BREATHER Plus

A randomised open-label 2-arm, 96-week 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 dolutegravir/tenofovir-based triple ART in virologically suppressed HIV-infected adolescents aged 12 to 19 years of age in sub-Saharan Africa

Version: 3.0
Date: 24-Mar-2023

MRC CTU at UCL ID: BREATHER Plus
ISRCTN #: 85058577
PACTR# PACTR202103692694276
Authorised by:

Name: A/Prof Adeodata Kekitiinwa-Rukyalekere
Role: Trial Chief Investigator

Signature: DocuSigned by: Prof Adeodata Kekitiinwa-Rukyalekere 62AD2265A94B43D...

Date: 28-Mar-2023

Name: Dr Debbie Ford
Role: Trial Statistician

Signature: DocuSigned by: Debbie Ford C79DC5F84BBB461...

Date: 27-Mar-2023

Name: Professor Sarah L. Pett
Role: Project Lead at MRC CTU at UCL

Signature: DocuSigned by: Sarah Pett EA0A520EC2DA404...

Date: 27-Mar-2023

GENERAL INFORMATION

This document was constructed using MRC CTU at UCL Protocol Template Version 9.0. The CTU endorses the Standard Protocol Items: Recommendations For Interventional Trials (SPIRIT) initiative. It describes the BREATHER Plus trial, coordinated by the Medical Research Council (MRC) Clinical Trials Unit (CTU) at University College London (UCL), and provides information about procedures for entering patients/participants into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but sites entering patients for the first time are advised to contact the trial team to confirm they have the most up-to-date version.

COMPLIANCE

International sites will comply with the principles of GCP as laid down by the ICH topic E6 (R2) and other applicable national regulations. The national regulations for participating sites are detailed below:
Kenya: Pharmacy and Poisons Board (PPB); National Commission for Science, Technology, and Innovation (NACOSTI).
South Africa: South African Health Products Regulatory Authority (SAHPRA).
Uganda: Uganda National Council for Science and Technology (UNCST); National Drug Authority (NDA).
Zimbabwe: Medical Research Council of Zimbabwe (MRCZ); Medicines Control Authority of Zimbabwe (MCAZ); Research Council of Zimbabwe (RCZ).

SPONSOR

UCL is the trial Sponsor and has delegated responsibility for the overall management of the BREATHER Plus trial to MRC CTU at UCL. Queries relating to UCL sponsorship of this trial should be addressed to Professor Max Parmar, MRC CTU at UCL Director, MRC CTU at UCL, Institute of Clinical Trials & Methodology, 90 High Holborn 2nd Floor, London, WC1V 6LJ, UK.

FUNDING

The trial is funded by the European and Developing Countries Clinical Trials Partnership [RIA2017MC-2005]. The MRC Clinical Trials Unit at UCL is supported by funding from the MRC (MC_UU_12023/22 and MC_UU_00004/03).

AUTHORISATIONS AND APPROVALS

This trial was approved by Research Ethics Committees/Institutional Review Boards in each of the participating countries (Kenya, South Africa, Uganda, Zimbabwe) and the UK (required by the Sponsor, UCL, although no participants are being enrolled in the UK); and by all required regulatory authorities in participating countries.

SAE/NE REPORTING

SAE/Notable Events are reportable within 1 working day of site awareness. SAE/NEs (apart from straight forward pregnancy notable events) should be submitted via the Adverse Event eCRF on the trial database. Pregnancy Notable Events should be submitted via the Pregnancy NE eCRF on the trial database.

As a back-up, if it is not possible to submit events via the trial database within the timeframe stated above, the associated worksheets for the event should instead be emailed to the MRC CTU within 1 working day of site awareness. **Any worksheets sent by email must be encrypted or transferred using other secure methods.**
For all SAE/NEs (irrespective of the method of reporting) - please ensure that an acknowledgement of receipt is received from the MRC CTU.
MRC CTU email:
mrcctu.bplata@ucl.ac.uk

RANDOMISATIONS

To randomise, please access the online trial database system. Training will be provided for DIRECT RANDOMISATION ONLINE as part of the site initiation and database training.
There will be no manual randomisation option.

TRIAL REGISTRATION

This trial has been registered with the International Standard Randomised Clinical Trial Number Register, where it is identified as ISRCTN85058577, and the Pan African Clinical Trial Registry where it is identified as PACTR202103692694276**.**

TRIAL ADMINISTRATION

Please direct all queries to the BREATHER Plus Trial Manager at MRC CTU at UCL in the first instance using mrcctu.bplata@ucl.ac.uk; clinical queries will be passed to the Trial Physician via the Trial Manager.

SPONSOR SITE

MRC Clinical Trials Unit at UCL
Infections Theme
90 High Holborn,
London
WC1V 6LJ
UK

Switchboard: +44 (0)20 7670 4700
Email: mrcctu.bplata@ucl.ac.uk

SPONSOR/MRC CTU AT UCL STAFF AND AFFILIATES

UK, Project Lead, and Trial Physician:	Professor Sarah L. Pett	Email:	s.pett@ucl.ac.uk
Trial Physician	Alasdair Bamford	Email:	a.bamford@ucl.ac.uk
Co-investigator, Trial Physician:	Anna Turkova	Email:	a.turkova@ucl.ac.uk
Co-Investigator, Trial Physician	Diana Gibb	Email:	diana.gibb@ucl.ac.uk
Clinical Project Manager	Margaret Thomason	Email:	m.thomason@ucl.ac.uk
Data Manager	Alex Green	Email:	alexandra.green@ucl.ac.uk
Trial Manager:	Rebecca Dodds	Email:	rebecca.dodds@ucl.ac.uk
Trial Statistician:	Debbie Ford	Email:	deborah.ford@ucl.ac.uk
Delegated Trial Statistician:	Jessica Kirk	Email:	j.kirk@ucl.ac.uk
Policy Coordinator:	Annabelle South	Email:	a.south@ucl.ac.uk

RECRUITING SITE STAFF

UGANDA: Trial Chief Investigator and Country Principal Investigator A/Professor Addy Kekitiinwa-Rukyalekere

Lead Site: Baylor College of Medicine Children's Foundation-Uganda
Block 5 Mulago Hospital
P.O Box 72052
Kampala
Uganda

Trial Chief Investigator and Country Principal Investigator:	A/Professor Adeodata (Addy) Kekitiinwa-Rukyalekere	Tel: Email:	+256 77246 2686 akekitiinwa@baylor-uganda.org
Co-investigator:	George Patrick Akabwai	Email:	GAkabwai@baylor-uganda.org
Co-investigator:	Pauline Mary Amuge	Email:	Amuge@baylor-uganda.org

UGANDA: Other site(s)

Joint Clinical Research Centre (JCRC)
Kampala
Plot 101 Lubowa Estates, off Entebbe road, P.O. Box 10005
Kampala
Uganda

Principal Investigator:	Professor Cissy Kityo Mutuluuza*	Tel: Email:	+256 417 723008 ckityo@jcrc.org.ug
Co-investigator:	Victor Musiime	Email:	vmusiime@jcrc.org.ug
Substudy lead:	Henry Mugerwa*	Email:	hmugerwa@jcrc.org.ug

*Neuropsychiatric Toxicity substudy lead

ZIMBABWE: Country Principal Investigators Dr Mutsa Bwakura-Dangarembizi

Lead Site: University of Zimbabwe Clinical Research Centre (UZCRC)
Number 2 Allan Wilson
Belgravia
Harare
Zimbabwe

Principal Investigator:	Dr Mutsa Bwakura-Dangarembizi	Tel: Email:	+263 4 705 986 mbwakura@uz-ctrc.org dangas@zol.co.zw
Co-investigator:	Kusum Nathoo	Email:	knathoo@mweb.co.zw
Co-investigator:	Hilda Angela Mujuru	Email:	hmujuru@mweb.co.zw

KENYA: Country Principal Investigator Professor Abraham Siika

Lead Site: Moi University Clinical Research Center (MUCRC)
Chandaria Cancer and Chronic Disease Center
Moi Teaching and Referral Hospital
Nandi Road
P.O Box 4606-30100 Eldoret Kenya

Principal investigator:	Professor Abraham Siika	Tel: Email:	+254 721 280 785 amsiika@africaonline.co.ke
Co-investigator:	Winstone Nyandiko	Email:	nyandikom@yahoo.com

SOUTH AFRICA: Country Principal Investigator Professor Moherndran (Mo) Archary

Lead Site: Enhancing Care Foundation (ECF) - King Edward VIII Hospital
Durban International Clinical Trials Research Site, Enhancing Care Foundation
Parkhome, King Edward VIII Hospital
Umbilo Road, Congella
Durban
South Africa

Principal Investigator:	Professor Moherndran (Mo) Archary	Tel: Email:	+ 27 031-260 4813. Archary@ukzn.ac.za
Co-investigator:	Rosie Mngqibisa	Email:	rosie@ecarefoundation.com

OTHER RESPONSIBLE INDIVIDUALS

SOCIAL SCIENCE SUBSTUDY LEADS

*London School of Hygiene and Tropical Medicine
Keppel St
Bloomsbury
London
WC1E 7HT
UK

¥Africa Health Research Institute
Nelson R. Mandela School of Medicine
3rd Floor
K-RITH Tower Building
719 Umbilo Road
Durban
South Africa

∞Uganda Virus Research Institute
P.O.Box 49
Plot 51-59 Nakiwogo Road
Entebbe
Uganda

Co-investigator:	Janet Seeley*∞	Email:	Janet.Seeley@LSHTM.ac.uk
Co-investigator:	Sarah Bernays*	Email:	sarah.bernays@lshtm.ac.uk
Co-investigator:	Nothando Ngwenya¥	Email:	Nothando.Ngwenya@ahri.org
Co-investigator:	Tamlyn Seunanden¥	Email:	tamlyn.seunanden@ahri.org
Co-investigator:	Stella Namukwaya∞	Email:	stella.namukwaya@mrcuganda.org

COMMUNITY SUPPORT/PATIENT AND PUBLIC INVOLVEMENT (PPI) LEADS

Fondazione PENTA ONLUS
Torre di Ricerca Pediatrica
Corso Stati Uniti
435127
Padova
Italy

Co-investigator:	Carlo Giaquinto	Email:	carlo.giaquinto@unipd.it
Co-investigator:	Magda Conway	Email:	magdasconway@gmail.com
Co-investigator:	Mercy Shibemba	Email:	mercy.shibemba@pentafoundation.org

HEALTH ECONOMICS SUBSTUDY LEADS

Centre for Health Economics
University of York
Heslington, York
YO10 5DD
UK

Co-investigator:	Paul Revill	Email:	paul.revill@york.ac.uk
	Simon Walker	Email:	Simon.walker@york.ac.uk

NB: throughout this document, “MRC CTU at UCL” is generally abbreviated to “CTU”.

SUMMARY OF TRIAL

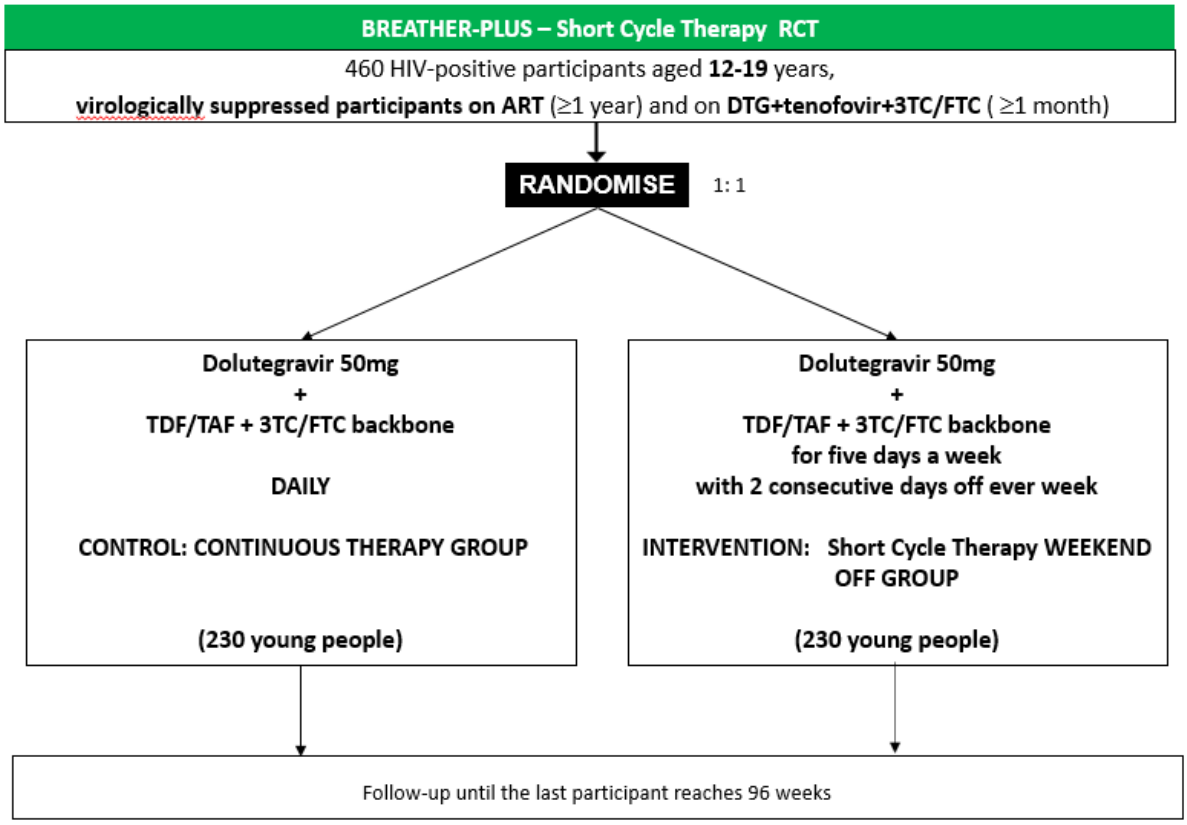
SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Acronym	BREATHER Plus
Long Title of Trial	A randomised open-label 2-arm, 96-week trial evaluating the efficacy, safety and acceptability of short cycle (5 days on, 2 days off) dolutegravir/tenofovir-based triple antiretroviral therapy (ART) compared to daily dolutegravir/tenofovir-based triple ART in virologically suppressed HIV-infected adolescents aged 12 to 19 years in sub-Saharan Africa
Version	3.0
Date	24-Mar-2023
ISRCTN #	85058577
PACTR #	202103692694276
Study Design	Open-label, randomised (1:1), multicentre, non-inferiority trial
Type of Participants to be Studied	HIV-infected, non-pregnant, non-breastfeeding adolescents aged 12 to 19 years of age, virologically-suppressed for at least one year, without any history of treatment failure, on 3-drug combination antiretroviral (ART) consisting of dolutegravir with a 2-drug NRTI backbone consisting of tenofovir and lamivudine/emtricitabine for at least 1 month. All participants will be recruited in sub-Saharan Africa
Setting	Kenya, South Africa, Uganda and Zimbabwe
Interventions to be Compared	**CT group:** Control group is continuous combination ART consisting of dolutegravir, with a tenofovir and lamivudine/emtricitabine backbone **SCT group:** Intervention group is short-cycle combination ART, consisting of dolutegravir, with a tenofovir and lamivudine/emtricitabine backbone. The SCT group will follow a cycle of 5 consecutive days on ART (Monday to Friday inclusive or Sunday to Thursday inclusive) and the same 2 consecutive days off every week (i.e. Saturday and Sunday, or Friday and Saturday)
Study Hypotheses	Dolutegravir-based SCT with a tenofovir and lamivudine/emtricitabine backbone will provide non-inferior sustained virological suppression compared to continuous dolutegravir-based ART with a tenofovir and lamivudine/emtricitabine backbone
Primary Outcome Measure(s)	The proportion of participants with confirmed virological rebound, defined as the first of 2 consecutive plasma HIV-RNA ≥50 copies/mL at any time up to the 96-week assessment

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Secondary Outcome Measure(s)	**Efficacy** (i) Proportion of participants with HIV-RNA ≥50 copies/mL at 48 and 96 weeks using a modified FDA snapshot algorithm (ii) The proportion of participants with HIV-RNA ≥1000 copies/mL (confirmed) by week 96 (iii) The number and type of HIV mutations at confirmed virological rebound (iv) HIV-RNA <50 copies/mL and no switch to second-line ART for treatment failure at 24, 48, 72 and 96 weeks
	Safety (i) Change in toxicity profile including change in metabolic parameters (lipids, HbA1c, phosphate), renal function (eGFR) from baseline to 96 weeks; change in anthropometric measures from baseline to 48 and 96 weeks (ii) Time to any new or recurrent WHO stage 3 or WHO stage 4 event or death (iii) Incidence of serious, grade 3, 4 and 5 and treatment-modifying adverse events (iv) The proportion of participants with any change from baseline ART regimen (v) Change in CD4+ and CD8+ T-cell count from baseline to 48 and 96 weeks
	Patient-reported outcomes (i) Adherence, acceptability, wellbeing and neuropsychiatric problems (e.g. depression, anxiety and sleep disturbance) (ii) Healthcare resource utilisation (as a sub-study outcome) (iii) Health-related quality-of-life (as a sub-study outcome)
Randomisation	Participants will be allocated 1:1 to one of the two groups
Number of Participants to be studied	N=460, with 230 in each group
Duration of Follow-up in the Trial	A minimum of 96 weeks per participant – individual follow-up will continue until the last participant reaches 96 weeks follow-up
Ancillary Studies/Substudies	Nested substudies: ▪ SCT pilot ▪ Adherence using the Medication Event Monitoring Systems (MEMSTM 6 TrackCap) ▪ Social science ▪ Neuropsychiatric toxicity ▪ Health economics
Sponsor	University College London (UCL)
Funder	EDCTP and the Medical Research Council (MC_UU_12023/26 and MC_UU_00004/03)

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
MRC CTU at UCL Project Leader	Professor Sarah L. Pett
Chief Investigator	Associate Professor Adeodata Kekitiinwa-Rukyalekere

TRIAL SCHEMA

Figure 1: Trial Entry, Randomisation and Treatment



Visits at screening, week 0 (randomisation), 4[Ω], 8, 16, 24, 32, 40, 48, 60, 72, 84, 96, and then every 12 weeks until the last participant reaches their 96 week visit.

[Ω]SCT group only

TRIAL ASSESSMENT SCHEDULE

Table 1: Trial Assessment Schedule

STUDY WEEK NUMBER	SCRN	RAND	0	1ϕ	2ϕ	3ϕ	4ϕ	4Ω	8	16	24	32	40	48	60	72	84	96	THEN EVERY¥
Clinical Assessments and dispensing requirements				PILOT				SCT only											
Informed consent/assent	•																		
Review eligibility	•	•																	
Demographics	•																		
Complete HIV viral load history and ART history	•	•																	
Complete medical history and HIV-1 infection confirmed		•																	
Contraception check	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
Clinical assessment		•					•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
Symptoms check		•					•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
Vital signs[a]			•						•		•			•				•	48 weeks and at close-out visit
Concomitant medication check and ART regimen review		•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
Adherence assessment with pill count				•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
AE and health utilisation assessment				•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
Dispense ART			•				•	•	•	•	•	•	•	•	•	•	•	•	12 weeks

STUDY WEEK NUMBER	SCRN	(RAND)	0	1ϕ	2ϕ	3ϕ	4ϕ	4Ω	8	16	24	32	40	48	60	72	84	96	THEN EVERY¥
Laboratory Assessments				**PILOT**				**SCT only**											
Biochemistry[b]			•															•	
Lipids[c]			•															•	
HbA1c			•											•				•	
Haematology[d]			•											•				•	
T cell bloods[e]			•											•				•	48 weeks
Real-time Plasma HIV-1 RNA[f]	•			•	•	•	(•)							•				•	48 weeks and at close-out visit
Urine pregnancy test[g]	•	•							•	•	•	•	•	•	•	•	•	•	12 weeks
Other Assessments				**PILOT**				**SCT only**											
Adherence questionnaire			•				•	•	•	•	•	•	•	•	•	•	•	•	12 weeks
Columbia-Suicide Severity Rating Scale (C-SSRS)	•	•							•		•			•		•		•	48 weeks, and at close-out visit
Mood survey[π]			•						•		•			•		•		•	48 weeks, and at close-out visit
Acceptability and wellbeing questionnaire (HATQoL)			•								•			•				•	48 weeks, and at close-out visit
QoL (EQ-5D)			•								•			•				•	And at close-out visit
MEMS caps (subset) rotating groups, gp 1 wk 8-wk to 32 ([1]); gp 2 wk 48 to wk 72([2])									•[1]	•[1]	•[1]	•[1]		•[2]	•[2]	•[2]			

STUDY WEEK NUMBER	SCRN	(RAND)	0	1ϕ	2ϕ	3ϕ	4ϕ	4Ω	8	16	24	32	40	48	60	72	84	96	THEN EVERY¥
Storage Samples≠				**PILOT**				**SCT only**											
Mandatory Plasma (10mL) stored for HIV RNA and/or potential resistance testing[f]			•				•	•	•	•	•	•	•	•	•	•	•	•	24 weeks and at close-out visit
Plasma (5mL) stored in case virological rebound detected[Σ]																			
Plasma 10mL (for bone, renal & inflammatory biomarkers) – optional/additional samples			•											•				•	
Urine for renal biomarkers* - optional/additional samples			•															•	
Estimated total blood draw per visit (in mL)	5		45	5	5	5	15	10	10	10	10	10	10	40	0	10	0	50	

a Vital signs: weight (not at W8), height (not at W8), waist circumference (not at W8), hip circumference (not at W8), resting pulse, sitting blood pressure

b Biochemistry: Creatinine in order to calculate eGFR (Cockgroft-gault formula) and Liver function tests (ALT, albumin, total bilirubin)

c Lipids (fasting (≥6 hrs) metabolic parameters): total cholesterol, HDL and calculated LDL cholesterol, triglycerides, and the phosphate

d Haematology: haemoglobin, white blood cells, absolute neutrophils, absolute lymphocytes, platelets

e T cell bloods: CD4 %, absolute CD4, CD8 %, absolute CD8, total lymphocytes

f Plasma HIV-1 RNA assays measured at local laboratories with a LLQ of <50 copies/mL, these assays at screening, week 48 and 96, and every 48 weeks (and more frequently if standard of care) until the last participant has reached 96 weeks, are run real time. If any real-time VL ≥50 copies/mL, participants will be recalled, and VL repeated at least 7 days after the rebound viral load ≥50 copies/mL with a plasma sample stored for possible HIV resistance testing; if the repeat viral load confirms viral rebound the following will apply: participants on SCT will return to CT; participants on the CT group will be managed as per local guidelines (SEE SECTION 5.5)

g Urine pregnancy test if female and of child bearing potential

π More detailed neuropsychiatric questionnaires are also used in the neuropsychiatric sub-study

ϕ **PILOT only:** Only in 15 participants in the control and SCT - HIV RNA, to be performed on Monday before 1st ART dose after weekend off for the SCT arm (this will be 72 hours after the last dose of ARVs for participants who take their medication in the morning; and approx. 60 hrs after the last dose if the participant takes their ARVs in the evening); HIV RNA will be run real time; a real-time HIV RNA sample at week 4 will only be run if the week 3 viral load is ≥50 copies/mL, this week 4 visit is denoted by **(Ω)**

¥ Participants will have a close-out visit within ±6 weeks of the last recruited participant reaching 96 weeks follow-up (with return for repeat viral load if the real-time viral load is ≥50 copies/mL when a plasma sample will be stored). The close-out visit will be their corresponding scheduled visit and will include all assessments required for that visit and any additional assessments as per table.

∑ At repeat visit for participants who are recalled following a real-time viral load which is ≥50 copies/mL (blood volumes are 5mL but are not drawn on the same day as the scheduled visit)

Ω Week 4 visit is performed in the SCT group **only**

€ All participants will be followed for a minimum of 96 weeks, and until the last participant has reached week 96

See the laboratory Manual of Operations for further details of laboratory related requirements. Appendix III also gives guidance on the blood draws, and the priority bloods in case of any difficulties with venepuncture.

LAY SUMMARY

BREATHER Plus is a study organised by an international group of researchers from the UK, Europe and Africa.

Background: The goal of HIV treatment is to make sure the HIV virus in the blood remains very low. This is called having an undetectable viral load (virological suppression). If this goal is achieved and sustained life-long, then people living with HIV can live a healthy life, with a normal life expectancy. However, it is challenging to take medication every single day for life. This may be an even bigger challenge for teenagers living with HIV. In an earlier study, called BREATHER, having weekends off HIV medicines using a combination that included an HIV treatment called efavirenz, was found to be safe and effective in HIV-infected young people. The young people taking part in the study also liked having weekends off their treatment.

What is the BREATHER Plus trial?
BREATHER Plus builds on what we found in the BREATHER study. But, instead of looking at weekends off efavirenz-based HIV treatment, we are looking at weekends off a different HIV treatment called dolutegravir.

In BREATHER Plus we will compare two different ways of taking HIV medicines that include the HIV medicine dolutegravir:

- **Short Cycle Therapy:** where people taking part will take all their HIV medicines during the week but stop taking them at weekends (either Friday and Saturday or Saturday and Sunday)
- **Continuous Treatment:** where people taking part will take all their HIV medicines every day without any interruptions

People joining BREATHER Plus will have an equal chance of being randomised to one of the two groups.

Who will be invited to join the BREATHER Plus trial?
We want 460 young people living with HIV from Kenya, South Africa, Uganda and Zimbabwe to be part of this trial. People taking part need to be 12 to 19 years old, HIV-1-infected, have undetectable HIV viral load for at least the last year, on combination antiretroviral therapy, and never have switched HIV medication in the past because of treatment failure. The combination antiretroviral therapy needs to include tenofovir, lamivudine (or emtricitabine) as the 'backbone' and dolutegravir. The participant needs to have been taking this 3-drug combination for at least a month before they can join. Also, participants can't join the trial if they are pregnant or breastfeeding. In order to join, adolescents who are at least 18 years old will need to sign a consent form. This describes what the trial is about and what is required of participants. Adolescents aged 12 to 17 years old will have to sign an assent form – this assent form describes what the trial is about and what is required of participants. The parent/guardian/carer of adolescents aged 12 to 17 years old will also need to sign a consent form. Each person taking part will stay in the trial for at least 96 weeks (about 2 years) although it may be longer, up to 4 years.

What will the trial involve?
Among the first people to enter the trial, 30 will be asked to come back every week for the first 4 weeks. There will be a blood test at each of these visits to measure the amount of HIV virus (viral load) in the blood. In general, people taking part in the trial will be asked to visit the clinic every 8 weeks in the first year and every 12 weeks in the second year, until the last participant has been in the trial for 96 weeks. We will ask questions about health, wellbeing and mood at some of the visits.

For girls who have started their periods, we will do a pregnancy test at each visit. At most visits, we will take a blood sample, some of which will be stored and looked at later to measure the amount of HIV virus in the blood. We will also take an additional blood and urine sample once a year, these stored samples will be used to look at the health of people's kidneys, heart and other organ systems. Everyone will get their viral load result once a year, like people not in the study. In a small group of people we will check how well their pills are being taken using a special pill bottle that triggers every time the bottle is opened and the pills are taken. These special bottles are called MEMSCAPS. The MEMSCAPS will be used in one small group of participants in the first year of the study for 6 months, and a different group in year 2 for 6 months. This is to closely monitor how well people manage to stick to taking their pills as they should in this trial. In addition to the main trial, there are two sub-studies (smaller studies within the main trial), which people taking part will be invited to take part in. Participants in BREATHER Plus will be invited to take part in a social science sub-study where there are interviews and focus groups to find out how people feel about HIV and their medication. In another sub-study, the mood (neuropsychiatric) sub-study, there are additional surveys to find out if young people are having problems with feeling down or worried or having sleep problems. These sub-studies require additional consent, so people can decide if they do or don't want to take part in these sub-studies, and whether someone chooses to join or not won't affect being in the BREATHER Plus trial.

What will the trial show us?

The trial will hopefully be able to show whether having weekends off dolutegravir-based HIV medicine with a 'backbone' that includes tenofovir and lamivudine/emtricitabine works as well as taking HIV medicines every day in HIV-infected young people. If the weekends off strategy works for dolutegravir-based HIV medicine partnered with the tenofovir and lamivudine/emtricitabine 'backbone', then we hope HIV guidelines will change to recommend this as an option for how people take this combination for their HIV treatment.

CONTENTS

ABBREVIATION

ABBREVIATION	EXPANSION
3TC	Lamivudine
AE	Adverse event
AR	Adverse reaction
ART	Antiretroviral therapy
ARV	Antiretroviral
AUC	Area under the curve
BID	Bis in die (twice a day)
BMI	Body Mass Index
CF	Consent Form
CHAI	Clinton Health Access Initiative
CI	Confidence interval
C-SSRS	Columbia-Suicide Severity Rating Scale
CT	Continuous Therapy
CTU	Clinical Trials Unit
DALY	Disability adjusted life-year
DBS	Dried blood spot
DTG	Dolutegravir
eCRF	Electronic Case Report Form
EDCTP	European Developing Countries Clinical Trials Partnership
EDTA	Ethylenediaminetetraacetic Acid
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration (US)
FDC	Fixed dose combination
FGD	Focus group discussions
f/ITAF	Emtricitabine or lamivudine with tenofovir alafenamide fumarate
FTC	Emtricitabine

ABBREVIATION	EXPANSION
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HBA1c	Haemoglobin A1c/glycosylated Haemaglobin
HDL	High-density lipoprotein
HIV	Human Immunodeficiency Virus
HSR	Hypersensitivity reaction
IAS-USA	International Antiviral Society-United States of America
IB	Investigator Brochure
ICER	Incremental cost-effectiveness ratio
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IDI	In-depth interviews
IDMC	Independent Data Monitoring Committee
IMP	Investigational medicinal product
ISF	Investigator Site File
INSTI	Strand transfer Integrase inhibitor
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention-to-treat
JCRC	Joint Clinical Research Centre
LDL	Low-density lipoprotein
LFTs	Liver function tests
MedDRA	Medical Dictionary for Regulatory Activities

ABBREVIATION	EXPANSION
MEMSTM	Medication Event Monitoring Systems
MOH	Ministry of Health
MOP	Manual of Operations
MRC	Medical Research Council
MRC CTU at UCL	Medical Research Council Clinical Trials Unit at University College London
MATE-1	Multidrug and Toxin Extrusion Transporter 1
NHB	Net Health Benefit
NRTI	Nucleoside reverse transcriptase inhibitor
NTD	Neural Tube Defects
OCT2	Organic Cation Transporter 2
OD	Once daily
OI	Opportunistic infection
PENTA	Fondazione Penta Onlus
PI	Principal Investigator
PIS	Patient Information Sheet
POPI	Protection of Personal Information
PPI	Patient and public involvement
PK	Pharmacokinetics
PTMG	Project Trial Management Group
QALY	Quality-adjusted life year
QMAG	Quality Management Advisory Group
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious adverse reaction
SCT	Short cycle therapy
SD	Standard deviation
SOP	Standard operating procedure

ABBREVIATION	EXPANSION
SmPC	Summary of Product Characteristics
SUSAR	Suspected unexpected serious adverse reaction
TAF	Tenofovir alafenamide fumarate
TB	Tuberculosis
TBA	To be appointed
TC	Total cholesterol
TDF	Tenofovir disoproxil fumarate
TFV-DP	Tenofovir-diphosphate
TLD	Fixed dose combination of TDF, lamivudine and dolutegravir
TLE	Fixed dose combination of TDF, lamivudine and efavirenz
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
TRU	Truvada – tenofovir disoproxil fumarate and emtricitabine
TSC	Trial Steering Committee
UAR	Unexpected adverse reaction
UCL	University College London
US	United States
UZCRC	University of Zimbabwe Clinical Research Centre
VL	HIV Viral load
WHO	World Health Organization
YPLHIV	Yong People Living with HIV
YTB	Youth Trial Board

1 BACKGROUND

1.1 IMPORTANCE AND RATIONALE

1.1.1 ADOLESCENTS WITH HIV-1-INFECTION

An estimated 2.1 million adolescents are living with HIV, with 85% of them in sub-Saharan Africa, and AIDS is a leading cause of death among adolescents in sub-Saharan Africa (1). The World Health Organization (WHO) has highlighted that the number of adolescents with perinatally-acquired HIV are increasing with improved survival on antiretroviral therapy (ART) and that, in addition, adolescents continue to be highly vulnerable to sexual acquisition of HIV-infection (2).

Adolescents have poorer treatment outcomes including higher loss to follow-up, lower treatment adherence, poorer virological suppression and higher mortality than older people living with HIV (3-7). Some of the adherence challenges in adolescents revolve around fear of disclosure associated with carrying/taking medication, HIV-stigma, and the burden of secrecy; this may be particularly difficult at weekends which are times for socialising with friends (8). Unstable lives, non-conducive to daily medication adherence, and relative lack of power in treatment decision-making also contribute to difficulties with daily-dosing (2).

In sub-Saharan Africa, there has been little research into strategies aimed at improving retention in care and adherence to ART in adolescents; and any studies have mostly focused on perinatally-infected adolescents, and provide no information on any differences in needs for perinatally- and horizontally-infected young people who have very different backgrounds and treatment experiences (9, 10). These findings highlight a need to develop novel strategies to increase the likelihood that adolescents will remain engaged in healthcare **and** virologically-suppressed on ART (11).

1.1.2 SHORT-CYCLE THERAPY

Small single-arm studies followed by randomised controlled trials (RCTs) in **adults** demonstrated that short cycle therapy (SCT), with 5- or 4-days-a-week ART, is sufficient to maintain virological suppression (12-19). In the BREATHER trial 199 (including 70 in Uganda) young people (aged 8-24 years) were randomised to SCT vs. Continuous Therapy (CT) with an efavirenz-600mg–based regimen (20, 21). By 144 weeks the estimated difference in viral load rebound (SCT-CT) was 1.9% (90% CI -6.6%, 10.4%; p=0.72), confirming the non-inferiority findings (margin 12%) at 48 weeks, and the durability of this approach (22). Immunological, safety and resistance profiles were similar between groups. Importantly, SCT participants expressed a strong preference for weekends-off ART, as it made socialising with friends much easier (20, 21); in a qualitative sub-study, SCT participants found SCT improved medication adherence during the week and reported relief from side-effects during weekends off (23). These studies are generally viewed as too small for guideline change, although the French HIV guidelines 2017 have endorsed a 4- or 5-days-a-week ART option in stable, virologically suppressed adults (24).

All SCT studies to date have included minimum 12-weekly viral load monitoring so results cannot be generalised to low- and middle-income countries with less frequent viral load monitoring. Additionally, most participants were taking efavirenz-based ART, thus results cannot be extrapolated to DTG-based regimens. The ANRS QUATUOR study, a France-wide RCT of CT vs. 4-days-a-week SCT which has enrolled predominantly middle-aged males reported the 48 week data in July 2019. The trial demonstrated virological non-inferiority of the 4 days on 3 days off per week approach vs. daily ART for 7 days a week, and 48% of the participants enrolled were on an integrase-inhibitor as the anchor drug (24). Further data, out to 96 weeks has shown the strategy is durable (25).

1.1.3 DOLUTEGRAVIR

DTG has been chosen as the third agent in the CT group (and, therefore, the experimental SCT group), because it has higher probability of virological suppression than other guideline-recommended third agents (= the 'gold standard' comparator) (26, 27), is safe, well tolerated (2, 27) has a high barrier to resistance (28) and critically, because it will be the preferred first-line ART in most of SSA by the end of this trial (29). WHO has now recommended DTG+2NRTI for first-line therapy in their guidelines (30) and >20 low- and middle-income countries have initiated DTG procurement, with CHAI predicting a 62% market share for DTG by 2022 (31).

Importantly, in September 2017 an agreement announced by South Africa and Kenya with UNAIDS, CHAI and BMGF allowed scale-up of generic DTG+tenofovir-based NRTI as a fixed dose combination costing just US$75/year, cheaper than efavirenz-based first-line ART (32).

While DTG SCT is yet to be assessed, DTG has the pharmacological attributes, with its very long effective intracellular half-life (33) and high genetic barrier to resistance (34) that make it a viable SCT option, especially when partnered with a tenofovir-containing backbone (given as tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide fumarate (TAF) partnered in fixed dose combination with lamivudine/emtricitabine, which too has a very long-intracellular half-life (60-180 hours) (32, 34).

1.1.4 DOLUTEGRAVIR AND NEURAL TUBE DEFECTS

Reproductive toxicology animal studies, including embryofetal development studies in rats and rabbits, showed no evidence of adverse developmental outcomes (34). However, in May 2018, a National Institutes of Health (NIH)-funded observational surveillance study in Botswana (TSEPAMO) evaluating the safety of ART regimens in pregnancy identified an increased risk of neural tube defects (NTD) amongst infants of women who initiated DTG-based regimens prior to pregnancy. The study reported 4 cases of NTD out of 426 infants in women who conceived on DTG (risk 0.9%), this compared to 14 out of 11,173 infants born to women receiving other (not DTG) regimens (risk 0.13%) (35). In a recent report including all data from August 2014 through to March 2021 there were 9 cases of NTD in 5860 deliveries where the mother was taking DTG at conception (0.15%), compared to 22 NTDs in infants of 22,475 women conceiving on non-dolutegravir regiments (0.11%), resulting in a non-significant estimated difference in NTD prevalence of 0.06% (95% CI -0.03 to 0.20) (36-38).

In the same study, there were two reported NTDs among 4581 deliveries in which the mother started dolutegravir during pregnancy (0.04%), compared to 87 NTDs among 119,630 deliveries (0.07%) in HIV-uninfected mothers (36), showing no increased risk of NTDs if dolutegravir was started post conception. The TSEPAMO study and other surveillance and reproductive studies are ongoing and more data on safety of dolutegravir for women of childbearing age and their infants are expected in the future.

Following these updated findings from Botswana (TSEPAMO) and other study data WHO has issued updated recommendations for the use of DTG-containing regimens in women of childbearing age. WHO guidelines now recommend DTG-containing regimens as the preferred option for first-line and second-line ART across all populations. There remains a need for close monitoring for pregnancy within the trial, to ensure pregnant adolescents return to daily ART where applicable, and that all pregnant adolescents receive appropriate advice and support. **Section 6.8** details how the risk of pregnancy will be minimised in girls who have reached menarche and enroll in the BREATHER Plus trial.

1.1.5 CONTINUOUS THERAPY AND SHORT CYCLE THERAPY – NUCLEOSIDE/NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITOR BACKBONE (N(T)RTI)

Tenofovir disoproxil fumarate (TDF) (39) with lamivudine (3TC) (40) or emtricitabine (FTC) (41) is the preferred 2NRTI backbone in adults. Recently, Gilead Sciences has developed a new prodrug of tenofovir, tenofovir alafenamide fumarate (TAF) (42). TAF has different mechanisms of excretion and a different pharmacokinetic profile than tenofovir-disoproxil-fumarate (TDF). Due to lower plasma exposure to tenofovir after intake of TAF versus TDF, TAF has smaller effects on bone mineral density than TDF (42); 48-week data in older adolescents confirms its short-term efficacy and safety (43).

In BREATHER Plus, participants will have the option, based on the availability, to take TDF or TAF with 3TC or FTC, preferably as a fixed-dose combination (FDC).

1.1.6 TB CO-INFECTION

Despite improved access to ART, TB remains a major co-infection in HIV-infected adolescents in low-income settings and a leading cause of morbidity and mortality, accounting for 30% of deaths in this population, with the highest burden in sub-Saharan Africa (44). Rifampicin is the cornerstone of drug-sensitive TB treatment; given drug-drug interactions between rifampicin and many antiretrovirals including DTG and TAF (45), specific dose adjustments need to be made. These are detailed in **Section 5.3.**

1.1.7 RELEVANT STUDIES UNDERWAY OR PLANNED

The BREATHER Plus Consortium is planning a concurrent RCT in the same population, the LATA trial (NCT05154747). LATA is exploring the safety, efficacy and tolerability of cabotegravir and rilpivirine long acting injectable dual therapy given every 8 weeks vs. CT with daily oral DTG-based triple therapy. Adolescents will only be able to enter one of these trials. Both trials together, will inform on novel approaches to ART in adolescents in SSA, and increase choice, with the ultimate aim of improving long-term outcomes for this population.

1.2 OBJECTIVES AND HYPOTHESES

The overarching objective of this trial is to evaluate an innovative and contemporary ART strategy in HIV-infected adolescents to provide choice for young people facing life-long treatment. Output from this RCT will provide evidence on efficacy, safety and acceptability of a novel treatment approach in HIV-infected adolescents in sub-Saharan Africa.

The BREATHER PLUS trial will evaluate the virological efficacy, safety, acceptability and Quality of Life of DTG-based Short-cycle Therapy with weekends off compared with Continuous Therapy with a DTG-based ART regimen, to optimise treatment for HIV-infected adolescents in sub-Saharan Africa. The backbone drugs will consist of tenofovir either as the TAF or TDF formulations partnered with either 3TC or FTC. Importantly for generalisability to low- and middle-income settings, the trial will be conducted using standard-of-care real-time viral load monitoring as recommended by the World Health Organization (currently annual in sub-Saharan Africa); with additional plasma samples taken for safety monitoring by the Independent Data Monitoring Committee (IDMC) but not returned to doctors/patients.

1.2.1 HYPOTHESES

Dolutegravir-based SCT (DTG SCT) with a tenofovir and lamivudine/emtricitabine backbone will provide non-inferior sustained virological suppression compared to continuous DTG-based ART with a tenofovir and lamivudine/emtricitabine backbone and will be superior with respect to secondary outcomes including toxicity, acceptability, and quality-of-life.

1.2.2 PRIMARY OUTCOME

The proportion of participants with confirmed virological rebound, defined as 2 consecutive plasma HIV-RNA ≥50 copies/mL at any time up to the 96-week assessment.

1.2.3 SECONDARY OUTCOMES

1.2.3.A EFFICACY

(i) Proportion of participants with HIV-RNA ≥50 copies/mL at 48 and 96 weeks using the FDA snapshot algorithm

(ii) The proportion of participants with HIV-RNA ≥1000 copies/mL (confirmed) by week 96

(iii) The number and type of HIV mutations at confirmed virological rebound*

(iv) HIV-RNA <50 copies/mL and no switch to second-line ART for treatment failure** at 24, 48, 72 and 96 weeks

*virological rebound is defined as two consecutive HIV-RNA ≥50 copies/mL. As obtaining resistance data is challenging when viral loads are very low, resistance testing may need to be restricted to samples with a higher VL where the chances of being able to sequence are greater; this will also be dependent on available technologies for testing.

**see Section 5.5 for more information

1.2.3.B Safety

(i) Change in toxicity profile including change in metabolic parameters (lipids, HbA1c phosphate), renal function (eGFR) from baseline to 96 weeks; change in anthropometric measures from baseline to 48 and 96 weeks

(ii) Time to any new or recurrent WHO stage 3 or WHO stage 4 event or death

(iii) Incidence of serious, grade 3, 4 and 5, and treatment-modifying (of any grade) adverse events

(iv) The proportion of participants with any change from baseline ART regimen

(v) Change in CD4+ and CD8+ T-cell count from baseline to 48 and 96 weeks

1.2.3.C PATIENT-REPORTED OUTCOMES

(i) Adherence, acceptability, wellbeing and neuropsychiatric toxicities and neuropsychiatric problems (e.g. depression, anxiety and sleep disturbance)

(ii) Healthcare resource utilisation (as a sub-study outcome)

(iii) Health-related quality-of-life (as a sub-study outcome)

1.2.4 SUB-STUDIES

Recognising the cost of conducting large trials, multiple parallel sub-studies are nested within the trial, and these include:

- **i.** Social science sub-study which will quantitatively and qualitatively assess adherence, acceptability and well-being among trial participants;
- **ii.** Neuropsychiatric toxicity sub-study. Specific objectives include: To compare neuropsychiatric toxicities, including depression, suicidality, anxiety and sleep disturbance longitudinally between randomised groups; to test practical and feasible tools to identify and monitor mental health illness among adolescents in busy over-stretched HIV clinics
- **iii.** Health economics. Specific objectives are to assess the costs and cost-effectiveness of SCT compared to CT

Optional biological specimens (blood and urine) are being stored at specified time points (see **Table 1**); these can be exploited in future, for example to further understanding of renal, metabolic and bone health.

2 SELECTION OF SITES/CLINICIANS

The trial Sponsor has overall responsibility for site and investigator selection.

2.1 SITE/INVESTIGATOR INCLUSION CRITERIA

Once a site has been identified as being compliant with the inclusion criteria (and not excluded), the trial team will provide the site with a copy of this protocol, and Investigator Brochures for the study IMPs (these are defined in **Section 5**).

To participate in the BREATHER Plus trial, investigators and clinical trial sites must fulfil a set of basic criteria that have been agreed by the BREATHER Plus Trial Management Group (TMG) and are defined below.

2.1.1 SITE PI'S QUALIFICATIONS

1. The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial at their site and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the Institutional Review Board (IRB) and/or the regulatory authorities.

2. The investigator(s) should be thoroughly familiar with the appropriate use of the IMP, as described in the protocol, in the current Investigator Brochures for the study IMPs and in other information sources provided by the Sponsor.

3. The investigator(s) should be aware of, and should comply with, the principles of GCP and the applicable regulatory requirements. A record of GCP training should be accessible for all investigators.

4. The investigator(s)/site should permit monitoring and auditing by the Sponsor or delegate, and inspection by the appropriate regulatory authorities.

5. The investigator is responsible for supervising any individual or party to whom the investigator delegates trial-related duties and functions conducted at the trial site.

6. If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution should ensure this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.

7. The investigator(s) should maintain a delegation log of appropriately-qualified persons to whom the investigator(s) have delegated significant trial-related duties.

8. The investigator(s) should sign an Investigator Statement, which verifies that the site is willing and able to comply with the requirements of the trial.

2.1.2 ADEQUATE RESOURCES

1. The investigator(s) should be able to demonstrate a potential for recruiting the required number of suitable participants within the agreed recruitment period (that is, the investigator regularly treats the target population).

2. The investigator(s) should have sufficient time to properly conduct and complete the trial within the agreed trial period.

3. The investigator (s) should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

4. The investigator(s) should ensure that all persons assisting with the trial are adequately informed about the protocol, the IMPs (storage, handling, administration, dispensing), and their trial-related duties and functions.

5. The site should have sufficient data management resources to allow prompt data entry (refer to the Data Management Plan for timelines). Sites that have previously participated in CTU-coordinated trials should have a proven track record of good data entry.

2.1.3 SITE ASSESSMENT

Each selected clinical trial site must complete a number of registration documents including the BREATHER Plus Site Evaluation Form, Investigator Statement, Signature and Delegation of Responsibilities Log, and staff contact details. The Investigator Statement verifies that the site is willing, and able to comply with the requirements of the trial. This will be signed by the Principal Investigator at the site. In addition, and in compliance with the principles of GCP, all site staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to CTU. CTU must be notified of any changes to trial personnel and/or their responsibilities. An up-to-date copy of this log must be stored in the Trial Master File (TMF) at the site and also at CTU.

2.2 APPROVAL AND ACTIVATION

Site training will be performed prior to the activation of the site and will include all processes for the trial including but not limited to protocol training, data management procedures, procedures for handling of investigational medicinal product, adverse event reporting procedures, procedures for laboratory samples, and frequency and expectations for any monitoring visits. A log of attendees will be kept in the TMF as a record of participants present at all types of training events.
On receipt of all the necessary documents at CTU, and once initiation training has been completed, sites will be sent a 'green light form' to confirm their readiness to open to BREATHER Plus. The CTU will review this form and provide written confirmation to the PI when the site is able open to enrolment. The 'green light form', and associated documents listed, will verify:

1. The site should conduct the trial in compliance with the protocol as agreed by the Sponsor and, if required, by the regulatory authorities. This protocol must also have been given a favourable opinion by the IRB.

2. The site PI or delegate should document and explain any deviation from the approved protocol, and communicate this with the trial team at CTU.

A list of activated sites may be obtained from the CTU Trial Manager.

3 SELECTION OF PATIENTS

There will be **no exceptions** to eligibility requirements at the time of randomisation**.** Questions about eligibility criteria should be addressed prior to attempting to randomise the participant.

The eligibility criteria are the standards used to ensure that only medically appropriate patients are considered for this study. Patients not meeting the criteria should not join the study. For the safety of the patients, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, it is important that no exceptions be made to these criteria for admission to the trial.

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

3.1 INCLUSION CRITERIA

1. HIV-1-infected
2. Aged 12 to 19 years
3. Aware of HIV status
4. On ART for ≥1 year
5. No previous regimen change for treatment failure
6. On ART consisting of DTG, tenofovir and lamivudine/emtricitabine for ≥1 month prior to screening
7. Virologically suppressed with all HIV-1 RNA viral loads <50copies/mL[a] in the last 12 months up to and including screening. Additionally there must be one result <50copies/mL[a] at least 12 months prior to screening and the viral load at trial screening must be <50 copies/mL
8. Girls who are sexually active must be willing to adhere to highly effective methods of contraception[b]
9. Written informed consent provided by participant (if aged 18 to 19 years) and/or carer/legal guardian (if participant aged 12 to 17 years) as appropriate
10. Written informed assent in participants aged 12 to 17 years

3.2 EXCLUSION CRITERIA

1. Females who are pregnant or breastfeeding
2. Females who plan to become pregnant during the trial follow-up or are unwilling to use a highly effective method of contraception[b] for the duration of the trial if sexually active
3. Moderate or High risk score on the Columbia-Suicide Severity Rating Scale
4. On treatment for any active TB
5. Contraindication to continued receipt of dolutegravir or any formulation of tenofovir, lamivudine/emtricitabine
6. Underlying medical condition that in the opinion of the Investigator precludes participation
7. Previous randomisation in the LATA trial

[a]If a historic viral load is from a diluted sample (maximum dilution 1:5), and below lower limit of quantification (LLQ), a calculated VL<100 copies/mL is allowed; if the viral load in the diluted sample is equal to the LLQ, the calculated VL should be below 50 copies/mL. If there are any viral loads measured on dried blood spots since the most recent viral load on plasma more than 12 months ago these must be below the LLQ for the assay used. **The screening sample viral load must always be <50 c/mL and cannot be done using a dry blood spot.**

[b]Highly effective contraception are injectable, implantable, oral and intrauterine contraceptives which have an expected failure rate <1% per year

3.3 NUMBER OF PARTICIPANTS

We aim to enrol 460 adolescents.

3.4 CO-ENROLMENT GUIDELINES

Participants will not ordinarily be permitted to participate in any other clinical intervention trial or research protocol whilst participating in the breather plus trial. Participation in other studies that do not involve an intervention may be acceptable, but this should be discussed first with the BREATHER Plus TMG via the CTU. Participants who have been randomised into BREATHER Plus are excluded from participating in the LATA trial.

3.5 SCREENING PROCEDURES & PRE-RANDOMISATION INVESTIGATIONS

3.5.1 CONSENT/ASSENT PROCEDURES

At or prior to the screening visit, participants and/or their parents/carers/guardians will be given an information sheet about the BREATHER Plus trial (a Patient Information Sheet (PIS)). Having been given adequate time to consider trial participation, and to ask any questions, participants and/or their parents/carers/guardians will be asked to give written informed consent/assent to the trial. If the adolescent is 18 years and above they are able to give consent to participate in the study without a carer's consent and will be approached directly to provide written informed consent. For younger adolescents, aged 12 to 17 years, the appropriate carer will be asked to give written informed consent. Younger adolescents will also be given trial information and counselling appropriate to their age and will be asked specifically to assent to participate in the trial. If the adolescent does not assent they cannot participate.

Written informed consent to join the trial and be randomised must be obtained from participants or their parents/carer/legal guardians (as appropriate) after they have received an explanation of the aims, methods, benefits and potential hazards of the trial and **before** any trial-specific procedures are performed or any blood is taken for the trial. It must be made completely and unambiguously clear that the participant (or parent/carer/legal guardian of a child) is free to refuse to participate in all or any aspect of the trial at any time and for any reason, without incurring any penalty or affecting their treatment (or that of their child). Consent must be sought again if/when a child's carer changes.

Signed consent forms must be kept by the investigator in the Investigator Site File (ISF), a copy placed in the participant's clinical records and a copy given to the participant or appropriate carer. The date of consent will also be documented on the appropriate trial worksheet/eCRF.

If a subject aged 18 years and above, is unable to read, an impartial witness should be present during the entire informed consent discussion. In this situation, the written informed consent form and any other written information will be read and explained to the subject. After the subject has orally consented to their participation in the trial, they should (if capable) sign and date the informed consent form. Following this, the witness should sign and personally date the consent form.

If a legally acceptable representative (as per ICH GCP E6(2)) for a participant aged 12 to 17 years is unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject's legally acceptable representative has orally consented to the subject's participation in the trial, they should (if capable) sign and date the informed consent form. Following

this the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject and/or the subject's legally acceptable representative, and that informed consent was freely given by the subject or the subject's legally acceptable representative.

The same process should be followed for a subject aged 12 to 17 years if they are unable to read, but need to provide their assent to participate.

3.5.2 SCREENING PROCEDURES

Screening procedures are described in **Table 1**, and include consent/assent, assessment of eligibility, complete medical and HIV history and a blood draw for HIV viral load. For female participants, menarche history and contraception use will be elicited and a urine pregnancy test (in all females who have reached menarche) will also be conducted. The Columbia-Suicide Severity Rating Scale should be completed at the screening visit. Anyone with a moderate or high score is excluded from participating/being eligible for the trial. They should be managed according to standard of care, with the greatest sense of urgency for anyone scoring 'high', indicating they are in imminent danger of attempting suicide.

At the screening visit the adolescent will be allocated a trial ID number. The trial register will record all persons who are screened for the trial. The register will be kept in a secure place in each clinical site and must be available for monitoring, audit and inspection. The management of the register will be the responsibility of the Principal Investigator at that site.

Screening forms should be entered on the trial database before the randomisation visit. Carers and adolescents will be given an appointment for a randomisation visit within 42 days of the screening visit; participants should be informed that they need to come to the randomisation visit fasted (no intake other than plain water for at least 6 hours).

If a participant who was found to be eligible at screening does not attend their randomisation visit within 6 weeks, they must be re-screened. Re-screening is also allowed if:

- A condition making the participant ineligible during screening has now resolved e.g. a medical condition or TB treatment previously causing ineligibility has resolved
- A participant who was identified as ineligible due to a moderate or high score on the screening or randomisation CSSRS questionnaire is no longer ineligible based on CSSRS - re-screening can only take place ≥3 months later (and only if the Investigator thinks it is safe to do so)

Participants will not ordinarily be permitted to participate in any other clinical intervention trial or research protocol whilst participating in the BREATHER Plus trial. Participation in other studies that do not involve an intervention may be acceptable, but this should be discussed first with the BREATHER Plus TMG via the CTU. Participants who have been randomised into BREATHER Plus are excluded from participating in the LATA trial.

4 RANDOMISATION

4.1 RANDOMISATION VISIT

Before randomisation the Investigator(s) should ensure the participant meets all eligibility criteria. This includes the participant having all screening blood tests completed and reviewed prior to randomisation. The randomisation visit may be scheduled at any time between 1 and 42 days (within 42 days) after the screening visit, providing that all laboratory test results from the screening visit are available, and the participant/carer feels they have had adequate time to consider trial participation. The 6 week window is to allow for screening HIV viral load results to be available in all sites, and for female participants to commence highly effective contraception if they are of child-bearing potential (i.e. have reached menarche and are sexually active). Highly effective contraception is a method(s) with an expected failure rate <1% per year; more details are found in the Manual of Operations. Highly effective contraception must be used in all sexually active girls who have reached menarche throughout the trial to avoid pregnancy.

Consent (and assent where applicable as described in **Section** Error! Reference source not found.) for randomisation into the trial must be verbally re-confirmed at the randomisation visit.

The assessments required on the day of randomisation (Day 0) will be performed as summarised in **Table 1** and are described in the section below.

4.2 RANDOMISATION PRACTICALITIES

The adolescent and their parent/carer/guardian (for participants aged 12 to 17 years of age) should be physically present together with a study clinician in the site clinic at the time of randomisation.

Further details on the method of randomisation can be found in **Section 9.1.** To randomise a participant the appropriate eCRFs must be completed on the trial database, accessible from the local clinical sites. The database will automatically check the participant's eligibility status. Only those with completed screening and randomisation eCRFs on the database will be able to be randomised. More details on this process are included in the trial MOP. The generated randomisation lists will be securely incorporated within this web-enabled trial database, and allocation concealed until the point of the current randomisation. The details of the participant's treatment allocation will be notified to the trial team at the clinical site via the online trial database.

If the main electronic randomisation system is not working, randomisations will not take place and the randomisation visit will need to be rescheduled. There will be no manual randomisation option.

The participant's open-label treatment allocation and the date of randomisation should be entered into the trial register at the site. The clinician should complete a prescription with the participant's details and trial medications as allocated. The prescription will be for 4 weeks for those randomised to the SCT group, and for 8 weeks for those randomised to the CT group. The pharmacist or pharmacy technician should ensure that the participant (and carer if appropriate) knows how to take the drugs before they leave the clinic.

4.2.1 ENROLMENT OF DIFFERENT MEMBERS OF THE SAME FAMILY/HOUSEHOLD INTO THE TRIAL

It is possible that multiple individuals from the same family/household will enter the BREATHER Plus trial at the same time. Where clinic staff are aware of this we will ensure that all family/household members are randomised to the same treatment group.

5 TREATMENT OF PARTICIPANTS

BREATHER Plus is a strategy study, exploring whether triple ART consisting of DTG with a tenofovir and lamivudine/emtricitabine backbone with 2 consecutive days without ART ("weekends off"), the SCT group, is as safe, effective and acceptable as taking daily ART (CT group) in virologically suppressed, non-pregnant, non-lactating adolescents aged 12 to 19 years.

The trial Investigational Medical Products (IMP) are as follows:

- Dolutegravir 50mg oral with tenofovir disoproxil fumarate (245mg)* co-formulated with either lamivudine (300mg) or emtricitabine (200mg) (TRU) oral in a fixed dose combination or separately

or

- Dolutegravir 50mg oral with tenofovir alafenamide fumarate (25mg) co-formulated with emtricitabine (200mg) or lamivudine (300mg) (f/ITAF) oral in a fixed dose combination or separately

Study IMP will be locally sourced through national programs.

*Tenofovir Disoproxil Fumarate (TDF) 300 mg (equivalent to 245mg of tenofovir disoproxil).

5.1 ANCHOR DRUG (DOLUTEGRAVIR)

The anchor drug in BREATHER Plus is dolutegravir. All participants must have been on DTG with the backbone of tenofovir and lamivudine/emtricitabine for at least 1 month prior to screening.

5.1.1 DTG (DOLUTEGRAVIR)

The Fixed Dose Combination (FDC) of DTG 50mg with a tenofovir-based backbone (see **Section 5.2** below) will be provided. The study drug must be stored in the original package in order to protect from moisture, and the bottle tightly closed. There is no required temperature storage condition for this product.

5.2 NRTI BACKBONE

All participants in the SCT and CT groups will use tenofovir (either TAF or TDF) plus 3TC/FTC as the backbone. The NRTI backbone will be provided as a FDC with dolutegravir, as described above.

5.3 INCIDENT TB MANAGEMENT

People with active TB are excluded from the study and should not be screened. Whilst the participant population is virologically suppressed and well, it is possible that incident TB may occur during trial follow-up. If incident TB occurs after enrolment on BREATHER Plus, and rifamycin (i.e. rifampicin) is used as part of the treatment, the participant should remain on the trial. Participants in the SCT group must return to daily therapy without any weekends off ART, until a minimum of 14 days after cessation of rifampicin, when they may restart SCT at their clinician's discretion. The following changes to ART must take place in both trial groups as detailed below.

5.3.1 DTG RECEIVED DURING TB TREATMENT:

The dose of DTG should be doubled, i.e. 100mg/day taken as DTG 50mg BID, for the duration of the rifampicin treatment, and for 14 days after the rifampicin has ceased.

5.3.2 NRTI BACKBONE RECEIVED DURING TB TREATMENT:

Participants on TAF (either taken separately or as part of a FDC) should be discussed on a case-by-case basis, as the guidelines in regards to management of the dosing of TAF during and post the cessation of rifampicin are evolving. The dose of 3TC or FTC does not need to be increased during TB-treatment.

5.3.3 COMPLETE ART-REGIMEN DURING TB TREATMENT

The dosing of the ART regimen is as follows:

- If participants are taking the Fixed Dose Combination of tenofovir disproxil fumarate (TDF) + 3TC or FTC + DTG they take one tablet of this FDC daily, and an additional DTG 50mg dose approximately 12 hours later. This means they will be taking DTG 50mg BID

Further details are found in the main and pharmacy Manual of Operations.

5.4 OPPORTUNISTIC INFECTION PROPHYLAXIS

The use of opportunistic infection prophylaxis should follow local guidelines.

5.5 MANAGEMENT OF VIRAL REBOUND, AND VIRAL FAILURE OF FIRST-LINE REGIMEN

Participants on SCT who have two consecutive HIV-RNA ≥50 copies/mL must return to CT, with additional adherence counselling. They may stay on their current regimen or may require a treatment change, at the discretion of the treating clinician. In general, participants on CT should be treated according to WHO/country-guidelines, which currently advise that a second-line regimen may be required where a patient has had adherence counselling and their HIV-RNA is confirmed ≥1000 copies/mL. The choice of second-line antiretroviral therapy should follow local guidelines. Second-line drugs must be taken daily with no weekend breaks.

5.6 DISPENSING OF ALL TRIAL MEDICATION AND ACCOUNTABILITY

Throughout the trial participants, or their parents/carers/guardians (as appropriate), will be provided with a supply of all antiretroviral drugs sufficient to last until their next clinic visit. Participants, or their carers, will be requested to return/bring all empty antiretroviral bottles, any bottles in use and any unused bottles to all follow-up clinic visits. Drugs will be provided for the trial as tablets. On no account should any drug assigned to a participant be used by anyone else. Any unused drug must be returned to the site if a participant withdraws from treatment.

All drug dispensed and returned to the site should be documented on a trial accountability/dispensing log. At each site, a named person (trial pharmacist or research nurse) will be required to maintain complete records of all study medication dispensed.

Procedures for drug labelling, accountability and destruction will be detailed in the BREATHER Plus Pharmacy Manual of Operations. CTU will monitor drug accountability at site visits.

5.7 OVERDOSE OF TRIAL MEDICATION

All participants should be counselled about the importance of taking the medications as prescribed. It is particularly important that participants understand how many pills of which type they should be taking. Participants must be told to come to the clinic immediately (during working hours; and to

attend emergency services if after hours) if they take too many pills, on the days they are meant to be taking pills i.e. every day of the week for the CT group, and 5 consecutive days per week with 2 days off in the SCT group. If a participant in the SCT group accidentally takes ART during the 2 days off, this does not constitute an 'overdose' of trial medication, providing the actual number of doses taken does not exceed what is recommended. As no specific adverse consequences of overdose with any of the trial medications have been described, participants will be managed on a case by case basis.

5.8 DOSE MODIFICATION, SUBSTITUTIONS, INTERRUPTION AND DISCONTINUATION

Toxicity will be managed in all randomised groups according to standard clinical practice. Blood tests, additional to those described in the trial schedule, may be requested at any time for clinical management of the participant. Wherever possible, any side-effects will initially be managed by symptomatic measures and administration of appropriate (non-contraindicated) medication. In particular, Grade 1-2 gastrointestinal side-effects, such as nausea (with or without vomiting), and diarrhoea will be managed by anti-emetics and or anti-diarrhoeal agents in the first instance. Interruption of or changes in ART will be avoided except in the event of Grade 3 or 4 toxicity that is considered at least possibly related to one or more of the ART drugs. Wherever possible, alternative ART drugs will be substituted from the same class.

Any substitutions away from tenofovir in the backbone, or the DTG anchor drug will necessitate a return to daily ART without any weekend breaks for SCT participants. Lamivudine and emtricitabine are considered interchangeable in this trial, so if lamivudine is substituted for by emtricitabine or vice versa, and the tenofovir and DTG continue, then the participant can continue on SCT, if that is what they were randomised to. More details on ART substitutions are provided in the Manual of Operations.

Alternate explanations for clinical or laboratory abnormalities that may at first appear to be related to a specific drug should be sought. The use of reduced doses of any drug is absolutely contraindicated.

Management of any other adverse events/toxicity should generally follow the criteria below, but clinicians should refer to the IB and use their clinical judgement as to the best management for the individual participant. Adverse events (clinical and laboratory) will be graded using the DAIDS (Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1) toxicity grading scale (Appendix I), with a minor modification of the neutrophil count grading to reflect norms in the African HIV infected population.

- Grade 1:
  - Continue study drugs
  - Routine monitoring
  - Manage using symptomatic measures and other concomitant medication, if appropriate

- Grade 2:
  - Continue study drugs
  - Manage using symptomatic measures and other concomitant medication, if appropriate
  - Work-up to exclude other causes

- Grade 3 or 4:
  - Request laboratory investigations if relevant, obtain repeat confirmatory laboratory results within 72 hours

- Continue study drugs pending receipt of the confirmatory laboratory tests/repeat observations, unless immediate need to substitute
- Work-up to exclude other causes
- Following confirmation of toxicity, and if there is no other obvious cause:
  - If not too sick, substitute immediately
  - Otherwise, stop all drugs and restart with substituted drugs when better

Although a complete halt of all ART may be required, the duration of time off ART should be minimised in order to reduce the risk of HIV viral rebound.

5.8.1 INFORMATION ON SPECIFIC ART DRUG TOXICITIES

The details below are for information, and do not constitute the reference safety information (RSI) for the trial. The RSI will be included within the Investigator Brochures for the study IMPs.

DTG (34)**:** The most commonly reactions were nausea (13%), diarrhoea (18%) and headache (13%). Neuropsychiatric AEs as listed below are common. Weight gain has been reported for all antiretroviral therapy including dolutegravir. In addition, DTG inhibits the renal organic cation transporter 2 (OCT2) and multidrug and toxin extrusion transporter 1 (MATE-1). Due to this inhibition, the tubular uptake of creatinine from the blood is decreased (creatinine secretory fraction dependent on OCT2 and MATE-1 transport), leading to increases in serum creatinine and decreased eGFR or creatinine clearance, without changing true GFR. A 10-14% decrease of creatinine clearance was observed in adult studies (34). These changes are not considered to be clinically relevant since they do not reflect a change in true GFR.

TAF (42) **and TDF** (39): The most frequently reported adverse reactions were headache (12%), nausea (6%), and fatigue (6%) for TAF.

3TC (40): Fatigue; fever; malaise; headache and reported as very common. .

FTC (41): In clinical trials of HIV infected adults, the most frequently occurring adverse reactions were diarrhoea (14.0%), headache (10.2%), elevated creatine kinase (10.2%) and nausea (10.0%). In addition to the adverse reactions reported in adults, anaemia (9.5%) and skin discolouration (31.8%) occurred more frequently in clinical trials involving HIV infected paediatric patients.

Table 2. Toxicities of the trial drugs.

TOXICITY				
DRUG	**VERY COMMON (≥ 1/10)**	**COMMON (≥ 1/100 TO < 1/10)**	**UNCOMMON (≥ 1/1,000 TO < 1/100)**	**RARE (≥ 1/10,000 TO < 1/1,000) OR VERY RARE (< 1/10,000)**
TLD (fixed dose combination of TDF, 3TC, DTG	Headache; Fatigue; fever; malaise; Nausea, diarrhoea, anorexia, dyspepsia	Decreases in renal function; proteinuria; renal insufficiency; increased creatinine; rash; itching; alopecia; raised creatine kinase; insomnia; anxiety; depression; abnormal dreams; cough; vomiting; Flatulence; Upper abdominal pain or cramps; abdominal pain; abdominal distension; dizziness; weight gain; weight change; Alanine aminotransferase (ALT) and/or Aspartate aminotransferase (AST) elevations;	Decrease in potassium in blood; Fanconi syndrome; lactic acidosis; renal failure; acute tubular necrosis; neutropaenia; anaemia; thrombocytopaenia; changes in fat distribution; accumulation of fat; loss of fat; lipodystrophy; arthralgia; myalgia; rhabdomyolysis; decreases in bone mineral density; osteoporosis; bone fractures Elevations of lipids; elevation of blood sugar; Suicidal ideation/thoughts*; suicide attempt (parasuicide)* *particularly in patients with a pre-existing history of depression or psychiatric illness. IRIS; hypersensensitivity; angioedema; allergic reaction; peripheral neuropathy; paraesthesia; pancreatitis; elevations of serum amylase and/or lipase; hypophosphataemia; hepatitis; transient elevations in liver function tests	Pure red cell aplasia; bone marrow failure; numbness and /or tingling of skin; acute hepatic failure; increased bilirubin; fatty liver; neural tube defects; dyspnoea

TOXICITY				
DRUG	**VERY COMMON (≥ 1/10)**	**COMMON (≥ 1/100 TO < 1/10)**	**UNCOMMON (≥ 1/1,000 TO < 1/100)**	**RARE (≥ 1/10,000 TO < 1/1,000) OR VERY RARE (< 1/10,000)**
DTG	Nausea, fatigue; diarrhoea and headache	Insomnia; anxiety; depression; abnormal dreams; Dizziness; Vomiting; Flatulence; Upper abdominal pain or cramps; abdominal pain; rash; itching (pruritus); Alanine aminotransferase (ALT) and/or Aspartate aminotransferase (AST) elevations transaminases; raised creatine kinase; weight gain; weight change; raised creatinine; rash; itching (pruritus);	Arthralgia; myalgia; rhabdomyolysis; decreases in bone mineral density; osteoporosis; bone fractures; hepatitis; elevations of lipids; elevation of blood sugar; suicidal ideation/thoughts*; suicide attempt (parasuicide)* *particularly in patients with a pre-existing history of depression or psychiatric illness; IRIS; hypersensensitivity; angioedema; allergic reaction	Acute hepatic failure; raised bilirubin; neural tube defects
3TC	Fatigue; fever; malaise; headache	Insomnia; cough; nasal symptoms; nausea; vomiting; abdominal pain or cramps; diarrhoea; rash; alopecia; arthralgia; Transient elevations of liver enzymes i.e. Alanine aminotransferase (ALT) and/or Aspartate aminotransferase (AST)	Neutropaenia and anaemia (both occasionally severe); thrombocytopaenia	Lactic acidosis; pancreatitis; elevation of lipase; pure red cell aplasia; peripheral neuropathy; angioedema; rhabdomyolysis; paraesthesia; hepatitis
FTC	Headache, diarrhoea, nausea	vesiculobullous rash, pustular rash, maculopapular rash, rash, pruritus, urticaria, skin discolouration (increased pigmentation); insomnia; abnormal dreams; hypertriglyceridaemia; hyperglycaemia; dizziness; elevated amylase; vomiting; abdominal pain, dyspepsia; allergic reaction; elevated creatine kinase; pain; asthenia; neutropaenia; elevated serum aspartate aminotransferase (AST) and/or elevated serum ALT, hyperbilirubinaemia	anaemia; angioedema	Lactic acidosis

TOXICITY				
DRUG	**VERY COMMON (≥ 1/10)**	**COMMON (≥ 1/100 TO < 1/10)**	**UNCOMMON (≥ 1/1,000 TO < 1/100)**	**RARE (≥ 1/10,000 TO < 1/1,000) OR VERY RARE (< 1/10,000)**
Tenofovir (TDF)	Headache; nausea; diarrhoea; anorexia; dyspepsia; hypophosphataemia; Asthenia	Vomiting; abdominal distension; flatulence; dizziness; fatigue; rash; itching; raised liver transaminases; upper abdominal pain or cramps; decreases in renal function; proteinuria; renal insufficiency; increased creatinine; hypokalaemia; Alanine aminotransferase (ALT) and/or Aspartate aminotransferase (AST) elevations;	decreases in bone mineral density; osteoporosis; bone fractures; IRIS; pancreatitis; elevations of serum amylase and/or lipase; Arthralgia; myalgia; rhabdomyolysis; Fanconi syndrome; renal failure; acute tubular necrosis, nephritis, nephrogenic diabetes insipidus; hepatitis; transient elevations in liver function tests; changes in fat distribution; accumulation of fat; loss of fat; lipodystrophy	Fanconi syndrome; lactic acidosis; angioedema; acute hepatic failure; increased bilirubin; fatty liver
Tenofovir (TAF)	Headache	Diarrhoea; vomiting; nausea; abdominal pain, abdominal distension; flatulence; dizziness; fatigue; rash; pruritus; Alanine aminotransferase (ALT) elevations; arthalgia;	IRIS; angioedema; urticaria	

[a]See **Section 1.1.4** and **Section 6.8**.

5.9 PROTOCOL TREATMENT DISCONTINUATION

In consenting to the trial, participants are consenting to a randomised strategy of DTG with a tenofovir and lamivudine/emtricitabine backbone given daily (CT group) or with weekends off (SCT), trial follow-up and data collection. However, an individual participant may stop the strategy early or be stopped early for any of the following reasons:

- Unacceptable toxicity or adverse event*
- Switching away from DTG and/or the tenofovir-based backbone*
- Inter-current illness that prevents further treatment*
- Any change in the patient's condition that justifies the discontinuation of treatment in the clinician's opinion*
- Inadequate compliance with the protocol treatment in the judgement of the treating physician*
- Withdrawal of consent for treatment by the participant or by the carer or withdrawal of assent by the participant.

*This would not be a reason to withdraw from the trial, the participant should continue with follow up visits

As participation in the trial is entirely voluntary, a participant may choose to discontinue the trial treatment at any time without penalty or loss of benefits to which they are otherwise entitled. Although the participant is not required to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason while fully respecting the participant's rights.

Participants should remain in the trial for the purpose of follow-up and data analysis (unless the participant withdraws their consent from all stages of the trial). See **Section 6.9** for details on early stopping of follow-up and withdrawal from follow-up. Further details are also available in the trial MOP.

5.10 COMPLIANCE & ADHERENCE

Participants in BREATHER Plus have been on DTG with tenofovir and lamivudine/emtricitabine triple therapy, for at least 1 month prior to study screening so we anticipate very low levels of non-compliance with the randomised strategy. Participants have also been on ART for at least 1 year, have no history of treatment failure, and are virologically suppressed; therefore adherence to ART is likely to be good. What may be a challenge for the study is ensuring that those randomised to SCT do adhere to 5-days on and 2 days off every week, especially as it is likely that daily adherence to ART (i.e. adherence 7-days a week) has been something that has been repeatedly reinforced. Additionally some participants in the CT group, having learned of the trial design/premise, may want to try weekends-off despite their randomised allocation. Suitable patient information (developed with young people) and fully informed consent/assent procedures will ensure that participants understand the trial requirements. All the clinical centres will provide adherence support and counselling, with participants in the SCT group having an extra early visit at 4 weeks. Participants will also be asked about missed doses and a pill count conducted at every study visit to monitor adherence. An adherence questionnaire will be completed to further monitor adherence at week 0, week 4 (pilot), week 4 (SCT group only), week 8 and every 8 weeks thereafter in the first year, and every 12 weeks in the second year, then at the close out visit.

An adherence sub-study will be carried out in a subset of 200 participants from selected sites (100 CT, 100 SCT). The Medication Event Monitoring Systems (MEMSTM 6 TrackCap) will be used to measure adherence to the protocol during weeks 8-32 and weeks 48-72, with the adherence caps devices rotating between participants as the study progresses. MEMScaps fit standard size medication bottles, and record the time and date of each opening as a presumptive dose. The Social Science qualitative sub-study (**Section 10.1**) will also capture information on adherence.

5.11 TREATMENT DATA COLLECTION

Information about all ART received by a participant, including formulation, frequency, dose and reasons for change will be documented in the participant's medical records and the Antiretroviral Log worksheet/eCRF for the trial.

5.12 NON-TRIAL TREATMENT

All necessary concomitant medications are allowed. If a medication with a known drug interaction to one of the trial medications is essential for a participant's management, then, if appropriate dose adjustment is not possible, the trial medication should be stopped and substituted with another ART combination (to avoid cessation of ART altogether), and the concomitant medication used. Where DTG or tenofovir are discontinued participants on SCT must return to continuous therapy.

6 ASSESSMENTS & FOLLOW-UP

See **Table 1** for a summary of all assessments and procedures.

All participants will be followed for at least 96 weeks. All participants will be seen at clinic at screening, week 0 (randomisation) and then at week 4 (SCT group only), week 8, and then every 8 weeks to week 48 and every 12 weeks thereafter, until the last participant reaches 96 weeks of follow-up. Once this milestone has been reached, participants would exit the trial and rollover into national ART programs. Visits will include evaluations from both physician/medical officers and nurses.

Trial visit schedules will be prepared for each participant at randomisation, and participants should be followed on the same schedule even if their trial medication is discontinued. The target dates for trial visits are determined by the date of randomisation and are not affected by subsequent events. Clinics may choose to re-schedule visits to allow for public holidays or other unavoidable circumstances that affect the scheduled visit date, but the re-scheduled visit should ideally be within +/- 7 days of the original scheduled visit date. If a participant is >7 days late for a trial visit, an unscheduled visit should be performed. Procedures and assessments conducted at the unscheduled visit should reflect those that should have been done at the missed visit.

Participants will be expected to attend on the scheduled day unless agreed in advance with the clinic. Participants will be given a card with the contact details for the trial research team so they can contact them if needed to re-arrange a visit. If they are unable to attend on the day, every effort should be made to complete the visit within 7 days of the scheduled date. If a scheduled visit is missed without notice then the clinic should endeavour to contact the participant by phone or by home visit.

The schedule of assessments (**Table 1**) defines the trial visits, necessary for data collection, but the participant may be seen more frequently for clinical care as needed, for example if the participant develops drug toxicity or other clinical events. At any such unscheduled visits, routine assessments as for a standard clinician and nurse visit will be performed. Other laboratory tests will be performed as clinically indicated.

Pregnancy testing in female participants who have reached menarche will be conducted every 8 weeks in the first year and every 12 weeks thereafter, for the duration of the trial. Pregnancy tests can also be performed outside of the mandated visits if indicated.

6.1 PILOT STUDY

Because there are no data on SCT with DTG, BREATHER Plus will include a pilot phase, which will be undertaken in 2-3 of the main clinical centres (which open first, are likely to recruit quickly and where HIV-RNA test results are available within 7 days). All procedures for Day 0 (randomisation) will be performed as detailed in **Table 1**.

Among the first participants randomised into the trial, 15 participants randomised to the SCT and 15 randomised to the control groups at these sites will have additional HIV viral load measurements at weeks 1, 2 and 3 (and a confirmatory viral load at week 4 following a single viral load above 50 copies/mL at week 3). If any participants in the in the SCT group do not have weekends off during the pilot phase, further participants will be included as needed. HIV viral load measurement in the SCT group will be performed after weekends off treatment. The independent IDMC will review these HIV

RNA assessments after all participants in the pilot phase have completed the pilot. This will be to determine whether the SCT group is safe to continue. Recruitment to the main trial will continue during pilot follow-up and review of the pilot data.

6.2 TRIAL ASSESSMENT SCHEDULE

See **Table 1** for a tabular summary of the details below. Once the last participant has reached their 96 week visit, all participants will exit the trial and rollover into national ART programs. This is likely to mean that those randomised to SCT will be expected to return to CT, until such time as SCT is incorporated into treatment guidelines, if BREATHER Plus demonstrates the efficacy and safety of this strategy.

6.2.1 AT THE RANDOMISATION/WEEK 0 VISIT THE FOLLOWING WILL BE PERFORMED

Randomisation:

- CSSRS ('triage' and 'past 2 months' versions)
- Urine pregnancy test
- Contraception check: use of highly effective contraception confirmed for sexually active female participants
- HIV-1 infection confirmed
- Screening HIV-1 RNA viral load <50copies/mL confirmed
- Medical history completed
- Concomitant medication check and ART history review
-
- Clinical assessment
- Symptoms check
- Eligibility assessed

Week 0:

- Haematology
- Biochemistry
- Lipids and phosphate
- HbA1c
- T-cell bloods
- Mandatory plasma sample storage
- Additional plasma sample storage (if consent/assent provided)
- Urine sample storage (if consent/assent provided)
- Vital signs
- HATQOL
- EQ5D
- Adherence questionnaire
- Mood survey
- ART dispensing

6.2.2 AT THE PILOT VISITS THE FOLLOWING WILL BE PERFORMED

Pilot Week 1:

- Real-time HIV VL test
- Adherence assessment with pill count
- Contraception check
- Adverse event and health utilisation assessment
- Concomitant medication check and ART regimen review

Pilot Week 2:

- Real-time HIV VL test
- Adherence assessment with pill count
- Contraception check
- Adverse event and health utilisation assessment
- Concomitant medication check and ART regimen review

Pilot Week 3:

- Real-time HIV VL test
- Adherence assessment with pill count
- Contraception check
- Adverse event and health utilisation assessment
- Concomitant medication check and ART regimen review

Pilot Week 4:

- Real-time HIV VL test (only if W3 VL result is ≥50 copies/mL)
- Adherence assessment with pill count
- Contraception check
- Adverse event and health utilisation assessment
- Concomitant medication check and ART regimen review

6.2.3 FROM WEEK 4 ONWARDS THE FOLLOWING WILL BE PERFORMED

Real-time HIV VL test: Weeks 48, 96 and then every 48 weeks (and more frequently if required by local guidelines, also for suspected treatment failure or at repeat visit following VL rebound)
Adherence assessment with pill count: Every visit
Contraception check: Every visit
Adverse event and health utilisation assessment: Every visit
Concomitant medication check and ART regimen review: Every visit
Haematology: Weeks 48 and 96
Biochemistry: Week 96
Lipids and phosphate: Week 96
HbA1c: Weeks 48 and 96
T-cell bloods: Weeks 48, 96 and then every 48 weeks
Mandatory plasma sample storage: Every visit up to week 96 and then every 24 weeks (also required if repeat visit for rebound VL or suspected treatment failure)
Additional plasma sample storage (if consent/assent provided): Weeks 48 and 96
Urine sample storage (if consent/assent provided): Week 96
Vital signs: Weeks 8 (weight, height, waist and hip circumference not required at W8), 24, 48, 96 and then every 48 weeks
HATQOL: Weeks 24, 48, 96 and then every 48 weeks
EQ5D: Weeks 24, 48 and 96
Adherence questionnaire: Every visit
Mood survey: Weeks 8, 24, 48, 72, 96 and then every 48 weeks
ART dispensing: Every visit (apart from Week 4 if sufficient supply dispensed at Week 0)
CSSRS (past 2 months): Weeks 8, 24, 48, 72, 96 and then every 48 weeks
Urine pregnancy test: Every visit (apart from Week 4)
Clinical assessment: Every visit
Symptoms check: Every visit
MEMSCap: Weeks 8, 16, 24 and 32 for group 1 and weeks 48, 60 and 72 for group 2

PHQ9, GAD-7 and Sleep questionnaires (part of neuropsychiatric toxicity sub-study): As indicated (i.e. selected for sub-study and eligible)

Study visits will take place every 8 weeks in the first year and every 12 weeks thereafter, until the last participant reaches week 96, reference **Table 1** for what clinical and pathology assessments need to be performed at these 12-weekly visits beyond week 96.

6.2.4 AT THE CLOSE OUT VISIT THE FOLLOWING WILL BE PERFORMED

When participants attend their close out visit, the site should complete all assessments according to their scheduled visit week. Close out visits should also include the following:

- CSSRS (past 2 months)
- Real time HIV VL (with a repeat VL where HIV-RNA ≥50 copies/mL)
- Vital signs
- HATQOL
- EQ5D
- Mood survey
- Urine pregnancy test
- Contraception check
- Mandatory plasma storage sample
- Clinical assessment
- Symptoms check
- Concomitant medication check and ART regimen review
- Adverse event and health utilisation assessment
- ART dispensing

Participants will have a close-out visit within ±6 weeks of the last recruited participant in 96 weeks follow-up (and always ≥96 weeks from randomisation). If the real-time viral load is ≥50 copies/mL at the close-out visit, participants will need to return for a confirmatory viral load and a stored sample for future resistance testing.

Sub-studies and procedure associated with each sub-study are detailed in **Section 10**.

6.3 PROCEDURES FOR ASSESSING EFFICACY

6.3.1 HIV VL TESTING (ALL PARTICIPANTS)

Plasma HIV VL will be measured in real-time at screening and weeks 48 and 96 (and 48-weekly thereafter) following WHO recommendations for annual VL monitoring, and results returned to clinicians for participant management. The mandated turn-around of VL results in the trial at weeks 48 and 96 (and 48-weekly thereafter) will be within 1 month. Participants with VL ≥50 copies/mL will be recalled back within the week 48 window (44-54 weeks), week 96 window (90-102 weeks) and 48 weekly (+/- 6 weeks of the scheduled visit date) thereafter to confirm their VL results. The repeat of the VL test, should be at least 7 days after the initial test result, and accompanied by intensified adherence counselling. 48 and 96 week visits should be arranged as close to the scheduled visit as possible (to allow for recall within the window if necessary). Clinicians should follow national guidelines if routine viral load testing is more frequent than annually, and must recall patients who have a real-time VL ≥50 copies/mL at any trial visit, ideally within ±6 weeks of the scheduled visit date). In addition, clinicians should request a real time VL for suspected treatment failure before switching a participant to second-line treatment if this occurs outside an annual visit, and plasma should additionally be stored for future resistance testing. VL will be assayed retrospectively in all

participants in batches of stored plasma samples for scheduled visits where a real-time VL was not measured.

All batched VL measurements done after randomisation will be performed using an assay platform within each country with lower limit of detection of no greater than 50 copies/mL in one or more designated laboratories. The IDMC will regularly review viral load measures during the trial (at the end of the pilot phase, after 6 and 12 months and then 6-12 monthly). These **additional** individual viral load test results will not be returned to doctors/patients following a public health approach, as the trial is designed to mimic the real-life setting in which it will be conducted.

A combination of retrospective batched viral loads and routine viral load test results are being used to monitor efficacy in the ODYSSEY trial (46) and completeness of viral load test results is very high (94% at the last IDMC, December 2019, Debbie Ford, personal communication).

6.3.2 Resistance testing (Subset of participants)

At the end of the trial, batched genotypic resistance testing will be performed retrospectively on stored samples from all participants who have met the primary outcome measure. Additional resistance tests will be performed on stored plasma samples in selected participants at the end of the trial to define in more detail the pattern of resistance development. As obtaining resistance data is challenging when viral loads are very low, resistance testing may need to be restricted to samples with a higher VL where the chances of being able to sequence are greater; this will be dependent on available technologies for testing.

All resistance test measurements will be performed by laboratories that have the most experience of HIV resistance testing and that have stringent quality assurance procedures in place. This will be within country wherever this is possible. Drug resistance mutations will be classified using the latest IAS-USA (47) definitions and drug susceptibility predicted using the latest version of the Stanford database algorithm (48).

The results of resistance tests performed in individual participants will be given to the treating physician when they become available, and after the last participant has completed 96 weeks follow-up.

6.3.3 CD4+ and CD8+ T-cell counts (All participants)

Blood will be collected at randomisation, weeks 48, and 96 and every 48 weeks after week 96, for determination of total and percentage CD4+ and CD8+ T-cell counts. These counts will be done using the standard assay in operation at each site laboratory according to quality-assured procedures.

6.3.4 Clinical Events (All participants)

A brief interim history, including information on hospital visits and AE assessment will be performed at each clinical visit. Where there is any clinical suspicion of a WHO Stage 3 or 4 disease event, sites will endeavour to investigate the participant to the fullest extent possible given local availability of imaging and laboratory investigations (particularly microbiology) in order to establish a clear diagnosis of the event. The list of these diseases and diagnostic criteria are provided in Appendix II**.**

6.3.5 Weight, Growth, Body Fat Distribution (All Participants)

All participants will have a targeted physical examination and vital signs (sitting blood pressure, resting pulse) at weeks 0, 8, 24, 48, and 96 and every 48 weeks thereafter. Anthropometric measurements (height, weight, waist circumference, hip circumference) will be measured at weeks

0, 24, 48, 96 and every 48 weeks thereafter. Vital signs and anthropometric measurements will also be collected at the close out visit.

6.4 PROCEDURES FOR ASSESSING SAFETY

The symptom checklist should be used to assess the interim medical history and AE. The checklist can become part of the source documentation completed by the nurse at each visit.

Blood will be drawn at specific trial visits to assess laboratory safety parameters as indicated in the schedule of trial assessments as described above and in **Table 1**. This includes full blood count, tests of renal and liver function, HbA1c, fasted lipids and phosphate. Urine samples for retrospective batched testing of protein:creatinine ratio, albumin:creatinine ratio, phosphate and other markers of renal function will be collected at weeks 0 and 96. Additional safety blood tests or investigations may be performed to investigate symptoms or monitor emergent laboratory test abnormalities as clinically indicated.

Adverse events (clinical and laboratory) will be graded using the DAIDS (Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1) toxicity grading scale (Appendix I), with a minor modification of the neutrophil count grading to reflect norms in the African HIV infected population. Laboratory measures (including any repeats) will all be captured on the database. Routine assessments will be graded automatically; although changes in creatinine will not be graded in themselves, instead creatinine will be used to calculate the estimated glomerular filtration rate (eGFR using the Cockcroft-Gault formula) – which more closely reflects renal function. Where the treating clinician believes a laboratory measure is clinically significant and it is grade 3 or 4 it should be reported as an AE.

6.5 PROCEDURES FOR ASSESSING ADHERENCE AND ACCEPTABILITY

Adherence to ART will be assessed in all participants at each visit by pill counts for tablets, and participants will complete a short adherence questionnaire at weeks 0, 8, and every 8 weeks in the first year and every 12 weeks thereafter, until the last participant reaches week 96, and at the close-out visit.

Acceptability and wellbeing questionnaires will be completed at weeks 0, 24, 48, 96 and every 48 weeks until the last participant reaches week 96, and at the close-out visit.

6.6 PROCEDURES FOR ASSESSING NEUROPSYCHIATRIC HEALTH

Strand transferase Integrase inhibitor (INSTI), including DTG, have been associated with neuropsychiatric toxicity, predominantly anxiety, depression and sleep disturbance including insomnia (49). The Neuropsychiatric (mood) questionnaire(s) and the Columbia suicidality questionnaire will be completed by the participants at weeks 0, 8, 24, 48, 72 and 96, and every 48 weeks until the last participant reaches week 96, and at the close-out visit, to assess any toxicity signals. The short mood questionnaire will be used to capture symptoms of depression, anxiety and sleep disturbance longitudinally in all participants, alongside clinical assessments for AEs. Additionally more extensive questionnaires will be used in a subset of participants, selected based on their responses to the short questionnaires and in participants who interrupt or switch off dolutegravir for neuropsychiatric toxicity or participant choice, allowing us to investigate and describe toxicities more comprehensively and to assess whether the more common toxicities are being identified by our simple screening questionnaires compared to the more detailed questionnaires being used in the Neuropsychiatric sub-study (**Section 10.2**).

The questionnaires will be administered/provided in the participant's own language by a member of the research team.

6.7 OTHER ASSESSMENTS

6.7.1 HEALTH ECONOMICS (ALL PARTICIPANTS)

Policymakers require information on the costs and health effects of alternative ART strategies when considering how to allocate limited resources to meet the population's health needs. We will estimate costs and cost-effectiveness of the trial's treatment strategies evaluated using a generic health measure (quality-adjusted life-years (QALYs)) to allow comparison with other interventions. Resource use and total costs from a health system perspective will be estimated using trial data and other sources (e.g. unit costs/prices) to be representative of general roll-out in African countries. Cost-effectiveness will be assessed using incremental cost-effectiveness ratios and compared to appropriate country specific cost-effectiveness thresholds.

A simple quality of life instrument (EQ-5D) will be used to estimate QALYs. Health care resource use including hospitalisations will be asked at every visit; concomitant medications are captured at every visit. Unit costs will be taken from published sources where available or sourced from trial co-investigators.

6.8 MANAGEMENT OF PREGNANCY

We will apply at a minimum the same safety management plan approach in this RCT as we have done in the ODYSSEY trial (46). The protocol, as currently written, excludes girls and women who are pregnant and breastfeeding and requires the avoidance of pregnancy for the duration of the study. Highly effective contraception which includes injectable, implantable, oral and intrauterine contraceptives which suppress ovulation and have an expected failure rate <1% per year (more details are provided in the Manual of Operations) must be used throughout the trial by sexually active girls who have reached menarche. Since all participants will be newly recruited, they will be counselled before trial entry regarding any risks of treatment based on the most up-to-date information.

Pregnancy testing for all girls post menarche will be performed at every scheduled clinical study visit (screening, randomisation, week 8 and then every 8 weeks in the first year and every 12 weeks thereafter, for the duration of the study). Any girl who suspects she may be pregnant (including anyone who has missed a period) between visits will be advised to contact the clinic and to come in for a pregnancy test.

Female participants who decide during the trial that they would like to become pregnant or are identified to be pregnant, need to discuss the risks and benefits of continuing dolutegravir with their health care providers. Although the updated data on the risk of neural tube defects shows that DTG is very safe, ART options should be discussed with the participant so that she has an informed choice.

Folic acid should be provided to pregnant participants throughout the first trimester. Any pregnancy that occurs in a trial participant will be followed-up to determine outcome (including premature termination) and status of mother and child up to 30 days of age, which must also be reported to MRC CTU at UCL on the Outcome of Pregnancy eCRF. The clinical team responsible will be informed of the mother's participation in the trial and will be asked to inform the MRC CTU at UCL if there is any suspicion of any adverse effect of the trial medication.

All participants in the SCT group, should return to taking continuous therapy during pregnancy and breastfeeding. Anyone in the SCT group can return to SCT once pregnancy and breast-feeding are over with the proviso that they must be on the dolutegravir, tenofovir, lamivudine/emtricitabine regimen.

Participants who become pregnant during their participation in the trial should be encouraged to remain on the trial, attending visits as per the schedule in **Table 1**.

Advice on breast-feeding should be given according to local or national guidelines. All infants will receive infant prophylaxis according to the current local standard-of-care. Follow-up of a child born to the partner of a male participant (who was taking trial treatment at the time of conception) will be according to local practice.

6.8.1 REPORTING INCIDENT PREGNANCY

Pregnancy occurring during participation in BREATHER Plus should be reported as a **notable event** to the CTU within one working day of site's awareness. Any event fulfilling the criteria of an SAE (e.g. pregnancy complication, congenital abnormality, birth defect or spontaneous abortion) should be reported within one working day of the site becoming aware of the event. Any SAE occurring in association with a pregnancy, which occurred during the trial and is brought to the investigator´s attention after the participant has completed the study and is considered by the investigator as possibly related to the investigational product, should also be reported as an SAE. All pregnancies will be reported to the Antiretroviral Pregnancy Register on an annual basis (50).

Any pregnancy that occurs in a trial participant will be followed up to determine the outcome (including premature termination) and status of mother and child up to 4 weeks of age, which must also be reported to the CTU on the pregnancy outcome worksheet/eCRF. The clinical team responsible will be informed of the mother's participation in the trial and will be asked to inform CTU if there is any suspicion of any adverse effect of the trial medication. This could mean following the participant beyond the end of the trial.

6.8.2 SUMMARY OF CURRENT DRUG SAFETY PROFILES OF NRTI IN PREGNANCY

The NRTIs, FTC and 3TC are generally considered to be safe in pregnancy and are widely used. The formulation of tenofovir, TDF, is also considered safe in pregnancy and widely used. Modifications to the NRTI combination may be made by the treating physician based on the assessment of risks of particular drugs in pregnancy and their likely antiretroviral efficacy. At present there are inadequate data regarding the use of TAF in pregnant women. Animal studies indicate no direct or indirect harmful effects with respect to reproductive toxicity (43). It will be left to the discretion of the treating physician, in discussion with the participant, as to whether the participant switches back to TDF if they are on TAF as part of their ART regimen.

6.9 EARLY STOPPING OF FOLLOW-UP

In consenting to the trial, participants and their carer are consenting to treatment according to the allocated treatment strategy as well as to trial follow-up visits and data collection. Although to be discouraged, a participant or their carer later may choose to discontinue participation. There are various categories of 'discontinuation':

1. Discontinue study treatment (i.e. dolutegravir, tenofovir, lamivudine/emtricitabine) both early and permanently and/or discontinue SCT
2. Discontinue scheduled BREATHER Plus trial visits

3. Object to being contacted in the future
4. Object to data being collected as part of the participant's routine care
5. Object to storage of existing blood samples (the participant must make the request in writing in order to withdraw consent for storage of existing blood samples)

If a participant chooses to discontinue their study treatment/strategy – as defined above, the clinician or nurse will explain the importance of remaining on trial follow-up or, failing this, of allowing routine clinic follow-up data to be used for trial purposes. Participants on SCT must return to continuous therapy if they discontinue trial ART or transfer to routine clinic follow-up.

If the participant does not wish to remain on trial follow-up, their decision must be respected. Prior to transferring to routine clinic follow-up, the participant will be asked to have assessments performed as appropriate for a close-out trial visit (as defined earlier in **Section 6.1.2**) although they would be at liberty to refuse any or all individual components of the assessment. It should be discussed with the participant and their carer whether they are willing to be contacted in the future, in order to collect routine data for the trial, or, if not, whether data may be collected from their medical notes. Even if a participant stops trial participation early, the medical data already collected during their participation in the trial will be kept and used in analysis, as consent cannot be withdrawn for data already collected. Consent for future use of stored samples already collected can be refused when stopping trial follow-up early (but this should be discouraged and should follow a discussion with the patient).

The CTU should be informed of all participants stopping participation in the trial early, regardless of the degree, using information captured on the Participant Status Worksheet/eCRF, and entered into the database. The reason for the participant stopping participation should be ascertained wherever possible. Participants stopping trial treatment early have a negative impact on a trial's data.

Participants may change their minds about stopping trial follow-up at any time and re-consent to participation in the trial. Participants who stop trial follow-up early will not be replaced; their data will be used up until the point of lost to follow-up and allowance has been made for loss to follow-up in the sample size calculations.

6.10 PARTICIPANT TRANSFERS

If a participant moves away from the area, every effort should be made for the participant to be seen at another participating trial site. A copy of the participant's medical record (the source documentation, including any trial worksheets/eCRFs) should be provided to the new site and the participant (and carer where appropriate) will need to sign a new consent/assent form. Once this has been done, the new site will take over responsibility for the participant; until this has been done, responsibility for the participant lies with the original site.

If transfer to another participating site is not possible (e.g. if the participant moves to a different country in which there is no BREATHER Plus site they can transfer their care to), then the participant should be considered as lost to follow-up; participants on SCT should be advised to return to continuous therapy.

6.11 LOSS TO FOLLOW-UP

For operational management in the clinic, a participant will be classified as 'lost to follow-up' (i.e. no further efforts to trace them are being made) when they have missed 3 scheduled clinic visits in year one or 2 consecutive clinic visits from year two onwards (with no contact being made with the

participant during this time). During this time period attempts should be made to contact the participant via phone (if available) and to follow-up with home visits, if at all possible. Subsequently, if the participant attends clinic and further data is received by CTU, the 'lost to follow-up' status will be reversed. Participants will have a close-out visit within ±6 weeks of the last recruited participant reaching 96 weeks follow-up (with return for confirmatory viral load if the close-out visit includes a real-time viral load which is ≥50 copies/mL). Participants who do not attend a close-out visit and are not known to have died will be classified as lost to follow-up in statistical reporting.

6.12 COMPLETION OF PROTOCOL FOLLOW-UP

Each participant will be in the trial until all participants have reached their 96 week visit, for participants enrolled early this means they will be in the trial for longer than 96 weeks. Depending on how long the trial takes to enrol fully, participants enrolling early may be in the trial for as long as 4 years. Participants will have a close-out visit within ±6 weeks of the last recruited participant reaching 96 weeks follow-up as described in **Section 6.2.4.** and with return for confirmatory viral load and plasma storage for potential resistance testing, if the real-time viral load at the close-out visit is ≥50copies/mL. After this point, each participant will rollover into the national ART programs, likely those on SCT will return to CT. It is possible that participants on SCT will want to continue on SCT and participants on CT may want to have weekends off their ART. However, once returned to the national ART programme, they will be managed according to SOC until such time as SCT for dolutegravir, tenofovir, lamivudine/emtricitabine is an acceptable way of taking ART, if BREATHER Plus shows this to be the case.

7 SAFETY REPORTING

The principles of GCP require that both investigators and Sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials. These procedures are described in this section of the protocol. **Section 7.1** lists definitions, **Section 7.2** gives details of the investigator responsibilities and **Section 7.3** provides information on CTU responsibilities.

7.1 DEFINITIONS

As the trial is funded by EDCTP, the definitions of the EU Directive 2001/20/EC Article 2 based on the principles of GCP apply to this trial protocol. These definitions are given in **Table 3**.

Table 3: Safety Definitions

TERM	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial participant to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: • Results in death • Is life-threatening[a] • Requires hospitalisation or prolongation of existing hospitalisation[b] • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly or birth defect • Is another important medical condition[c]

[a]The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

[b]Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation.

[c]Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the

participant or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation or development of drug dependency.

7.1.1 MEDICINAL PRODUCTS

An investigational medicinal product (IMP) is defined as the tested investigational medicinal product and the comparators used in the study (EU guidance ENTR/CT 3, April 2006 revision). The IMP in this trial are dolutegravir, tenofovir (the TDF or TAF formulations) and lamivudine/emtricitabine.

Adverse reactions include any untoward or unintended response to drugs. Reactions to an IMP should be reported appropriately.

7.1.2 ADVERSE EVENTS

Adverse Events include:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition (even though it may have been present prior to the start of the trial) detected and/or worsening after trial drug administration
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the study treatment
- Overdose of medication with signs and symptoms associated

7.1.3 DISEASE-RELATED EVENTS

Disease-related events are those considered to be related to HIV infection and are categorised according to WHO staging of HIV infection. All adverse events meeting the definitions above should be reported, regardless of their relationship to HIV. All deaths should be reported as fatal SAEs.

7.2 INVESTIGATOR RESPONSIBILITIES

See **Section 6.4** above. The **agreed timelines** for reporting grade 3 or 4 AEs, WHO stage 3 or 4 events and grade 1 or 2 AEs which lead to modification of ART on the trial database, is 7 working days. SAEs and notable events (defined in **Section 7.2.1.D**) should be entered onto the trial database within 1 working day of the investigator becoming aware of the event.

Suicidal Ideation or Behaviours

The following are always reportable as an AE from the point of randomisation and should be reported on the database:

- New suicidal ideation with method, intent, plan or behaviour
- A preparatory act towards imminent suicidal behaviour
- A non-fatal suicide attempt
- A new moderate/high risk score on the C-SSRS
- Complete suicide
- Any suicidal ideation or behaviour that led to ART modification

Completed suicide, suicidal behaviour, new suicidal ideation with intent or a new high-risk score on the C-SSRS is always an SAE and must be reported on the database within 1 working day of the site becoming aware. A new moderate-risk score on the C-SSRS or new suicidal ideation with method but no intent or plan must be assessed for seriousness based on standard SAE criteria.

7.2.1 Investigator Assessment

7.2.1.A Seriousness

When an AE or AR occurs, the investigator responsible for the care of the patient must first assess whether or not the event is serious using the definition given in **Table 3**. If the event is serious, then an SAE/notable event form must be completed and CTU notified within 1 working day.

7.2.1.B Severity or Grading of Adverse Events

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded using the toxicity gradings in Appendix I.

7.2.1.C Causality

The investigator must assess the causality of all clinically significant AEs in relation to ART used since randomisation using the definitions in **Table 4**. There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR.

Table 4. Assigning type of SAE through causality

Relationship	Description	SAE Type
Unrelated	There is no evidence of any causal relationship	Unrelated SAE
Unlikely	There is little evidence to suggest that there is a causal relationship (for example, the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (for example, the patient's clinical condition, other concomitant treatment).	Unrelated SAE
Possible	There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (for example, the patient's clinical condition, other concomitant treatments).	SAR
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	SAR
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	SAR

If an AE is considered to be related to trial treatment and drug is stopped (refer also to **Section 5**), this should be recorded on the Antiretroviral Log.

7.2.1.D Notable Events

Pregnancy

Any pregnancy occurring during participation in the trial should be reported as a notable event by completing the Pregnancy Notable Event eCRF on the trial database within 1 working day of the site becoming aware. If there is an adverse outcome for example pregnancy complications and elective terminations for medical reasons, these must be reported as an adverse event. Spontaneous

abortions/miscarriages, congenital abnormality or birth defect are SAEs and, as for any event fulfilling the criteria of an SAE, should be reported on the AE eCRF within 1 working day of the site becoming aware of the event.

Liver events
Any suspected case of drug induced liver injury (regardless of drug received) should be entered on the AE eCRF (with notable event indicted) on the trial database within 1 working day of the site becoming aware. These events should be discussed with the Clinical Reviewer on a case-by-case basis. Additional information may then be requested from the sites. Any results of liver investigations, including liver biopsy and/or imaging should be reported. The usual criteria should be used to assess whether the event additionally meets the criteria of a grade 3/4 AE, SAE or ART-modifying AE (or none of these).

7.2.1.E Expectedness
For SAEs, if there is at least a possible involvement of IMP given during the trial, the trial physician at MRC CTU (to whom responsibility is delegated by the Sponsor) will assess the expectedness of the event. An unexpected adverse reaction is one not previously reported in the current approved Investigator Brochure for the study IMP) or one that is more frequent or more severe than previously reported. The current approved reference safety information can be obtained by emailing the CTU at mrcctu.bplata@ucl.ac.uk. The definition of an unexpected adverse reaction (UAR) is given in **Table 4.** Please see also **Table 2** for a list of expected toxicities associated with the study IMP. If a SAR is assessed as being unexpected, it becomes a SUSAR.

7.2.1.F Notification
The CTU should be notified of all SAEs and Notable Events within 1 working day of the investigator becoming aware of the event.

Investigators should notify CTU of all reportable AEs occurring from the time of randomisation until the later of 30 days after the last dose of IMP or close-out visit. Any subsequent events that may be attributed to IMP received in the trial should be reported to the national reporting schemes in accordance with relevant requirements in that country.

7.2.1.G Notification Procedure
For SAE/notable events, the AE worksheet (or Pregnancy Notable Event eCRF), must be completed by an investigator (named on the Signature List and Delegation of Responsibility Log, who is responsible for the participant's care; this will be either the Principal Investigator or another medically qualified person with delegated authority for SAE reporting). Due care should be paid to the grading and causality of the event, as outlined above. In the absence of the responsible investigator, the event worksheet should be completed and signed by a member of the site trial team. The responsible investigator should subsequently check the event, and make changes as appropriate.

The minimum criteria required for reporting an SAE are the trial number name of investigator reporting, the event, and why it is considered serious.

1. An AE should be entered on the AE eCRF on the trial database, and marked as being an SAE and/or a notable event. The additional questions required for SAEs (as per the SAE worksheet) should be completed. Pregnancies should be reported on a Pregnancy Notable Event eCRF. As back-up, if it is not possible for the event information to be entered on to the trial database within 1 working day of the site becoming aware of the event, completed event worksheets must be sent securely to the CTU using the email address: mrcctu.bplata@ucl.ac.uk.

Updated information should be recorded on the study worksheets and entered on the trial database as information becomes available. The relatedness to the received medications should be updated based on newly available information and the course of the event should be updated.

2. Follow-up: patients must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow-up should continue after completion of protocol treatment if necessary

3. Staff should follow their institution's procedure for local notification requirements.

7.3 MRC CTU AT UCL RESPONSIBILITIES

Medically-qualified staff at CTU and/or the Chief Investigator (or a medically-qualified delegate) will review all reportable events received which will include making an assessment of expectedness for SARs. Non-serious AEs or ART-modifying events will be batched and reviewed at specific time points as detailed in the Safety Management Plan.

The CTU is undertaking the duties of trial Sponsor (on behalf of UCL) and is responsible for the reporting of SUSARs and other SARs to the regulatory authorities and the research ethics committees, as appropriate. This responsibility is delegated to country PIs, and site PIs where appropriate, for relevant reporting requirements in individual countries.

The CTU will keep all investigators informed of any safety issues that arise during the course of the trial. MRC CTU at UCL, will prepare Annual Safety Reports in the form of a Development Safety Report (DSUR) on behalf of the Sponsor, which will be submitted to the Competent Authorities and Ethics Committees in each country participating in the trial.

7.4 RESPONSIBILITIES OF COUNTRY PRINCIPAL INVESTIGATOR

The country Principal Investigator's site is responsible for the reporting of all necessary events to the regulatory authorities in that country, although the Sponsor will retain oversight of this.

8 QUALITY ASSURANCE & CONTROL

8.1 RISK ASSESSMENT

The Quality Assurance (QA) and Quality Control (QC) considerations have been based on a formal Risk Assessment, which acknowledges the risks associated with the conduct of the trial and how to address them with QA and QC processes. QA includes all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled. This Risk Assessment has been reviewed by CTU's Research Governance Committee (RGC) and has led to the development of a Data Management Plan (DMP), Safety Management Plan and Monitoring Plan which will be separately reviewed by CTU's Quality Management Advisory Group (QMAG).

8.2 CENTRAL MONITORING AT MRC CTU AT UCL

Each site will be responsible for its own data entry and local trial management. Data will be entered into the trial database directly at the site. The site will retain the original source documentation for these data (e.g. participant clinic notes/worksheets/eCRFs). Automated queries will be generated via the eDC; CTU staff will also generate manual queries as needed. Changes required will be made on the original source documentation and entered into the database at the site. CTU will also send reminders for any overdue and/or missing data with regular inconsistency reports of errors.

Other essential trial issues, events and outputs will be detailed in the Monitoring Plan that is based on the trial-specific Risk Assessment.

8.3 ON-SITE MONITORING

Staff from CTU or local monitors will visit clinical sites to validate and monitor data. The frequency, type and intensity for routine monitoring and the requirements for triggered monitoring will be detailed in the Monitoring Plan. This plan will also detail the procedures for review and sign-off.

A detailed site initiation visit with training will be performed at each study site by staff from CTU as well as local staff who will be specifically trained for this role. The site initiation visits will include training in the administration and side-effects of study drugs, as well as the trial procedures.

Typical monitoring visits will include completion of the following as a minimum:

- Verify completeness of the Investigator Site File
- Confirm adherence to protocol
- Review eligibility verification and consent procedures
- Look for missed clinical event reporting
- Verify – in a sample as detailed in the monitoring plan – the completeness, consistency and accuracy of data being entered on worksheets/eCRFs from the patient's medical record
- Verify – in a sample as detailed in the monitoring plan – the completeness of the protocol defined stored sample biobank
- Evaluate drug accountability
- Provide additional training as needed

The monitors will require access to all participant's medical records including, but not limited to, laboratory test results and prescriptions. The site investigator (or delegated deputy) should work with the monitor to ensure that any problems detected are resolved.

8.3.1 Direct Access to Patient Records

Participating investigators should agree to allow trial-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required. Participants' consent for this must be obtained. Such information will be treated as strictly confidential and will in no circumstances be made publicly available.

8.3.2 Source data

Investigator(s) and institutions should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail).The following data should all be verifiable from source documents i.e. the patient's medical records:

- Signed consent and assent forms (where applicable)
- Dates of visits including dates of any trial specimens were taken and processed in the laboratory
- Eligibility and baseline values
- Adverse events of any grade that lead to modification of HIV treatment
- Serious adverse events, severe (grade 3/4) and 5 adverse events, and WHO stage 3/4 events
- Pharmacy/clinic drug logs.

Not all such information will be monitored; rather the monitoring plan will describe a risk-based approach to monitoring based on ongoing random samples of patient clinical and laboratory data which may be increased if issues are identified.

For this trial, the eCRFs will not be the source document for any data elements. A source data log will be established as part of the green light process with each site. This log will define the source documents and the data therein, together with location of these source documents and any applicable plans for transmission of source data between the site and the Sponsor/delegated institution.

8.3.3 Confidentiality

We plan to follow the principles of the General Data Protection Regulation and the UK Data Protection Act 2018 regardless of the countries where the trial is being conducted. In particular, the investigator must assure that participants' anonymity will be maintained and that their identities are protected from unauthorised parties. Participants will be assigned a trial identification number and a 3-letter code, and this will be used on all worksheets/eCRFs and in the trial database; participants will not be identified by their name. The investigator will keep securely a participants' trial register showing identification numbers, names and date of birth. This unique trial number will identify all laboratory specimens, case record forms, and other records and no names will be used, in order to maintain confidentiality. All records will be kept in locked locations. Clinical information will not be released without written permission, except as necessary for monitoring by the trial monitors.

9 STATISTICAL CONSIDERATIONS

9.1 METHOD OF RANDOMISATION

Participants will be randomised 1:1 to continue on continuous ART (CT) or to move to short-cycle therapy (SCT). Randomisation will be stratified by centre and mode of infection (horizontal or vertical).

The randomisation list will be prepared by staff at CTU under the direction of the Trial Statistician using permuted blocks with variable size. These computer-generated sequentially numbered lists will be incorporated securely into the trial database (accessed over the web at each site), concealed from local staff. Allocation will be made after eligibility has been confirmed by local site staff through the web-enabled database. Only the next randomisation will be provided, the remainder of the list will be concealed (ensuring allocation concealment). Delegated member(s) of staff at each site will be responsible for carrying out the randomisation process using a secure electronic system within the trial database.

9.2 OUTCOME MEASURES

9.2.1 PRIMARY OUTCOME MEASURE

The proportion of participants with confirmed virological rebound, defined as the first of 2 consecutive plasma HIV-RNA ≥50 copies/mL at any time up to the 96-week assessment

9.2.2 SECONDARY OUTCOME MEASURES

9.2.2.A EFFICACY

(i) Proportion of participants with confirmed HIV-RNA ≥50 copies/mL at 48 and 96 weeks using the modified FDA snapshot algorithm

(ii) The proportion of participants with confirmed HIV-RNA ≥1000 copies/mL defined as 2 consecutive plasma HIV-RNA ≥1000 copies/mL at any time up to the 96-week assessment

(iii) The number/type of HIV mutations at confirmed HIV-1 RNA ≥50copies/mL

(iv) The proportion of participants with HIV-RNA <50 copies/mL and no switch to second-line ART for treatment failure at 24, 48, 72 and 96 weeks

9.2.2.B SAFETY

(i) Change in toxicity profile including change in metabolic parameters (lipids, HbA1c, phosphate), renal function (eGFR) from baseline to 96 weeks; change in anthropometric measures from baseline to 48 and 96 weeks

(ii) Time to any new or recurrent WHO stage 3 or WHO stage 4 event or death

(iii) Incidence of serious, grade 3, 4 and 5 and treatment-modifying (of any grade) adverse events

(iv) The proportion of participants with any change from baseline ART regimen

(v) Change in CD4+ and CD8+ T-cell count from baseline to 48 and 96 weeks

9.2.2.C PATIENT-REPORTED

(i) Adherence, acceptability, wellbeing and including neuropsychiatric problems (e.g. depression, anxiety and sleep disturbance)

(ii) Healthcare resource utilisation (a sub-study outcome)

(iii) Health-related quality-of-life (a sub-study outcome)

9.2.3 PROTECTION FROM BIAS

BREATHER Plus is an open-label trial exploring treatment optimisation through **reduced** ART exposure. To counter the possibility of bias, objective outcome measures have been chosen where possible. The primary endpoint (virological rebound) will be measured by laboratory staff blinded to randomised allocation, as will other secondary outcomes based on laboratory tests (HIV resistance, CD4+ T-cell count, tests of renal function, bone profiles and lipids).

For SAEs clinicians will report events using a structured clinical narrative. Designated Trial Clinicians at the CTU will review these events.

Every effort will be made to minimise loss to follow-up and to ascertain outcomes completely thus avoiding bias from differential ascertainment between the randomised groups. Participants lost to follow-up will be traced using home visits and mobile phone contact numbers taken at recruitment.

9.3 SAMPLE SIZE

Non-inferiority of SCT will be assessed by the difference between the SCT group and the CT group in the estimated proportion of participants with viral rebound (defined as the first of two consecutive HIV-1 RNA ≥50 copies/mL) **by week 96.**

The BREATHER Plus trial was designed with a fixed non-inferiority margin of 10%. At the design stage it was estimated a total of 460 participants (230 per group) would provide 90% power, 2-sided alpha of 5%, to demonstrate non-inferiority of SCT vs. CT, assuming 11% of participants met the primary endpoint of confirmed HIV-RNA ≥50 copies/mL by the 96 week assessment in both groups (22) and allowing for 10% loss to follow-up.

Should the viral rebound rate in the control group be substantially different to 11% by 96 weeks, we will modify the non-inferiority margin (currently set at 10%) using the Smooth Away From Expected (SAFE) frontier (51). The choice of non-inferiority margin will be based on the observed viral rebound rate in the control group.

Provided that the observed viral rebound rate in the control arm is not lower than 9%, a 95% two-sided confidence interval will be computed for the difference in viral rebound between SCT and CT groups and a 10% non-inferiority margin will be used. If the observed rate of viral rebound in the control arm is less than 9%, a 99% two-sided confidence interval will be computed for the difference in viral rebound between SCT and CT groups; the non-inferiority margin will depend on the control event rate as shown in Figure 2 and Table 5. If the upper bound of the respective confidence interval is no higher than the selected non-inferiority margin, then the null hypothesis will be rejected and SCT will be declared non-inferior to control.

Figure 2: Choice of non-inferiority margin based on observed confirmed viral rebound risk using the Smooth Away From Expected (SAFE) frontier

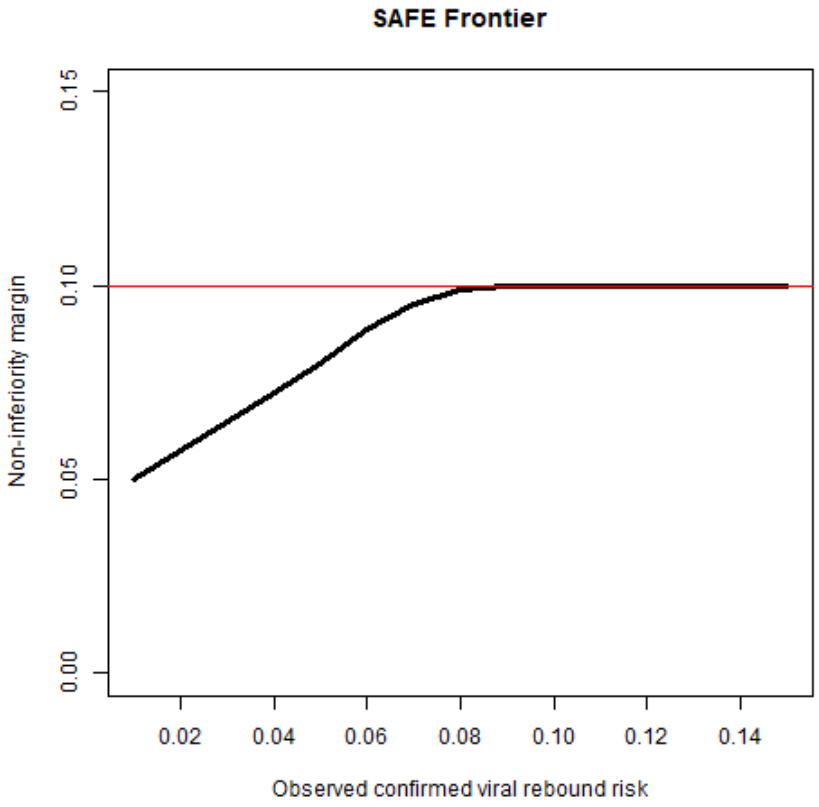


Table 5: Choice of non-inferiority margin and significance level based on observed confirmed viral rebound risk using the Smooth Away From Expected (SAFE) frontier

Observed confirmed viral rebound risk (P0)*	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%
NI Margin	5.0%	5.8%	6.5%	7.3%	8.0%	8.9%	9.5%	9.9%	10.0%	10.0%	**10.0%**	10.0%	10.0%	10.0%	10.0%
Significance level	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	2.5%	2.5%	**2.5%**	2.50%	2.50%	2.50%	2.50%
Power	95.8%	89.1%	84.4%	81.4%	80.4%	81.1%	82.2%	83.3%	84.6%	85.0%	**86.3%**	86.6%	85.7%	84.0%	82.0%
Type 1 error	2.50%	2.28%	2.36%	2.53%	2.67%	2.76%	2.86%	2.74%	2.69%	2.71%	**2.69%**	2.66%	2.64%	2.63%	2.61%
P (change margin)**	100%	100%	100%	99.9%	99.0%	95.0%	84.8%	67.8%	47.5%	29.0%	**15.5%**	12.1%	6.1%	2.8%	1.2%

The column in bold corresponds to the sample size calculation assumption made at the design stage.

*The choice of non-inferiority margin and significance level will depend on the observed confirmed viral rebound risk. The power, type 1 error and probability of changing margin depend on the true control event risk.

**The probability of changing the margin is the probability that, for a given true control event risk, the observed control event risk will be lower than 9%, hence leading to using a non-inferiority margin in the analysis different from the originally planned 10%.

9.4 ESTIMAND

Table 6: The estimand for the primary outcome

Treatments	The comparison is between the SCT group and the CT group (control)
Population	The population of interest is HIV-1 infected adolescents aged 12 to 19 years in Kenya, South Africa, Uganda, and Zimbabwe that meet the inclusion/exclusion criteria as defined in section 3
Endpoint	Proportion of children with confirmed viral rebound, defined as the first of 2 consecutive HIV-1 RNA≥50 c/mL at any time up to the 96-week assessment
Population-level summary measure	Difference in proportions (SCT - CT)
Intercurrent events	
Any treatment modification including: • change in any ART component; • ART dose modification; • ART discontinuation; • Return to continuous ART in the SCT group	Treatment policy
Missed doses of treatment	Treatment policy
Died	Hypothetical

9.5 INTERIM MONITORING & ANALYSES

An Independent Data Monitoring Committee (IDMC) Charter will be drawn up that describes the membership of the IDMC, relationships with other committees, terms of reference, decision-making processes, and the timing and frequency of interim analyses (with a description of stopping rules and/or guidelines).

9.5.1 PILOT STUDY

Among the first participants randomised into the trial, 15 participants randomised to SCT and 15 randomised to CT at sites participating in the pilot study will have viral load measurements at weeks 1, 2, and 3 (and a confirmatory viral load at week 4 following a single viral load above 50 copies/mL at week 3) with these measures in the SCT group being performed after weekends off treatment. Should any participants randomised to SCT in the pilot not take weekends off treatment, additional SCT participants will be recruited. The independent IDMC will review these HIV RNA assessments after all participants in the pilot phase have completed this pilot. This will be to determine whether the SCT group is safe to continue. Recruitment to the trial will continue during pilot follow-up and review of the pilot data.

9.5.2 INTERIM ANALYSES

The IDMC will meet to review unblinded data for randomised comparisons within 6 months and 12 months of the trial starting. They will review data on enrolment, safety, adherence to randomised strategies and effectiveness, in strict confidence. Batch runs of viral loads will be planned prior to IDMC meetings and should include test results for visits up to 3-4 months prior to the IDMC meeting date; by the 12 month review meeting viral loads 8 and 16 weeks after enrolment should be available on ~140 participants, with 24-week viral loads on ~70 participants. The frequency of future IDMC meetings will be at the discretion of the IDMC, but are likely to be every 6-12 months. Monitoring guidelines will be specified in the IDMC charter; the trial will not stop early for non-inferiority

See **Section 14.4** for details on the requirements for membership of the oversight committees.

9.6 ANALYSIS PLAN (BRIEF)

The analyses will be described in detail in a full Statistical Analysis Plan. This section summarises the main issues.

For the primary analysis, the two treatment groups will be compared in the intention-to-treat (ITT) population, defined as all randomised patients excluding those demonstrably randomised in error; where randomisation in error will be judged by the participant meeting a major violation of the eligibility criteria. The comparison will be of the cumulative probability of virological rebound by week 96 (as defined above). To allow for censoring, the survival curve for each combination of strata and randomised group will be calculated using a Cox model adjusting for stratification factors (as appropriate where sufficient participants are enrolled across strata) and randomised group. The average cumulative failure function (1-survival curve) for each randomised group will be estimated by standardisation (52) as a weighted average of the corresponding stratum-specific cumulative failure functions with weights equal to the prevalence of that stratum in the whole ITT population. Similarly, the average difference between the cumulative failure functions at week 96 is the point estimate for the difference in overall probability of virological rebound between the SCT group and the CT group. A 2-sided bias-corrected CI (significance level as per table 5) for the difference in the probability of virological rebound by week 96 (SCT-CT) will be calculated with bootstrap standard errors. The bootstrapping will sample 10,000 times and be stratified by adjustment factors. SCT will be considered non-inferior to CT if the upper limit of the respective confidence interval for the difference SCT-CT is less than the non-inferiority margin selected as per Table 5).

For analysis of the primary outcome and other virological outcomes, except for the FDA snapshot analysis, multiple imputation will be applied if either of the following criterium is met:

- 5% of all HIV-1 RNA measurements at scheduled visits are missing

Or

- 10% of confirmatory real-time HIV-1 RNA measurements are missing.

Once a criterium for imputation is met, missing HIV-1 RNA measurements at scheduled visits will be multiply imputed.

The primary analysis will be repeated

i. In a pre-defined per-protocol population (i.e. Per-protocol analysis is a comparison of treatment groups that includes follow-up in patients complying with randomised strategy)
ii. Considering the secondary outcome measure confirmed HIV-RNA ≥1000 copies/mL by week 96

Real time viral loads at weeks 48 and 96 (with confirmatory testing for anyone with HIV-RNA ≥50 copies/mL) will allow us to compare the proportion with virological rebound at 48 and 96 weeks using the modified FDA snapshot algorithm – as detailed in the Statistical Analysis Plan. The estimated difference in proportion with virological rebound between SCT and CT arms will be computed with 95% CI by the Mantel-Haenszel weighted mean of proportions in each stratum.

Other secondary outcome measures will be compared for superiority between the SCT and CT groups using appropriate statistical methods in the intention-to-treat population. Adjusted models, adjusting for stratification factors (as appropriate), and unadjusted analyses will be presented.

For adverse events and WHO stage 3/4 events, randomised groups will be compared in terms of time to first event using Cox proportional hazards regression models. Rates will be presented and Poisson regression used to calculate the incidence rate ratios for SCT vs. CT groups, allowing for more than one event in the same participant. Binary outcomes including the proportion of participants with resistance at failure and the proportion suppressed with no switch to second-line for treatment failure (by timepoint) will be compared between arms using the Chi-squared test or Fisher's exact test, as appropriate. The 95% confidence interval for the difference in proportion will be provided. Logistic regression will be used for analyses adjusting for stratification factors.

For analysis of continuous variables (laboratory and anthropometric measures), the mean change from baseline over follow-up will be calculated using linear mixed models (unstructured covariance) with random intercept for participant and fixed effects for randomised group and visit weeks, including interactions between randomised group and visit week, adjusting for baseline. Adjusted analyses will be performed adjusting for stratification factors (as appropriate) in addition to baseline.

A Statistical Analysis Plan will be written and approved by the Trial Management Group (TMG), Trial Steering Committee (TSC) and the IDMC before the first interim analysis (after pilot review) is reviewed by the IDMC.

9.6.1 SUBGROUP ANALYSIS

Subgroup analyses are planned by the randomisation stratification factors, provided there are sufficient participants in the corresponding sub-groups.

9.6.2 HEALTH ECONOMIC ANALYSIS

The cost-effectiveness of the BREATHER Plus trial groups (using a generic measure of health including DALYs-averted and QALYs gained) will be considered. Given the scarcity of health care resources, especially in Africa, decision makers need to understand the magnitude of health benefits that different policy options would generate across the population. This requires quantifying health gains of interventions and health forgone by other patients elsewhere within the system (i.e. health opportunity costs), as a result of limited resources consequentially being unavailable for other priorities. Net health benefit (NHB, health gains from an intervention, less associated health opportunity costs) provides a useful summary measure of population health improvement.

Standard costing and cost-effectiveness analysis methods will be used. For costing this will involve an ingredients approach whereby resource use recorded in the trial is combined with unit costs/prices as reflective of general healthcare use in South Africa, Zimbabwe, Kenya and Uganda. A health

service provider perspective will be used in the base case analyses. Analyses will be conducted across and within country. Further details are provided in the Statistical analysis plan for the sub-study.

10 ANCILLARY STUDIES

10.1 SOCIAL SCIENCE SUB-STUDY

The social science sub-study will quantitatively and qualitatively assess key secondary trial outcomes of adherence, acceptability and well-being among adolescents participating in BREATHER Plus on short cycle therapy versus daily oral ART. The specific objectives include:

- To evaluate adherence on SCT compared to daily ART in the trial population;
- To evaluate the acceptability of treatment strategy and wellbeing among adolescents on SCT compared to daily ART in the trial population.
- To qualitatively explore adolescents' experiences of SCT ART compared to daily ART in a sub-sample of the trial population.

All participants will complete adherence questionnaires as part of the wider trial procedures. In addition, questionnaires on acceptability and wellbeing are completed at weeks 0, 24, 48, and 96, then 48-weekly and at the close-out visit. Questionnaires on Quality of Life are completed at weeks 0, 24, 48, 96 and the close-out visit.

Participants in South Africa will be invited to participate in the in-depth interviews (IDI) and focus group discussions (FGD). Participants in Uganda will be invited to take part in focus group discussions (FGD). There is a separate information statement and consent form for the IDI and FGD. The IDI and FGD guides are based on previous experiences with the BREATHER trial (20-22) and other studies, and with input from PPI stakeholders including the Youth Trial Boards (YTB) for this trial.

10.1.1 IN-DEPTH INTERVIEWS

The in-depth interviews are likely to focus on i) acceptability and attitudes towards CT/SCT and the impact on wellbeing, ii) experience of CT/SCT, iii) impact of CT/SCT on relationships, including sexual behaviour and contraception, and iv) experience of side effects including neuropsychiatric side effects. The in-depth interviews in South Africa will conducted in reiterative waves to allow for analysis to take place before more date is collected. In South Africa we will purposefully select up to 30 participants for two longitudinal in-depth interviews: 15 each from the 2 trial arms and stratified by sex, and age. Our previous work in BREATHER (20-22) demonstrated the value of this longitudinal approach to develop trust with participants and generate rich data on sensitive topics such as adherence, disclosure and sexual behaviour. The longitudinal approach also allows us to explore changes over time, including treatment fatigue and the extent to which this differs across the treatment strategies. These qualitative findings will be used alongside the quantitative data to assess adherence, acceptability and wellbeing and will potentially provide context which will contribute to explaining the results.

10.1.2 FOCUS GROUP DISCUSSIONS

Four to twelve FGDs will be conducted using participatory approaches in each country in years 1 and 2 in Uganda and years 2 and 3 in both countries with trial participants, non-trial clinic patients, and health care providers (in separate groups) to explore the feasibility of SCT and preferences for alternative ways of accessing therapy.

IDIs and FGDs will be conducted in the first language of the participants, digitally recorded, transcribed and translated. A thematic coding approach will be used, analysis will be conducted by the country specific research teams, and regular (electronic) meetings across the Ugandan and South African teams will support data interpretation. This will provide a more comprehensive understanding of young peoples' lived experience of ART and their adherence behaviour across the

trial groups and in the different settings/countries, as well as the acceptability of interventions and whether the interventions are feasible. The qualitative data will be stored on a secure computer server at each study site and only authorised study staff will have access to this information. These data will be managed according to site policies that adhere to GDPR/Protection of Personal Information (POPI) regulations. Data sharing between sites for analysis purposes will only involve pseudo-anonymized data through a secure data enclave after seeking permission from the study PI and the site data manager as per the Institutes' data sharing policies.

10.2 NEUROPSYCHIATRIC SUB-STUDY

The neuropsychiatric toxicities of DTG are increasingly recognised. However, there is paucity of data on their toxicity profile in an adolescent population. The main aim of this sub-study are to:

- Compare neuropsychiatric problems (key secondary trial outcomes), including depression, suicidality, anxiety and sleep disturbance longitudinally between the randomised groups;
- Test practical and feasible tools to identify and monitor mental health illness among adolescents in busy over-stretched HIV clinics.

This work will complement the social science research being conducted on acceptability, adherence and wellbeing by drilling down on key factors that affect mental health. Further details of the neuropsychiatric toxicity sub-study will be available in a sub-study specific Manual of Operations.

10.2.1 QUESTIONNAIRES USED IN THE NEUROPSYCHIATRIC TOXICITY SUB-STUDY

Short, simple neuropsychiatric questionnaires and the Columbia-Suicide Severity Rating Scale will be completed by all trial participants at weeks 0, 8, 24, 48, 72 and 96, and then every 48 weeks and at the close-out visit. In addition, the standard clinical assessments at each visit will capture grade 3 and 4 and ART-modifying neuropsychiatric adverse events, of any grade.

Participants will be invited to join the neuropsychiatric sub-study as follows;

(i) A random sample of participants identified ≥24 weeks after trial entry and reporting depression, anxiety or sleep disturbance on the short questionnaires (approx. 10 per trial group for each of depression, anxiety and sleep disturbance

(ii) Any participant stopping DTG for neuropsychiatric toxicity or patient choice up to 96 weeks; and

(iii) A random sample of participants identified 24-48 weeks after trial entry and selected for the absence of reported symptoms on the short questionnaires (n=~36 (≥18 per trial group)). If no additional toxicities were identified among 36 participants, we would be 95% confident that the short questionnaires captured toxicities of ≥10% prevalence

This will allow us to investigate neuropsychiatric problems/toxicities in detail, including among participants modifying treatment; and to explore whether the short questionnaires provide appropriate and feasible tools for identifying neuropsychiatric problems/toxicities including lower grade toxicities which may be associated with dissatisfaction with treatment and may even lead to treatment changes, non-adherence and or/loss to follow-up, or whether significant toxicities are being missed. Following an early analysis pooled across treatment groups of the longer questionnaires in participants who reported no neuropsychiatric problems/toxicities on the short questionnaires, additional questions may be added to the short questionnaires.

The longer questionnaires employed in this sub-study will include more detailed assessments of depression, anxiety, and sleep quality.

11 REGULATORY & ETHICAL ISSUES

11.1 COMPLIANCE

11.1.1 REGULATORY COMPLIANCE

International sites will comply with the principles of GCP as laid down by the ICH topic E6 (R2) and other applicable national regulations (i.e. Kenya, Uganda, South Africa and Zimbabwe regulations).

11.1.2 SITE COMPLIANCE

An agreement will be in place between the site and the CTU, setting out respective roles and responsibilities (see **Section 13**).

The site will inform the CTU as soon as they are aware of a possible serious breach of compliance, so that the CTU can work with the local site to report the breach as required to the local regulatory authorities and the UCL Research Ethics Committee. For the purposes of this regulation, a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects in the trial, or
- The scientific value of the trial.

11.1.3 DATA COLLECTION & RETENTION

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data are contained in source documents and are defined by EU guidelines as all information in original records that are used for the reconstruction and evaluation of the clinical trial. Source documents are the first place where the source data are recorded. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail). Each data element should only have one source.

Worksheets, clinical notes, and administrative documentation should be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for a minimum of 25 years (or longer if this is a local regulatory requirement) after the end of the trial. During this period, all data should be accessible, with suitable notice, to the competent or equivalent authorities, the Sponsor and other relevant parties in accordance with the applicable regulations. The data may be subject to an audit by the competent authorities. Medical files of trial participants should be retained for a minimum of 25 years (or longer if this is a local regulatory requirement) after the end of the trial.

11.2 ETHICAL CONDUCT OF THE STUDY

11.2.1 ETHICAL CONSIDERATIONS

Patients in BREATHER Plus are participating in a randomised clinical trial. It is expected that the experimental group will be non-inferior to the control group with respect to virological suppression. The main risk to patients is of viral rebound on SCT. If participants do have confirmed virological rebound, this may be because they have developed resistance to one or more components of the ART regimen. This may mean they can only subsequently achieve virological suppression using a second-line ART regimen, which is more difficult to take than the first-line regimen with more pills and more potential for drug-drug interactions. However, the researchers in the BREATHER Plus

consortium think this risk is low, and participants are being monitored much more closely than standard-of-care.

The study visits are more frequent than SOC but the amount of extra blood taken is modest for most participants as detailed in **Table 1**.

At trial entry participants must agree to the storage samples taken for viral load testing, and resistance testing including in the event of virological rebound. Annual blood and urine storage samples are only taken with consent, participants can opt out of storage samples, and still participate in the trial. No genetic testing will be performed. The additional blood and urine samples will be used to explore changes in end-organ disease e.g. renal function, metabolic changes, bone health) and inflammation levels, and where applicable the levels of the ART drugs. These are donated by participants to the trial, and no novel discovery is expected.

Pregnancy should be avoided during participation in the trial. There is very frequent pregnancy testing (every 8 weeks in the first year and every 12 weeks thereafter) in girls who have reached menarche, even if they are not sexually active. Contraception will be provided to all sexually active girls, and boys will be counselled about the use of condoms with their female partners. If a girl becomes pregnant during the trial, she will be counselled about continuing use of DTG or substituting it for efavirenz for the first trimester of pregnancy; participants in the SCT group will return to continuous therapy for the duration of pregnancy and breastfeeding. With the further data from Botswana and other countries, the risk of neural tube defect in babies of females who become pregnant while on DTG is now much lower than first thought when this risk was reported in 2018, and no different to any other ART used in pregnancy.

Participants will be reimbursed for reasonable travel costs for themselves and their carer (if applicable) as detailed in the patient information statement. These costs have been decided in collaboration with participating sites.

Data that are collected in the trial are of a sensitive nature, and will be de-identified as described in the protocol. Qualitative data collected as part of the Social Science sub-study will be stored in accordance with GDPR/POPI guidelines.

Publication of data will be based on group – not individual – data, as will feedback of overall results to participants and their carers (as applicable). It is envisaged that the YTBs will play a major role in ensuring the format of the feedback on the trial results are ‘youth friendly’.

The contract with supporting pharmaceutical companies will specify that the supply of study drugs will be limited to the duration of the trial in each individual patient, although provision will be available for a short-term extension to cover the period while the patient makes the transition to the national treatment programme. Importantly as TLD is now the WHO recommended first-line ART regimen, this ART combination will be available to participants after the trial is over.

Participants will be informed fully of the trial randomisation, known risks and possible benefits by means of a patient information sheet for carers and older children/adolescents, and this will be reinforced by discussions with the trial research teams at the individual sites prior to enrolment.

Patients’ confidentiality will be maintained throughout the trial. Data submitted into the database from trial sites, and samples sent to central testing facilities, will be identified only by trial identifiers (as well as date of blood draw for blood samples).

11.2.2 FAVOURABLE ETHICAL OPINON

Following main IRB approval and before initiation of the trial at each clinical site, the protocol, all informed consent forms, and information materials to be given to the prospective participant will be submitted to each appropriate ethics committee for approval. Any further amendments will be submitted and approved by each ethics committee.

The study has been developed with Patient and Public Involvement (PPI) to ensure that its design is feasible and acceptable to potential participants, and to ensure its outcomes and potential impact are relevant to the population who may benefit from its results. PPI also helps to ensure transparency and accountability throughout this research. PPI activity will continue for the duration of the study, including dissemination of study results. The PPI involvement in BREATHER Plus is via Youth Trial Boards (YTB) in participating countries.

The rights of the participant to refuse to participate in the trial without giving a reason must be respected. After the participant has entered into the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. The reason for doing so, however, should be recorded; the participant will remain within the trial for the purpose of follow-up and for data analysis by the treatment option to which they have been allocated. Similarly, the participant must remain free to change their mind at any time about the protocol treatment and trial follow-up without giving a reason and without prejudicing his/her further treatment.

11.3 COMPETENT AUTHORITY APPROVALS

This protocol will be reviewed by/submitted to the national competent or equivalent authority, as appropriate in each country where the trial will be run. Current country-specific authorisations will be adhered to.

The progress of the trial and safety issues will be reported to the regulatory agency or equivalent in accordance with local requirements and practices in a timely manner. Safety reports, including expedited reporting and SUSARS, will be submitted to the competent authority in accordance with each authority's requirements in a timely manner.

11.4 OTHER APPROVALS

The protocol will be submitted by those delegated to do so to the relevant local departments for approval as required in each country. A copy of the relevant local approval and of the template PIS and Consent Form (CF) on local headed paper should be forwarded to the CTU before any patients are enrolled at the site.

11.5 END OF TRIAL

The end of the trial will be when all participants have attended their final study visit (including follow-up for VL HIV-RNA ≥50 copies/mL), retrospective viral load testing is complete and the database has been locked.

11.5.1 SAMPLE STORAGE AND DESTRUCTION

Specimens for which patients have consented will be stored and used for analyses as specified in the BREATHER Plus protocol, patient information sheet and consent; once analyses are complete, any of these samples that remain will be disposed of according to standard laboratory procedures and guidelines in the respective countries. Stored samples will be kept for up to 5 years after the end of

the trial, and then destroyed as per the laboratory manual of operations. Evidence of sample destruction will be kept in the TMF and by CTU. Samples may be shipped internationally. Any shipments and sample labelling will comply with GDPR.

12 INDEMNITY

University College London holds insurance against claims from participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a research facility/clinic, the research facility/clinic continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the research facility/clinic's duty of care, or any negligence on the part of research facility/clinic employees. Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to UCL's Insurers, via CTU.

Research facility/clinics selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

13 FINANCE

The trial is supported by grant funding from the European Developing Countries Clinical Trials Partnership. The MRC Clinical Trials Unit at UCL is supported by funding from the MRC (MC_UU_12023/22 and MC_UU_00004/03).

The trial will be coordinated by CTU. A written agreement with the site PI and CTU will outline the funding arrangements to sites.

Financial reporting is required to EDCTP and is the overall responsibility of the CI of BREATHER Plus.

14 OVERSIGHT & TRIAL COMMITTEES

There are a number of committees involved with the oversight of the trial. These committees are detailed below.

14.1 SITE TRIAL MANAGEMENT TEAMS (TMT)

A Trial Management Team (TMT) will be formed at each site to conduct the day-to-day management of the trial at the site. This will include the investigators and trial staff at the site. These groups will meet every one to two weeks and will be chaired by the Principal Investigator or Co-Principal Investigator at the site. The group will discuss issues related to the progress of the trial at the site and to ensure that the trial is running well.

There will be a similar Trial Management Team formed to conduct the day-to-day management of the trial at the CTU. This will include the CTU Project Lead, trial statistician, trial physician, clinical project manager, trial manager and data manager. The group will meet at least once per month, although may meet more often if required.

14.2 TRIAL MANAGEMENT GROUP (TMG)

A Trial Management Group (TMG) will be formed comprising the Trial Chief Investigator; site Principal Investigators, Co-Investigators and Trial Managers, other Lead Investigators (clinical and non-clinical) and members of CTU and PPI contributors. The TMG will be responsible for the day-to-day running and management of the trial. It will hold regular teleconference, approximately at monthly intervals, at which sites will summarise progress and challenges and bring up for discussion any difficulties, as well as discuss and decide matters of general importance for the trial. This group will be chaired by the Trial Chief Investigator and all decisions regarding the overall running of the trial will be made in this forum with the exception of matters of fundamental importance to the viability of the trial or that require major changes to the protocol. These will be referred to the Trial Steering Committee (TSC). The full details can be found in the TMG Charter.

14.3 TRIAL STEERING COMMITTEE (TSC)

The Trial Steering Committee (TSC) has membership from the TMG plus independent members, including the Chair and PPI contributors. The role of the TSC is to provide overall supervision for the trial and provide advice through its independent Chair. The ultimate decision for the continuation of the trial lies with the TSC. Further details of TSC functioning are presented in the TSC Charter.

There will be an independent Chair of the BREATHER Plus Trial Steering Committee who has experience of previous Trial Steering Committees. There will be at least 4 other independent representatives, including least one community representative, making a total 5 independent members including the Chair. The trial team will be represented by the EDCTP BREATHER PLUS/LATA coordinator and BREATHER Plus CI, Kekitiinwa-Rukyalekere (Uganda), and the UK Project Lead (Pett).

Whenever possible, the BREATHER Plus Youth Trials Boards (see under PPI) will liaise with the patient representatives on the TSC, and will feedback concerns and questions from the community. The Sponsor will ensure they are updated with the latest developments in the trial and the wider scientific community. All the sites currently have active patient participation groups.

14.4 INDEPENDENT DATA MONITORING COMMITTEE (IDMC)

An IDMC will be formed. The IDMC will be the only group who sees the confidential, accumulating data for the trial. Reports to the IDMC will be produced by CTU statisticians. The frequency of the envisaged IDMC meetings is detailed in **Section 9.4.** The IDMC will consider data using the statistical analysis plan and will advise the TSC (see **Section 9.4**). The IDMC can recommend premature closure or reporting of the trial, or that recruitment be discontinued.

14.5 ROLE OF STUDY SPONSOR

UCL is the Sponsor of BREATHER Plus and delegates this responsibility to CTU to oversee the implementation of the study by ensuring that arrangements are put into place for adequate management, monitoring, analysis and reporting of the trial.

15 PATIENT AND PUBLIC INVOLVEMENT

Patient and Public Involvement (PPI) in research is defined by INVOLVE (an advisory group established by the NIHR) as research being carried out 'with' or 'by' members of the public rather than 'to', 'about' or 'for' them. INVOLVE intends 'public' to include patients, potential patients, carers and other users of health and social care services, as well as people from organisations that represent people who use services. In some cases, this may include involvement of a trial's participants in guidance or oversight of a trial. PPI involvement in BREATHER Plus is a key workpackage for the trial and this workpackage is funded.

15.1 SET UP OF YOUTH TRIAL BOARDS (YTB)

The aim of the YTB is to ensure the voices of Young people living with HIV (YPLHIV) are heard, and they contribute meaningfully to the development, delivery and dissemination of paediatric clinical trials. Youth Trial Boards are already functional in Uganda, South Africa and Zimbabwe, and we will support and work with these existing groups from the start to ensure BREATHER-Plus is conducted and communicated in a way that is relevant and acceptable to YPLHIV. A YTB is being established in Kenya using lessons learnt from existing YTBs, to contribute to capacity building activities and support future research. The YTBs will include both male and female participants as well as representation across different ages between 15-19 years. Broad terms of reference (ToR) will be developed by the YTB working with the leads of the workpackage.

15.2 PROTOCOL DESIGN AND STUDY SETUP

The role of the YTBS is to drive the development of the information statement and consent forms for the main trial..

15.3 PPI IN THE ONGOING RUNNING OF STUDY

The YTBs will be overseen by Magda Conway (representing Fondazione PENTA ONLUS), with a coordinator for each YTB. Each YTB will meet 2-3 times a year, while continuing to liaise with trial sites in advocating for trial participants and responding to pertinent issues arising during trial progress. It is expected that representatives of the YTBs will attend annual meetings of the consortium and receive an update on progress and findings (when available) of the trial. These meetings will provide a face-to-face opportunity to seek input from YTBs.

15.4 INTERPRETING AND PLANNING DISSEMINATION OF STUDY RESULTS

The YTBs will be the key interface between YPLHIV and this trial, and will liaise with their peers to explore the findings, possible barriers for roll-out and solutions to these. When we have results, we will invite some key stakeholders, including the YTBs, to work with us in developing key messages about the results and audience-friendly ways to communicate those messages to different audiences. Based on the stakeholder mapping and communication strategy, we will develop and distribute a variety of tools to communicate the results to our different audiences. What these tools are will depend on the strategy, but are likely to include, at a minimum: Participant meetings to communicate the results; Press releases for local, national and international media; Journal articles for Open Access publication in high impact journals; News articles for partner websites, written in plain language; PowerPoint slide sets for scientific audiences; Briefing papers summarising the results for policymakers.

Other tools we have positive experience of using for previous trials include: Training videos for health workers (distributed online and via DVDs); Films about the results (targeted at the public, or health workers, or policymakers); Infographics; Social media; Animations.

15.5 DOCUMENTING THE INPUT OF PPI

The description of the PPI input will be included in the main publication, and subsequent publications as appropriate. The YTB will be acknowledged on all publications arising.

16 PUBLICATION AND DISSEMINATION OF RESULTS

BREATHER Plus trial data are not the property of individual participating investigators or health care facilities where the data were generated. UCL has delegated the custodianship of the data and storage specimens, to the BREATHER Plus TSC for the duration of the trial while the TSC is in place. Once the TSC is dissolved, custodianship will revert to UCL, following CTU standard operating procedures..

It is anticipated that a number of opportunities will arise for publication during the course of and following completion of the BREATHER Plus trial. Publications include papers (including abstracts) for presentation at national and international meetings, as well as the preparation of manuscripts for peer-reviewed publication. In order to avoid disputes regarding authorship, it is important to establish a consensus approach that will provide a framework for all publications derived in full or in part from this clinical trial. The following approach is derived from *The Lancet* and from the publication policies used in other clinical trials coordinated by CTU:

- All publications are to be approved by the TMG and TSC before submission for publication. Any publication arising before the end of the trial (not by randomised groups) will also be approved by the IDMC in order to ensure that the primary objective of the trial (the randomised comparison) is not compromised. In particular, no analyses by randomised group of any outcome (primary, secondary or other) in either the main trial or associated sub-studies will be conducted or presented before the end of the trial, other than those for interim review by the IDMC. The TMG and TSC will resolve problems of authorship and maintain the quality of publications.

In line with MRC policy that the results of publicly-funded research should be freely available, manuscripts arising from the trial will be submitted to peer-reviewed journals which enable Open Access via Europe PubMed within six months after the official date of final publication. All conference presentations will be made available as soon as possible after the event via the BREATHER Plus website. All publications will acknowledge the trial's funding sources.

- For all publications, the TMG will nominate a chairperson or approve an individual’s request to chair a manuscript writing committee. The chair will usually be the primary or senior author. The chairperson is responsible for identifying fellow authors and for determining with that group the order of authorship that will appear on the manuscript. The TSC will resolve any problems of authorship and maintain the quality of publications.

- A list of investigators to be presented in at the end of the paper will be maintained by the CTU. These investigators who contributed to the investigation being reported but who are not members of the writing committee will be named in any manuscript arising. In principle, sub-study reports should include all investigators for the main study, although in some instances where a smaller number of investigators have made any form of contribution, it may be appropriate to abbreviate the listing. All authors named in the masthead of any publication arising from the main study or sub-studies must have a made a substantive academic or project management contribution to the work that is being presented. “Substantive” must be defined by a written declaration of exactly what the contribution of any individual is believed to have been. In addition to fulfilling the criteria based on contribution, additional features that will be considered in selecting an authorship group will include the recruitment of patients who contributed data to any set of analyses contained in the manuscript and/or the conduct of analyses (laboratory and statistical), leadership and coordination of the project in the absence of a clear academic contribution.

The data derived from this clinical trial are considered the property of the Sponsor (UCL) with custodianship delegated to the BREATHER Plus TSC. The presentation or publication of any data collected by the participating investigators on patients entered into this trial is under the direct control of the TMG and TSC (and the IDMC before the end of the trial). This is true whether the publication or presentation is concerned directly with the results of the trial or is associated with the trial in some other way. However, although individual participating investigators will not have any inherent right to perform analyses or interpretations or to make public presentations or seek publication of any of the data other than under the auspices of and with the approval of the TMG and TSC (and the IDMC before the end of the trial), they will be encouraged to develop sub-studies or propose analyses subject to the approval by the TMG and TSC (and the IDMC before the end of the trial). Any requests for access to raw data will be welcomed as long as they are scientifically valid and do not conflict with the integrity of the trial or ongoing analyses by the trial team

Outcome data by randomised group will not be revealed to the participating investigators until the data collection phase and full primary analysis of the trial has been completed. This policy safeguards against possible bias affecting the data collection. The IDMC will be monitoring the outcome results and may recommend that the trial be stopped for safety reasons or if a definitive answer is reached earlier than the scheduled end of the trial.

17 DATA AND/OR SAMPLE SHARING

During the trial data will be shared according to the CTU's Data Sharing SOP which describes a controlled access approach, based on the following principles:

- No data should be released that would compromise an ongoing trial or study.
- There must be a strong scientific or other legitimate rationale for the data to be used for the requested purpose.
- Investigators who have invested time and effort into developing a trial or study should have a period of exclusivity in which to pursue their aims with the data, before key trial data are made available to other researchers.
- The resources required to process requests should not be under-estimated, particularly successful requests which lead to preparing data for release. Therefore adequate resources must be available in order to comply in a timely manner or at all, and the scientific aims of the study must justify the use of such resources.
- Data exchange complies with Information Governance and Data Security Policies in all of the relevant countries.

Data will be available for sharing after the publication of the main trial results, and in line with Horizon 2020 open research data pilot. Researchers wishing to access BREATHER Plus data should contact the Trial Management Group (mrcctu.bplata@ucl.ac.uk) in the first instance.

18 PROTOCOL AMENDMENTS

Protocol v1.0 04-Mar-2020	
Protocol v2.0 18-Mar-2020	
Changes Made	**Sections Updated**
Changes to names listed as site leads	Page 4: Recruiting Site Staff
Protocol v3.0	
Changes Made	**Sections Updated**
Typographical corrections and amendments for consistency and clarity	Throughout
Updates to trial administration including: - Protocol version and date - PENTA and UZCRC logos - Grant codes - Addition of trial PACTR number - Trial team members - Site addresses - Site staff contact details	Cover page Page 2: Funding Page 3: Trial Registration Page 4: Sponsor/MRC CTU at UCL Staff and Affiliates Page 4: Recruiting Site Staff Page 6: Other Responsible Individuals Page 7: Summary of Trial Section 13: Finance
Clarification of safety secondary outcome, with addition of grade 5 captured under serious adverse events	Page 7: Trial Summary Section 1.2.3.B: Safety Secondary Outcomes Section 9.2.2.B: Safety Outcome Measures
Update from 8-weekly to 12-weekly visits from year two onwards	Page 10: Trial Schema Page 11: Trial Assessment Schedule Page 15: Lay Summary Section 1.2.3A: Efficacy Secondary Outcomes Section 5.10: Compliance and Adherence Section 6: Assessments and Follow Up Section 11.2.1: Ethical Considerations
Clarification of assessment timepoints and separation of assessments required in line with the data collection on eCRFs	Page 11: Trial Assessment Schedule Section 6.2: Trial Assessment Schedule Section 10.1: Social Science Sub-Study
Change in timelines for MEMScap sub-study completion to between weeks 8-32 (group 1) and weeks 48-72 (group 2)	Page 11: Trial Assessment Schedule Section 5.10: Compliance and Adherence
Addition of updated data on dolutegravir and pregnancy	Section 1.1.4: Dolutegravir and Neural Tube Defects
Confirmation of LATA dosing schedule and addition of clinicaltrials.gov registration number	Section 1.1.7: Relevant Studies Underway or Planned
Confirmation of virological rebound definition	Section 1.2.3A: Efficacy Secondary Outcomes
Clarification of PI qualification requirements and site assessment procedures	Section 2.1: Site/Investigator Inclusion Criteria
Clarification of approval and activation process	Section 2.2: Approval and Activation
Inclusion criteria split into two separate criteria for clarity	Section 3.1: Inclusion Criteria
Confirmation of acceptable dilution factors to meet viral load eligibility criteria	Section 3.2: Exclusion Criteria
Clarification of re-screening criteria	Section 3.5.2: Screening Procedures

Clarification of randomisation procedures, including removal of MRC CTU manual randomisation	Page 3: Randomisations Section 4.2: Randomisation Practicalities
Removal of references to pharmaceutical collaborator and confirmation that IMP will be sourced through national ART programmes	Section 5: Treatment of Participants Section 7.3 MRC CTU at UCL Responsibilities
Clarification for incident TB management	Section 5.3.1: Incident TB Management
Clarification of accountability requirements	Section 5.6: Dispensing of All Trial Medication and Accountability
Table 2 updated to align with the Investigator Brochures	Section 5.8.1: Information of Specific ART Drug Toxicities
Clarification regarding procedures for laboratory safety assessments	Section 6.4: Procedures for assessing safety
Clarification of reporting timelines for pregnancies to the Antiretroviral Pregnancy Register	Section 6.8.1: Reporting Incident Pregnancy
Clarification of lost to follow up definition	Section 6.11: Loss to Follow-Up
Confirmation of the procedure and timelines for MRC CTU being notified of reportable events	Section 7.2: Investigator Responsibilities
Clarification of requirements of reporting suicidal ideation or behaviours	Section 7.2: Investigator Responsibilities
Clarification for procedures for assessing expectedness	Section 7.2.1.E: Expectedness
Clarification of procedures for reporting pregnancy notable events, including that spontaneous abortions/miscarriages, congenital abnormality or birth defect are SAEs	Section 7.2.1D: Notable Events
Additional guidance on definition and documentation of source data	Section 8.3.2: Source Data
Pre-specification of non-inferiority margin dependent on control event rate	Section 9.3: Sample Size
Correction of viral load timepoints that will be reviewed by the IDMC and number of participants	Section 9.4.2: Interim Analysis
Clarification of analysis to be used for outcomes and clinical events	Section 9.5: Analysis Plan (Brief)
Update to process for in-depth interviews	Section 10.1.1: In-Depth Interviews. It will not be possible to explore and differences according to mode of transmission (vertical vs. horizontal) as so few horizontally infected participants have been enrolled.
Clarification of TMG meeting schedule	Section 14.2: Trial Management Group (TMG)
Update to references	Section 19: References

19 REFERENCES

1. All In to #EndAdolescentAIDS 2015 [Online]. Available from: https://www.unaids.org/sites/default/files/media_asset/20150217_ALL_IN_brochure.pdf [Accessed 3 July 2019].

2. World Health Organization 2016. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach - Second edition [Online]. Available from: http://www.who.int/hiv/pub/arv/arv-2016/en/ [Accessed 3 July 2019].

3. Auld AF, Agolory SG, Shiraishi RW, Wabwire-Mangen F, Kwesigabo G, Mulenga M, 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 Morbidity and mortality weekly report. 2014;63(47):1097-103.

4. Evans D, Menezes C, Mahomed K, Macdonald P, Untiedt S, Levin L, 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.

5. Grimsrud A, Balkan S, Casas EC, Lujan J, Van Cutsem G, Poulet E, et al. Outcomes of antiretroviral therapy over a 10-year period of expansion: a multicohort analysis of African and Asian HIV programs. Journal of acquired immune deficiency syndromes. 2014;67(2):e55-66.

6. Lamb MR, Fayorsey R, Nuwagaba-Biribonwoha H, Viola V, Mutabazi V, Alwar T, et al. High attrition before and after ART initiation among youth (15-24 years of age) enrolled in HIV care. Aids. 2014;28(4):559-68.

7. Nachega JB, Hislop M, Nguyen H, Dowdy DW, Chaisson RE, Regensberg L, et al. Antiretroviral therapy adherence, virologic and immunologic outcomes in adolescents compared with adults in southern Africa. Journal of acquired immune deficiency syndromes. 2009;51(1):65-71.

8. Kawuma R, Bernays S, Siu G, Rhodes T, Seeley J. 'Children will always be children': exploring perceptions and experiences of HIV-positive children who may not take their treatment and why they may not tell. African journal of AIDS research: AJAR. 2014;13(2):189-95.

9. Adejumo OA, Malee KM, Ryscavage P, Hunter SJ, Taiwo BO. Contemporary issues on the epidemiology and antiretroviral adherence of HIV-infected adolescents in sub-Saharan Africa: a narrative review. Journal of International AIDS Society. 2015;18:20049.

10. Kenny J, Mulenga V, Hoskins S, Scholten F, Gibb DM. The needs for HIV treatment and care of children, adolescents, pregnant women and older people in low-income and middle-income countries. Aids. 2012;26 Suppl 2:S105-16.

11. Reisner SL, Mimiaga MJ, Skeer M, Perkovich B, Johnson CV, Safren SA. A review of HIV antiretroviral adherence and intervention studies among HIV-infected youth. Topics in HIV medicine : a publication of the International AIDS Society, USA. 2009;17(1):14-25.

12. Alvarez J, De Truchis P, Emuri A, Assoumou L, Landman R, Mathez D, et al. Efficacy of antiretroviral drugs during intermittent maintenance treatment with a 4-days-a-week regimen despite low plasma concentrations (ANRS 162-4D trial) (P081). Journal of International AIDS Society. 2016;19(Suppl 7).

13. 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.

14. Leibowitch J, Mathez D, de Truchis P, Ledu D, Melchior JC, Carcelain G, 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 journal: official publication of the Federation of American Societies for Experimental Biology. 2015;29(6):2223-34.

15. 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 journal: official publication of the Federation of American Societies for Experimental Biology. 2010;24(6):1649-55.

16. Reynolds SJ, Kityo C, Hallahan CW, Kabuye G, Atwiine D, Mbamanya F, 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. Rudy BJ, Sleasman J, Kapogiannis B, Wilson CM, Bethel J, Serchuck L, 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.

18. Cohen CJ, et al. FOTO study: the 48 week extension to assess durability of the strategy of taking efavirenz, tenofovir and emtricitabine fi ve days on, two days off (FOTO) each week in virologically suppressed patients. 5th IAS Conference, Cape Town; July, 2009. Abstr MOPE B063.

19. de Truchis P, Assoumou, L., Landman R. Efficacy of a maintenance four-days-a-week regimen, the ANRS162-4D trial. 21st International AIDS Conference (AIDS); 2016 Jul 18-22; Durban, South Africa. Poster presentation THPEB 063 2016.

20. Butler K, Inshaw J, Ford D, Bernays S, Scott K, Kenny J, 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. 2016;20(49):1-108.

21. Group BT. 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):e421-30.

22. Turkova A, Moore CL, Butler K, Compagnucci A, Saidi Y, …, Ford D*, Babiker A*, Gibb DM* (* equal contribution) (2018) Weekends-off efavirenz-based antiretroviral therapy in HIV-infected children, adolescents and youg adults (BREATHER): Extended follow-up results of a randomized, open-label, non-inferiority trial. PloS One 13 (4): e0196239.

23. 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(2):e012934.

24. Landman R, de Truchis P, Assoumou L, Lambert S, Bellet J, et al, 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 Feb;9(2):e79-e90. doi: 10.1016/S2352-3018(21)00300-3].

25. Landman R et al. *W96 efficacy of 4/7 days maintenance ART strategy: ANRS-170 QUATUOR trial.* Conference on Retroviruses and Opportunistic Infections, abstract 419, 2021.

26. Kanters S, Vitoria M, Doherty M, Socias ME, Ford N, Forrest JI, et al. Comparative efficacy and safety of first-line antiretroviral therapy for the treatment of HIV infection: a systematic review and network meta-analysis. Lancet HIV. 2016;3(11):e510-e20.

27. Patel DA, Snedecor SJ, Tang WY, Sudharshan L, Lim JW, Cuffe R, et al. 48-week efficacy and safety of dolutegravir relative to commonly used third agents in treatment-naive HIV-1-infected patients: a systematic review and network meta-analysis. PloS one. 2014;9(9):e105653.

28. Brenner BG, Wainberg MA. Clinical benefit of dolutegravir in HIV-1 management related to the high genetic barrier to drug resistance. Virus research.. 2017;239:1-9.

29. Clinton Health Access Initiative. HIV Market Report: The State of the HIV Treatment, Testing, and Prevention Markets in Low- and Middle-Income Countries, 2017-2022. ISSUE 9, September 2018.

30. World Health Organization 2019. Update of recommendations on first- and second-line antiretroviral regimens [Online]. Available from: https://apps.who.int/iris/bitstream/handle/10665/325892/WHO-CDS-HIV-19.15-eng.pdf?ua=1. [Accessed 25 November 2019].

31. UNAIDS 2018. New high-quality antiretroviral therapy to be launched in South Africa, Kenya and over 90 low-and middle-income countries at reduced price [Online]. Available from: http://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2017/september/20170921_TLD. [Accessed 3 July 2019].

32. 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. The Journal of antimicrobial chemotherapy. 2015;70(10):2810-5.

33. Lamorde M, Schapiro JM, Burger D, Back DJ. Antiretroviral drugs for prevention of mother-to-child transmission: pharmacologic considerations for a public health approach. Aids. 2014;28(17):2551-63.

34. MRC CTU at UCL (Sponsor) Investigator's Brochure for Dolutegravir (DTG) as part of the antiretroviral therapy partnered with Tenofovir disoproxil fumarate, Lamivudine, Dolutegravir (TLD) for BREATHER Plus and LATA protocols, version 1.0 dated 04-May-2021.

35. Zash R, Makhema J, Shapiro RL. Neural-Tube Defects with Dolutegravir Treatment from the Time of Conception. N Engl J Med. 2018;379(10):979-81.

36. Zash, R., et al., Surveillance for Neural Tube Defects following Antiretroviral Exposure from Conception, the Tsepamo study (Botswana). AIDS 2018; Amsterdam, 23-27 July 2018.

37. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health. 2018;6(7):e804-e10.

38. Zash R, Diseko M, Jacobson D.L, et al. Neural-Tube Defects and Antiretroviral Treatment Regimens in Botswana. The New England Journal of Medicine. 2019(381):827-40.

39. MRC CTU at UCL (Sponsor) Investigator's Brochure for Tenofovir Disoproxil Fumarate (TDF) for the treatment of HIV-1 infection, in combination with other antiretroviral therapy, in the BREATHER Plus and LATA protocols, version 1.0 dated 23-Jun-2022.

40. MRC CTU at UCL (Sponsor) Investigator's Brochure for Lamivudine (3TC) for the treatment of HIV-1-infection in combination with other antiretroviral therapy in the BREATHER Plus and LATA protocols, version 1.0 dated 23-Jun-2022.

41. MRC CTU at UCL (Sponsor) Investigator's Brochure for Emtricitabine (FTC) for the treatment of HIV-1-infection in combination with other antiretroviral therapy in the BREATHER Plus and LATA protocols, version 1.0 dated 23-Jun-2022.

42. MRC CTU at UCL (Sponsor) Investigator's Brochure for Tenofovir Alafenamide Fumarate (TAF) for the treatment of HIV-1-infection in combination with other antiretroviral therapy in the BREATHER Plus and LATA protocols, version 1.0 dated 23-Jun-2022.

43. Gaur, A.H., et al., Safety, efficacy, and pharmacokinetics of a single-tablet regimen containing elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide in treatment-naive, HIV-infected adolescents: a single-arm, open-label trial. Lancet HIV, 2016. 3(12): p. e561-e568.

44. World Health Organization, Global Tuberculosis report 2015 [Online]. Available from: http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf?ua=1, 2015: Geneva. [Accessed 3 July 2019].

45. Custodio JM et al. Twice daily administration of tenofovir alafenamide in combination with rifampin: potential for tenofovir alafenamide use in HIV-TB coinfection. 16th European AIDS Conference (EACS). October 25-27 2017. Milan. Oral abstract PS13/4.

46. ClinicalTrials.gov 2017. A randomised trial of dolutegravir (DTG)-based antiretroviral therapy vs. standard of care (SOC) in children with HIV infection starting first-line or switching to second-line ART [Online]. Available from: https://clinicaltrials.gov/ct2/show/NCT02259127?term=odyssey&cond=Hiv&rank=1. [Accessed 3 July 2019].

47. International Antiviral Society-USA. HIV Drug resistance Mutations [Online]. Available from: www.iasusa.org/resources/hiv-drug-resistance-mutations/ [Accessed 3 July 2019].

48. Stanford University. HIV Drug Resistance Database [Online]. Available from: https://hivdb.stanford.edu/ [Accessed 3 July 2019].

49. Christian Hoffmann, Josep M Llibre. Neuropsychiatric Adverse Events with Dolutegravir and Other Integrase Strand Transfer Inhibitors. AIDS Rev. 2019;21(1):4-10. doi: 10.24875/AIDSRev.19000023.

50. Antiretroviral Pregnancy Registry Steering Committee, Antiretroviral Pregnancy Registry International Interim Report for 1 January 1989 through 31 January 2017 [Online]. Available from: www.APRegistry.com. [Accessed 3 July 2019].

51. 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. Research Square; 2022. DOI: 10.21203/rs.3.rs-2175825/v1

52. Morris, T.P., Walker, A.S., Williamson, E.J. et al. Planning a method for covariate adjustment in individually randomised trials: a practical guide. Trials, 2022; 23(328) DOI: 10.1186/s13063-022-06097-z

DocuSign

Certificate Of Completion

Envelope Id: 1362D5111FBF454B9FEB931879359298
Status: Completed
Subject: Complete with DocuSign: BP_Protocol_v3.0 24Mar2023.docx
Source Envelope:
Document Pages: 88
Signatures: 3
Certificate Pages: 5
Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Enabled
Time Zone: (UTC) Dublin, Edinburgh, Lisbon, London

Envelope Originator:
Alexandra Green
90 High Holborn 2nd Floor London
London, London WC1V 6LJ
alexandra.green@ucl.ac.uk
IP Address: 128.40.217.97

Record Tracking

Status: Original
27 March 2023 | 10:43
Holder: Alexandra Green
alexandra.green@ucl.ac.uk
Location: DocuSign

Signer Events	Signature	Timestamp
Sarah Pett s.pett@ucl.ac.uk Professor of Infectious Diseases, Project Lead of BREATHER Plus, and CI of LATA Security Level: Email, Account Authentication (Optional)	DocuSigned by: Sarah Pett EA0A520EC2DA404... Signature Adoption: Pre-selected Style Using IP Address: 144.82.114.150	Sent: 27 March 2023 | 10:53 Viewed: 27 March 2023 | 11:29 Signed: 27 March 2023 | 11:29
Electronic Record and Signature Disclosure: Accepted: 22 February 2022 | 16:34 ID: f6085741-aefe-4fed-8547-3b1808096cd8		
Debbie Ford deborah.ford@ucl.ac.uk Statistician MRC CTU at University College London Security Level: Email, Account Authentication (Optional)	DocuSigned by: Debbie Ford C79DC5F84BBB461... Signature Adoption: Pre-selected Style Using IP Address: 144.82.114.153	Sent: 27 March 2023 | 11:29 Viewed: 27 March 2023 | 12:05 Signed: 27 March 2023 | 12:07
Electronic Record and Signature Disclosure: Not Offered via DocuSign		
Prof Adeodata Kekitiinwa-Rukyalekere akekitiinwa@baylor-uganda.org Security Level: Email, Account Authentication (Optional)	DocuSigned by: Prof Adeodata Kekitiinwa-Rukyalekere 62AD2265A94B43D... Signature Adoption: Pre-selected Style Using IP Address: 41.210.143.73	Sent: 27 March 2023 | 12:07 Viewed: 28 March 2023 | 18:34 Signed: 28 March 2023 | 18:34
Electronic Record and Signature Disclosure: Accepted: 28 March 2023 | 18:34 ID: 138629a6-6f8c-4c5d-bed4-8e239f925ec6		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	27 March 2023 | 10:53
Certified Delivered	Security Checked	28 March 2023 | 18:34
Signing Complete	Security Checked	28 March 2023 | 18:34
Completed	Security Checked	28 March 2023 | 18:34

Payment Events	Status	Timestamps

Electronic Record and Signature Disclosure

ELECTRONIC RECORD AND SIGNATURE DISCLOSURE

From time to time, MRC Clinical Trials Unit at UCL (we, us or Company) may be required by law to provide to you certain written notices or disclosures. Described below are the terms and conditions for providing to you such notices and disclosures electronically through the DocuSign system. Please read the information below carefully and thoroughly, and if you can access this information electronically to your satisfaction and agree to this Electronic Record and Signature Disclosure (ERSD), please confirm your agreement by selecting the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

Getting paper copies

At any time, you may request from us a paper copy of any record provided or made available electronically to you by us. You will have the ability to download and print documents we send to you through the DocuSign system during and immediately after the signing session and, if you elect to create a DocuSign account, you may access the documents for a limited period of time (usually 30 days) after such documents are first sent to you. After such time, if you wish for us to send you paper copies of any such documents from our office to you, you will be charged a $0.00 per-page fee. You may request delivery of such paper copies from us by following the procedure described below.

Withdrawing your consent

If you decide to receive notices and disclosures from us electronically, you may at any time change your mind and tell us that thereafter you want to receive required notices and disclosures only in paper format. How you must inform us of your decision to receive future notices and disclosure in paper format and withdraw your consent to receive notices and disclosures electronically is described below.

Consequences of changing your mind

If you elect to receive required notices and disclosures only in paper format, it will slow the speed at which we can complete certain steps in transactions with you and delivering services to you because we will need first to send the required notices or disclosures to you in paper format, and then wait until we receive back from you your acknowledgment of your receipt of such paper notices or disclosures. Further, you will no longer be able to use the DocuSign system to receive required notices and consents electronically from us or to sign electronically documents from us.

All notices and disclosures will be sent to you electronically

Unless you tell us otherwise in accordance with the procedures described herein, we will provide electronically to you through the DocuSign system all required notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you during the course of our relationship with you. To reduce the chance of you inadvertently not receiving any notice or disclosure, we prefer to provide all of the required notices and disclosures to you by the same method and to the same address that you have given us. Thus, you can receive all the disclosures and notices electronically or in paper format through the paper mail delivery system. If you do not agree with this process, please let us know as described below. Please also see the paragraph immediately above that describes the consequences of your electing not to receive delivery of the notices and disclosures electronically from us.

How to contact MRC Clinical Trials Unit at UCL:

You may contact us to let us know of your changes as to how we may contact you electronically, to request paper copies of certain information from us, and to withdraw your prior consent to receive notices and disclosures electronically as follows:
To contact us by email send messages to: s.assam@ucl.ac.uk

To advise MRC Clinical Trials Unit at UCL of your new email address

To let us know of a change in your email address where we should send notices and disclosures electronically to you, you must send an email message to us at s.assam@ucl.ac.uk and in the body of such request you must state: your previous email address, your new email address. We do not require any other information from you to change your email address.

If you created a DocuSign account, you may update it with your new email address through your account preferences.

To request paper copies from MRC Clinical Trials Unit at UCL

To request delivery from us of paper copies of the notices and disclosures previously provided by us to you electronically, you must send us an email to s.assam@ucl.ac.uk and in the body of such request you must state your email address, full name, mailing address, and telephone number. We will bill you for any fees at that time, if any.

To withdraw your consent with MRC Clinical Trials Unit at UCL

To inform us that you no longer wish to receive future notices and disclosures in electronic format you may:

i. decline to sign a document from within your signing session, and on the subsequent page, select the check-box indicating you wish to withdraw your consent, or you may;

ii. send us an email to s.assam@ucl.ac.uk and in the body of such request you must state your email, full name, mailing address, and telephone number. We do not need any other information from you to withdraw consent.. The consequences of your withdrawing consent for online documents will be that transactions may take a longer time to process..

Required hardware and software

The minimum system requirements for using the DocuSign system may change over time. The current system requirements are found here: https://support.docusign.com/guides/signer-guide-signing-system-requirements.

Acknowledging your access and consent to receive and sign documents electronically

To confirm to us that you can access this information electronically, which will be similar to other electronic notices and disclosures that we will provide to you, please confirm that you have read this ERSD, and (i) that you are able to print on paper or electronically save this ERSD for your future reference and access; or (ii) that you are able to email this ERSD to an email address where you will be able to print on paper or save it for your future reference and access. Further, if you consent to receiving notices and disclosures exclusively in electronic format as described herein, then select the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

By selecting the check-box next to 'I agree to use electronic records and signatures', you confirm that:

- You can access and read this Electronic Record and Signature Disclosure; and
- You can print on paper this Electronic Record and Signature Disclosure, or save or send this Electronic Record and Disclosure to a location where you can print it, for future reference and access; and
- Until or unless you notify MRC Clinical Trials Unit at UCL as described above, you consent to receive exclusively through electronic means all notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you by MRC Clinical Trials Unit at UCL during the course of your relationship with MRC Clinical Trials Unit at UCL.