 copies/mL, by site) was chosen; resistance testing was not performed if no VLs met this threshold. Drug resistance mutations were classified according to the International Antiviral Society–USA (IAS-USA)[28] and drug resistance scores were defined according to the Stanford University HIV drug resistance database[29].

Participants received TLD as a fixed-dose combination of tenofovir disoproxil fumarate (300 mg), lamivudine (300 mg) and dolutegravir (50mg), provided by national programmes. The protocol allowed for use of emtricitabine (200 mg) instead of lamivudine and/or tenofovir alafenamide (25 mg) instead of tenofovir disoproxil fumarate, but only TLD was used. Participants randomised to SCT could choose their two consecutive days off TLD i.e. Friday/Saturday or Saturday/Sunday, and continued this cycle. Participants randomised to CT continued daily ART. Participants on SCT who had confirmed real-time VL ≥50 copies/mL, who became pregnant, had incident tuberculosis or switched off TLD returned to CT. At the end of pregnancy and breastfeeding or on completion of tuberculosis treatment, participants could restart SCT. Changes to treatment regimen in both arms were at the discretion of the treating clinician.

In a Medication Event Monitoring Systems (MEMS Cap) sub-study, participants were randomly selected across Ugandan and Kenyan sites to measure adherence to allocated treatment strategy during weeks 8-32 (targets, 50 SCT; 50 CT) and weeks 48-72 (targets, 50 SCT; 50 CT). 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 VLs ≥50 copies/mL) by week 96. Secondary efficacy outcomes were confirmed VL ≥1000 copies/mL by week 96, VL <50 copies/mL and no switch to second-line ART for treatment failure at 24, 48, 72 and 96 weeks, VL ≥50 copies/mL at weeks 48 and 96 using a modified Food and Drug Administration (FDA) snapshot algorithm (Statistical Analysis Plan, Appendix 21)[30], and HIV genotypic drug resistance at confirmed viral rebound. Safety outcomes were: change in lipids, HbA1c, phosphate, eGFR, CD4+ and CD8+ T-cell counts from baseline to 96 weeks, change in anthropometric measures from baseline to 48 and 96 weeks, time to any new or recurrent World Health Organization (WHO) stage 3 or 4 event or death, incidence of serious, grade ≥3 clinical events, treatment-modifying adverse events, and the proportion of participants with any change from baseline ART regimen. Patient-reported outcomes were: adherence, acceptability and neuropsychiatric toxicities.

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, 2-sided alpha of 5%, to demonstrate non-inferiority of SCT versus CT, assuming 11% of participants met the primary endpoint by week 96 in both groups and allowing for 10% loss to follow-up. Prior to the trial opening, a decision was made to use the Smooth Away From

Expected (SAFE) non-inferiority frontier[31] 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 pre-specified such that, for any observed control event rate less than 9%, the non-inferiority margin would be less than 10%, with the margin decreasing as the control event risk declined (Appendix 2). To maintain the nominal type I error rate to ≤0.03 (2-sided alpha<0.06), a 5% 2-sided significance level was planned if the event rate was 9% or higher and a 1% significance level if the event rate was lower than 9%; this maintained power at ≥80% for a control event rate between 1-15%, holding the sample size and other assumptions fixed. The SAFE frontier was only defined for analyses of the primary endpoint.

All participants with post-baseline VL data were included in the intent-to-treat (ITT) analysis. The proportion of participants experiencing confirmed viral rebound was estimated by arm and clinical centre using adjusted Kaplan-Meier methods (no adjustment was made for mode of infection due to 97% participants acquiring HIV vertically) and censoring at the end of the week 96 window (week 102 – 1 day) or last viral load <50 copies/mL if not seen ≥102 weeks. The average cumulative failure function for each randomised arm was calculated using standardisation[32], as a weighted average of the corresponding centre-specific estimates. The difference in the probability of confirmed viral rebound (SCT-CT) at week 96 was then estimated, with a 2-sided 95% or 99% confidence interval calculated using bias-corrected percentiles of bootstrap estimates (10,000 samples, stratified by centre/arm). SCT should be considered non-inferior to CT if the upper limit of the CI of the difference is less than the selected non-inferiority margin. Sensitivity analyses included an equivalent analysis unadjusted for centre, and use of a flexible parametric model[33] allowing a time-varying effect of SCT.

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

The difference between arms in the proportion of participants with confirmed VL ≥1000 copies/mL by week 96 was estimated in the same way as the primary outcome. The proportion of participants with cross-sectional VL <50copies/mL at each timepoint was compared between arms using an unadjusted test of proportions. The modified FDA snapshot algorithm was used to estimate the proportions with VL ≥50 copies/mL at weeks 48 and 96 and compared between arms using a

Cochran-Mantel-Haenszel test; if a participant changed ART regimen or treatment strategy (SCT to CT), was lost to follow-up, died or withdrew before or during the respective visit window, their most recent VL prior to regimen or strategy change or end of follow-up was used to define their outcome (rebound if VL ≥50 copies/mL or no virologic data if most recent VL was <50 copies/mL and prior to the window). In an exploratory analysis, we considered time spent above different VL thresholds from first post-baseline VL to the end of the week 96 window, using last observation carried-forward.

Adverse events were compared between arms considering time-to-first-event in a Cox regression model. Change from baseline in continuous variables was evaluated using linear mixed models with random intercept for participant and fixed effects for baseline measure, arm and visit week, including interaction terms between arm and visit week.

For participants in the MEMS Cap sub-study, an adherent week was defined as daily openings in the CT group, and as 5 days on (daily openings) Monday-Friday or Sunday-Thursday with the corresponding 2 days off (no openings) Friday-Saturday or Saturday-Sunday in the SCT group.

Further details are in the protocol (Appendix 20) and Statistical Analysis Plan (Appendix 21).

The trial was registered in ISRCTN (#:85058577).

Role of the funding source

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

RESULTS

Five hundred and twenty-four ALHIV were screened for eligibility (Figure 1), of whom 54 were ineligible. Between 29-June-2022 and 10-May-2023, 470 ALHIV were randomised (239 SCT, 231 CT). Three participants did not have a post-baseline VL (1 death and 1 withdrawal in SCT; 1 withdrawal in CT), and a further seven participants (4 SCT, 3 CT) withdrew before the week 96 assessment. VL data at scheduled time-points in follow-up were almost complete up to and including week 96 (43/2390 (2%) measurements missing in SCT and 29/2310 (1%) measurements missing in CT). Follow-up VL measurements were available after all initial VL of ≥50 copies/mL. Median (IQR) follow-up was 117 weeks (108-120). The last participant visit was on 27-May-2025.

Of the 470 randomised, 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. Baseline characteristics were similar between arms (Table 1): median (IQR) age was 16.5 years (14.6-18.1), with 140 (30%) aged below 15 years; 263 (56%) participants were female; 454 (97%) acquired HIV vertically; median CD4+ T-cell count was 878 cells/mm^3 (690-1119). Median pre-trial ART exposure was 11.8 years (8.6-14.1), including 2.5 years (2.1-3.2), minimum 1 month, on dolutegravir (Appendix 3).

Thirty-four participants (23 SCT, 11 CT) met the week 96 primary endpoint (Table 2, Figure 2, Appendix 4), with an estimated probability of confirmed viral rebound of 9.9% (95% CI 6.4-14.3) in SCT versus 4.8% (2.6-7.8) in CT. The estimated difference (SCT–CT) in proportion with confirmed viral rebound was 5.1% (99% CI -0.8-11.5, bootstrap p=0.034). The upper bound of the two-sided 99% confidence limit was above the 8% non-inferiority margin (pre-specified for a control event rate of 5%, Appendix 2) (Figure 2), and there was evidence at the 5% significance level that participants in the SCT arm were at higher risk of confirmed viral rebound than participants in the CT arm. Results from the sensitivity analyses were similar (Figure 2). In the per-protocol analysis there were 20 SCT events and 10 CT events (1 CT event dropped due to self-reported adherence, 2 SCT events dropped after ART break >7 days, 1 SCT event dropped after switch to CT) (Figure 2). There was no evidence of a difference in the effect of strategy (SCT versus CT) by clinical centre or by age-group (Appendix 5). 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 SCT versus 2.2% (95% CI 0.9-4.3) in CT (bootstrap p=0.086) (Appendix 6).

At last VL measurement, 19/23 participants in the SCT arm who experienced confirmed viral rebound by week 96 had VL <50 copies/mL, including 10/14 who were on CT at last VL (1 of the 4

unsuppressed on CT had returned to CT before meeting the primary endpoint) and 9/9 who were on SCT at last VL (8 met the primary endpoint based on retrospective VL testing). This compared with 8/11 participants in the CT arm (1/3 unsuppressed met the primary endpoint at close-out) (Appendix 7). Between week 96 and close-out an additional 2 SCT participants and 1 CT participant had confirmed viral rebound (Appendix 8).

By week 96, ten participants (5 SCT, 5 CT) had confirmed VL ≥1000 copies/mL, with an estimated difference in proportion between arms (SCT-CT) of 0.0% (95% CI -2.6 to 2.7; bootstrap p=0.99) (Table 2, Appendix 9). No participants switched to second-line ART (Table 3, Appendix 17) and based on cross-sectional VLs, proportions suppressed <50 copies/mL were similar by arm at weeks 24, 48, 72 and 96 (Table 2, Appendix 11). Based on the modified FDA snapshot algorithm, and treating return from SCT to CT as failure, failure was not significantly higher in the SCT arm compared to the CT arm at 48 weeks or 96 weeks (Table 2, Appendix 12). In an exploratory analysis, 4.2% of time to week 96 in the SCT arm vs 2.4% of time in the CT arm was spent with VL at ≥50 copies/mL (p=0.011). Corresponding estimates for time spent ≥200 copies/mL were 2.4% SCT versus 1.4% CT (p=0.083) and for time spent ≥1000 copies/mL were 1.5% SCT versus 0.8% CT (p=0.177) (Appendix 13).

Of 34 participants reaching the primary endpoint, resistance results were available for 28 (17 SCT, 11 CT). One participant in the CT arm 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 SCT arm and two participants in the CT arm had major non-nucleoside reverse transcriptase inhibitor-related resistance mutations. No major protease inhibitor-related resistance mutations were identified (Table 2, Appendix 15).

By end of follow-up, there were 4 new or recurrent WHO stage 3/4 events or deaths (2 in each arm, Table 3). This included one death in an SCT participant 8 days after randomisation, judged unrelated to ART or HIV. There were 16 SAEs in 15 participants in the SCT arm compared with 22 in 16 participants in the CT arm (HR 0.91 [95%CI 0.45-1.85] p=0.80); 16 SAEs (8 SCT, 8 CT) were pregnancy related (Appendix 16). Similar proportions of participants experienced adverse events grade 3 and above (23 SCT versus 23 CT; p=0.94, Table 3). There were 5 neuropsychiatric toxicity events in 5 participants, all CT (Appendix 16).

At week 96, twenty SCT participants were receiving CT: six had previously reached the primary endpoint (based on real-time VL measurements), eight reverted at pregnancy, five reverted due to

stopping highly effective contraception and one due to participant choice. One additional participant (SCT) reverted to CT before withdrawing prior to week 96. A further 4 participants reverted to CT at week 96 (1 ART change, 3 met the primary endpoint) and 5 post week 96 (2 confirmed viral rebound, 2 pregnancy, 1 stopping highly effective contraception).

In both trial arms, mean CD4+ and CD8+ T-cells declined by week 96 but changes were similar by arm (Table 3); only 2 (1%) SCT, 8 (4%) CT had CD4+ T-cells <350 10^6 cells/L at week 96. There was a small significant decline in the eGFR in the CT arm compared to SCT, but only one participant (SCT), switched off TLD for reduced eGFR (at week 109). There were no significant differences between arms in weight or total cholesterol (Table 3). Further results are summarised in Appendix 18/19.

Self-reported adherence on the 7-day recall questionnaire was high in both arms: at 96% visits in the SCT arm and 96% visits in the CT arm, participants reported taking all prescribed ART (according to strategy) in the previous 7 days. In the MEMS Cap sub-study the proportion of participants who opened their pill bottle Monday to Thursday was similar in both arms (SCT 92%, CT 92%, Figure 3). 71% of SCT weeks were adherent to SCT strategy, with 20% of weeks non-adherent due to >2 tablets missed and the remainder (9%) non-adherent due to tablets taken on > 5 days or 2 days missed in the week other than Friday/Saturday or Saturday/Sunday. 71% of CT weeks were adherent to CT strategy, with 16% non-adherent due to one day off ART and 13% due to ≥2 days off ART.

At 96 weeks 201/218 (92%) participants randomised to SCT reported that stopping ART at weekends was a lot easier, 14/218 (6%) a little easier, 2/218 (1%) no difference and one participant reported it was a little more difficult.

DISCUSSION

BREATHER Plus evaluated weekends-off ART in adolescents on dolutegravir/tenofovir-based ART over 96 weeks. It is the first trial to consider SCT in the context of 6-12 monthly VL monitoring, aligning with standard-of-care in Africa, and only the second trial to consider SCT on three-drug ART using dolutegravir, which is now the predominant anchor drug globally. BREATHER Plus demonstrated that SCT was inferior to CT in maintaining viral suppression below 50 copies/mL over 96 weeks in ALHIV.

In contrast, ANRS-170 QUATUOR, demonstrated non-inferiority of SCT, but included only 73 participants on SCT using dolutegravir and enrolled a long-term adherent population, of mostly older men, who were seen at week 4, 12 and 12-weekly thereafter with real-time VLs measured at each study visit, with results available for clinicians and participants[22]. Such frequent VL monitoring may have reduced the risk of failure through identifying viral 'blips' and providing adherence counselling at these time-points to help participants resuppress quickly without returning to continuous therapy.

Other small trials with promising results on newer regimens have used bictegravir/emtricitabine/tenofovir alafenamide[23,34]; tenofovir alafenamide (10-fold higher intracellular levels of tenofovir than TDF), a regimen not widely available in sub-Saharan Africa.

Reassuringly, in both arms of BREATHER Plus most participants (~80%) who experienced confirmed viral rebound were virologically suppressed at close-out with no change to ART regimen. There was only one participant with a major integrase resistance mutation (CT arm). However, these findings should not be interpreted as evidence that SCT can be recommended in routine practice for ALHIV. Persistent VL <50 copies/mL should be strived for and at least <200 copies/mL for prevention of transmission. Exploratory analysis suggested that SCT participants were at risk of onward transmission of HIV (VL ≥200 copies/mL)[35,36] for a higher proportion of follow-up than CT participants. It is also unclear what effect periods of increases in VL have, even when temporary, in regard to accumulation of resistance mutations and the size of the viral reservoir including in sanctuary sites like the brain. There is a risk this might ultimately lead to treatment failure on TLD[35].

The MEMS Cap sub-study, conducted at three of five centres, suggested that total prescribed tablets taken fell below 95% with evidence in the SCT arm of more than two days off being taken in around 20% of monitored weeks. However, comparison of MEMS Cap data (Figure 3) with similar data collected in the BREATHER trial[19] showed that adherence was similar, if not better in BREATHER Plus

(84% SCT and 89% CT tablets were taken Monday-Thursday in BREATHER, versus 92% and 92% in BREATHER Plus). In BREATHER Plus ~10% missed tablets in addition to taking weekends-off may have contributed to inferior virological suppression in SCT versus CT. Since BREATHER demonstrated very similar virological suppression in SCT versus CT[37], it is possible that differences in the ART regimen (dolutegravir-based in BREATHER Plus which has a shorter half-life than efavirenz used in BREATHER[38,39]) and/or reduced frequency of VL monitoring (6-12 monthly in BREATHER Plus versus 12-weekly in BREATHER) contributed to the inferiority of SCT in BREATHER Plus.

Confirmed virological rebound in the BREATHER Plus CT arm was low (2% by 48 weeks, 5% by 96 weeks). However, in the ANRS-QUATUOR 170 study, which defined all confirmed ≥50 copies/mL by week 48 as virological failure in their primary analysis, aligning closely with our primary endpoint, failure rates by 48 weeks were still numerically lower in the control arm (1.3% in the modified Intention-to-Treat Population and 0.7% in the sub-group on INSTI-based ART) compared to BREATHER Plus[22].

BREATHER Plus is the first trial to use the SAFE non-inferiority frontier, where the non-inferiority margin is pre-specified dependent on the control event rate[31]. The event rate in the CT arm was considerably lower than estimated at the design stage; by considering this scenario prior to opening to 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 ALHIV spanning the adolescent age-range, although we were unable to recruit planned numbers of adolescents who had acquired HIV horizontally who may face additional challenges with adherence[40]. It is likely that outcomes would be similar or worse among adolescents who have acquired HIV during their teenage years, since our trial population was stable and on long-term treatment.

Generalisability of our findings to the real-world is also limited by the carefully controlled nature of a clinical trial. The frequency of visit attendance in BREATHER Plus did not align with routine care, including 8-weekly visits in year 1, reduced to 12-weekly from year 2. Participants were contacted following a late or missed visit, and visit attendance was very high. VLs were measured in real-time 6-12 monthly and adherence counselling was provided at first raised VL≥50 copies/mL. Adolescents typically have lower adherence to ART than adults[3,5,7] and in real-world settings, it is likely that adherence to a SCT strategy among ALHIV would be poorer than in a trial, particularly if VL

monitoring was less frequent. This could lead to even greater difference between SCT and CT outcomes.

In conclusion, BREATHER Plus clearly demonstrates that SCT is inferior to CT for ALHIV in Africa receiving TLD and monitored using 6-12 monthly VL testing. While SCT may be desirable in terms of cost reduction and Quality-of-Life, the importance of virological suppression for long term health and prevention of onward transmission (vertical and horizontal) remains fundamental to population health. As such, we urge caution in recommending dosing 4-5 days per week[41] in low- and middle-income countries to extend antiretroviral drug supplies in the current funding climate. It may be that the costs of additional adherence support and viral load monitoring to ensure SCT is implemented safely and effectively, will outweigh the savings in drug costs. The impact of these findings on policy in the context of existing reassuring data from smaller, mainly adult trials requires careful consideration, and an Evidence-to-Decision framework[42] should be applied when reviewing available data for guideline development. Our results also emphasise a continued need to assess strategies to support adherence and to safely simplify ART for adolescents including daily and weekly oral options, dual therapy and long-acting agents in real-world settings.

Contributors

ARK, MBD, CK, AS, MA, DF and SLP designed the study. ARK, MBD, CK, AS, MA, GPA, HAM, HM, CK, RM, RN, RBO, GM, CW and TA were responsible for study conduct and data collection at clinical research sites. AJ and DF had access to all the data. AJ performed the statistical analysis and AJ and DF verified the data reported. DF, SLP, AB and AJ wrote the first draft of the paper, which all authors critically reviewed. All authors approve the final manuscript and had responsibility for the decision to submit for publication.

Data sharing

The BREATHER Plus data are held at the Medical Research Council Clinical Trials Unit at University College London, which encourages optimal use of data by using 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 EDCTP2 programme (grant number RIA2017MC-2005-BREATHER Plus), funded by the European Union, UK Medical Research Council and Swedish International Development Cooperation Agency. The Medical Research Council Clinical Trials Unit at University College London receives core support from the UK Medical Research Council (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, Professor Kusum Nathoo (University of Zimbabwe Clinical Research Centre), who passed away in June 2025, for her enormous contribution to the BREATHER Plus trial.

REFERENCES

1. Organisation WH. 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 [Accessed 16 Dec 2024.
2. UNAIDS. The Urgency of Now: AIDS at a Crossroads. 2024. https://www.unaids.org/sites/default/files/media_asset/2024-unaids-global-aids-update_en.pdf (accessed [Accessed 16 Dec 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**(47): 1097-103.
4. Chappell E, Lyall H, Riordan A, et al. The cascade of care for children and adolescents with HIV in the UK and Ireland, 2010 to 2016. *Journal of the International AIDS Society* 2019; **22**(9): e25379.
5. Evans D, Menezes C, Mahomed K, et al. Treatment outcomes of HIV-infected adolescents attending public-sector HIV clinics across Gauteng and Mpumalanga, South Africa. *AIDS research and human retroviruses* 2013; **29**(6): 892-900.
6. Fokam J, Takou D, Njume D, et al. Alarming rates of virological failure and HIV-1 drug resistance amongst adolescents living with perinatal HIV in both urban and rural settings: evidence from the EDCTP READY-study in Cameroon. *HIV medicine* 2021; **22**(7): 567-80.
7. Grimsrud A, Balkan S, Casas EC, et al. Outcomes of antiretroviral therapy over a 10-year period of expansion: a multicohort analysis of African and Asian HIV programs. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2014; **67**(2): e55-e66.
8. Initiative CHA. 2024 HIV Market Report, 2024.
9. Ananworanich J, Hirschel B. Intermittent therapy for the treatment of chronic HIV infection. *Aids* 2007; **21**(2): 123-34.
10. 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 clinical trials* 2007; **8**(1): 19-23.
11. Rudy BJ, Sleasman J, Kapogiannis B, et al. Short-cycle therapy in adolescents after continuous therapy with established viral suppression: the impact on viral load suppression. *AIDS research and human retroviruses* 2009; **25**(6): 555-61.
12. 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. *The FASEB Journal* 2010; **24**(6): 1649-55.

13. 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. *The FASEB Journal* 2015; **29**(6): 2223-34.

14. de Truchis P, Assoumou L, Landman R, et al. Four-days-a-week antiretroviral maintenance therapy in virologically controlled HIV-1-infected adults: the ANRS 162-4D trial. *Journal of Antimicrobial Chemotherapy* 2018; **73**(3): 738-47.

15. 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. *Journal of Personalized Medicine* 2023; **13**(4): 583.

16. 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**(4): e10307.

17. 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**(12): 1633-41.

18. Bellagamba R, Giancola ML, Tommasi C, et al. Randomized clinical trial on efficacy of fixed-dose efavirenz/tenofovir/emtricitabine on alternate days versus continuous treatment. *Aids* 2019; **33**(3): 493-502.

19. 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 Technology Assessment (Winchester, England)* 2016; **20**(49): 1.

20. Bernays S, Paparini S, Gibb D, Seeley J. When information does not suffice: young people living with HIV and communication about ART adherence in the clinic. *Vulnerable children and youth studies* 2016; **11**(1): 60-8.

21. Turkova A, Moore CL, Butler K, et al. 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**(4): e0196239.

22. Landman R, de Truchis P, Assoumou L, et al. 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. *The lancet HIV* 2022; **9**(2): e79-e90.

23. Hsin-Yun Sun Y-TL, Wen-Chun Liu, Chiao-Wen Huang, Ching-Hua Kuo, Chien-Ching Hung. Five-day-on-two-day-off (FOTO) vs daily BIC/FTC/TAF: a proof-of-concept randomized clinical trial. IAS AIDS 2024. Germany; 2024.

24. Katongole F, Arumugam T, Jennings A, et al. 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. *Contemporary clinical trials* 2025: 107963.

25. Zash R, Makhema J, Shapiro RL. Neural-tube defects with dolutegravir treatment from the time of conception. *New England Journal of Medicine* 2018; **379**(10): 979-81.

26. Zash R, Holmes L, Makhema J, et al. Surveillance for neural tube defects following antiretroviral exposure from conception. 22nd International AIDS Conference; 2018; 2018. p. 23-7.

27. Pereira G, Kim A, Jalil E, et al. No occurrences of neural tube defects among 382 women on dolutegravir at pregnancy conception in Brazil. JOURNAL OF THE INTERNATIONAL AIDS SOCIETY; 2019: JOHN WILEY & SONS LTD THE ATRIUM, SOUTHERN GATE, CHICHESTER PO19 8SQ, W …; 2019. p. 93-.

28. Society-USA IA. HIV Drug resistance Mutations. www.iasusa.org/resources/hiv-drug-resistance-mutations/ (accessed [Accessed 26 June 2025.

29. University S. HIV Drug Resistance Database https://hivdb.stanford.edu/ (accessed [Accessed 26 June 2025.

30. Administration UFaD. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-immunodeficiency-virus-1-infection-developing-antiretroviral-drugs-treatment.

31. 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**(1): 556.

32. Morris TP, Walker AS, Williamson EJ, White IR. Planning a method for covariate adjustment in individually randomised trials: a practical guide. *Trials* 2022; **23**(1): 328.

33. Royston P, Parmar MK. Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. *Statistics in medicine* 2002; **21**(15): 2175-97.

34. Chivite I, Lazzari ED, Sempere A, et al. Safety, Tolerability, and Efficacy of a BIC/FTC/TAF Dose Reduction Strategy. CROI Conference on Retroviruses and Opportunistic Infections; 2025; San Francisco, CA; 2025.

35. 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. *The Lancet* 2023; **402**(10400): 464-71.

36. Services DoHaH. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents

in Adults and Adolescents With HIV. https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-arv (accessed [Accessed 30 June 2025.

37. BREATHER T, Group T. Weekends-off efavirenz-based antiretroviral therapy in HIV-infected children, adolescents, and young adults (BREATHER): a randomised, open-label, non-inferiority, phase 2/3 trial. *The lancet HIV* 2016; **3**(9): e421-e30.

38. Osman N, Mesplede T, Quashie PK, Oliveira M, Zanichelli V, Wainberg MA. Dolutegravir maintains a durable effect against HIV replication in tissue culture even after drug washout. *Journal of Antimicrobial Chemotherapy* 2015; **70**(10): 2810-5.

39. 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. *Clinical pharmacology & therapeutics* 2007; **81**(4): 557-66.

40. 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**(4): e0250218.

41. 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**(7): F1-F4.

42. Alonso-Coello P, Schünemann HJ, Moberg J, et al. GRADE Evidence to Decision (EtD) frameworks: a systematic and transparent approach to making well informed healthcare choices. 1: Introduction. *bmj* 2016; **353**.

Table 1 – Baseline characteristics

	SCT (N=239)	CT (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%)
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)
Creatinine clearance (eGFR), mL/min	116 (100, 131)	113 (99, 132)	114 (100, 131)
CD4+ T-cell count, 10^6 cells/L	866 (678, 1085)	909 (693, 1163)	878 (690, 1119)
CD8+ T-cell count, 10^6 cells/L	708 (533, 859)	728 (544, 962)	716 (540, 902)
Weight, 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)

Count (%) or median (IQR) presented. 2 participants missing baseline CD4+/CD8+ T-Cell results.

Other modes of infection include sexual contact (4 SCT, 3 CT), blood product (2 SCT), cross contamination at birth (1 CT), suspected malicious infection (1 SCT), suspected sharing of sharps with infected relative (1 CT). One participant (SCT) with 'Other' mode of acquisition (blood product) randomised in vertical stratum in error.

Table 2 – BREATHER plus efficacy outcomes

	SCT	CT	SCT vs CT
Participants	239	231	
Participants with confirmed HIV-1 RNA ≥50 c/mL by 96 weeks	23	11	
Estimated probability of confirmed HIV-1 RNA ≥50 c/mL	0.099 (95% CI 0.064-0.143)	0.048 (95% CI 0.026-0.078)	0.051 (99% CI -0.008-0.115) p=0.034
Participants with confirmed HIV-1 RNA ≥1000 c/mL by 96 weeks	5	5	
Estimated probability of confirmed HIV-1 RNA ≥1000 c/mL	0.022 (95% CI 0.008-0.043)	0.022 (95% CI 0.004-0.044)	0.000 (95% CI -0.026-0.027) p=0.987
Participants with HIV-1 RNA <50 c/mL and no switch to second-line at week 48	223	221	
Estimated probability of HIV-1 RNA <50 c/mL and no switch to second-line	0.95 (95% CI 0.92-0.98)	0.97 (95% CI 0.95-0.99)	-0.02 (95% CI -0.06-0.02) p=0.270
Participants with HIV-1 RNA <50 c/mL and no switch to second-line at week 96	215	211	
Estimated probability of HIV-1 RNA <50 c/mL and no switch to second-line	0.92 (95% CI 0.89-0.96)	0.93 (95% CI 0.90-0.96)	-0.01 (95% CI -0.05-0.04) p=0.781
Participants with HIV-1 RNA ≥50 c/mL at 48 weeks, modified FDA snapshot	7	4	
Estimated probability of HIV-1 RNA ≥50 c/mL, modified FDA snapshot	0.03 (95% CI 0.01-0.06)	0.02 (95% CI 0.00-0.04)	0.01 (95% CI -0.02-0.04) p=0.45
Participants with HIV-1 RNA ≥50 c/mL at 96 weeks, modified FDA snapshot	14	9	
Estimated probability of HIV-1 RNA ≥50 c/mL, modified FDA snapshot	0.06 (95% CI 0.03-0.10)	0.04 (95% CI 0.02-0.07)	0.02 (95% CI -0.02-0.06) p=0.33
Participants with major resistance mutation(s) following confirmed HIV-1 RNA ≥50 c/ml by week 96			
INSTI	0/17 (0.00)	1/10 (0.10)	
NRTI	0/17 (0.00)	1/11 (0.09)	
NNRTI	3/17 (0.18)	2/11 (0.18)	
PI	0/17 (0.00)	0/11 (0.00)	

INSTI - integrase strand transfer inhibitors; NNRTI - non-nucleoside analogue reverse transcriptase inhibitors; PI - protease inhibitors; NRTI - nucleos(t)ide analogue reverse transcriptase inhibitors

99% CI used for SCT vs CT estimated probability of confirmed HIV-1 RNA ≥50 c/mL according to SAFE non-inferiority framework. 95% CI used in all other cases presented here.

SCT vs CT estimate presented as risk difference (SCT-CT) in all cases. Probability for primary analysis and confirmed HIV-1 RNA ≥1000 based on adjusted Kaplan-Meier estimate; p-values/CIs based on bootstrap resampling, adjusted for site. P-value/CIs for HIV-1 RNA <50 at each week based on unstratified test of proportions at each week. Proportion, as well as p-value/CIs, for modified FDA snapshot based on Cochran–Mantel–Haenszel test, stratified by site.

Resistance testing was not possible for 6 SCT participants with insufficient viremia at/after confirmed viral rebound (5 with all VL<200, 1 with all VL<400). All had resuppressed by close-out visit. The integrase region failed to amplify for one participant in the CT group. They had no other resistance mutations.

Table 3 – BREATHER plus safety outcomes

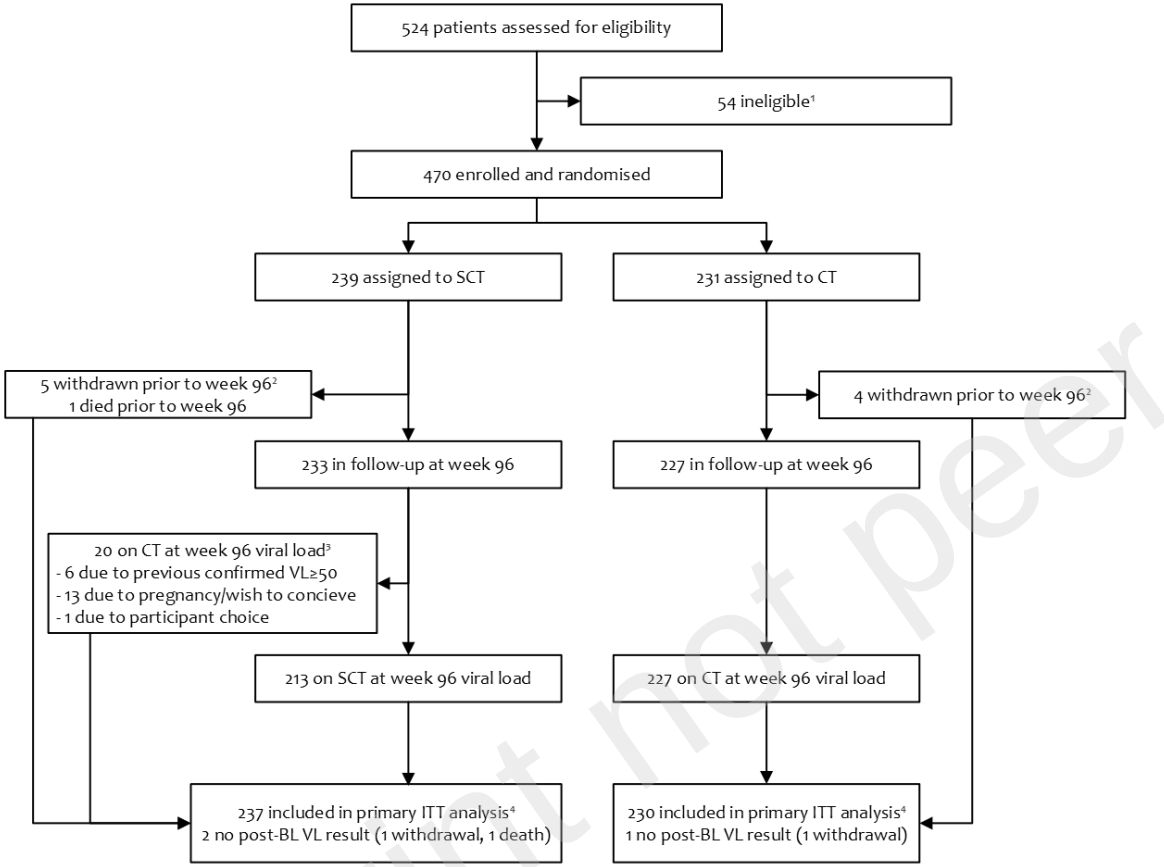
	SCT	CT	SCT vs CT
Participants	239	231	
Adverse events [participants] over all follow-up			
WHO stage 3 or 4 events or death	2 [2]	2 [2]	HR 0.98 (95% CI 0.14-6.95) p=0.982
Serious adverse events	16 [15]	22 [16]	HR 0.91 (95% CI 0.45-1.85) p=0.801
Grade ≥3 clinical/lab adverse events	26 [23]	32 [23]	HR 0.98 (95% CI 0.55-1.75) p=0.944
ART-modifying adverse events	2 [2]	4 [4]	HR 0.50 (95% CI 0.09-2.71) p=0.420
Participants with a change in baseline ART regimen (%)*	2 (1)	2 (1)	Fisher's exact p=1.00
Safety laboratory measures			
Estimated mean change from baseline total cholesterol at week 96, mmol/L	0.08 (95% CI 0.01-0.15)	0.03 (95% CI -0.04-0.10)	0.05 (95% CI -0.05-0.16) p=0.288
Estimated mean change from baseline eGFR at week 96, mL/min	0.25 (95% CI -2.11-2.62)	-3.46 (95% CI -5.85--1.06)	3.71 (95% CI 0.34-7.08) p=0.031
Estimated mean change from baseline CD4+ T-cell count at week 48, 10^6 cells/L	-69.1 (95% CI -100.8--37.4)	-52.4 (95% CI -84.5--20.3)	-16.7 (95% CI -61.8-28.4) p=0.469
Estimated mean change from baseline CD4+ T-cell count at week 96, 10^6 cells/L	-80.9 (95% CI -112.7--49.2)	-90.9 (95% CI -123.1--58.7)	10.0 (95% CI -35.3-55.2) p=0.666
Estimated mean change from baseline CD8+ T-cell count at week 48, 10^6 cells/L	-98.7 (95% CI -128.2--69.1)	-81.8 (95% CI -111.7--51.8)	-16.9 (95% CI -59.0-25.2) p=0.432
Estimated mean change from baseline CD8+ T-cell count at week 96, 10^6 cells/L	-64.6 (95% CI -94.3--34.9)	-59.6 (95% CI -89.6--29.5)	-5.0 (95% CI -47.2-37.2) p=0.816
Anthropometric measures			
Estimated mean change from baseline weight at week 48, kg	2.62 (95% CI 2.23-3.02)	2.35 (95% CI 1.95-2.75)	0.27 (95% CI -0.28-0.83) p=0.334
Estimated mean change from baseline weight at week 96, kg	4.33 (95% CI 3.94-4.73)	4.35 (95% CI 3.95-4.75)	-0.02 (95% CI -0.57-0.54) p=0.957

ART-modifying adverse events reported were chronic kidney disease (SCT), pulmonary tuberculosis (CT), disseminated tuberculosis (CT) and two cases of suicidal ideation (both CT). Count of participants with a change in baseline ART regimen excludes any modification limited to dose of ART (2 CT participants had tuberculosis AE resulting in doubling dose of dolutegravir).

HR: hazard ratio; presented HRs are based on time to first event, adjusted for site.

All change from baseline estimates based on model adjusted for site. Mean baseline values in change from baseline model: baseline total cholesterol=3.36mmol/L; baseline eGFR=116.8mL/min; baseline CD4+ T-cell count=923 10^6 cells/L; baseline CD8+ T-cell count=749 10^6 cells/L; baseline weight=49.5kg

Figure 1 – CONSORT Diagram



SCT - short-cycle therapy; CT - continuous therapy; ITT - intention-to-treat; BL - baseline; VL - viral load

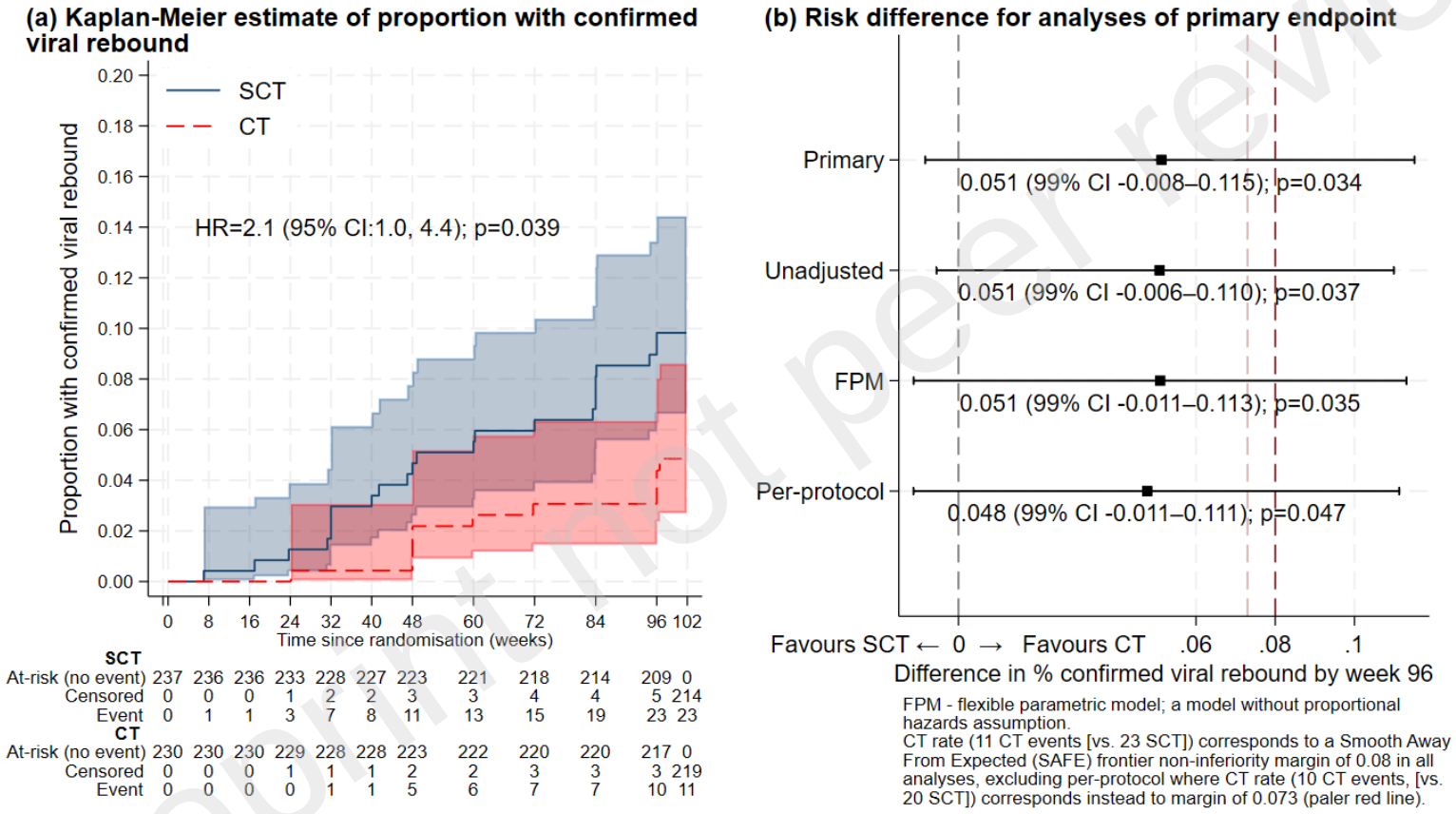
[1]Reasons for ineligibility (not mutually exclusive): 22 screening VL not <50 c/mL, 6 no consent/assent (participant aged 12-17), 5 previous treatment failure, 5 HIV-1 infection not confirmed, 5 moderate/high risk of suicide, 4 pregnant, 4 no consent (participant aged 18-19), 3 not all VL<50c/mL in last 12m, 1 randomisation >42d after screening, 1 most recent VL ≥12m prior to screening not <50c/mL, 1 not on highly effective contraception, 1 underlying medical condition, 1 not on DTG + TDF/TAF + 3TC/FTC for ≥1m prior to screening, 1 on treatment for active TB

[2]No participants who withdrew had met the primary endpoint

[3]1 further SCT participant withdrawn prior to week 96 was on CT at withdrawal

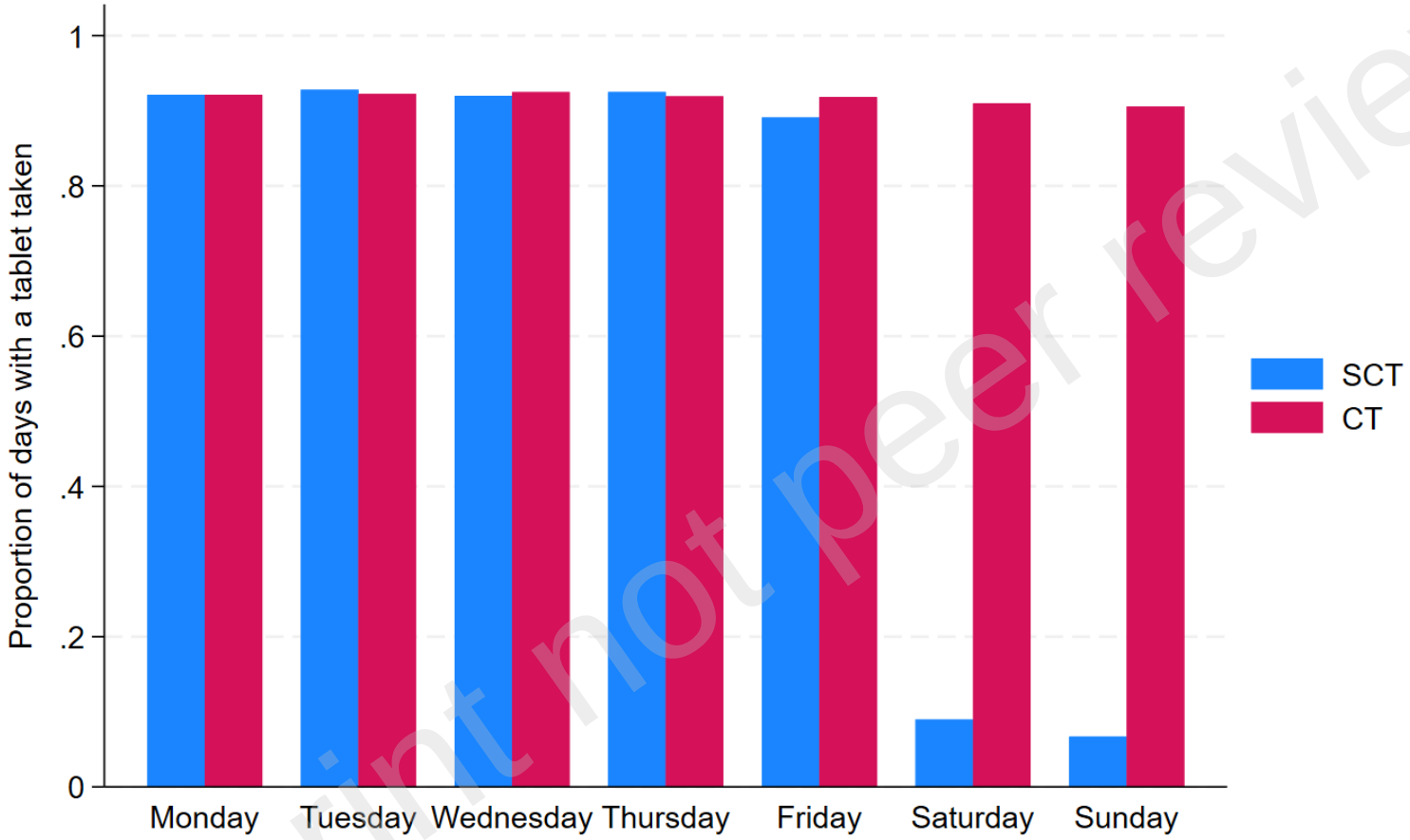
[4]Participants with a post-baseline VL result but withdrawn/died prior to week 96 contributed up to their last VL result

Figure 2 – Kaplan-Meier estimate of proportion of participants with confirmed viral rebound, and risk difference for analysis (primary, unadjusted, flexible parametric model and per-protocol) of the primary endpoint



FPM - flexible parametric model; a model without proportional hazards assumption.
CT rate (11 CT events [vs. 23 SCT]) corresponds to a Smooth Away From Expected (SAFE) frontier non-inferiority margin of 0.08 in all analyses, excluding per-protocol where CT rate (10 CT events, [vs. 20 SCT]) corresponds instead to margin of 0.073 (paler red line).

Figure 3 – MEMS cap sub-study, proportion of days with a tablet taken, by day of the week



Only 2 (2%) participants reported taking Friday and Saturday off instead of Saturday and Sunday (both options were permitted per protocol).