RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL TO EVALUATE THE EFFECT OF RALTEGRAVIR INTENSIFICATION (1.200 MG QD) ON THE GUT MICROBIOTA OF CHRONICALLY HIV-1 INFECTED SUBJECTS OVER TIME: THE RAGTIME STUDY

Code: RAGTIME

Version 2, 10th February 2017

EudraCT: 2016-002722-36

Sponsor:

Fundació Lluita contra la SIDA
Hospital Universitari Germans Trias i Pujol
Carretera de Canyet s/n
08916 Badalona (Barcelona)

Principal / Coordinator Investigator: Dr. Roger Paredes, MD, PhD

Co-Investigators:

Dr. José Ramón Santos, MD, PhD
Dra. Patricia Echeverria Bermudez, MD, PhD
Dr. Bonaventura Clotet Sala, MD, PhD
Dr. Boris Revollo Barriga

SIGNATURES

The coordinating investigator and the sponsor of the study:
Randomized, double-blind, placebo-controlled clinical trial to evaluate the effect of Raltegravir intensification (1.200 mg QD) on the gut microbiota of chronically HIV-1 infected subject over time: THE RAGTIME STUDY

Declare that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirements.

Modifications to this protocol must be submitted prior agreement of the coordinator / principal investigator and sponsor.

Principal / Coordinator Investigator: Dr. Roger Paredes, MD, PhD

[redacted]

Signature and Date: 10/02/2017

Sponsor: Bonaventura Clotet, PhD, MD
Fundació Lluita contra la SIDA

[redacted]

Signature and Date: 10/02/2017

1 GENERAL INFORMATION

1.1 TITLE

Randomized, double-blind, placebo-controlled clinical trial to evaluate the effect of Raltegravir intensification (1.200 mg QD) on the gut microbiota of chronically HIV-1 infected subject over time: THE RAGTIME STUDY

1.2 CODE

RAGTIME

1.3 PROTOCOL VERSION AND DATE

Version 2, 10th February 2017. Any modification of the protocol must also bear the amendment number and date.

1.4 SPONSOR

Lluita contra la SIDA Foundation
Germans Trias i Pujol Hospital
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Person authorized by the sponsor to sign the protocol and amendments:
Bonaventura Clotet Sala, president of Lluita contra la SIDA Foundation

1.5 MONITOR

Cristina Herrero
FLS-Research Support
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1.6 PRINCIPAL / COORDINATING INVESTIGATOR

Dr. Roger Paredes
Principal Investigator, HIV Unit,
Lluita contra la SIDA Foundation
Hospital Universitari Germans Trias i Pujol, Badalona (Barcelona)
[redacted]

1.7 SITES AND INVESTIGATORS

Dr. Roger Paredes (principal investigator)
Dr. José Ramón Santos (co-investigator)
Dra. Patricia Echeverria (co-investigator)
Dr. Bonaventura Clotet (co-investigator)
Dr. Boris Revollo (co-investigator)
Isabel Bravo (study nurse/data management)

HIV Unit, Lluita contra la SIDA Foundation
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1.8 TECHNICAL SERVICES INVOLVED

Biochemistry, hematology, quantitative HIV-1 RNA levels, HLA typing, CD4 counts will be all performed at Hospital Germans Trias i Pujol.

Analysis of composition, structure and function of the intestinal microbiome will be performed in the IrsiCaixa AIDS Research [redacted]

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2 ABBREVIATIONS

AE	Adverse event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
AST	Aspartat aminotransferase
ART	Antiretroviral Therapy
CRF	Case Report Form
CRO	Clinical Research Organisation
DAIDS	Division of acquired immune deficiency syndrome
DNA	Deoxyribonucleic Acid
DSUR	Development update safety report
EC	Ethics Committee
ELISA	Enzyme-linked immunosorbent assay
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GI	Gastrointestinal
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HPV	Human papillomavirus
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICH	International Conference of Harmonisation
IL	Interferon gamma
INSTI	Integrase strand transfer inhibitor
IP-10	Interferon gamma-induced protein 10
I-FABP	Intestinal Fatty Acid Binding Protein
IMP	Investigational medicinal product
INR	International normalized ratio
LPS	Lipopolysaccharide
LBP	LPS-binding protein
LEFSe	Linear discriminant analysis Effect Size
LOPD	Ley Orgánica de protección de datos
MSD	Merck Sharp and Dohme
NNRTI	Non-nucleoside reverse-transcriptase inhibitors

OTU	Operative Taxonomic Unit
PBMC	Peripheral Blood Mononuclear Cell
PI	Protease inhibitor
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
RNA	Ribonucleic acid
RAL	Raltegravir
rDNA	Ribosomal Deoxyribonucleic Acid
SmPC	Summary of product characteristics
SAE	Serious adverse evente
SUSAR	Serious unexpected adverse reaction
UAE	Unexpected adverse event
UNAIDS	United Nations Program on HIV/AIDS

3 BACKGROUND INFORMATION

The gut microbiome is essential for the maturation of the neonatal immune system and the adequate development and function of adult immune responses[1]. **HIV-1 infection** in children and adults exerts a rapid and severe **depletion of gut-associated lymphoid tissue**, which damages the intestinal barrier, allowing **translocation of gut commensal bacteria** into the systemic circulation[2–5]. Bacterial translocation causes **chronic inflammation** and **immune activation**, which lead to **immune deterioration** and **premature aging** of HIV-1-infected subjects, including metabolic disturbances, cardiovascular diseases, cognitive disorders and HIV-associated cancers[6–8]. Persistence of residual HIV-1 replication in the presence of ART has been associated to incomplete HIV-1 suppression in gut lymphatic tissues due to suboptimal tissular penetration of PI/s or NNRTIs[9].

In previous work in our institute[10]**,** we have observed that **HIV-1 infection is independently associated with significant reductions in the gut microbiome richness**, which is, in turn, **inversely correlated with systemic inflammation**. Reduced microbial richness, for example, has been associated with intestinal inflammatory diseases as well as with metabolic syndrome, diabetes and obesity and correlated with metabolic markers[11–13].

Recovering bacterial richness might thus have a positive impact on immune activation, chronic inflammation and the overall health of HIV-infected individuals. However, achieving that goal will possibly require, alongside potential bacterial supplementations, the use of **ART with high penetration into gut lymphoid tissue** to limit as much as possible the continued damage exerted by residual HIV replication on the GALT[14]. Antiretroviral drugs with higher intestinal penetration like raltegravir may be more effective at recovering the intestinal microbiome composition and function than those with lower gut penetration like darunavir or the NNRTIs. Thereby, **raltegravir intensification could be associated with increases in intestinal microbial richness, implying an improvement on intestinal and overall health.**

Despite the lack of evidence on that regard, previous studies from our group and others would favor that hypothesis. Residual HIV-1 replication in plasma can be deterred by ART intensification with raltegravir[15], which is, in part, due to the high penetration of raltegravir in intestinal tissues[9]. Moreover, raltegravir intensification decreases peripheral CD8 T-cell activation CD45RA (-) and creates a transient CD4 T-cell redistribution, which revert after raltegravir withdrawal[16].

The project presented here will be the **first prospective, randomized evaluation of the effect of ART on the structure and function of the gut microbiome**. This study provides a unique opportunity to understand the benefits of ART with high intestinal penetration on the gut microbiome. It is thus a key study to understand the bidirectional interactions between the microbiome and the host in people living with HIV/AIDS.

HIPOTHESIS

1. ART intensification with raltegravir leads to increases in gut bacterial richness, could possibly affect its composition and/or function.

2. The gut microbiome structure and composition correlates with markers of inflammation, coagulation, enterocyte damage and T-cell activation, maturation and immune senescence.

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4 TRIAL OBJECTIVE AND PURPOSE

4.1 PRIMARY OBJECTIVE

To evaluate the ability of raltegravir intensification to increase gut microbiome richness and modify the gut microbiome composition of people living with HIV receiving stable ART.

4.2 SECONDARY OBJECTIVE

To correlate changes in the gut microbiota composition and richness with markers of inflammation, coagulation, enterocyte damage, bacterial translocation and T-cell maturation, activation, exhaustion and senescence during raltegravir intensification.

5 TRIAL DESIGN

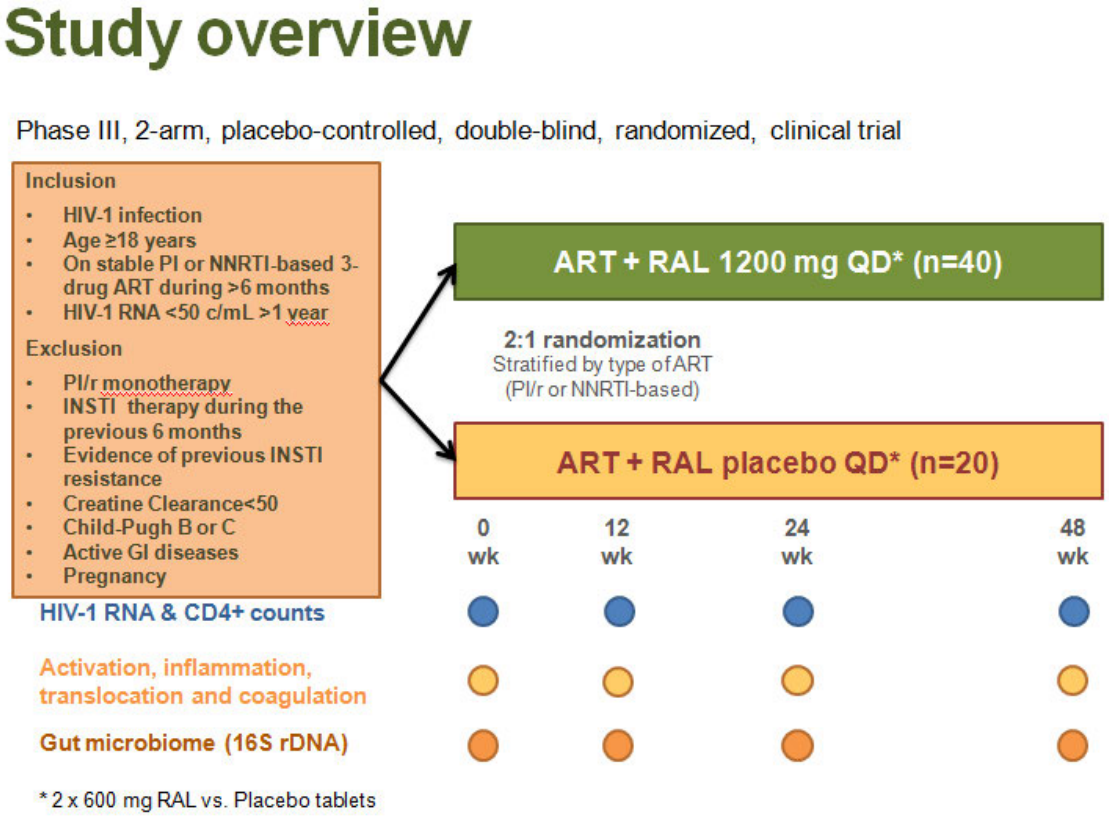
5.1 TYPE OF TRIAL

Phase III, prospective, randomized, double-blind, 2-arm, placebo-controlled clinical trial, with a non-authorized investigational medicinal product.

5.2 DESCRIPTION OF THE DESIGN

The trial was conducted in a sample of 60 chronically HIV-1-infected adults patients on a stable 3-drug ART with PI/r/c or NRTI/NNRTI and HIV-1 RNA suppression <50 copies/mL, will be randomized 2:1 to intensify their current ART with RAL 1200 mg QD (Intensification arm, n=40) vs. placebo (placebo arm, n=20).

Subjects will be followed prospectively during 48 weeks.



5.3 ENDPOINTS

5.3.1 Primary endpoint(s)

- Increase in bacterial richness (observed species) at week 48, relative to baseline (week 0).

5.3.2 Secondary endpoints

- Longitudinal changes in:
  - Gut bacterial composition
  - Gut bacterial function
  - Other estimators of richness and diversity: Chao1, ACE, Shannon, 1/Simpson.

- Association of the gut microbiome composition and richness with:
  - Inflammation: IL-6, IP-10

- Coagulation: D-Dimer
- Enterocyte damage: Intestinal Fatty Acid Binding Protein (I-FABP)
- Bacterial translocation and monocyte activation: LPS-binding protein (LBP), soluble CD14.
- Maturation, activation, exhaustion and immune senescence in CD4+ and CD8+ T-cells: CD3+, CD4+, CD8+, CD45RA, CCR7, CD28, CD27, HLA-DR, CD38, PD-1, CD57.
- CD4 and CD8+ counts
- CD4+/CD8+ ratio

• In case of changes in the microbiome, the qquantification of viral reservoir will be done in PBMCs.

5.4 MEASURES TO AVOID BIAS

5.4.1 Randomization

Once eligibility has been confirmed, study participants will be assigned sequentially into the two treatment groups (intensification with RAL or placebo) through a randomization schedule based on the randomization plan using dedicated computer software.
Merck Sharpe & Dohme Co. will provide to the un-blinded correspondent with the CID randomization schedule that will list the grouping description and ID number. The randomization will be centralized in the CRO FLS-Research Support. The investigator
will phone to the CRO to inform about the eligibility of a patient and if he/she is on
treatment with PI/r/c or NRTI/NNRTI-based. The CRO will add the patient to the randomization list, they will assign a code number to the patient and will inform to the Pharmacy Service the group where the patient has been assigned.
The distribution between the branches A (intensification with RAL) and B (placebo) will be 2:1.
It will be impossible for the investigators to know which group will be assigned to a patient before his/her inclusion in the study.

5.4.2 Stratification

Randomization will be stratified by type of ART (PI/r/c or NRTI/NNRTI-based) and CD4 nadir (> 300 or < 300) at screening. In this way, the distribution of these parameters between the study groups will be balanced.

5.4.3 Blinding

A double-blind/masking technique will be used. Reformulated raltegravir and placebo will be packaged identically so that blind/masking is maintained. The subject, the investigator and Sponsor personnel or delegate(s) who are involved in the treatment or clinical evaluation of the subjects are unaware of the group assignments.
The Pharmacy Service will dispense the raltegravir /placebo to the patient.
The study drug (raltegravir or placebo) will be labelled with identical labels including: the
protocol identifying number, Hospital Universitari Germans Trias i Pujol, subject number in
the clinical trial, administration route, XX tablets of RAL or placebo, posology: 2 tablet/24 h, coordinator investigator, sponsor, store at room temperature, batch number, expiration date and “for clinical trial use only”. They will be storage at the Pharmacy Service of Germans Trias i Pujol Hospital.

5.4.4 Unblinding procedures

When the investigator or sub-investigator needs to identify the drug used by a subject and the dosage administered in case of emergency e.g., the occurrence of serious adverse experiences, he/she will contact the un-blinded correspondent in the center and make
a request for emergency unblinding. As requested by the investigator or sub -investigator the un-blinded correspondent will provide the information to him/her promptly and report unblinding to the sponsor. The un-blinded correspondent will make a record promptly however, the investigator or sub-investigator must enter the intensity of the adverse experiences observed, their relation to study drug, the reason thereof, etc., in the medical chart etc., before unblinding is performed.
In the event that unblinding has occurred, the circumstances around the unblinding (e.g., date and reason) must be documented promptly, and the Monitor notified as soon as possible. Only the principal investigator or

delegate and the respective subject's code should be unblinded. Trial site personnel and Sponsor personnel directly associated with the conduct of the trial should not be unblinded.

5.5 FORESEEN CALENDAR

- Clinical trial submission: October 2016
- First patient first visit: December 2016
- Inclusion period: 36 weeks
- Follow-up period: 48 weeks
- Last patient last visit: 30/09/2018 (End of study)
- Final report submission: September 2019

5.6 END OF TRIAL

The date of the end of the trial will be to the last visit of the last patient.

The trial will be prematurely stopped if there are withdrawals because of intolerance or toxicity more than 20%.

If the study must be interrupted prematurely, all non-used materials should be returned to the sponsor, at the *Lluita Contra la SIDA Foundation.* The principal investigator will keep the investigator file and a copy of the completed CRF.

In case there were no patients included in the study, the sponsor will take care of all materials.

5.7 SOURCE DATA

Source documents are the patient's medical records, the results obtained through blood tests as routine clinical and laboratory results obtained from blood samples and fecal samples collected on the same day that the blood test.

Study data will be collected trough a Case Report Form (CRF).

6 TRIAL INVESTIGATIONAL PRODUCT(S)

6.1 EXPERIMENTAL AND CONTROL TREATMENTS

Study treatment consists to intensify current ART with reformulated raltegravir 1200 mg QD versus placebo:

- Raltegravir arm: Current ART + Raltegravir 1200 mg. It is an antiretroviral drug used in general HIV management. The formulation of the active principal components corresponds to the non-marketed formulation.

- Placebo arm: Current ART + RAL-placebo: The placebo was created by Merck Sharpe & Dohme co. to match the active product.

6.2 SUPPLY, PACKAGING, LABELING AND STORAGE

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

All antiretroviral treatments will be administered by the Pharmacy Service of the participating sites.

Clinical Supplies will be provided by the Merck Sharpe & Dohme co. as follows:

Bottle ID **Product Name & Potency Dosage Form**
- Reformulated raltegravir 600 mg (Film-coated Tablet)
- Placebo to match reformulated raltegravir 600 mg (Film-coated Tablet)

Reformulated raltegravir/placebo will be sent to the Pharmacy Service of site, where reception, units, batch number and expiration date will be confirmed. The investigational medication will be supplied and labeled under GMP in order to support a double-blind trial and in compliance with regulatory requirements. No conditioning nor labeling will be required in the Pharmacy Service.
All investigational products will be storage at the Pharmacy Service of Germans Trias i Pujol Hospital.

It is not required special storage conditions.

6.3 DOSE, INTERVAL, ROUTE AND METHOD OF ADMINISTRATION

The route of study drug administration is oral.

- Reformulated Raltegravir 1200 mg (2 tablets at the same time x 600mg) once daily plus current ART during 48 weeks from randomization.
- RAL- Placebo (2 tablets at the same time of placebo) once daily plus current ART during 48 weeks from randomization.

Reformulated raltegravir/placebo can be taken with or without food.

If a subject misses a dose and it is less than 12 hours before the next dose, the missed dose should be skipped and the normal dosing schedule resumed. The subject should not double the next dose in order to compensate for what has been missed.

Acceptable antiretroviral drugs in the background regimen will include a PI boosted with cobicistat/ritonavir or NNRTI/NRTI based 3-drug ART.

6.4 DRUG ACCOUNTABILITY

No returned drug accountability will be performed.

To perform the drug accountability of the dispensed product, the records of the Pharmacy Service of the site will be used.

6.5 ARM DESCRIPTION

Patients will continue on their usual antiretroviral regimen and at randomization moment will be added during 48 weeks reformulated raltegravir or placebo according to randomization. The rest of the antiretroviral treatment will remain unchanged.

- RAL arm: ongoing ART + RAL 1200 mg QD (2 x 600 mg)
- Placebo arm: ongoing ART + RAL placebo QD (2 tablets of RAL placebo)

6.6 MODIFICATION OF THE TREATMENT REGIMEN

No changes in treatment regimes are foreseen during the study period, except changes in the antiretroviral regimen as the NRTI/NNRTI by PI/r/c or the PI/r/c by NRTI/NNRTI.

In case of failure of one of the regimens, a new regimen will be decided using a resistance test.

In case adverse events to the medication occur, the investigator will decide if it is necessary to replace it.

At the end of the study, the change of treatment will be done at the discretion of the physician.

6.7 CONCOMITANT TREATMENTS

All other treatments, including over the counter treatments, apart from the study medication which is administered during the study period will be considered concomitant treatments and should be documented in the CRF.

It is remembered that the patients who participate in the study should not continue any concomitant treatment without the knowledge and permission of the investigator.

All participants will be discouraged to initiate any concomitant treatment with antibiotics, nutritional supplements, or drugs that modulate immunity. Subjects requiring some of these treatments for their clinical management during follow-up will not be excluded from the analyses, but the type, duration and reasons for intake will be recorded and taken into account in association analyses.

In the IMPD/IB of the IMP (appendix I - II), detail on pharmacological interacions and dose recomendations with other drugs are specified.

6.8 COMPLIANCE

Treatment adherence will be self-reported by the patient by answering the adapted SERAD questionnaire (included in the CRF).

The investigator is to ask the patient about treatment adherence to antiretroviral and concomitant treatment and this data is to be written in the clinical record. This data is to guarantee the compliance.

No pill count will be performed to assess compliance.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 INCLUSION CRITERIA

1. Age ≥18 years old
2. Documented HIV infection
3. Stable 3-drug antiretroviral treatment including PI/r/c or NNRTI for at least 6 months.
4. Plasma HIV-1 RNA load <50 copies/mL for at least 12 months.
5. Signed Informed Consent

7.2 EXCLUSION CRITERIA

1. PI/r monoterapy
2. INSTI therapy during the previous 6 months
3. Evidence of previous INSTI resistance
4. Creatine clearance <50 mL/min
5. Child- Pugh B or C
6. History of active uncontrolled GI disorders or diseases including:
   - **6.1.** Major surgery of the GI tract, with the exception of cholecystectomy and appendectomy, in the previous 5 years.
   - **6.2.** Any major bowel resection at any time
   - **6.3.** Any chronic digestive disease such as peptic ulcer, Crohn's disease, ulcerative colitis, coeliac disease, confirmed intolerance to lactose or indeterminate colitis.
   - **6.4.** Persistent infectious gastroenteritis, colitis or gastritis; persistent or chronic diarrhea of unknown etiology; *Clostridium difficile* infection (recurrent) or Helicobacter pylori infection (untreated);
   - **6.5.** Irritable bowel syndrome (moderate-severe)
   - **6.6.** Chronic constipation
   - **6.7.** Active proctitis
7. Antibiotic therapy within the previous 2 months
8. In women, pregnancy or breastfeeding*.

* Female subjects of childbearing potential must not be pregnant, not be planning a pregnancy or breast-feeding. Sexually active women must be willing to use an effective method of contraception (hormonal contraception, diaphragm, intra-uterine device (IUD), or anatomical sterility in self or partner) from baseline until the end of the clinical trial. Sexually active men in heterosexual relationships must be willing to use an effective method of contraception (anatomical sterility in self) or agree on the use of an effective method of contraception by his partner (hormonal contraception, diaphragm, intra-uterine device, or anatomical sterility) from baseline until the end of the clinical trial.

** condom use is considered as an additional method of contraception only and cannot be the only method of contraception used as not been considered an effective method by the Clinical Trial Facilitation Group (CTFG) guidelines.*

7.3 SUBJECT WITHDRAWAL CRITERIA

7.3.1 Early subject withdrawal

The patients will complete the clinical study before the stipulated time in the following circumstances:

- Virological failure: it is defined as≥ 200 copies/mL in 2 consecutive determinations, or a single HIV-1 RNA values > 1000 copies/mL.
- Interruption of treatment due to adverse events, intolerance or poor adherence during the study.

- Concurrent process or illness which in the opinion of the investigator requires the withdrawal of the patient.
- Protocol deviation which in the opinion of the sponsor requires the withdrawal of the patient.
- The patient withdraws consent to participate in the study.
- Other

7.3.2 Medical approach to withdrawal

In all cases, 'end of study form' is to be filled. Detailed information will be given about the date and reasons of the discontinuation to the sponsor. The investigator will facilitate the necessary medical support.

7.3.3 Follow-up after early withdrawal

That is, as a general rule, all patients who discontinue treatment prematurely will undergo a clinical examination and all tests specified in the visit.

In the presence of virological failure, the new antiretroviral treatment regimen will be constructed based on the physicians' criteria.

7.3.4 Replacement of patients

Patients withdrawn by protocol deviation will be replaced by new patients until the expected number of patients. Patients withdrawn by other reasons will not be replaced.

7.4 PRE-RANDOMIZATION / PRE-BASELINE LOSSES

Data from patients that do not meet the selection criteria after completing the screening visit will not be considered for the study. These data will not be collected.

8 TRIAL CONDUCTION AND RESPONSE EVALUATION

8.1 CRITERIA FOR RESPONSE EVALUATION

8.1.1 Primary parameter

Bacterial richness (observed species)*.

8.1.2 Secondary parameters

- Gut bacterial composition*
- Gut bacterial function*
- Other estimators of richness and diversity: Chao1, ACE, Shannon, 1/Simpson.
- Inflammation: IL-6, IP-10
- Coagulation: D-Dimer
- Enterocyte damage: Intestinal Fatty Acid Binding Protein (I-FABP)
- Bacterial translocation and monocyte activation: LPS-binding protein (LBP), soluble CD14.
- Maturation, activation, exhaustion and immune senescence in CD4+ and CD8+ T-cells: CD3+, CD4+, CD8+, CD45RA, CCR7, CD28, CD27, HLA-DR, CD38, PD-1, CD57.**
- CD4 and CD8+ counts
- CD4+/CD8+ ratio
- In case of changes in the microbiome, the quantification of viral reservoir will be done in PBMCs.

***Analysis of the composition, structure and function of the intestinal microbiome**.

- Structure and compositon of the microbiome. DNA will be extracted and purified from fecal samples using the PowerSoil ® kit (MOBIO Laboratories, Carlsbad, USA) and cryopreserved at -80ºC until amplification. The purified DNA will be amplified using Illumina-tagged primers to amplify the V3 and V4 16S ribosomal DNA (rDNA) regions, as described elsewhere [7,8]. PCR reactions will be performed in triplicate to preserve diversity. Pooled triplicates will be sequenced in a MiSeq platform, which will produce more than 30.000 sequences per each 16S measurement, ensuring adequate sampling depth. Bioinformatic analyses will be performed with the latest versions of the most frequently used pipelines. For example, 16S rDNA sequences will be analyzed with MOTHUR or equivalent software. The analysis procedure will include filtering sequences of poor quality, sequencing error correction, elimination of artifact chimeric sequences with UCHIME, taxonomic clustering of sequences, establishment of operational taxonomic units (Operative Taxonomic Units, OTU) and classification and annotation of OTUs using algorithms described in the Greengenes or SILVA databases. This will result in a detailed description of the composition of the intestinal bacteriome, including the relative abundance of the species detected.

- Function of the bacteriome. The software PICRUSt [5] will be used to predict the gene content and their corresponding functions. The gene content will be inferred from the abundance of each bacteria in the intestinal bacteriome according to the 16S rDNA information. PICRUSt has demonstrated excellent correlation with more costly and computationally intensive shotgun metagenomics approaches. Samples will be stored, however, to allow potential future metagenomics studies, not budgeted in this proposal.

****Immune markers**

- Plasma markers will be assessed by Luminex and or ELISA technology using EDTA-plasma samples. PBMC-associated markers will be analyses using multicolor flow cytometry. In all cases cryopreserved samples, will be stained and acquired on a Fortesa (BD Biociences) cytometer and analyzed using commercial (Flow Jo) and on-site developed software (Ourflow).

8.2 TRIAL DEVELOPMENT

After accepting the participation and meeting selection criteria during the screening visit, patients would be assessed the baseline visit. Study visits would be at screening, entry (BL) and weeks 12, 24 and 48. Visits should be performed within a 2-weeks window.

Blood and fecal specimens will be obtained and stored at the points specified in the flow chart of the study.

Plasma samples and PBMC will be stored at –80ºC.

Viral load, CD4 cells count and a complete biochemistry and hematology will be performed at all visits.

8.3 CLINICAL RECORD AND PHYSICAL EXAM

Demographic and HIV infection-related data will be collected in order to characterize the study population: sex, age, time since HIV diagnosis, risk factor and history of opportunistic infections or tumors, active acute or chronic diseases related or not with HIV-infection, date and type of past surgical interventions, ART history, nadir and current CD4+ counts, HBV or HCV co-infection, history of HPV infection, allergies, current abdominal transit, history of altered abdominal transit, current or past antibiotic therapy, dosing and duration of antibiotic therapy during the previous 6 months.

Patients will be asked for all medication, antiretroviral or not, that he/she may have taken (including over the counter medication), within one week before inclusion and during the study.

A complete physical examination will be performed at the baseline visit, including weight and height. In the follow-up, a physical exam will be performed according to patient's symptoms.

8.4 LABORATORY TESTS

Patients will fast for at least 8 hours prior to assessment, in the points specified in the flow chart of the study (section 8.5). The following parameters will be quantified, as needed:

- **Blood count:**
  - Hemoglobin
  - Red blood count
  - Hematocrit
  - White cell blood count
  - Neutrophil count
  - Lymphocyte count
  - Platelets
  - Prothrombin time
- **Blood biochemistry:**
  - Albumina
  - Glucose
  - Urea
  - Creatinine
  - Estimated glomerular filtration rate (CKD-EPI)
  - Ionogramme: sodium, potassium
  - Total Bilirubin
  - Cholesterol (HDL, LDL and total)
  - Triglycerides
  - Liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT, alkaline phosphatase
- **Pregnancy test in women (test strip)**

- **Immunology:**
  - CD4 lymphocytes count
  - CD4 lymphocytes percentage
  - CD8 lymphocytes count
  - CD8 lymphocytes percentage
  - CD4/CD8 ratio
  - HLA-DR
- **Virology:** Plasma HIV-1 viral load.
- **Genotypic testing in plasma at virological failure.**
- **Whole blood:** a blood sample (5 x 10 mL EDTA tubes will be collected at each visit, from which irsiCaixa will obtain 4 plasma vials and as many PBMCs as possible). Plasma markers will be assessed by Luminex and or ELISA technology using EDTA-plasma samples. PBMC-associated markers will be analyses using multicolor flow cytometry. In all cases cryopreserved samples, will be stained and acquired on a Fortesa (BD Biociences) cytometer and analyzed using commercial (Flow Jo) and on-site developed software (Ourflow). In case of changes in the microbiome, the quantification of viral reservoir will be done in PBMCs.
- **Stool samples** will be collected at each visit. This will allow accounting for natural variation in gut microbiota composition. Samples will be used for taxonomic and functional / metagenomic analysis of the bacterial and viral microbiome and soluble & cellular markers of immune activation, inflammation and bacterial translocation.

Note: Before the beginning of the study, all labs will facilitate to the sponsor and to the investigator a list of the reference normal values of the parameters assessed.

8.5 ASSESSMENTS FLOW-CHART

VISITS	SCR	BL	W12	W24	W48	VF
Window			+/- 2 weeks	+/- 2 weeks	+/- 2 weeks	
Inclusion/exclusion criteria	√					
Patient informed form and Informed consent	√					
Pregnancy test	√[B]			√		
Randomization		√				
Clinical visit		√	√	√	√	
Adherence questionnaire		√	√	√	√	
HIV-1 Viral load	√[C]	√	√	√	√	√
Biochemistry	√[C]	√[C]	√	√	√	
Hematology	√[C]	√[C]	√	√	√	
CD4/CD8 cells count		√[C]	√	√	√	
Stool sample		√	√	√	√	
Whole blood simple for IRSI[A]		√	√	√	√	
Adverse Events		√	√	√	√	
Concomitant medication		√	√	√	√	
Genotypic testing						√

[A] **blood sample** (5 x 10 mL EDTA tubes, from which irsiCaixa will obtain 4 plasma vials and as many PBMCs as possible)
[B] Women of childbearing potential, within 10 days prior to randomization.
[C] Parameters performed within 2 months prior to Baseline will be accepted.

9 ADVERSE EVENTS

9.1 DEFINITION

Adverse event: (AE) Medical event presented by a patient or clinical research subject administered a pharmaceutical product, and which does not necessarily have a causal relation to the treatment.

Serious adverse event: (SAE) Medical event classified as such and which, regardless of the dose involved:

- causes patient death,
- produces a life-threatening situation for the patient,
- requires or prolongs in hospital admission,
- produces important or persistent incapacitation/handicap, or constitutes a congenital defect or anomaly,
- needs action to prevent any of above situations, or
- is considered medically significant

Examples of such events are intensive care in an Emergency Service or in the home in a patient with allergic bronchospasm; blood dyscrasias or seizures not giving rise to hospital admission, or the development of drug dependency or abuse.

Unexpected adverse event: (UAE) AE related to the product in investigation the nature or intensity of which does not coincide with the information available on the product administered (IB or SmPC).

Serious Unexpected Adverse Reaction: (SUSAR) SAE related to the product in investigation the nature or intensity of which does not coincide with the information available on the product administered (IB or SmPC).

9.2 MONITORING, RECORDING AND REPORTING OF ADVERSE EVENTS

An AE is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the CRF rather than the individual signs or symptoms of the diagnosis or syndrome.

An overdose, accidental or intentional, whether or not it is associated with an AE, or abuse, withdrawal, sensitivity or toxicity to an investigational product should be reported as an AE. If an overdose is associated with an AE, the overdose and AE should be reported as separate terms.

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject's clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, or other appropriate tests and procedures.

All AEs will be recorded by the Investigator from the time the subject signs informed consent until the last study visit or to 28 days after the last dose of IMP in case of early withdrawal while patient is receiving IMP. AEs and SAEs will be recorded on the AE page of the eCRF and in the subject's source documents. All SAEs must be reported to the Sponsor (see contact details at the end of the paragraph) within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form (Appendix IV, can be found in the Investigator's File), or approved equivalent form.

9.3 DOCUMENTATION RELATED TO AE AND SAE

Each AE and SAE to take place during the study should be documented in the medical records of the subject in accordance with standard clinical practice of the researcher, and in the CRF. For each SAE, an independent set

of SAE forms will be used. Only if there are multiple SAE at the time of the initial report and these are temporary and / or clinically interrelated can they be registered on the same set of SAE forms.

The researcher should try to make a diagnosis of the event based on the signs, symptoms and / or other clinical information. An AE diagnosis has to be recorded per line or a sign/symptom if the diagnosis is not available. If a diagnosis subsequently becomes available, this then should be entered and the sign/symptom crossed out, initialed and dated by the investigator.

SAE pages found in the investigator's file shall be completed as precisely as possible, printed and shall be signed by the investigator before being sent to the sponsor. It is very important that the researcher completing the SAE form provides their opinion in regard to the relationship of the event to the study drug.

9.4 EVALUATION OF ADVERSE EVENTS

9.4.1 Description of the imputability criteria

Relatedness is an assessment made by a study clinician of whether or not the event is related to the study agent. Degrees of relatedness will be categorized according to current DAIDS-approved guidelines (Appendix V). Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, January 2010), the relationship categories that will be used for this study are:

Related: There is a reasonable possibility that the AE may be related to the study agent(s) suggested by:

- A plausible, reasonable time sequence exists in relation to administration of the drug or its plasma or tissue concentrations.
- The observed manifestation coincides with the known adverse reactions profile of the implicated drug.
- The event cannot be or unlikely be explained by a concurrent disease or by other drugs or chemical substances.
- Response to withdrawal is clinically plausible, i.e., the condition improves on discontinuing administration of the drug.

Not Related: There is not a reasonable possibility that the AE is related to the study agent(s).

9.4.2 Adverse events severity/intensity

For both AEs and SAEs, the Investigator must assess the severity / intensity of the event.

Grading will be performed using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014].

Citation: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014]. Available from: http://rsc.techres.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf

In case of events or laboratory abnormalities not included in the table, the following scale will be used:

Grade 1 (mild): Symptoms causing no or minimal interference with usual social & functional activities

Grade 2 (moderate): Symptoms causing greater than minimal interference with usual social & functional activities

Grade 3 (severe): Symptoms causing inability to perform usual social & functional activities

Grade 4 (potentially life-threatening): Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

Grade 5 (death): Any AE where the outcome is death.

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is *not* the same as "serious" which is based on subject/event *outcome* or *action* criteria associated with events that pose a threat to a subject's life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

9.4.3 Seriousness

A SAE is any AE occurring at any dose that:

- Results in death;
- Is life-threatening (i.e., in the opinion of the Investigator, the subject is at immediate risk of death from the AE);
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay);
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions);
- Is a congenital anomaly/birth defect;
- Constitutes an important medical event.

Important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events **not considered** to be SAEs are hospitalizations for:

- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
- A procedure that is planned (i.e., planned prior to starting of treatment on study); must be documented in the source document and the CRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.
- An elective treatment of a pre-existing condition unrelated to the studied indication.
- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, both the AE page/screen of the CRF and the SAE Report Form must be completed.

For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to IMP, action taken regarding IMP, and outcome.

9.4.4 Duration

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event.

9.4.5 Action Taken

The Investigator will report the action taken with IMP as a result of an AE or SAE, as applicable (e.g., discontinuation of IMP) and report if concomitant and/or additional treatments were given for the event.

9.4.6 Outcome

Any SAE will be followed preferably until:

- Resolution of the event;
- Stabilization of the event; or
- Resetting the baseline situation of the event, in case baseline situation is available.

Otherwise, they will continue until:

- The event can be attributed to products other than the study medication or factors unrelated to the study; or
- It is unlikely to obtain further information.

9.5 PROCEDURE FOR REPORTING ADVERSE EVENTS

9.5.1 Immediate reporting by Investigator to sponsor

The investigator will immediately notify the study sponsor of any serious and/or unexpected adverse events.

The report will be realized during the first 24 hours since the start of the serious adverse event. Notification will be made by means of the adverse events reporting form contained in Appendix IV of this protocol.

Contact details for Sponsor: [redacted]

Contact details for Pharmacovigilance: Pharmacovigilance Department of MSD Spain (fax to 915716466).

All adverse events will be recorded, regardless of the imputability (i.e., causal) relationship involved, in the corresponding adverse events description form. The latter is found in the CRF of each participant in the study.

The Sponsor will provide investigators with a copy of the annual periodic safety report e.g. Development Update Safety Report (DSUR) at the time of submission to the Regulatory Authority and Ethics Committee.

9.5.2 Reporting to Regulatory Authorities and the Ethics Committee

The sponsor will inform the Spanish Drug Agency (Ministry of Health), the competent authorities of the autonomous region and the Ethics Committees implicated in the clinical trial about any important information of security of the investigational medicinal product.

The sponsor will inform the Spanish Drug Agency (Ministry of Health) of any SUSAR which may be related to the study treatment.

The sponsor will inform competent authorities of the implicated autonomous region of any SUSAR which may be related to the study treatment, and that have been happened in patients in its autonomous region.

The Sponsor will inform relevant Regulatory Authorities;

- Of all relevant information about SUSAR that are fatal or life-threatening as soon as possible, and in any case no later than 7 days after knowledge of such a case. Relevant follow-up information for these cases will be subsequently be submitted within an additional eight days

- Of all other SUSAR as soon as possible, but within a maximum of 15 days of first knowledge by the investigator.

The sponsor will keep a detailed register of all the adverse events notified by the investigators.

All adverse events will be notified in table form in the final report of the clinical trial.

9.6 ABNORMAL LABORATORY PARAMETERS

An abnormal laboratory parameter shall be considered an AE if the abnormality:

- results in withdrawal from the study
- requires treatment, dose modification or investigational drug interruption or any other therapeutical intervention
- is considered clinically important

Regardless of their severity, only laboratory abnormalities that meet criteria of seriosness should be recorded as SAE.

If the laboratory abnormality is part of a diagnosis or syndrome, only the syndrome or diagnosis will be included as AE or SAE. If the laboratory abnormality is not part of a diagnosis or syndrome, it shall be recorded as AE or SAE.

Clinically significant changes in safety parameters that are associated with the disease under study will not be rated as AE or SAE, unless the investigator judges that are more severe than expected given the patient's condition.

9.7 PREGNANCY

The cases of pregnancy shall be recorded as AE and should only be considered as SAE only if they meet any seriousness criteria. Pregnancy is also a protocol deviation requiring premature termination of the patient. The investigator will provide medical support to the pregnant patient.

10 STATISTICS

Statistical analysis will be conducted by IrsiCaixa. A computerized database for the study will be created, which will be used for data analysis. Statistical analysis will be carried out using R.

A general analysis of all variables in the study will be conducted, including demographic and clinical characteristics at baseline.

10.1 STATISCAL ANALYSIS

- Taxonomic and functional diversity within (alpha-diversity) and between samples (beta-diversity) will be determined. Rarefaction curves will be generated to evaluate sampling efficiency. Diversity measures, reflecting the richness of the microbiome in terms of number of species and abundance of each species will be calculated. This will enable comparisons between groups and timepoints. Ordination methods including principal component analysis and phylum abundance within individuals will be used to check for significant differences between groups. Bacterial abundance and functional profiles will be analyzed with LEFSe. Univariate correlations involving >2 groups will be done applying ANOVA plus Tukey-Kramer post-hoc tests and Benjamini-Hochberg correction; 2-group comparisons will be performed with the Welch's t-test. The relationship between the microbiome and markers of immune activation, inflammation, tissue damage and bacterial translocation will require the application of nonlinear Kernel regression methods.

- Subjects requiring antibiotics for their clinical management during follow-up will not be excluded from the analyses, but the type, duration and reasons for antibiotic intake will be recorded and taken into account in association analyses. Results in HIV-1-infected subjects will be compared to the gut microbiome profiles of healthy HIV-1-negative individuals previously reported by the EU FP7-funded MetaHIT consortium. We will also use microbial ecology theory to describe ecological networks between microbes and between them and the host (degree distribution, clustering coefficient, distance, centrality, community structure, connectivity index, etc.).

For extensive examples of microbiome analyses available and performed previously by our group at irsiCaixa, please see: Noguera-Julian, M. et al. Gut microbiota linked to sexual preference and HIV infection. EBioMedicine (2016) http://dx.doi.org/10.1016/j.ebiom.2016.01.032.

10.2 SAMPLE SIZE DESCRIPTION

This is a pilot exploratory study with no formal sample size calculation. This is, in fact, the first study addressing the effects of antiretroviral therapy intensification on the human gut microbiome. A total of 60 patients will be included in this trial during an inclusion period of 36 weeks. 40 patients in the intensification arm (raltegravir 1200 mg) and 20 patients in placebo arm.

10.3 DEVIATION OF STATISTICAL PLAN

Any deviation from that presented statistical plan will be described and justified in the final report.

11 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Investigators and institutions will allow the monitoring, and audits by the Health Authorities or the Sponsor giving direct access to data and original source documents.

Access to personal patient information will be restricted to the Study physician / staff. To allow monitorings, audits and inspections, access to data to Health Authorities (Spanish Agency for Medicines and Health Products), the Ethics Committee and personnel authorized by the Sponsor, is guaranteed while maintaining the confidentiality thereof according to current legislation.

12 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 STUDY MONITORING

In accordance with applicable regulations and Good Clinical Practice (GCP), the monitor will visit or contact the center on a regular basis. The duration, nature and frequency of visits / contacts depend on the monitoring plan.

During these contacts, the monitor shall:

- monitor and evaluate the progress of the study;
- examining the data collected;
- carry out a verification of the source documents;
- identify any problems and find solutions;

The goal of the monitoring activity is to verify that:

- the rights and welfare of subjects are respected;
- survey data are accurate, complete and verifiable with the help of original documents;
- the study is performed according to the protocol and any amendment adopted, GCPs and regulations.

The investigator must agree to:

- grant to monitor direct access to all relevant documentation;
- devote part of his/her time and staff time to the monitor in order to discuss the results of the monitoring, as well as any other possible aspect.

The monitor should also contact the center before starting the study with the aim to discuss with staff the Protocol and procedures for data collection.

12.2 AUDITS AND INSPECTIONS

Sponsor can carry out an audit of quality control at its sole discretion. In this case, the investigator should agree to grant the auditor direct access to all relevant documentation and devote part of his/her time and staff time to the auditor in order to discuss the results of the monitoring, as well as any other possible aspect.

Moreover, regulatory authorities may also inspect the study. In this case, the investigator should agree to give the inspector direct access to all relevant documentation and devote part of his/her time and staff time to the inspector in order to discuss the results of the supervision, as well as any other possible aspect.

12.3 CASE REPORT FORM

Study data will be collected through a Case Report Form.

Accurate and reliable data collection is ensured by checking and cross checking the CRF front site records conducted by the study monitor (verification of source documents). The data collected will be added to a computer database which will be reviewed for possible inconsistencies to be resolved by the research team of the study in each site.

13 ETHICS

13.1 GENERAL CONSIDERATIONS

The clinical trial will be conducted according to the principles of the Declaration of Helsinki, Fortaleza, Brasil, octubre 2013.

This study will be conducted according to Spanish regulations and the required documentation prior to the start will be:

- Protocol acceptance by the sponsor and the coordinating investigator
- Protocol approval by the Ethics Committee.
- Protocol authorization from the Spanish Drug Agency (Ministry of Health)

All subjects will be guaranteed continued medical and nursing supervision throughout the duration of the study.

This study will conform to the standards of "Good Clinical Practice".

13.2 PATIENT INFORMATION SHEET AND INFORMED CONSENT

Informed consent will be obtained before including the patient in the trial (Appendix II). The investigator is to inform the patient of the nature, duration and purpose of the study, as well as of all the obstacles and inconveniences which – within reason – may be expected from it. Furthermore, the patient is to receive information in writing. The participating patients must be legally competent to give informed consent, with the possibility of taking decisions at his/her own free will. The patient has the right to leave the study at any time.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA HANDLING

The processing of the data to be compiled by the study sponsor during the trial will be subject to current legislation as regards data protection (LOPD, Ley Orgánica 15/1999, de 13 de diciembre de protección de datos de carácter personal). The patient will be identified in the records by the corresponding code number only. The patient is to be guaranteed anonymity, and is to be informed that all communication will take place between him/her and the investigator – not the sponsor of the trial.

Data transmitted to third countries and other countries will in no case contain personal data. In the event that such transfer occurs, it will be for the same purposes of the study described and ensuring confidentiality at least to the level of protection of the law in Spain.

14.2 RECORD KEEPING

14.2.1 Investigator file and document retention

The investigator must keep the investigator file with the proper and accurate records to enable the study to be fully documented and data subsequently verified.

The Investigator's study file will contain the protocol and its amendments, CRFs, questionnaires' forms, EC approval and authorization from the health authorities, samples of the patient information sheet and informed consent, staff curriculum, signatures' delegation log and listing of subjects, as well as other appropriate documents and correspondence.

Clinical source documents from subjects (usually predefined by the project to record key efficacy and safety parameters or documents that are not in the clinical record of the hospital) will be filed indicating the number of patient without personal data.

The investigator should retain these documents at least 25 years, according to RD 1090/2015, provided that the promoter does not express a greater period.

14.2.2 Source documents and basic data

Patient participation in the study will be included on medical records, including assigned code number and identification of the different study visits that will take place throughout the study. At the end of the study, a copy of the CRF will be placed on the site.

15 FINANCING AND INSURANCE

15.1 SOURCE OF FINANCING

This Investigator initiative study will be funded by Lluita contra la SIDA Foundation in collaboration with Merck Sharp And Dohme.

15.2 INSURANCE POLICY

In accordance with Royal Decree 1090/2015, of 4 December, the trial sponsor has a policy of liability insurance with Zurich Insurance Company PLC Branch in Spain established in Barcelona (Appendix III). The sponsor shall extend this policy or another with equivalent coverage until the end of the trial. The policy will cover the damages to the people that could be set as a result of the trial by an insured amount of 300,000 € per patient tested to a maximum of € 3,000,000 per year and clinical trial. This policy also covers the responsibilities of the sponsor, the principal and his/her collaborators, as well as the hospital or site where they carry out the clinical trial.

The sponsor agrees to pay the premiums to cover the liability pertaining to the trial. It is presumed, unless proven otherwise, that damage affecting the health of the person subject to testing during implementation and in the following year the completion of treatment, have occurred as a result of the trial. However, once the year ended, the test subject is required to prove the link between the trial and damage.

The site and the principal investigator undertake to inform the sponsor of any claim or legal, real or potential action if known, linkable to trial.

16 PUBLICATION POLICY

The publication of the trial results shall meet the requirements set out in Article 42 of Royal Decree 1090/2015. The results of the present study will be submitted for being presented in an international meeting related to HIV infection and antiretroviral treatment (ie: CROI, ICAAC, IAS…). Publication of the results in an international medical journal within the first quartile of impact factor (AIDS, Antimicrobial Agents Chemotherapy, Journal Antimicroial Chemotherapy…) will be attempted.

APPENDIX I: INVESTIGATOR BROCHURE

APPENDIX II: PATIENT INFORMATION AND WRITTEN INFORMED CONSENT

APPENDIX III: INSURANCE

APPENDIX IV: ADVERSE EVENT REPORTING FORM

APPENDIX V: DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS