EZ-FV-030

A cross-sectional, observational study to characterise the transition to dolutegravir-based regimens in South Africa in terms of the emergence of obesity, viral re-suppression and integration into routine programme care

Short Title:	CHARACTERISE
Protocol Version and Approval Date:	Version 1.2, 06 April 2022
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Funder:	Unitaid
Principal Investigator:	Dr Bronwyn Bosch Ezintsha, Faculty of Health Sciences University of the Witwatersrand Building C, Sunnyside Office Park 32 Princess of Wales Terrace Parktown, Johannesburg, 2193

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

A cross-sectional, observational study to characterise the transition to dolutegravir-based regimens in South Africa in terms of the emergence of obesity, viral re-suppression and integration into routine programme care

I, the undersigned, am responsible for the conduct of the trial at this site and affirm that:

- I agree to personally conduct or supervise the study.
- I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein, as per any approved protocol amendments, as per ICH Good Clinical Practice (GCP) and all applicable national requirements and laws.
- I will not deviate from the protocol without prior review and written approval from the Institutional Review Board or Ethics Committee, except where necessary to prevent immediate danger to the participant.
- I will ensure that the requirements related to obtaining informed consent are in accordance with ICH Guidelines for GCP Section and local requirements.
- I agree to document adverse events that occur during the study, to maintain adequate and accurate records and make those records available, in accordance with ICH Guidelines for GCP, South African GCP and other local requirements. I agree to promptly report to the Ethics Committee all changes in the research activity and all unanticipated problems involving risk to the participants.
- I have sufficient time to properly conduct and complete the trial within the agreed trial period, and I will ensure that any qualified staff at my site(s) who are involved in the trial conduct are adequately trained regarding the protocol, and their responsibilities for the foreseen duration of the trial to conduct the trial properly and safely. If I delegate any of my trial activities, I will document this on a Delegation of Activities Form. I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
- I understand that the study may be terminated, or enrolment suspended at any time by me if it becomes necessary to protect the best interest of the participants.

_____ 07 Apr 2022

Dr Bronwyn Bosch Date

SPONSOR SIGNATORY APPROVAL PAGE

A cross-sectional, observational study to characterise the transition to dolutegravir-based regimens in South Africa in terms of the emergence of obesity, viral re-suppression and integration into routine programme care

I, the undersigned have read this protocol and I approve the design of this trial:

_____ 07 Apr 2022

Nonkululeko Mashabane Date
Head of Research Operations
Ezintsha, University of the Witwatersrand

TABLE OF CONTENTS

ABBREVIATIONS AND TERMS

Term	Definition
AE	Adverse event
BMI	Body mass index
CVD	Cardiovascular disease
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form
GCP	Good Clinical Practice
HbA1C	Glycosylated haemoglobin
HREC	Human Research Ethics Committee
ICF	Informed consent form
LMIC	Low- to middle-income countries
NCD	Non communicable diseases
OSA	Obstructive sleep apnoea
PLHIV	People living with HIV
POPIA	Protection of Personal Information Act
SAP	Statistical Analysis Plan
TEE	Tenofovir disoproxil fumarate/emtricitabine/efavirenz
TLD	Tenofovir disoproxil fumarate/lamivudine/dolutegravir
TLE	Tenofovir disoproxil fumarate/lamivudine/emtricitabine
WBIS	Weight Bias Internalization Scale
WHO	World Health Organization
Wits	University of the Witwatersrand

SYNOPSIS

Title	A cross-sectional, observational study to characterise the transition to dolutegravir-based regimens in South Africa in terms of the emergence of obesity, viral re-suppression and integration into routine programme care
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Background	The ADVANCE clinical trial compared three recommended first-line regimens (two containing dolutegravir) head-to-head and demonstrated virological non-inferiority at 48- and 96-weeks respectively[1,2], paving the way for the mass-introduction of dolutegravir-containing regimens across low- and- middle-income countries. The dolutegravir-containing regimens in ADVANCE were very well tolerated and demonstrated remarkable viral re-suppression in patients with viraemia when adherence measures were instituted, even in the presence of genotypically-documented resistance[1,2]. Across Africa, including South Africa, and in many other low- and middle-income countries (LMIC), the combination of tenofovir disoproxil fumarate/lamivudine (or emtricitabine) /dolutegravir (TLD) has been rolled out to millions of patients, much of this with Unitaid support to research, programmes and communities. Most ADVANCE patients have since transitioned out of the study and are on TLD in South African public sector clinics in central Johannesburg. One of the unanticipated findings of ADVANCE and the concomitant Unitaid-supported NAMSAL[3] study in Cameroon, as well as analyses of registration studies and observational studies, was the consistent finding that patients on dolutegravir experience significant weight gain and new-onset obesity[4]. It remains unclear whether this is a feature of the integrase inhibitor class (and aggravated by tenofovir alafenamide), or whether other factors are at play - it is possible that HIV infection itself may predispose to weight gain in successfully treated patients, and other antiretrovirals may alter weight trajectories. The signal has been met with alarm by the public health community, as many countries where TLD is being rolled out are experiencing a parallel obesity epidemic. Obesity is strongly associated with adverse outcomes, including diabetes, cardio-vascular-disease (CVD), sleep apnoea, gastrointestinal and muscular-skeletal disorders, asthma, poor pregnancy outcomes, many cancers, mental health issues, and poor COVID-19 outcomes. In many countries with large antiretroviral programmes, these concurrent epidemics have significant public health and financial implications, and clarification of the extent of the obesity signal is urgent. Weight gain on ADVANCE was greatest with more advanced HIV (low CD4, high viral load) and among women; other studies have confirmed this signal, as well as suggesting that Black patients are more predisposed. For South Africa, where the majority of those on TLD are Black women and many initiated with advanced disease, this has significant implications for future programme design. The study

	was extended to 192 weeks, utilizing Unitaid and ViiV funding, with continued drug donations from Gilead and ViiV. The study has been highly influential in regard to guidelines and is expected to yield in excess of 30 papers once complete. The 192-week manuscript is being written, but preliminary data suggest continued weight gain in all arms, even among men, but continued excellent suppression. Moreover, South African public health policy makers and academics have become increasingly aware that attention needs to be paid to obesity (41% among adult women, and 11% among men in the general population), associated type-2 diabetes (South Africa's second-commonest cause of death after tuberculosis, and now the commonest cause of death among women), and cardiovascular risk, all in the context of a background HIV epidemic. Almost a quarter of SA women, 80% of whom are Black, aged 15-49 had HIV in 2020 and one in five women had severe obesity (as opposed to 3% of men), and almost half of women over 15 years were hypertensive, rising to 78% by age 55 years[1]. According to the South African Demographic and Health Survey (2016), type 2 diabetes is also more common in Black SA women than men, and in 2016 was the leading cause (7.2%) of death in women[5]. There are reports from central Africa of high levels of diabetes experienced within those initiating or switched to TLD. Remarkably, women on ADVANCE had absolute baseline weights greater than menprior to ART initiation (evidence of a background obesity epidemic among women), and the majority were classified as being overweight or having obesity after 96 weeks of ART, irrespective of regimen. These overlapping common conditions are classic ''syndemics'', a relatively new global health research priority.
Rationale	The introduction of dolutegravir has been one of the most significant and successful changes to HIV programmes in the last decade, affecting tens of millions of patients in low- and middle-income countries (LMIC). The remarkable tolerability and resistance profile that dolutegravir has conferred on modern antiretroviral combination regimens has significantly contributed to progress to the last ''90'' of UNAIDS' 90-90-90 targets[6]. Unitaid has been a major part of this, heavily investing in both facilitatory research, as well as community preparedness. The introduction has been accompanied by two unexpected observations – first, a positive indicator noting viral re-suppression rates (almost all patients with viral rebound on dolutegravir will re-suppress); and a second, less positive trend of treatment-emergent obesity (most women, and many men, will develop obesity on dolutegravir). Both observations have raised important questions that require public health consideration, and for both observations, the ADVANCE study is in a unique position to add vital additional long-term African data to assist policy makers, planners, patients and health providers in understanding and designing future programmes. In addition, we have access to a further highly characterised cohort of patients initiated between 8-10 years previously, with similar baseline measurements, who were transitioned to TLD,

allowing us to compare to the more recently initiated ADVANCE cohort, and allowing us to predict what will occur to the over 5 million South Africans and, indeed, the tens of millions of Southern Africans initiated on efavirenz and TDF-containing antiretrovirals in the last 20 years, and now transitioning to TLD.

Primary questions relating to safety, weight gain and viral suppression on treatment are:

- Was the excellent tolerability and safety seen in ADVANCE over 4 years continued in the subsequent years? How does this compare to patients who had been on antiretroviral care for longer on efavirenz-based regimens within the state sector who then transitioned to TLD.
- How much weight are people gaining on treatment, and what is the trajectory – is it more rapid over an initial period, or a continuous trajectory over time? How does this compare to the long-term efavirenz-based patients on legacy regimens?
- Is the weight gain leading to additional health problems, and if so, what are those problems? Does this differ from the patients on long-term therapy?
- How significantly will the issues of treatment emergent weight gain, and the additional health problems associated with it, add to the public health burden, and how can we prepare and programme for this, particularly in LMIC countries, where there are already concurrent non communicable disease (NCD) epidemics?
- It is encouraging that ADVANCE has been able to establish that it is now possible to achieveviral re-suppression routinely in a tightly regulated environment like a clinical trial. However, in a real world scenario like a resource-constrained government clinic, the development of resistance may be more likely as monitoring and adherence is less intense. In a real-world environment, are there opportunities to turn suppression failure trajectories around given the re-suppression opportunities this drug regimen provides?
- What was the experience of patients movin from the clinical trial environment to a “real world” setting in the public sector clinics?

To provide this data and answer the questions raised, we propose accessing the ADVANCE participants, as well as well-characterised patients who have previously exited other studies, who were on tenofovir disoproxil fumarate/lamivudine or emtricitabine/efavirenz (TLE or TEE) and are now utilising the state dolutegravir antiretroviral programme. We will describe the general safety, long-term weight gain trajectory and associated obesity-related complications, as well as experience transitioning to the above state programmes in order to inform and guide future monitoring and screening programmes. We believe this is particularly important, as it appears that the burden of these complications will be disproportionately borne by South African women, who already are experiencing an obesity epidemic, and in ADVANCE and other studies experienced the most severe weight gain. Additionally, we

propose to identify those with virological failure, the pattern of resistance, and the response to a routine adherence intervention.

Finally, we want to describe the complex experience of these patients, their health providers, community members and advocates, and programme implementers, of dolutegravir introduction during the COVID-19 pandemic. Specifically, new regimens and long-acting antiretroviral agents are on the horizon, and it will be critical to be clear on lessons around mass regimen transition ahead of this change.

Proposal

We propose an observational study to address:

- *Long term trajectory and consequences of weight gain and obesity on patients initiating and transitioning to TLD*. The ADVANCE cohort is unique in this regard – it represents a cohort of diverse African patients who have been on dolutegravir on the continent the longest. In addition, our second cohort of patients were similarly characterised at baseline, and drawn from the same community, re-entering the same health system at study exit, while moving from TEE to TLD at approximately the same time as the ADVANCE patients. The extensive characterisation of both men and women, as well as important predictors of weight trajectory (baseline weight, CD4 and viral load), allow us to start modelling what the current roll-out of dolutegravir will look like in several years from now. Importantly, it will inform which, if any, metabolic and other complications should be monitored, and potentially at which thresholds.
- *Levels of viral suppression, and prevalence of resistance in those without suppression, in patients that have transitioned to TLD.* There is significant interest at the prospect of the programme simplicity of ''TLD to TLD'' – of focusing on adherence challenges in viraemic patients, rather than changing drug regimens. Current WHO recommendations recommend a switch 'to appropriate regimen'' with prolonged viraemia, but there is considerable doubt whether this is necessary, as genotype testing in ADVANCE and other studies suggest no resistance occurring to dolutegravir, and additional data accruing from studies such as NADIA[3], VISEND[7] and ARTIST[8] suggest that nucleoside backbones can be recycled successfully in most cases.The ADVANCE patients again represent patients who have been on dolutegravir the longest– and hence have had the most time to develop resistance – and screening them for viraemia, resistance, and then re-suppression with an intensive adherence intervention, will again provide invaluable data on whether a ''TLD to TLD'' approach is appropriate.
- *Patient, nurse, civil society and programme manager perceptions of the TLD expansion, against the backdrop of safety concerns about neural tube defects and weight gain.* The mass transition to TLD was supposed to be done alongside a community, patient and healthcare education

	programme, that emphasized viral suppression at the point of transition (to avoid switching viraemic patients with possible functional dual- or mono-therapy), informed consent around the issue of the neural tube defect risk, and finally, counselling about weight gain and emergent obesity, and mitigation strategies. In the end, the rollout coincided partly with the onset of the initial COVID-19 wave in South Africa, and one of the world's most severe lockdowns, meaning that the impact on both education, the speed, experience and fidelity of the rollout, and monitoring has been very difficult to assess. We will utilise the ADVANCE cohort, as well as a similarly well-described cohort of patients previously on tenofovir disoproxil fumarate/lamivudine (or emtricitabine)/efavirenz (TLE or TEE)[2], who have transitioned to routine care on TLD, with whom we have continued contact. This will allow us to compare medium (5 year in the ADVANCE cohort) and longer (9 year in the other cohort) metabolic and virologic data of those on long-term antiretrovirals, and their experience in the transition to TLD. In addition, these patients, as well as the health care workers providing them care, the activists and community members involved in advocacy and education, and the programme managers coordinating care, can give us their experience with the transition, in focus group and individual discussions as part of a sub-study. As we are anticipating futuremoves to other regimens, including long-acting agents that may be far more complex than the transition to TLD, these lessons may prove invaluable.
Design	This is a single centre, follow-up, observational, cross-sectional study reviewing two cohorts of patients who have transitioned to routine care on tenofovir disoproxil fumarate/lamivudine/dolutegravir (TLD). The first cohort will include ADVANCE patients, in which participants were randomised to one of three arms including either DTG+TAF/FTC, DTG+TDF/FTC or EFV/TDF/FTC. The second cohort will include patients previously on TLE or TEE who have since transitioned to routine care on TLD. The medium (5-year data in the ADVANCE cohort) and longer (9-year data in the other cohort) metabolic and virologic consequences of those on long-term antiretrovirals will be described and compared in the above cohorts. After obtaining informed consent from potential participants, a single cross-sectional, baseline visit will be conducted for each participant. Demographic data, clinical history, and details of previous and concomitant medications will be collected. Questionnaires including a food diary, Weight Bias Internalization Scale (WBIS) and Berlin questionnaire will be administered. Bone density and weight distribution will be assessed through use of a dual-energy X-ray absorptiometry (DXA) scan, and cardiac function assessed by conduction of a baseline electrocardiogram (ECG). Laboratory evaluations will include a renal function test, urea and electrolytes, liver function test, glycated haemoglobin (HbA1C), plasma HIV-1 RNA (viral load), lipid panel, serum glucose, and DNA extraction for genotyping in those with

	unsuppressed viral loads above 1000 copies/mL. Plasma samples will be stored locally for possible future analysis. After the baseline visit, participants who are suitable for one, or more, sub-study will be identified. A randomly selected sub-group for each of the following sub-studies and additional investigations will be drawn from eligible participants within each cohort: • Sleep evaluation: actigraphy and polysomnography • Glucose metabolism evaluation: oral glucose tolerance test (OGTT) including assessment of glucose, insulin, and C-peptide to estimate insulin sensitivity and beta cell function • Experiences of users and providers in the roll out of TLD in South Africa. Abnormalities detected in the assessments will be managed by on-study medical personnel with referral as appropriate.
Population	**<u>Observational Cohort Population</u>** Adults of at least 18 years of age with HIV-1 infection who are currently on the TLD state programme who satisfy the below eligibility criteria. The following eligibility criteria will be used to select study participants for the main study (baseline visit only): • *<u>Inclusion criteria (baseline visit/main study):</u>* 1. Able and willing to provide written or electronic informed consent for the baseline visit prior to any study-specific assessment or procedure. 2. Age at least 18 years at the time of signing the informed consent form. 3. Previously enrolled in the ADVANCE trial and have been on the TLD state programme for more than one year **[Cohort 1]** or, part of an existing cohort of patients initiating TLE (and switched to TEE) more than 9 years ago, who have been transitioned to TLD within the state programme for more than one year **[Cohort 2].** 4. Access to a reliable telephone or other device permitting information transfer. • *<u>Exclusion criteria (baseline visit/main study):</u>* 1. Personnel (e.g., investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study. 2. Any physical, mental, or social condition, that, in the Investigator's judgment, might interfere with the completion of the baseline assessments and evaluations. The Investigator should make this determination in consideration of the volunteer's medical history. 3. Participant is judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to

<table>
<tr><td></td><td colspan="2">cause harm to self or seriously interfere with the validity of the study results.

Additional eligibility criteria will be used to identify study participants who are eligible for random selection for any of the sub-studies:
• Inclusion criteria (sub-studies):
1. Enrolled into main study and completed baseline visit.
2. Able and willing to provide written or electronic informed consent for the relevant sub-study.
3. Identified as high risk for development of OSA based on the Berlin Questionnaire responses [Sleep sub study only].

1. Exclusion criteria (sub-studies): Self-reported diabetic or on treatment for diabetes mellitus (Type 1 or 2) [Glucose metabolism sub-study only].
2. Serum glucose and/or HbA1C assessment at baseline consistent with a diagnosis of diabetes mellitus [Glucose metabolism sub-study only].

Participants may be enrolled into more than one sub-study.</td></tr>
<tr><td>Treatment</td><td colspan="2">No treatment will be administered during this observational study.</td></tr>
<tr><td rowspan="2">Objectives and Endpoints</td><td>Primary Objective

Characterisation of weight gain and metabolic consequences associated with long-term TLD use</td><td>Primary Endpoint

• Weight measurement with body mass index (BMI) calculation
• Blood pressure (BP)
• Plasma glucose, oral glucose tolerance test and C-peptide measurements
• Lipid panel
• Liver function tests
• DXA
• ECG</td></tr>
<tr><td>Secondary Objectives</td><td>Secondary Endpoints</td></tr>
</table>

	• Assessment of factors contributing to, and induvial perception on weight gain	• Food diary questionnaire • Weight Bias Internalization Scale (WBIS)
	• Description of the presence of viraemia	• Plasma HIV-1 RNA levels
	• Description of the presence and patterns of HIV-1 resistance mutations in participants with viral loads > 1000 copies/mL	• HIV-1 genotyping
	• Description and occurrence of associated sleep quality and disorders	• Berlin Questionnaire for risk of sleep apnoea • Polysomnography (PSG) in randomly selected 20% of participants at high risk of developing obstructive sleep apnoea (OSA) based on the Berlin Questionnaire
	Exploratory Objectives • The association between various specialised sleep and glucose metabolism parameters, as well as patient experiences with the transition to TLD, will be explored through the sub-studies detailed below.	
Sample Size	Observational Cohort: 375 participants in total. Enrolled participants will be assessed for their suitability for various sub-studies. From the eligible participants, up to 120 will be randomly selected to participate in each sub-study. The same participant may participate in more than one sub-study. No formal sample size calculation was performed for this observational study.	
Duration	The duration of participation for each participant will either be a single visit (of approximately 5 hours), or two or more visits depending on the presence of baseline viraemia and whether they are enrolled into any sub-studies. Participants selected for the sleep study will have an overnight admission at the Wits Faculty of Health Sciences sleep laboratory. Enrolment to the study is expected to complete within 6 months.	

Statistical Analysis	Participants enrolled in the main, and various sub-studies, will be summarised. Demographic data and other baseline characteristics will be summarised overall and per cohort, using simple descriptive statistics. All primary and secondary endpoints will be summarised descriptively overall, per cohort and for various risk factors as deemed appropriate in this exploratory study. Associations between weight gain, metabolic risk and presence of sustained viral re-suppression may be explored. Additionally, any HIV-1 resistance mutations noted on genotyping will be described in detail.

1. SCHEDULE OF ASSESSMENTS

All participant enrolled will form part of the main study. Main study assessments, questionnaires, and procedures may be performed or administered in any order.

Specific sub-study questionnaires and assessments are detailed in Sections 7.2 and in the sub-study-specific plans.

Procedure / assessment / questionnaire	**Visit 1**	**Visit 2 If viraemic VL ≥ 1000**	**Visit 2 If viraemic VL < 1000**
Month(s)	**May – Jun 22**	**Jul – Aug 22**	**Jul – Aug 22**
Informed consent[1]	X		
Demographic data	X		
Medical and surgical history	X		
Previous and concomitant medications	X		
Height measurement	X		
Weight measurement	X		
Blood pressure, heart rate, temperature	X		
Symptom-led physical examination (per investigator discretion)	X	X	X
Creatinine clearance (Cockcroft-Gault formula); UECr	X		
Liver function test (total and indirect bilirubin, ALT, AST, GGT, ALP, albumin, and total protein)	X		
HbA1C (glycated haemoglobin)	X		
Plasma HIV-1 RNA	X	X	X
Plasma glucose	X		
Lipid panel (total cholesterol, triglycerides, HDL, and LDL)	X		
Oral Glucose Tolerance Test (OGTT) / Glucose Loading[2]	X----------X		
C-peptide, insulin[2]	X----------X		
Plasma for storage[3]	X		
HIV-1 genotyping		X	
DXA	X		
ECG[4]	X		
Polysomnography (PSG) in randomly selected 20% of patients[5]	X----------X		
Adherence intervention		X	X
Questionnaires: Food diary, Weight Bias Internalization Scale (WBIS), Berlin questionnaire			
Selection of participants for sub-study(ies)[6]	X----------X		

ECG = electrocardiogram

1 Written or electronic informed consent to be provide prior to any screening or study-specific assessments, questionnaires or procedures are conducted.

2 Glucose metabolism assessed as part of the relevant sub-study in eligible patients (described in Section 7.3.2.2 below)

3 Optional plasma storage

4 A standard 12-lead ECG will be conducted after the participant has rested in the supine position for 10 minutes.

5 This will be based on those identified as high risk for development of obstructive sleep apnoea (OSA) from the Berlin Questionnaire

6 Study team personnel will review each participant's baseline data and identify participants who are eligible for participation in one, or more, sub-study. Invited participants will be randomly selected from the set of eligible participants for each sub-study. Participants who decline the invitation to participate in one, or more, sub-study, will be replaced by a randomly selected, matched-cohort participant.

2. INTRODUCTION

2.1 Background

The introduction of dolutegravir has been one of the most significant and successful changes to HIV programmes in the last decade, affecting tens of millions of patients in low- and middle-income countries (LMIC). The remarkable tolerability and resistance profile that dolutegravir has conferred on modernantiretroviral combination regimens has significantly contributed to progress to the last ''90'' of UNAIDS' 90-90-90 targets[6]. Unitaid has been a major part of this, heavily investing in both facilitatory research, as well as community preparedness.The introduction has been accompanied by two unexpected observations – first, a positive indicator noting viral re-suppression rates (almost all patients with viral rebound on dolutegravir will re-suppress); and a second, less positive trend of treatment-emergent obesity (most women, and manymen, will develop obesity on dolutegravir). Both observations have raised important questions that require public health consideration, and for both observations, the ADVANCE study is in a unique position to add vital additional long-term African data to assist policy makers, planners, patients and health providers in understanding and designing future programmes. In addition, we have access to a further highly characterised cohort of patients initiated between 8-10 years previously, with similar baseline measurements, who were transitioned to TLD, allowing us to compare to the more recently initiated ADVANCE cohort, and allowing us to predict what will occur to the over 5 million South Africans and, indeed, the tens of millions of Southern Africans initiated on efavirenz and TDF-containing antiretrovirals in the last 20 years, and now transitioning to TLD.

The ADVANCE clinical trial compared three recommended first-line regimens (two containing dolutegravir) head-to-head and demonstrated virological non-inferiority at 48- and 96-weeks respectively, paving the way for the mass-introduction of dolutegravir-containing regimens across low- and middle-income countries (LMIC)[1,2].

The dolutegravir-containing regimens in ADVANCE were very well tolerated and demonstrated remarkable viral re-suppression in patients with viraemia when adherence measures were instituted, even in the presence of genotypically-documented resistance[1,2]. Across Africa, including South Africa, and in many other low- and middle-income countries (LMIC), the combination of tenofovir disoproxil fumarate/lamivudine (or emtricitabine) /dolutegravir (TLD) has been rolled out to millions of patients, much of this with Unitaid support to research, programmes and communities. Most ADVANCE patients have since transitioned out of the study and are on TLD in South African public sector clinics in central Johannesburg.

One of the unanticipated findings of ADVANCE and the concomitant Unitaid-supported NAMSAL[3] study in Cameroon, as well as analyses of registration studies and observational studies, was the consistent finding that patients on dolutegravir experience significant weight gain and new-onset obesity[4]. It remains unclear whether this is a feature of the integrase inhibitor class (and aggravated by tenofovir alafenamide), or whether other factors are at play - it is possible that HIV infection itself may predispose to weight gain in successfully treated patients, and other antiretrovirals may alter weight trajectories. The signal has been met with alarm by the public health community, as many countries where TLD is being rolled out are experiencing a parallel obesity epidemic. Obesity is strongly associated with adverse outcomes, including diabetes, cardio-vascular-disease (CVD), sleep apnoea, gastrointestinal and muscular-skeletal disorders, asthma, poor pregnancy outcomes, many cancers, mental health issues, and poor COVID-19 outcomes. In many countries with large

antiretroviral programmes, theseconcurrent epidemics have significant public health and financial implications, and clarification of the extent of the obesity signal is urgent.

Weight gain on ADVANCE was greatest with more advanced HIV (low CD4, high viral load) and among women; other studies have confirmed this signal, as well as suggesting that Black patients are more predisposed. For South Africa, where the majority of those on TLD arelack women and many initiated with advanced disease, this has significant implications for future programme design. The study was extended to 192 weeks, utilizing Unitaid and ViiV funding, with continued drug donations from Gilead and ViiV. The study has been highly influential in regard to guidelines and is expected to yield in excess of 30 papers once complete. The 192-week manuscript is being written, but preliminary data suggest continued weight gain in all arms, even among men, but continued excellent suppression.

Moreover, South African public health policy makers and academics have become increasingly aware that attention needs to be paid to obesity (41% among adult women, and 11% among men in the general population), associated type-2 diabetes (South Africa's second-commonest cause of death after tuberculosis, and now the commonest cause of death among women), and cardiovascular risk, all in the context of a background HIV epidemic. Almost a quarter of SA women, 80% of whom are Black, aged 15-49 had HIV in 2020 and one in five women had severe obesity (as opposed to 3% of men), and almost half of women over 15 years were hypertensive, rising to 78% by age 55 years[1]. According to the South African Demographic and Health Survey (2016), type 2 diabetes is also more common in Black SA women than men, and in 2016 was the leading cause (7.2%) of death in women[5]. There are reports from central Africa of high levels of diabetes experienced within those initiating or switched to TLD. Remarkably, women on ADVANCE had absolute baseline weights greater than men prior to ART initiation (evidence of a background obesity epidemic among women), and the majority were classified as being overweight or having obesity after 96 weeks of ART, irrespective ofregimen. These overlapping common conditions are classic ''syndemics'', a relatively new global health research priority.

Primary questions relating to safety, weight gain and viral suppression on treatment are:

- Was the excellent tolerability and safety seen in ADVANCE over 4 years continued in the subsequent years? How does this compare to patients who had been on antiretroviral care for longer on efavirenz-based regimens within the state sector who then transitioned to TLD.

- How much weight are people gaining on treatment, and what is the trajectory – is it more rapid over an initial period, or a continuous trajectory over time? How does this compare to the long-term efavirenz-based patients on legacy regimens?
- Is the weight gain leading to additional health problems, and if so, what are those problems? Does this differ from the patients on long-term therapy?
- How significantly will the issues of treatment emergent weight gain, and the additional health problems associated with it, add to the public health burden, and how can we prepare and programme for this, particularly in LMIC countries, where there are already concurrent non communicable disease (NCD) epidemics?
- It is encouraging that ADVANCE has been able to establish that it is now possible to achieve viral re-suppression routinely in a tightly regulated environment like a clinical trial. However, in a real world scenario like a resource-constrained government clinic, the development of resistance may be more likely as monitoring and adherence is less intense. In a real-world environment, are there opportunities to turn suppression failure trajectories around given the re-suppression opportunities this drug regimen provides?

- What was the experience of patients movin from the clinical trial environment to a "real world" setting in the public sector clinics?

2.2 Study Rationale

To provide this data and answer the questions raised, we propose accessing the ADVANCE participants, as well as well-characterised patientswho have previously exited other studies, who were on tenofovir disoproxil fumarate/emtricitabine/efavirenz (TEE) and are now utilising the state dolutegravir antiretroviral programme. We will describe the general safety, long-term weight gain trajectory and associated obesity-related complications, as well as experience transitioning to the above state programmes in order to inform and guide future monitoring and screening programmes. We believe this is particularly important, as it appears that the burden of these complications will be disproportionately borne by South African women, who already are experiencing an obesity epidemic, and in ADVANCE and other studies experienced the most severe weight gain. Additionally, we propose to identify those with virological failure, the pattern of resistance, and the response to a routine adherence intervention.

Finally, we want to describe the complex experience of these patients, their health providers, community members and advocates, and programme implementers, of dolutegravir introduction during the COVID-19 pandemic. Specifically, new regimens and long-acting antiretroviral agents are on the horizon, and it will be critical to be clear on lessons around mass regimen transition ahead of this change.

We propose an observational study to address:

- *Long term trajectory and consequences of weight gain and obesity on patients initiating and transitioning to TLD*. The ADVANCE cohort is unique in this regard – it represents a cohort of diverse African patients who have been on dolutegravir on the continent the longest. In addition, our second cohort of patients were similarly characterised at baseline, and drawn from the same community, re-entering the same health system at study exit, while moving from TEE to TLD at approximately the same time as the ADVANCE patients. The extensive characterisation of both men and women, as well as important predictors of weight trajectory (baseline weight, CD4 and viral load), allow us to start modelling what the current roll-out of dolutegravir will look like in several years from now. Importantly, it will inform which,if any, metabolic and other complications should be monitored, and potentially at which thresholds.
- *Levels of viral suppression, and prevalence of resistance in those without suppression, in patients that have transitioned to TLD.* There is significant interest at the prospect of the programme simplicity of ''TLD to TLD'' – of focusing on adherence challenges in viraemic patients, rather than changing drug regimens. Current WHO recommendations recommend aswitch 'to appropriate regimen'' with prolonged viraemia, but there is considerable doubt whether this is necessary, as genotype testing in ADVANCE and other studies suggest no resistance occurring to dolutegravir, and additional data accruing from studies such as NADIA[3], VISEND[7] and ARTIST[8] suggest that nucleoside backbones can be recycled successfully in most cases.The ADVANCE patients again represent patients who have been on dolutegravir the longest– and hence have had the most time to develop resistance – and screening them for viraemia, resistance, and then re-suppression with an intensive adherence intervention, will again provide invaluable data on whether a ''TLD to TLD''

approach is appropriate.

- *Patient, nurse, civil society and programme manager perceptions of the TLD expansion, against the backdrop of safety concerns about neural tube defects and weight gain.* The mass transition to TLD was supposed to be done alongside a community, patient and healthcare education programme, that emphasized viral suppression at the point of transition (to avoid switching viraemic patients with possible functional dual- or mono-therapy), informed consent around the issue of the neural tube defect risk, and finally, counselling about weight gain and emergent obesity, and mitigation strategies. In the end, the rollout coincided partly with the onset of the initial COVID-19 wave in South Africa, and one of the world's most severe lockdowns, meaning that the impact on both education, the speed, experience and fidelity of the rollout, and monitoring has been very difficult to assess.

We will utilise the ADVANCE cohort, as well as a similarly well-described cohort of patients previously on tenofovir disoproxil fumarate/lamivudine (or emtricitabine)/efavirenz (TLE or TEE)[2], who have transitioned to routine care on TLD, with whom we have continued contact. This will allow us to compare medium (5 year in the ADVANCE cohort) and longer (9 year in the other cohort) metabolic and virologic data of those on long-term antiretrovirals, and their experience in the transition to TLD. In addition, these patients, as well as the health care workers providing them care, the activists and community members involved in advocacy and education, and the programme managers coordinating care, can give us their experience with the transition, in focus group and individual discussions as part of a sub-study. As we are anticipating future moves to other regimens, including long-acting agents that may be far more complex than the transition to TLD, these lessons may prove invaluable.

3. OBJECTIVES AND ENDPOINTS

Primary	
Objectives	**Endpoints**
Characterisation of weight gain and metabolic consequences associated with long-term TLD use	• Weight measurement with BMI calculation • Blood pressure (BP) • Plasma glucose, HbA1C • Lipid panel • Liver function tests • DXA • ECG

Secondary	
Objectives	**Endpoints**
• Assessment of factors contributing to, and induvial perception on weight gain	• Food diary questionnaire • Weight Bias Internalization Scale
• Description of the presence of viraemia	• Plasma HIV-1 RNA levels
• Description of the presence and patterns of HIV-1 resistance mutations in participants with viral loads > 1000 copies/mL	• HIV-1 genotyping
• Description and occurrence of associated sleep quality and disorders	• Berlin Questionnaire for risk of sleep apnoea • Polysomnography (PSG) in randomly selected 20% of participants at high risk of developing OSA based on the Berlin Questionnaire
Exploratory Objectives	
• The association between various specialised sleep and glucose metabolism parameters, as well as patient experiences with the transition to TLD, will be explored through the sub-studies detailed below.	

4. STUDY DESIGN

4.1 Overall Design

This is a single centre, follow-up, observational, cross-sectional study reviewing two cohorts of patients who have transitioned to routine care on tenofovir disoproxil fumarate/lamivudine/dolutegravir (TLD). The first cohort will include ADVANCE patients, in which participants were randomised to one of three arms including either DTG+TAF/FTC, DTG+TDF/FTC or EFV/TDF/FTC. The second cohort will include patients previously on TLE or TEE who have since transitioned to routine care on TLD.

The medium (5-year data in the ADVANCE cohort) and longer (9-year data in the other cohort) metabolic and virologic consequences of those on long-term antiretrovirals will be described and compared in the above cohorts.

After obtaining informed consent from potential participants, a single cross-sectional, baseline visit will be conducted for each participant. Demographic data, clinical history, and details of previous and concomitant medications will be collected. Questionnaires including a food diary, Weight Bias Internalization Scale (WBIS) and Berlin questionnaire will be administered. Bone density and weight distribution will be assessed through use of a dual-energy X-ray absorptiometry (DXA) scan, and cardiac function assessed by conduction of a baseline electrocardiogram (ECG).

Laboratory evaluations will include a liver function test, glycated haemoglobin (HbA1C), plasma HIV-1 RNA (viral load), lipid panel, C-peptide, both serum glucose and oral glucose tolerance test, and DNA extraction for genotyping in those with unsuppressed viral loads above 1000 copies/mL. Plasma samples will be stored locally for possible future analysis.

After the baseline visit, participants who are suitable for one, or more, sub-study will be identified. A randomly selected sub-group for each of the following sub-studies and additional investigations will be drawn from eligible participants within each cohort:

- Sleep evaluation: actigraphy and polysomnography
- Glucose metabolism evaluation: oral glucose tolerance test (OGTT) including assessment of glucose, insulin, and C-peptide to estimate insulin sensitivity and beta cell function
- Experiences of users and providers in the roll out of TLD in South Africa.

Abnormalities detected in the assessments will be managed by on-study medical personnel with referral as appropriate.

4.2 Study Treatments

No treatment will be administered during this observational study. Participants taking medication(s) at the time of screening and enrolment, will continue to take this throughout their participation in the study.

4.3 Study Duration

The duration of participation for each participant will either be a single visit (of approximately 5 hours), or two or more visits depending on whether they are enrolled into any sub-studies.

Total duration will be from 1 to 60 days.

Participants selected for the sleep study will have an overnight admission at the University of the Witwatersrand (Wits) Faculty of Health Sciences sleep laboratory.

4.4 Individual Participant Withdrawal

A participant may be withdrawn from the study for any of the following reasons:

- At the request of the participant (withdrawal of informed consent), irrespective of the reason for this
- At the request of the primary care provider if he or she thinks the study is not in the best interest of the participant
- At the discretion of the Investigator if he or she believes that continuation in the study would be detrimental to the participant’s well-being in any way, or
- At the discretion of the Institutional Review Board/Ethics Committee if they believe that continuation in the study would be detrimental to the participant’s well-being in any way.

Participants will be considered withdrawn if they state an intention to withdraw, fail to return for visits, or are lost to follow-up for any other reason. For participants who are lost to follow-up, the Investigator will attempt to trace the participant, and will demonstrate "due diligence" by documenting all steps taken to contact the participant (e.g., dates of telephone calls, home visit, etc.) in the source documents.

If a participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such withdrawal of consent.

If a participant withdraws from the study, he or she may request destruction of any samples taken and not tested. This must be documented by the Investigator in the site study records.

Participants who withdraw or are withdrawn will not be replaced.

5. STUDY POPULATION

Adults of at least 18 years of age with HIV-1 infection who are currently on the TLD state programme who satisfy the below eligibility criteria.

The following eligibility criteria will be used to select study participants for the main study (baseline visit only):

- *Inclusion criteria (baseline visit/main study):*
    1. Able and willing to provide written or electronic informed consent for the baseline visit prior to any study-specific assessment or procedure.
    2. Age at least 18 years at the time of signing the informed consent form.
    3. Previously enrolled in the ADVANCE trial and have been on the TLD state programme for more than one year **[Cohort 1]**
       or,
       part of an existing cohort of patients initiating TLE (and switched to TEE) more than 9 years ago, who have been transitioned to TLD within the state programme for more than one year **[Cohort 2].**
    4. Access to a reliable telephone or other device permitting information transfer.

- *Exclusion criteria (baseline visit/main study):*
    1. Personnel (e.g., investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study.
    2. Any physical, mental, or social condition, that, in the Investigator's judgment, might interfere with the completion of the baseline assessments and evaluations. The Investigator should make this determination in consideration of the volunteer's medical history.
    3. Participant is judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

Additional eligibility criteria will be used to identify study participants who are eligible for random selection for any of the sub-studies:

- Inclusion criteria (sub-studies):
    1. Enrolled into main study and completed baseline visit.
    2. Able and willing to provide written or electronic informed consent for the relevant sub-study.
    3. Identified as high risk for development of OSA based on the Berlin Questionnaire responses **[Sleep sub study only].**

- Exclusion criteria (sub-studies):
    1. Self-reported diabetic or on treatment for diabetes mellitus (Type 1 or 2) **[Glucose metabolism sub-study only]**.

2. Serum glucose and/or HbA1C assessment at baseline consistent with a diagnosis of diabetes mellitus **[Glucose metabolism sub-study only]**.

Participants may be enrolled into more than one sub-study.

5.1 Cohorts

The following well-described cohorts of participants will be enrolled (375 participants in total):

- Cohort 1: ADVANCE patient cohort, who have been on the TLD state programme for more than one year.
- Cohort 2: existing cohorts of patients initiating TLE (and subsequently switched to TEE) more than 9 years ago and who have since transitioned to TLD within the state programme for more than one year.

5.2 Recruitment

Potential participants may volunteer in response to advertised clinical trial information or will be contacted in compliance with POPIA regulations and invited to participate in the informed consent process (Section 11.2) and attend the baseline visit. Written informed consent will be obtained prior to any screening, and study-specific assessments and procedures.

The full recruitment strategy will be outlined in the relevant SOP.

5.3 Main Study Eligibility Criteria (Baseline Visit)

The following eligibility criteria will be used to select study participants for the main study (baseline visit only).

5.3.1 Inclusion criteria

1. Able and willing to provide written or electronic informed consent for the baseline visit prior to any study-specific assessment or procedure.
2. Age at least 18 years at the time of signing the informed consent form.
3. Previously enrolled in the ADVANCE trial and have been on the TLD state programme for more than one year **[Cohort 1]**
   or,
   part of an existing cohort of patients initiating TLE (and switched to TEE) more than 9 years ago, who have been transitioned to TLD within the state programme for more than one year **[Cohort 2].**
4. Access to a reliable telephone or other device permitting information transfer.

5.3.2 Exclusion criteria

1. Personnel (e.g., investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study.
2. Any physical, mental, or social condition, that, in the Investigator's judgment, might interfere with the completion of the baseline assessments and evaluations. The Investigator should make this determination in consideration of the volunteer's medical history.

3. Participant is judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

5.4 Sub-Study Eligibility Criteria (Subsequent Optional Visit(s))

The following additional eligibility criteria will be used to identify study participants who are eligible for random selection for any of the sub-studies. Participants may be enrolled into more than one sub-study.

5.4.1 Inclusion criteria

1. Enrolled into main study and completed baseline visit.
2. Willing to provide written or electronic information consent for the relevant sub-study.
3. Identified as high risk for development of OSA based on the Berlin Questionnaire responses **[Sleep sub study only].**

5.4.2 Exclusion criteria

1. Self-reported diabetic or on treatment for diabetes mellitus (Type 1 or 2) **[Glucose metabolism sub-study only]**.
2. Serum glucose and/or HbA1C assessment at baseline consistent with a diagnosis of diabetes mellitus **[Glucose metabolism sub-study only]**.

5.5 Participant Identification

All volunteers who provide informed consent to participate in the study will be allocated a unique sequential screening number which will be used as the primary identifier for the duration of the study for enrolled participants.

5.6 Co-enrolment Guidelines

Participants may be co-enrolled in other research studies if these are observational in nature or include behavioural interventions only. Other co-enrolments require approval by the Principal Investigator after consideration of possible confounding effects and participant safety and well-being regarding blood draw volumes and exposure to multiple assessments.

6. STUDY VISITS

A brief screening assessment will be conducted for all study participants who provide informed consent for participation in the study. Those who are eligible for enrolment will continue into a baseline visit where all main study questionnaires will be administered, and assessments and procedures conducted.

Participants who are eligible for various sub-studies will be identified by the study team after review of their baseline visit results. Sub-study participants (up to 120 participants) will be randomly selected from these eligible participants and will be invited to provide separate informed consent for each sub-study. An additional visit or visits will be conducted for each sub-study. The number of study visits for each participant will depend on the number of sub-studies in which they participate. All study visits

(including the screening/baseline visits) will be completed for each participant within a maximum of 60 days.

Questionnaires to be administered, and assessments and procedures to be conducted at each study visit (main and sub-studies) are detailed in Section 1. Details regarding each questionnaire, assessment and procedure are described in Section 7.

7. STUDY ASSESSMENTS, QUESTIONNAIRES, AND PROCEDURES

No study-specific assessments will be performed, or information gathered, until the potential participant has given written, informed consent (Section 11.2) for screening assessments and (if found to be eligible) for study participation.

The timing of all assessments and procedures is detailed in the Schedule of Assessments (Section **Error! Reference source not found.**).

7.1 Main Study

Screening and baseline assessments are expected to be performed on the same day but may be spread over two or more separate days given the expected duration of the visit (approximately 5 hours).

7.1.1 Enrolment (Visit 1)

Review of the main study eligibility criteria will be performed for all participants providing informed consent for the main study (baseline visit).

7.1.2 Baseline assessments, questionnaires, and procedures (Visit 1)

The following main study (baseline) evaluations will be performed. These may be performed in any sequential order during the baseline visit. All questionnaires, surveys and rating scales will be administered in accordance with the specific guidelines associated with validated versions of the tools and will be included in the Study Assessments Manual.

Participants will be required to fast for 8 hours (water permitted *ad libitum*) prior to the drawing of blood for serum glucose assessments and lipid panel. If additional baseline assessments are scheduled for the same day as the blood draw, this will be performed as one of the first procedures of the visit and participants will be given a meal at the research unit before proceeding with the remaining assessments.

7.1.2.1 Demographics, and social background and habits data

The following will be collected:

- Sex, age, race, and country of origin
- Town of residence
- Use of tobacco products, alcohol, and illicit/street drugs.

7.1.2.2 Medical and surgical history

General information related to past and current relevant medical conditions and surgical procedures will be collected, as well as pregnancy and lactation status (for female participants only).

7.1.2.3 Previous and concomitant medications

Details of previous and ongoing medications will be collected.

7.1.2.4 Physical examination and vital signs

Height and weight (as measured in light indoor clothing or underwear only, but without shoes) will be measured, and body mass index (BMI) derived. Only blood pressure will be necessary to collect as part of vital signs.

A symptom-directed physical examination will be performed by the investigator where necessary.

7.1.2.5 Factors contributing to weight and perception of weight gain

- Food diary
- Weight Bias Internalization Scale (WBIS)[9]

7.1.2.6 Sleep evaluation

Sleep quality and associated disorders will be evaluated using the:

- Berlin Questionnaire: a self-administered questionnaire designed to identify subjects at high risk for obstructive sleep apnoea[10].
- Polysomnography in a randomly selected 20% of patients in those identified to be at high risk of development of OSA

7.1.2.7 Laboratory assessments

Blood sampling will be performed to collect samples for the following laboratory assessments:

- Serum chemistry: serum sodium, potassium, chloride, bicarbonate
- Liver function: total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH)
- Renal function: urea, creatinine, estimated creatinine clearance (Cockcroft-Gault method)
- Lipid panel: total cholesterol, triglycerides, HDL, and LDL cholesterol
- Glucose metabolism: plasma glucose, HbA1C
- HIV virological suppression: plasma HIV-1 RNA

An additional plasma (50 mL) sample will be stored for possible future analysis.

Details regarding blood sample collection, handling and processing, and the assays (where applicable) will be documented in the Laboratory Manual.

7.2 Viraemia confirmation visits (Visit 2)

These visits will only be conducted for participants with unsuppressed viral loads at baseline. In instances where a single baseline HIV-1 RNA level is ≥ 1000 copies/mL, a repeat viral load and HIV genotyping will be done, and an assessment of adherence and intensive adherence counselling done. Once genotyping results are available, the participant may be recalled for review of results and referral back into the state ART programme for ongoing care. In instances where a single baseline HIV-1 RNA

level is < 1000 copies/mL, adherence counselling will be done, and the participant recalled for a repeat viral load to assess re-suppression.

7.3 Sub-Studies

Participants may be invited to take part in more than one sub-study, or none. Up to 120 participants will be enrolled into each sub-study.

7.3.1 Screening and informed consent

Separate informed consent will be obtained for each sub-study for participants invited to take part in these.

7.3.2 Sub-study visit(s)

7.3.2.1 Sleep evaluation

Participants who are selected and provide consent for participation in the sleep sub-study, will have the following additional assessments:

- Actigraphy
- Overnight polysomnography (PSN) at the Wits Faculty of Health Sciences Sleep Laboratory.

Further details can be found in the Sleep Sub-Study Plan.

7.3.2.2 Glucose metabolism evaluation

Participants with normal serum glucose and HbA1C at the baseline visit, and who are not on treatment for diabetes mellitus (Type 1 or 2), will be randomly selected for participation in the glucose metabolism sub-study. Those who provide consent for participation will attend a single visit where an OGTT, including measures of glucose, insulin, and c-peptide to estimate insulin sensitivity and beta-cell function, will be performed.

Further details can be found in Glucose Metabolism Sub-Study Plan.

7.3.2.3 Review of experiences of users and providers in the roll out of TLD in South Africa

This qualitative sub-study seeks to understand the roll out of TLD in South Africa since its introduction in 2019. To achieve this, we will perform in-depth interviews and focus group discussions with various key informants and stakeholders. The purpose of in-depth interviews will be to understand the roll out from a guideline's perspective and how some of that has been put into practice. The focus group discussions will seek to ascertain how the target population of the programme (people living with HIV) have reacted to the transition, their perceptions, and practical constraints.

Further details can be found in the Qualitative Assessment of User and Provider Experiences in the Roll-out of TLD in South Africa Sub-Study Plan.

7.3.3 Telephonic follow-up

Participants taking part in sub-studies will be contacted telephonically within 28 days of completion of their last sub-study visit to inform them of the results of their assessments and conduct adherence counselling where necessary. Abnormalities detected in assessments (suggesting undiagnosed medical issues) will be managed by on-study medical personnel with referral as appropriate.

7.4 Biohazard Containment

Precautions will be employed by all personnel in the handling of blood and urine specimens collected during this study.

All biological specimens will be transported using packaging mandated by national and regional regulations. Details of these procedures will be described in the Laboratory Manual and will comply with relevant IATA Dangerous Goods Regulations.

8. SAFETY MONITORING

8.1 Responsibilities for Ensuring the Safety of Study Participants

8.1.1 Principal Investigator

The Principal Investigator has a personal responsibility to closely monitor study participants and an inherent authority to take whatever measures necessary to ensure their safety, including ensuring that procedures and expertise are available to cope with medical emergencies during the study.

8.1.2 Study Sponsor

The Sponsor has an institutional responsibility to ensure participant safety and undertakes to promptly notify the Wits Human Research Ethics Committee (HREC) of findings that could adversely affect the safety of participants included in the study, impact the conduct of the study, or alter the HREC's approval of, or favourable opinion to continue, the study.

8.2 Adverse Events

8.2.1 Definitions

Some of the assessments and procedures used in this study may be associated with rare discomfort or adverse events (AEs), e.g., blood sampling, six-minute walk test, injection of contrast media, skin irritation from electrodes used in polysomnography.

For this study, AEs associated with study assessments or procedures will be recorded in the eCRF for each participant. The following information be recorded for each AE:

- a description of the AE
- the dates of onset and resolution of the event
- the characteristics of the event (seriousness, severity in accordance with the DAIDS Grading Table Version 2.1, Jul 2017)[11]
- the action taken in response to the event (including treatment required)
- the outcome of the event.

9. STATISTICAL CONSIDERATIONS

Details of the statistical analyses and their presentation will be documented in the Statistical Analysis Plan (SAP).

9.1 Sample Size Determination

No formal sample size was calculated for this exploratory study. On-study findings may suggest that larger sample sizes are required to further analyse endpoints in specific groups, and further planning for this may be performed.

9.2 Analysis and Presentation of Data

9.2.1 Disposition, demographic, and background data

Participants enrolled in the main, and various sub-studies, will be summarised. Demographic data and other baseline characteristics will be summarised overall and per cohort.

9.2.2 Primary and secondary endpoints

All primary and secondary endpoints will be summarised descriptively overall, per cohort and for various risk factors as deemed appropriate in this exploratory study. Associations between weight gain, metabolic risk and presence of sustained viral re-suppression may be explored. Additionally, any HIV-1 resistance mutations noted on genotyping will be described in detail.

9.2.3 Safety data

All AEs will be listed.

9.2.4 Exploratory analyses

Details of exploratory endpoints and analyses to be performed will be included in the SAP.

10. STUDY MONITORING

Study conduct will be monitored by a research site monitor. Review of individual participant records, including consent forms, electronic case report forms (eCRFs), supporting data, questionnaire responses, and laboratory specimen records will be performed as detailed in the Clinical Monitoring Plan, to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect the site files to ensure that good clinical practice requirements are being followed.

The Clinical Monitoring Plan will describe these activities and will take into consideration necessary adaptations to be made if physical access to the site is limited at any stage due to pandemic restrictions.

11. ETHICAL CONSIDERATIONS

11.1 Ethical Considerations

The study will be conducted according to GCP (including South African GCP Guidelines [**Error! Reference source not found.**]), the Belmont Report, the Declaration of Helsinki, and South Africa legal requirements regarding clinical research. The study protocol and relevant supporting documents will be submitted for review and approval by the Wits HREC responsible for oversight of research conducted at the study site. The study protocol will be registered with the South African National Clinical Trial Registry (www.sanctr.gov.za), National Human Research Ethics Committee (www.ethicsapp.co.za) and www.ClinicalTrial.gov. Six-monthly progress reports will be submitted to the HREC for the duration of the study, and as requested. Upon completion or premature termination

of the study, the Investigator will provide the HREC with a summary of the study's outcome, and any reports required.

11.2 Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out. The consent form will describe the purpose of the study, the assessments, questionnaires, and procedures to be performed, and the risks and benefits of participation. Separate consent forms will be available for the main study and all sub-studies. Participation in the sub-studies is voluntary and consent will be sought for these after completion of the baseline visit of the main study for participants who are eligible for further enrolment into sub-studies. Separate consent will be required for each sub-study in which a participant is enrolled.

Potential participants will have the opportunity to have any questions answered before and after signing the informed consent forms (ICFs). The informed consent process and all questions raised will be documented.

The study staff who conduct the informed consent process will also sign the ICFs. A copy of the consent form(s) will be given to the participant, and this fact will be documented in the participant's record.

Any participant who is rescreened should be reconsented and eligibility for the study must be re-checked prior to enrolment.

11.3 Blood Volume

The volume of blood to be drawn from participants taking part in the main study is less than 100 mL. For a participant taking part in all additional sub-studies, the total blood volume across the entire study will not exceed 250 mL over 60 days.

11.4 Study Records and Confidentiality

The study site will establish a standard operating procedure for confidentiality protection. The site will ensure that study records, including ICFs, locator forms, case report forms, notations of all contacts with the participant, and all other source documents are stored in a secure manner.

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality.

All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring, and auditing by the HREC.

The Principal Investigator or designee and all employees and co-workers involved with this study may not disclose or use, for any purpose other than performance of the study, any data, record, or other unpublished confidential information disclosed to those individuals for the purpose of the study.

All computers used during the study conduct will be password-protected, and records will only be accessible to authorised study staff.

11.5 Participant Remuneration

For each day of protocol-related study procedures, the participants will receive compensation for travel costs to and from the clinic and inconvenience incurred, as per local regulating body recommendations.

12. ADMINISTRATIVE CONSIDERATIONS

12.1 Protocol Amendments

Any protocol amendments will be prepared by the Sponsor and submitted to the HREC in accordance with their requirements.

Approval must be obtained from the HREC before the implementation of any changes, except for changes necessary to eliminate an immediate hazard to participants, or changes that involve logistical or administrative aspects only (e.g., change in contact information).

12.2 Clinical Data Records

A log of names, signatures and initials of all staff authorised to enter data into a participant's clinic file and eCRF will be kept.

The Investigator will maintain paper or electronic source documentation for all study participants. Protocol-specific participant information will be captured in an eCRF. The Clinical Data Management System will comply with guidelines and requirements for electronic systems used in clinical research.

Data validation and quality control procedures will be detailed in the Data Management Plan.

All deviations from this study protocol will be documented in the Trial Master File and included in the final study report. An assessment of the significance of each protocol deviation will be presented in the clinical study report.

12.3 Record Retention

All source data, clinical records and laboratory data relating to the study will be archived for a minimum period after completion of the study in accordance with South African GCP guidelines, Sponsor and Funder requirements. Data will be available for retrospective review or audit by arrangement with the appropriate representative at the archiving organisation (e.g., Sponsor Head).

12.4 Discontinuation of the Study

The Sponsor, the Funder, the Principal Investigator, and the HREC independently reserve the right to discontinue the study at any time for safety or other reasons. This will be done in consultation with the Funder, Sponsor and Principal Investigator where practical. In the event of premature termination or suspension of the study, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension. Following such a decision, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the participants' interest and safety. Ongoing participants will be followed up telephonically to ensure that feedback related to the results of their assessments, questionnaires and procedures is provided.

12.5 Publication Policy

A dissemination plan will be developed with all project partners prior to study completion. After study completion, results will be disseminated using the following strategies: written methods (i.e., publications in peer reviewed scientific journals), presentations at scientific conferences and workshops, in person dissemination of results to the research participants, and using electronic methods such as the project website and electronic media to publish results.

12.6 Study Audits

Audits may be carried out by the SAMRC, Sponsor, or HREC quality assurance representatives. All documents pertinent to this study must be made available for such inspections after adequate notice of the intention to audit is provided.

13. REFERENCES

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APPENDICES

APPENDIX 1: STUDY GOVERNANCE

The following Investigators and Institutional Affiliations are established. Designees may be provided, as appropriate.

Name	**Role**	**Institution**
Dr Bronwyn Bosch	Principal Investigator	Ezintsha, University of the Witwatersrand Building C Sunnyside Office Park 32 Princess of Wales Terrace Parktown, Johannesburg South Africa
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