Simplification from Tenofovir plus Lamivudine or Emtricitabine plus Ritonavir-Boosted-Protease Inhibitor to Ritonavir-Boosted-Atazanavir plus Lamivudine in Virologically-Suppressed-HIV-Infected Adults with Osteopenia: a pilot study

Primary investigator:
J.L Blanco
Co-investigators:
J.F. Rojas, E. Martinez, J.M. Gatell

Sponsor's Protocol Code Number: Osteosimply014
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1. STUDY SYNOPSIS

Protocol Number:	Osteosimply014
EudraCT Number:	2014-002720-27
Investigational Medicinal Products:	Atazanavir, ritonavir, lamivudine
Study Title:	Simplification from Tenofovir plus Lamivudine or Emtricitabine plus Ritonavir-Boosted-Protease Inhibitor to Ritonavir-Boosted-Atazanavir plus Lamivudine in Virologically-Suppressed-HIV-Infected Adults with Osteopenia: a pilot study
Phase of Study:	Phase IV
Objectives:	**Primary:** To assess the change in BMD by dual-energy X-ray (DXA) absorptiometry in HIV-infected adults with hip or spine T-score < -1.0 by DXA at week 48 after switching to r/ATV plus lamivudine. **Secondary:** To assess the effects of switching on: o Proportion of patients free of virologic failure (confirmed VL≥ 50 copies/mL) at 48 weeks o Adverse effects at 48 weeks o Bone turnover markers (BTMs): urinary N-terminal telopeptide of type-1 collagen (NTX), and bone-specific alkaline phosphatase at 48 weeks. o Estimated glomerular filtration rate (eGFR) and phosphorus in blood sample; (ii) proximal tubule dysfunction parameters: glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG in urine samples. at week 48 when compared to baseline
Study Design	A 48-week, open label, non comparative prospective trial in stable chronic HIV-infected patients having achieved complete virological suppression for more than 24 weeks (HIV-1 RNA <50 c/ml) switching from an ARV regimen containing TDF+3TC or FTC+r/PI to r/ATV+3TC Study visits will take place at screening, baseline,

	weeks 4, 12, 24, and 48.
Indication:	Chronic HIV-1 infection
Planned Sample Size:	45
Summary of Eligibility Criteria:	Inclusion criteria: o Adult (≥18 years old) HIV-1-infected subjects. o Provision of written, informed consent o Hip or spine T-scores <-1.0 measured by dual-energy X-ray absorptiometry (DXA) (in the previous 24 weeks) o On stable cART based on TDF+3TC or FTC+r/PI for at least 24 weeks. o Having plasma HIV-1 RNA <50 copies/mL for at least the previous 24 weeks, including at least two samples. Exclusion criteria: o Pregnancy, breast-feeding status or plans for pregnancy in the short term. o Primary genotypic resistance mutations and/or previous virological failures to ATV or 3TC/FTC o Chronic hepatitis B infection o Patients with indication for therapy for the prevention of bone fractures. o 25-OH vitamin D deficiency (< 10ng/mL), o Hypogonadism (low total testosterone according to local reference range), untreated. o Hypothyroidism (low T4 and increased thyroid stimulating hormone levels according to local reference ranges) o Hyperparathyroidism (increased parathyroid hormone level with hypercalcaemia according to local reference ranges) o Having received oral corticosteroids or inhaled fluticasone (daily doses higher than 5 mg/d prednisone equivalent for 3 months or more) o Using anti-resorptive therapy (Calcium and vitamin D supplements are encouraged but not mandated) o BMI lower than 19. o A patient with a history or current evidence of any condition, therapy, or laboratory abnormality, or other circumstance that might confound the results of the study, or interfere with the patient's participation for

	the full duration of the study o Unable to complete study procedures
Number of Study Centres	o 1
Duration of Treatment:	o 48 weeks
Dose and Route of Administration:	o All patients will switch to the following regimen: atazanavir 300 mg plus ritonavir 100 mg plus lamivudine 300 mg, once daily, orally for 48 weeks. o
Criteria for Evaluation:	o Virological suppression, safety, physical examination, and laboratory parameters.
Primary Endpoint:	o Change in BMD by dual-energy X-ray (DXA) absorptiometry at week 48.
Secondary Endpoints:	o Proportion of patients free of virologic failure (confirmed VL≥ 50 copies/mL) at 48 weeks o Proportion of patients with adverse effects at 48 weeks o Changes in bone turnover markers (BTMs): urinary N-terminal telopeptide of type-1 collagen (NTX), and bone-specific alkaline phosphatase at 48 weeks. o Changes from baseline to week 48 in: (i) estimated glomerular filtration rate (eGFR) and phosphorus in blood sample; (ii) proximal tubule dysfunction parameters: glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG in urine samples.

2. GENERAL STUDY INFORMATION

Sponsor:	Fundació Clínic per a la Recerca Mèdica Rosselló, 149 08036 Barcelona, Spain
Primary Investigator:	José Luis Blanco Hospital Clínic de Barcelona Villarroel 170, 08036 Barcelona, Spain Telephone number: +34 93 2275430
Co-investigators	Esteban Martínez MD, PhD José M Gatell MD, PhD Jhon F Rojas MD
Statistics and Data Management:	Elisa De Lazzari, Statistician
Monitor:	Joan Albert Arnaiz MD, PhD CTU Clinic

3. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

3TC	Lamivudine
ATV	Atazanavir
ACTG	AIDS Clinical Trial Group
AE	Adverse event
ALT	Alanine transaminase
cART	Combined Antiretroviral therapy
ARV	Antiretroviral
AST	Aspartate transaminase
BMD	Bone mineral density
BTM	Bone turnover marker
CRF	Case report form
CYP450	Cytochrome P450
DXA	Dual-energy X-ray absorptiometry
FTC	Emtricitabine
HAART	Highly Active Antiretroviral therapy
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
ICH GCP	International conference of harmonization good clinical practice
LDH	Lactate dehydrogenase
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NAG	N-acetyl-beta-D-glucosaminidase
NTX	N-terminal telopeptide
OD	Once daily
r/PI	Ritonavir-boosted Protease inhibitor
RTV	Ritonavir
SAE	Serious adverse event
TDF	Tenofovir disoproxil fumarate

4. FLOW CHART OF ASSESSMENTS

Time of Visit	**Screen**	**Baseline**	**Follow-up visits**				
Week	**-4**	**0**	**4**	**12**	**24**	**36**	**48**
Clinical							
Informed consent	x						
Inclusion / exclusion criteria	x	x					
Demographic data	x						
Physical examination / Vital signs	x						
Targeted physical examination		x	x	x	x	x	x
Concomitant medication		x	x	x	x	x	x
Safety/ Adverse events		x	x	x	x	x	x
Switching to r/ATV+3TC		x					
Other tests							
DXA	x				x		x
Laboratory (blood)							
Total and free testosterone, TSH and free-T4	x						
HIV RNA viral load	x	x	x	x	x	x	x
Biochemistry*		x	x	x	x	x	x
Blood test to exclude pregnancy	x						
Full blood count		x	x	x	x	x	x
CD4/CD8+ cell count		x					x
Calcium/phosphate**	x						
eGFR and phosphorus		x		x	x		x
bone-specific alkaline phosphatase		x	x		x		x
Laboratory (urine)							
BTMs***		x	x		x		x
glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG		x		x	x		x

* *Biochemistry:* urea, electrolytes (including sodium, potassium, chloride, bicarbonate,calcium, magnesium and phosphate), creatinine, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, total protein, bone-specific alkaline phosphatase [ALP], gamma glutamyltransferase [GGT], bilirubin),

**If abnormal, determine parathyroid hormone

***BTMs: NTX

5. BACKGROUND

5.1. Management of low bone mineral density in HIV-infected adults

The increased life expectancy for a majority of patients with access to combined antiretroviral therapy (cART) treatment has shown up that HIV infected patients continue to suffer many conditions, not all directly related to untreated HIV infection. Multiple factors contribute to non-AIDS conditions, with lifestyle, continued immunosuppression (despite cART), immune activation, inflammation, co-infections (e.g.HCV), and long term exposure to ART all implicated. These co-morbidities are becoming increasingly important for HIV-infected persons because are the major barrier to the goal of 'normal' life expectancy for cART-treated, HIV-infected patients. (1). Among these conditions, bone disease, particularly osteoporosis, has attracted increasing attention, particularly as the HIV infected population ages. Low bone mineral density (BMD) predisposes to fractures of the hip, spine and forearm - all more prevalent in HIV-infected adults (2). Antitretroviral therapy is associated with significantly greater loss of BMD (3). Tenofovir (TDF) particularly induces a greater decline in BMD–and increases bone turnover markers (BTMs)- than other ARV drugs -that trends to stabilize after 1 year of treatment- (4), and a large, retrospective cohort study reported more minimal trauma fractures with TDF based ART (5). Optimal management of low BMD in HIV-infected adults remains unclear. Potential strategies include bisphosphonate therapy and ART switch strategies. Switching TDF to abacavir (6) or to raltegravir (7) have been proposed.

Once started, lifelong cART continuation is recommended, however concerns about long-term toxicity and sustainability of cART have been raised. Recently, new simpler therapeutic approaches that may limit the number of drugs have been evaluated both as initial therapy as well as maintenance therapy after virologic control. Maintenance monotherapy with a ritonavir-boosted PI (r/PI), the drugs with higher genetic barrier to resistance, is the most widely investigated strategy (8,9). A novel strategy based on dual therapy with a r/PI plus lamivudine (3TC) has been recently evaluated due to the finally lower efficacy of monotherapy with ritonavir-boosted Darunavir or Lopinavir compared to standard cART.

Simplification strategy is widely referred into the Spanish Guidelines for the Use of Antiretroviral Treatment in HIV-infected Individuals (15). Ritonavir-boosted atazanavir is a once daily r/PI that has shown good tolerability with limited impact on lipid and glucose metabolism. In dual therapy with 3TC is the only rPI so far that has recently proven, in simplification strategy, to be safe, well tolerated and with rare virological failure without resistance selection (10,11). This combination is supported by a recent analysis of prevalence of transmitted drug resistance in Spain that have shown the very low rate of primary resistance for these specific drugs (3TC – 0.7% and ATV-0.1%) (16)

5.2. Rationale for Performing the Study

The aim of this pilot study is to evaluate if the strategy of switching from a stable triple therapy based on TDF plus lamivudine/emtricitabine (3FTC) plus rPI to a dual therapy of 3TC plus r/ATV in HIV-infected adults is safe, maintains a good control of HIV replication, increases BMD and decreases BTMs.

6. STUDY OBJECTIVES

6.1. Primary Objective

- To assess the change in BMD by dual-energy X-ray (DXA) absorptiometry in HIV-infected adults with hip or spine T-score < -1.0 by DXA at week 48 after switching to r/ATV plus lamivudine.

6.2. Secondary Objectives

To assess the effects of switching on:

- Proportion of patients free of virologic failure (confirmed VL≥ 50 copies/mL) at 48 weeks
- Adverse effects at 48 weeks
- bone turnover markers (BTMs): urinary N-terminal telopeptide of type-1 collagen (NTX), and bone-specific alkaline phosphatase at weeks 4, 24 and 48.
- (i) estimated glomerular filtration rate (eGFR) and phosphorus in blood sample; (ii) proximal tubule dysfunction parameters: glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG in urine samples. at week 48 when compared to baseline.

7. SELECTION CRITERIA

7.1. Total Number of patients

A pilot study including forty-five (45) eligible participants.

7.2. Inclusion criteria:

- HIV-1-infected subjects with age ≥18 years old.
- Provision of written, informed consent
- Hip or spine T-scores <-1.0 by dual-energy X-ray absorptiometry (DXA) (in the previous 24 weeks)
- Stable cART based on TDF+3TC or FTC+r/PI for at least 24 weeks.
- Having plasma HIV-1 RNA <50 copies/mL for at least the previous 24 weeks, including at least two samples.

7.3. Exclusion criteria:

- Pregnancy, breast-feeding status or plans for pregnancy in the short term.
- Primary genotypic resistance mutations and/or previous virological failures to ATV or 3TC/FTC
- Chronic hepatitis B infection
- Patients with indication for therapy for the prevention of bone fractures.
- 25-OH vitamin D deficiency (< 10ng/mL),
- Hypogonadism (low total testosterone according to local reference range),
- Hypothyroidism (low T4 and increased thyroid stimulating hormone levels according to local reference ranges)
- Hyperparathyroidism (increased parathyroid hormone level with hypercalcaemia according to local reference ranges)
- Having received oral corticosteroids or inhaled fluticasone (daily doses higher than 5 mg/d prednisone equivalent for 3 months or more)
- Using anti-resorptive therapy (Calcium and vitamin D supplements are encouraged but not mandated)

- BMI lower than 19.
- A patient with a history or current evidence of any condition, therapy, or laboratory abnormality, or other circumstance that might confound the results of the study, or interfere with the patient's participation for the full duration of the study
- Unable to complete study procedures.

8. STUDY DESIGN

8.1. Design

This is an open-label, 48-week prospective, non comparative clinical trial. Forty five (45) eligible subjects will be allocated to switch their current cARV regimen to r/ATV + 3TC.

8.2. Interventions and Assessments

Once the individual has fulfilled the inclusion/exclusion criteria, has understood the study aims and procedures, accepted its terms and signed the informed consent, he/she will be included in the study. The study will then start following the schedule detailed below.
Virological failure was defined as the first of two consecutive HIV-RNA levels >37 copies/mL. A GRT will be performed in these patients. After VF, treatment will be modified at the investigator's decision.
A trial stopping rule was planned by assuming that an acceptable total virological failure rate should not have exceeded 12.5% (six patients). Therefore, study termination was planned if more than six virological failures were observed.

Assessments

Clinical visit: safety, adverse events, full blood count, biochemistry and HIV viral load, will be assessed at baseline, weeks 4, 12, 24, 36 and 48. (CD4/CD8 at baseline and w48)

Bone: Parathyroid hormone levels will only be determined after screening if serum calcium or phosphate levels are abnormal. Total and free testosterone, TSH and free-T4 will be performed at prescreening (week: -4). DXA will be performed at pre-screening, weeks 24 and 48. BTMs will be assessed at baseline and weeks 4, 24 and 48 : urinary N-terminal telopeptide of type-1 collagen (NTX) (a peptide fragment released during bone resorption), bone-specific alkaline phosphatise (an isoform of alkaline phosphatase (ALP), a serum marker of bone formation). The 10-year risks of osteoporotic and hip fractures will be estimated at screening using the WHO FRAX(R) algorithm (using Spain-specific dataset)

Kidney: eGFR, and phosphorus in blood sample; and, proximal tubule dysfunction parameters: glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG in urine samples will be performed at baseline and weeks 12, 24 and 48.

Plasma samples will be stored for possible subsequent testing of viral load if necessary after each visit.

Study conduction, recruitment and follow-up:

The study will be conducted at a single center: Infectious Diseases Department, Hospital Clinic, University of Barcelona

Recruitment will take 24 weeks and duration of patient follow-up will be 48 weeks

9. STUDY INVESTIGATIONAL MEDICINAL PRODUCTS

All participants will switch their current ARV regimen to a r/ATV + 3TC once daily. Medication will be used under the approved conditions detailed in the Summary of Product Characteristics (SmPC).

For the management of Drug-drug interactions, contraindications and drug-related adverse events , please refer to the SmPC.

Any changes in the ARV regimen will be made by the investigator and recorded in the CRF.

9.1. Provision of Antiretroviral drugs during the Study

All ARV drugs will be obtained by prescription from the study participant's treating doctor.

The budget impact of this simplification strategy, usually done in our clinical practice in candidate patients, is associated with net savings on hospital ARV drug budget, since all 45 patients will be switched from a triple to a double therapy regiment thus reducing acquisition costs.

10. VISIT SCHEDULE

Screening Visit (week -4)

Eligibility Screening Form

An eligibility screening form should be completed at the screening visit. Participant details will be entered on the study participant identification log including whether or not the participant was included, and if excluded, the reason why.

Eligibility of participants

Prior to screening, participants must be on stable and well-tolerated ART including a TDF+3TC or FTC+r/PI for at least 24 weeks prior to randomisation.

Informed consent should be obtained and forms signed and dated by the participant prior to any study-related procedures being performed.

The screening procedures will be completed in one visit.

Assessments (results to be recorded in the CRF)

Clinical eligibility will be measured by inclusion criteria and exclusion criteria as listed below.

Inclusion criteria:

- Provision of written, informed consent
- HIV-1-infected subjects with age ≥18 years old.
- Hip or spine T-scores <-1.0 by dual-energy x-ray absorptiometry (DXA) (in the previous 24 weeks)
- Stable cART based on TDF+3TC or FTC+r/PI for at least 24 weeks.

- Having plasma HIV-1 RNA <50 copies/mL for at least the previous 24 weeks, including at least two samples.

Exclusion criteria:

- Pregnancy, breast-feeding status or plans for pregnancy in the short term.
- Primary genotypic resistance mutations and/or previous virological failures to ATV or 3TC/FTC
- Chronic hepatitis B infection
- Patients with indication for therapy for the prevention of bone fractures.
- 25-OH vitamin D deficiency (< 10ng/mL),
- Hypogonadism (low total testosterone according to local reference range), untreated.
- Hypothyroidism (low T4 and increased thyroid stimulating hormone levels according to local reference ranges)
- Hyperparathyroidism (increased parathyroid hormone level with hypercalcaemia according to local reference ranges)
- Having received oral corticosteroids or inhaled fluticasone (daily doses higher than 5 mg/d prednisone equivalent for 3 months or more)
- Using anti-resorptive therapy (Calcium and vitamin D supplements are encouraged but not mandated)
- BMI lower than 19.
- A patient with a history or current evidence of any condition, therapy, or laboratory abnormality, or other circumstance that might confound the results of the study, or interfere with the patient's participation for the full duration of the study
- Unable to complete study procedures

Other screening assessments:

Demographic data: date of birth, age, gender, race,
Full physical examination, medical history concomitant medications information will be obtained by the study doctor
DXA (obtained in the previous 24 weeks)
Blood and urine samples will be taken for lab test

Safety

For potentially child-bearing female participants a blood test will be done to exclude pregnancy.

HIV antibodies/Western blot/proviral DNA (if report not available prior to study entry)
HIV RNA viral load
Biochemistry – urea, electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate), creatinine, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, total protein, alkaline phosphatase [ALP], gamma glutamyltransferase [GGT], bilirubin, creatine kinase

Fasting metabolic parameters
total cholesterol, triglycerides, HDL/LDL cholesterol and calculated VLDL cholesterol
Lab results will be recorded on the screening page of the CRF.

Baseline Visit (week 0)

Baseline assessments will be recorded in the CRF: vital signs, physical examination, new concomitant medications, and adverse events

Blood samples will be taken for full blood count, biochemistry – urea, electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate), creatinine, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, total protein, , gamma glutamyltransferase [GGT], bilirubin, creatine kinase, bone-specific alkaline phosphatase [ALP],CD4+/CD8+ lymphocyte count, total cholesterol, triglycerides, HDL and LDL cholesterol, glucose.

Urine samples will be collected for BTMs and glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG

Study participants will be provided with one (1) month's supply of study medication, sufficient to cover 4 weeks

Week 4

Week 4 assessments will be recorded in the CRF: vital signs, physical examination, new concomitant medications, and adverse events

Blood samples will be taken for full blood count, biochemistry – urea, electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate), creatinine, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, total protein, alkaline phosphatase [ALP], gamma glutamyltransferase [GGT], bilirubin), creatine kinase, bone-specific alkaline phosphatase [ALP], CD4+ lymphocyte count, HIV RNA viral load ,total cholesterol, triglycerides, HDL and LDL cholesterol.

Urine samples will be collected for BTMs and glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG.

At Week 4, study participants will be provided with study medication for at least two (2) months plus an additional two (2) weeks supply to allow for the allowable study window (±7 days).

Weeks 12, 24, 36 and 48

All clinical assessments will be recorded in the CRF: vital signs, physical examination, new concomitant medications, and adverse events.

Blood samples will be taken for full blood count, biochemistry – urea, electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate), creatinine, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, total protein, alkaline phosphatase [ALP], gamma glutamyltransferase [GGT], bilirubin, creatine kinase, bone-specific alkaline phosphatase [ALP] (weeks 24 and 48),CD4+ lymphocyte count, HIV RNA viral load, total cholesterol, triglycerides, HDL and LDL cholesterol.

Urine samples will be collected for BTMs (weeks 24 and 48) and glucose, protein, albumin, creatinin, phosphorus, beta-2 microglobuline and NAG when specified.

DXA will be performed on weeks 24 and 48.
Bone-specific alkaline phosphatase [ALP] will be determined at baseline and at week 48.

Study Visit Windows

- Baseline and Week 4 visits have a visit window of ±2 days.
- Week 12, 24, 36 and 48 visits have a visit window of ±7 days.

Pregnancy

Despite the known effects of the study drugs (atazanavir, ritonavir and lamivudine), and their relative safety during pregnancy, women should avoid becoming pregnant during the course of this trial. If a participant becomes pregnant, she will be monitored until the end of the pregnancy. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child which must be reported to the sponsor(BMS)

Blood and Urine Specimen Management

Safety parameters will be assessed by the local laboratory. Local laboratory reference ranges will apply. Safety parameter blood samples will be transported according to the local laboratory requirements for measurement of: urea, electrolytes (including, sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate), creatinine, liver function tests - alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin, total protein, bone-specific alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), bilirubin and creatine kinase. Lipids: triglycerides, total cholesterol, HDL, LDL, VLDL cholesterol, glucose

HIV parameters will be assessed by Local laboratory and local reference ranges will apply. HIV parameter blood samples will be transported to according to the local laboratory requirements for measurement of: full blood count, CD4/CD8+ lymphocyte count and percentage and HIV viral load.

11. ADVERSE EVENTS

11.1 Definitions

An adverse event is any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a casual relationship with the study treatments. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

Adverse events observed by the Investigator, or reported by the subject, and any remedial action taken, will be recorded in the subject's CRF and should be verifiable in the subject's notes throughout the study. The nature of each event, time of onset after drug administration, duration and severity will be documented together with the Investigator's opinion of the causal relationship to the treatment (unrelated, unlikely, possible, probable, and definite).
All subjects experiencing adverse events, whether considered associated with the use of the study medication or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed.
Procedures such as surgery should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as the adverse event and the resulting appendectomy noted on the CRF.
Planned procedures such as surgery planned prior to the subject's enrolment into the study need not be reported as adverse events if these are documented as planned at the screening visit.

Clinically significant changes in physical examination and blood safety profiles should also be recorded as adverse events.

11.2 Assessment of Intensity

Severity should be recorded and graded according to the AIDS Clinical Trial Group (ACTG) Grading Scale.

Note: There is a distinction between the gravity and the intensity of an adverse event. Severe is a measure of intensity; thus, a severe reaction is not necessarily a serious adverse event. For example, a headache may be severe in intensity but would not be classified as serious unless it met one of the criteria for serious events.

All events deemed to be Grade 4 (potentially life threatening) according to the ACTG grading scale should be routinely reported as a serious adverse event. However there may be occasions where in the investigator's clinical judgement they do not consider the event to be life threatening therefore they do not consider the event to meet the definition of an SAE. In these cases the investigator must document clearly in the participants source documentation that the Grade 4 event has been assessed and why in their clinical judgement they do not consider the event to be life threatening.

11.3 Assessment of Causality

The relationship to study drug of each adverse event will be assessed using the following definitions:
DEFINITE: distinct temporal relationship with drug treatment. Known reaction to agent or chemical group, or predicted by known pharmacology. Event cannot be explained by subject's clinical state or other factors.
PROBABLE: reasonable temporal relationship with drug treatment. Likely to be known reaction to agent or chemical group, or predicted by known pharmacology. Event cannot easily be explained by subject's clinical state or other factors.
POSSIBLE: reasonable temporal relationship with drug treatment. Event could be explained by subject's clinical state or other factors.
UNLIKELY: poor temporal relationship with drug treatment. Event easily explained by subject's clinical state or other factors.
UNRELATED: the event occurs prior to dosing. Event or intercurrent illness is due wholly to factors other than drug treatment.

11.4 Collection and follow up of Adverse Events

All adverse events, however minor, will be documented in the CRF whether or not the Investigator concludes the event to be related to drug treatment.

The adverse event reporting period will be from consent until the subjects final study visit. In addition, any untoward event that may occur subsequent to the reporting period that the Investigator assesses as possibly, probably or definitely related to the study drug medication should also be reported as an Adverse Event.

Adverse events may be directly observed, reported spontaneously by the subject or by questioning the subject at each study visit.

All adverse events should be followed up until they are resolved or the subject's participation in the study ends (i.e. until the final CRF is completed for that subject). In addition, all serious and non-serious adverse events assessed by the Investigator as possibly related to the investigational medication should continue to be followed even after the subject's participation in the study is over. Such events should be followed until resolution, or until no further change can reasonably be expected. Deaths occurring more than 30 days after the final dose, which are considered to be unrelated to the study medication, should not be reported as a Serious Adverse Event.

Rash and any associated symptoms should be reported as adverse events (see section 4.3)

11.5 Serious Adverse Events (SAE)

A serious adverse event is any untoward medical occurrence that at any dose:
i) Results in death
ii) Is life threatening
iii) Requires in patient hospitalisation or prolongation of existing hospitalisation

iv) Results in persistent or significant disability/ incapacity
or
v) Is a congenital anomaly/ birth defect.

Additionally, important medical events that may not results in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Example of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs. An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE
Any component of a study endpoint that is considered related to study therapy (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported) should be reported as SAE.

The SAE should be reported immediately to the sponsor and BMS (study supported).

NOTE: bilirubin fractionation should be performed if testing is available. If testing is unavailable, record presence of detectable urinary bilirubin on dipstick indicating direct bilirubin elevations and suggesting liver injury. If testing is unavailable and a subject meets the criterion of total bilirubin ≥2xULN, then the event should still be reported as an SAE. If INR is obtained, include values on the SAE form. INR elevations >1.5 suggest severe liver injury..

Deadline for SAE notification

All SAEs, whether or not deemed drug-related or expected, and pregnancies must be reported to BMS within **24 hours** of first becoming aware of the event. All SAEs related related to the use of Atazanavir must be emailed to Bristol-Myers Squibb: SAE Email Address: Worldwide.Safety@BMS.com
SAEs must be recorded on a SAE form (BMS can provide), MEDWATCH or CIOMS form; Pregnancies on a Pregnancy Surveillance Form.
All SAEs should be also reported to local Ethic Committee.
Pharmacovigilance aspects will be conducted according to Appendix 3 described in this protocol.

SAE Reconciliation

The investigator will reconcile the clinical database SAE cases transmitted to BMS Global Pharmacovigilance (GPV&E). Frequency of reconciliation will be determined prior to study commencement but will occur no less than once prior to study database lock. BMS GPV&E will e-mail upon request from the investigator, the GPV&E reconciliation report. Requests for reconciliation should be sent to aepbusinessprocess@bms.com. The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the investigator determines a case was not transmitted to BMS GPV&E, the case will be sent immediately.

12. Sample size and Statistical analysis

12.2 Statistical analysis

This is an open-label pilot study. All patients included in the study will be included in the safety population. All analyses will be performed after all participants have completed the study, permanently withdrawn or been lost to follow-up.

Descriptive statistics will be used for continuous and ordinal variables. For continuous data, mean change from baseline will be calculated for participants with baseline and at least 1 follow-up visit.

Changes on BMD from baseline at week 48 (and 24), will be assessed using random-effects regression model. Patient's characteristics (age, sex, weight, BMI, smoking, units of alcohol), antiretroviral therapy, tenofovir duration, concomitant medication, hepatitis C coinfection and laboratory markers will be assessed using a linear regression as a potential predictors of changes in bone mineral density. Those that were associated ($p<0.05$) will then be tested for their independent association with greater increase in BMD using stepwise regression. Correlations between changes in BTMs and changes in BMD at week 48 will be calculated using Spearman's rank correlation coefficient. An additional multivariate model will be constracted including the changes in the BTMs at weeks 4 and 24 as a covariates.

Analyses will be primarily by intent-to-treat. As this study is designed to evaluate the biological effects of an intervention, a per-protocol analysis will also be performed. An interim analysis will be done at week 24, study will be stopped if more than six virological failures were observed.

12.3 Sample size

This is a non-comparative pilot study planning to include 45 patients. Switching from TDF to RAL in osteopaenic HIV-infected patients virological suppressed achieved a 2.5% increase in BMD at 48 weeks (7). Assuming a standard deviation (SD) of 3.5%, in order to detect a 2% increase from baseline with 90% power and $\alpha=0.025$ in two-sides, 45 patients would be required (allowing for 10% losses to follow-up).

13. Data Quality, Ethics, Patient protection

The study will be conducted guided by the Declaration of Helsinki and under the ICH GCP guidelines on clinical research with medicines.

13.1 Data recording

The investigators will be provided with CRF folders for the input of study data. These should be completed according to the printed set of instructions accompanying these folders. Participants will not be identified by name on any CRFs, but rather by a site-specific 'Study Identifier' and 'Name Code'.

13.2 Data transfer to Study Sponsor

The completed study CRFs should be transferred to the Study Sponsor Statistician once source data verification has been performed.

Any data queries generated will be addressed in consultation with study monitor.

13.3 Confidentiality and Personal Data

All data will be managed in accordance with the ICH GCP guidelines and under Spanish regulations on protection of personal data (LEY ORGÁNICA 15/1999, DE 13 DE DICIEMBRE, DE PROTECCIÓN DE DATOS DE CARÁCTER PERSONAL)

13.4 Monitoring Arrangements

The purpose of monitoring is to verify the rights and wellbeing of human subjects are protected; that trial data is accurate, complete and verifiable with source data; that the trial is conducted in compliance with the protocol, GCP and the applicable regulatory requirements.
A monitor will conduct regular site visits for the purpose of monitoring various aspects of the study. The Investigator must agree to allow the study monitor and authorised representatives of the Sponsor, to inspect all CRF and corresponding source documents, e.g. original medical records, subject records and laboratory raw data, access to the clinical supplies, dispensing and storage areas and agree to assist with their activities if requested. The Investigator should provide adequate time and space for monitoring visits.
The monitor will query any missing or spurious data with the Investigator, which should be resolved in a timely manner. A monitoring log will be maintained recording each visit, the reason for the visit, the monitor's signature and Investigator's or designee's confirmation signature.

13.5 Ethics Approval

The study protocol, subject information and consent form, the Investigator brochure, or other reference safety information, subject recruitment procedures, information about payments and compensation available to the subjects and documentation evidencing the Investigator's qualifications should be submitted to the Ethics Committee for ethical review and approval according to local regulations, prior to the study initiation. Any changes, which may need to be made, will be submitted in the form of numbered and dated protocol amendments in accordance with local regulations.

13.6 Regulatory

As required by Spanish regulations, authorisation by the Spanish competent Authorities (AEMPS) will be obtained, prior to study initiation.

13.7 Insurance Provisions

The sponsor will take out appropriate insurance cover for this trial in accordance with Spanish regulations.

13.8 Funding

The study conduction is funded by a grant provided by BMS.

14 Publications and presentations

The study will be registered in a publicly available data base before the inclusion of the first patient. The final data will be presented at one or more scientific meetings and published in a peer-reviewed journal. No patient data will be presented that could permit identification of any study participant. No other publication will be made before the primary manuscript has been agreed upon and accepted for publication and without prior approval of the principal investigator.

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