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**Isala study:**  
**Analytical treatment interruption in HIV positive patients with low viral reservoir to evaluate the potential of a functional cure**

**Initial Protocol**  
**Version 6.0, 23 May 2018**



Vrije  
Universiteit  
Brussel



Universitair Ziekenhuis Gent

**Sponsor:** Institute of Tropical Medicine  
Nationalestraat 155  
B-2000 Antwerpen - Belgium

**Coordinating Investigator:** Dr. Eric Florence

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<b>Sponsor:</b>	Institute of Tropical Medicine, Antwerp, Belgium
<b>Coordinating Investigator:</b>	Eric Florence Department of Clinical Sciences Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium
<b>Telephone:</b>	+32 3 247 66 42
<b>Fax:</b>	+32 3 247 64 10
<b>Email:</b>	<a href="mailto:eflorence@itg.be">eflorence@itg.be</a>

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This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits her/himself to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the Declaration of Helsinki, Good Clinical [Laboratory] Practice (GC[L]P) and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

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### **COORDINATING INVESTIGATOR:**

Title, Name: Dr Eric Florence \_\_\_\_\_ Date: \_\_\_\_\_  
Signed: \_\_\_\_\_

### **DIRECTOR OF THE INSTITUTE OF TROPICAL MEDICINE:**

Title, Name: Prof. Dr. Bruno GRYSSELS \_\_\_\_\_ Date: \_\_\_\_\_  
Signed: \_\_\_\_\_

**PRINCIPAL INVESTIGATOR ITM:**

Title, Name: Dr Eric Florence \_\_\_\_\_ Date: \_\_\_\_\_  
Signed: \_\_\_\_\_

**PRINCIPAL INVESTIGATOR UNIVERSITY HOSPITAL GHENT (UZG):**

Title, Name: Prof. Dr. Linos Vandekerckhove \_\_\_\_\_ Date: \_\_\_\_\_  
Signed: \_\_\_\_\_

**PRINCIPAL INVESTIGATOR UNIVERSITY HOSPITAL BRUSSELS (UZB):**

Title, Name: Prof. Dr. Sabine Allard \_\_\_\_\_ Date: \_\_\_\_\_  
Signed: \_\_\_\_\_

**PRINCIPAL INVESTIGATOR UNIVERSITY HOSPITAL SAINT-PIERRE (UMC SINT-PIETER) :**

Title, Name: Prof. Dr. Stéphane De Wit \_\_\_\_\_ Date: \_\_\_\_\_  
Signed: \_\_\_\_\_

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## SYNOPSIS

<b>HYPOTHESIS</b>	Analytical interruption of longstanding antiretroviral therapy (ART) in HIV-1 infected patients with a very low proviral reservoir results in “functional cure”.
<b>DESIGN</b>	Prospective observational study.
<b>STUDY SITE &amp; POPULATION</b>	HIV-1 infected patients with normal peripheral blood CD4 T-cell counts and undetectable viral load will be recruited in four Belgian HIV reference centers: Institute of Tropical Medicine Antwerp, University Hospital Ghent, University Hospital Brussel, Saint-Pierre University Hospital.
<b>DURATION</b>	9 months screening + 48 weeks clinical study + 12 weeks post study. (23 months in total until last patient visit)
<b>OBJECTIVES AND ENDPOINTS</b>	The primary objective is to evaluate the proportion of patients with low proviral reservoir that have a sustained viral suppression after 48 weeks of treatment interruption.
<b>INCLUSION &amp; EXCLUSION CRITERIA</b>	HIV-1 infected adults with peripheral blood CD4 T-cell counts $\geq 500/\mu\text{L}$ and plasma viral load $< 50$ copies/ml will be evaluated for inclusion. HBV or HCV co-infected patients and patients with ongoing HIV-related diseases or a history of antiretroviral drug resistance or AIDS event will be excluded.
<b>SCREENING, RECRUITMENT &amp; RANDOMIZATION</b>	Two-step screening: viral reservoir measurement and analytical treatment interruption. There is no randomization foreseen
<b>INTERVENTION</b>	Interruption of antiretroviral treatment in HIV-1 infected patients with low viral reservoir.
<b>FOLLOW-UP</b>	Intensive clinical and laboratory follow-up during 48 weeks followed by 12 weeks post intervention.
<b>ANALYTICAL METHODS</b>	Total HIV PCR for plasma viral RNA, for cell-associated viral RNA and DNA will be quantified using droplet (digital PCR droplet). Viral outgrowth assay (limiting dilution culture of CD4 T cells).
<b>SAFETY</b>	Safety of the intervention will be evaluated by recording (Serious) Adverse Events and grading laboratory parameters and vital signs. Data will be monitored by means of a sponsor regulated pharmacovigilance system.

## **1. INTRODUCTION**

### **1.1 Background**

Combined antiretroviral treatment (cART) induces a rapid decline in HIV plasma viral load (pVL), which after several years of cART reaches a plateau of 1-10 copies per ml plasma [1]. Nevertheless, proviral DNA, which represents the HIV reservoir, may persist at levels of hundreds or thousands of copies per million CD4-T cells in peripheral blood. The establishment of this proviral reservoir is complex and involves, among others, naïve, central memory, transitional and effector memory T cells [2].

The HIV reservoir is widely distributed, not only in blood, but also in lymphoid organs, the central nervous system, the intestinal tract and genital organs [3–5]. Measuring proviral DNA for clinical purposes can readily be performed on peripheral blood mononuclear cells (PBMC), CD4-T cells or subsets thereof. The magnitude of the proviral DNA reservoir is not necessarily predictive for the occurrence and extent of viral rebound following cART interruption since a high proportion of integrated HIV DNA has been shown to be replication incompetent in viral outgrowth assays (VOA) [6]. Therefore, quantifying the replication capacity of the HIV reservoir, by optimally stimulating CD4<sup>+</sup> T cells in a limiting dilution VOA, currently represents the most reliable ex vivo parameter of viral fitness [7,8]. Cell-associated unspliced HIV RNA (usRNA) has recently been proposed as a marker/indicator of residual viral transcription in cART-treated subjects with pVL below the targeted clinical limit of 50 copies/ml. Indeed, slightly elevated levels of PBMC-associated usRNA among patients with pVL below detection level but with suboptimal treatment adherence were shown to be predictive of therapy failure in the future [8]. Cell-associated viral DNA, usRNA and viral outgrowth capacity need therefore to be evaluated in the development of novel HIV therapeutic strategies.

HIV research is challenged by the concept of inducing “functional cure” or HIV remission, i.e. interventions aiming at keeping the viral load at low or undetectable levels after interrupting cART in infected patients. It has been suggested that so called “elite controllers” (EC) may provide important clues in this quest [9]. By definition EC are able to maintain their pVL “spontaneously” below the lower limit of detection i.e. without ever being treated with cART (or other regimens) [10,11]. Importantly, reports have recently emerged describing patients maintaining pVL below the lower limit of detection after interruption of long-time cART. These patients have been referred to as “secondary controllers” [12] or “post treatment controllers” (PTC) [13]. Conceptually, this status may provide additional and potentially more relevant information to develop strategies aiming at HIV cure.

The undetectable pVL in EC has been shown to be associated with low proviral DNA in peripheral blood CD4<sup>+</sup> T cells and other reservoirs (e.g. the gut-associated lymphoid tissue) and with low or absent intracellular viral RNA. Some genetic host factors (e.g. the HLA types B27 and B57) as well as viral genetic characteristics (e.g. defects in Nef, Vif or other genes) are known to be associated with the EC phenotype. In the majority of the EC, HIV-specific CD8<sup>+</sup> T- cell response has been shown to be qualitatively superior to the response seen in progressors. With this regard, HIV-specific CD8<sup>+</sup> T cells targeting conserved viral proteins (e.g. Gag), exhibiting a polyfunctional cytokine production pattern, recognizing a broad array of epitopes and/or characterized by a high avidity have been shown to correlate with a better clinical outcome. However, the most consistent finding in EC is the presence of CD8<sup>+</sup> T cells with a high “virus-suppressive/cytolytic” activity. Consequently, even in the absence of viral genetic defects, the replication capacity of HIV strains isolated from EC has been shown to be lower than what is seen in progressors [14].



Several large studies in chronically HIV infected subjects showed that interruption of long-term cART not only resulted in prompt rebound of plasma viremia but was also clinically harmful in the long-term, especially when performed repetitively in subjects with low nadir CD4<sup>+</sup> T cells [15,16]. However, observational studies [17–20] and clinical trials [21,22] evidenced that interruption of cART that was initiated during primary HIV infection (PHI), resulted in delayed viral rebound and disease progression [23]. Interestingly, in some of these patients, pVL remained below the lower limit of detection during many months or years post treatment interruption (TI). Steingrover et al. identified 4 out of 24 patients initiating cART during PHI, who maintained pVL < 50 copies/ml for at least 48 weeks after interruption of long-term cART [24]. In analogy, Hocqueloux et al. described 5 out of 32 PHI patients with VL < 50 copies/ml for a median of 75 months after TI [25]. Within the French ANRS PRIMO cohort, 164 patients interrupted cART that was initiated during PHI. pVL remained <50 copies/mL in 14/164 subjects for a median of 4.5 years [26]. Additional PTC were described in the European seroconverter CASCADE cohort [27]. However, these cohorts of PTC do not provide insight in the underlying immune and/or viral mechanisms contributing to the control of viral replication post TI.

Although no viral rebound (i.e. pVL > 400 copies/ml) was documented in the 14 PTC described in the French VISCONTI cohort, six of these patients showed intermittent blips in pVL (i.e. pVL elevation between 50 and 400 copies/ml) during the 48-113 months of TI. In contrast with EC, the CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation state as well as the HIV-specific T-cell responses were shown to be weaker in PTC. Moreover, no overrepresentation of “protective” HLA alleles (e.g. B27 and B57) was observed in this population. In addition, ex-vivo CD8<sup>+</sup> T-cell mediated HIV suppressive activity, which is commonly observed with EC, was completely absent in the PTC population from the VISCONTI study. Importantly, the cellular proviral DNA in PTC was very low following TI. Very remarkably, the cellular proviral DNA tended to further decrease over time in the absence of cART in some of these patients. Nevertheless, HIV could be cultured *in vitro* from autologous CD4<sup>+</sup> T cells of all PTC. Unfortunately, the viral replication capacity, as measured by limiting dilution (outgrowth) assay or kinetic (fitness) assay, was not evaluated [13].

Four patients followed at ITG, who initiated cART during the chronic viremic phase, durably maintained control over viral replication after interruption (for varying reasons) of long-term cART [12]. No particularities were highlighted when evaluating the HIV-specific T-cell responses. Although no HLA-association or CCR5Δ32 polymorphism was observed, all four PTC had a low viral reservoir when compared to non-controllers, as assessed by PBMC-associated total HIV DNA. In addition, no intracellular viral unspliced mRNA species could be measured, suggesting a status of “silent” provirus. Finally, VOA from autologous CD4<sup>+</sup> T cells repeatedly failed in one patient and showed delayed kinetics and low fitness in two others. With respect to viral characteristics the latter secondary controllers were rather similar to EC (low proviral load, low intracellular HIV mRNA and difficult-to-culture viruses). They were also clearly different compared to viremic progressors or aviremic subjects under continuous cART (from whom HIV could be easily cultured *in vitro* and who had higher HIV DNA and intracellular HIV mRNA). Five years following TI and although lacking viral rebound, two PTC were restarted on cART because of low CD4<sup>+</sup> T cell counts [12]. Similarly, the SALTO study showed that 7 out of 95 chronically-infected HIV patients were able to control viral replication below the 400 copies/mL 12 months after long-term cART interruption [28]. Again, in these patients, the only associated predictive factor for viral control in this early treated chronic population was a low level of blood cell associated HIV DNA.

Thus, recent data provide sufficient evidence that a small proportion of HIV-1 infected subjects is able to spontaneously maintain viral suppression after interruption of long-term cART. This observation challenges the common wisdom that cART needs to be taken lifelong to prevent viral rebound and disease progression [29]. Although most PTC described in the medical literature so far initiated cART in the acute phase of the infection, there is emerging evidence that some patients, who started cART in the chronic progressive phase of the infection, are also able to control viremia following TI. Based on the observations in the VISCONTI, PRIMO and SALTO cohorts it is estimated that between 5 to 15 % of patients initiated on cART at the acute phase of the disease might spontaneously control viral replication following TI [13].

Since it has repeatedly been argued that analytical treatment interruption (ATI) in selected patient subgroups may be acceptable to evaluate interventions aimed at functional cure [30,31], prospective studies looking into possible mechanisms and predictors of PTC are urgently needed. The data summarized above suggest that at least some patients with normal CD4<sup>+</sup> T-cell, high nadir CD4<sup>+</sup> T cells and an exceptionally low proviral load after prolonged periods of cART, initiated either in the acute or chronic phase of the disease, might spontaneously control viral replication after ATI. In depth prospective studies on clinical and laboratory characteristics, including total HIV DNA, intracellular RNA and VOA, in post-treatment controllers and non-controllers might provide clues to understand the nature of control of viral replication after ATI. Obviously, candidates for ATI should carefully be selected (e.g. to avoid HIV superinfection or other clinical complications) and closely monitored following TI, with prompt re-initiation of cART according to preset criteria. Ultimately, well-designed ATI trials might reveal modifiable factors that could inspire novel treatment strategies aiming at functional cure.

## **1.2 Rationale**

Over the decennia, the face of HIV infection has changed from a fatal to a chronic disease with near normal life expectancy, provided cART is maintained. However, HIV cannot be eradicated as it persists as proviral DNA in long-lived host cells, mainly memory CD4<sup>+</sup> T cells. Therefore, in most patients, treatment interruption results in prompt reactivation of the proviral reservoir, rebound in plasma viremia to pre-treatment levels and ensuing disease progression. Hence, today, it is generally accepted that cART needs to be taken lifelong [32]. Currently, about 10 million HIV-infected patients worldwide, including around 4,000 in Flanders, are treated with cART. This number will increase as most recent guidelines recommend earlier treatment initiation, given the benefits of cART in terms of blocking disease progression, prevention of transmission and reducing co-morbidity [33]. Moreover, despite major improvement in antiretroviral drug efficacy, tolerability and pill burden, several drugs, especially protease inhibitors and nucleoside analogues, are associated with long-term toxicities[34].

In addition to the cumulative cART toxicity, the difficulties to access treatment in some areas of the world, the dislike expressed by patients about lifelong drug use on a daily base, the development of drug resistance and the cost associated with life-long cART, create an urgent need for an HIV cure. Complete elimination of HIV proviral DNA reservoir (also known as “sterilizing” cure) has so far possibly only been accomplished in one patient, also known as the “Berlin patient”[35]. Strategies towards a “functional” HIV cure (i.e. very low HIV reservoir and sustained control of viremia in the absence of cART) are currently being

developed [36]. These strategies remain however experimental and might need years before entering into clinic [37].

Interestingly, we and others have observed that a small proportion of patients, with long-standing cART history, were able to spontaneously control viral replication for a long period of time following TI [12]. An exceptionally low proviral DNA reservoir was shown to be a common characteristic of these PTC. Very low or absent viral replication capacity (as measured by intracellular HIV RNA and ex vivo outgrowth assay) might also be involved in the PTC phenotype. Eventually, these recently identified PTC may constitute an interesting model towards functional HIV cure [38].

To our knowledge, so far, no clinical trial has been performed prospectively to quantify the viral reservoir in order to identify patients in whom cART could safely be interrupted. We will therefore prospectively evaluate about 640 cART treated HIV-1 positive patients in order to select a subgroup of individuals with extremely low HIV reservoirs, defined as an undetectable total HIV DNA and cell-associated HIV RNA (see section 5.1.3 for details) **(first strategic goal)**. In those patients harboring low HIV reservoir, an analytical treatment interruption will be proposed **(second strategic goal)**. Clinical and laboratory parameters (including VOA at baseline) of ensuing controllers and non-controllers (PTC vs. non-PTC) should allow us to identify predictors of functional cure after ATI **(third strategic goal)**.

The innovative approach taken in this trial should help to take decisions on interrupting cART in future HIV cure strategies and lead to the identification of clinical and laboratory parameters correlating with PTC.

## **2. STUDY DESIGN**

### **2.1 General study design**

This is a single arm multi-centric non-randomized (prospective) study. The participants will be included step-wise. The study will be divided in two parts. The first one will aim at quantifying the HIV reservoir in a large group of patients under ART with strictly predefined inclusion and exclusion criteria. The second step encompasses an analytical treatment interruption among participants with the lowest viral reservoir. Both steps will be subjected to a specific informed consent procedure.

### **2.2 Sub-studies**

Sub-studies will be implemented concurrently with the main study pending approval by the steering committee.

A laboratory committee will be set up to overlook and monitor the organization of the substudies. This committee will be composed of one investigator from each of the participating center and will advise the steering committee on the prioritization of ancillary projects.

Separate approval of the sub-study protocol has to be obtained from the institutional review board (IRB) of the Institute of Tropical Medicine (ITM) and the relevant independent ethics committees (IECs). A separate informed consent procedure will be required in case of extra sampling or procedures.

## **3. STUDY OBJECTIVES**

The project hypothesis is that treatment interruption (TI) of longstanding combined antiretroviral therapy in participants with a very low proviral reservoir results in “functional cure”.

The **primary objective** is to determine the proportion of post treatment controllers (PTC) i.e. patients under cART with low baseline peripheral blood proviral DNA that will show sustained viral suppression at 48 weeks post TI .

The **secondary objectives** are:

Confirmation of the safety of a TI strategy in selected HIV patients

Assessment of the viral reservoir magnitude prior and after TI

Assessment of viral and host dynamics in those participants with viral rebound post TI.

The **exploratory objective** is:

Identification of predictors (clinical and/or laboratory) of PTC phenotype

### **3.1 Primary endpoint**

Assessment of the proportion of participants with pVL below the lower limit of detection (<50 HIV RNA copies/ml plasma) 48 weeks following interruption of antiretroviral treatment.

### **3.2 Secondary endpoints**

1) Safety of interrupting antiretroviral treatment will be reported according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

2) Evaluation of the reservoir replenishment upon interruption of antiretroviral treatment by quantifying the viral reservoir at baseline (i.e. just before TI), during TI and at viral rebound.

3) Assessment of the kinetics of HIV viral load rebound after TI based on the repetitive pVL measurements.

### **3.3 Exploratory endpoint**

Determination of various potential predictors of PTC by evaluating demographic, clinical and biological parameters. (see section 8.3).

## **4. SUBJECTS, POPULATION & SELECTION**

### **4.1 Settings, selection & recruitment:**

The participants will be recruited during a 21 months period from the patients regularly followed at the HIV reference centers of each partner (ITM, UZG, UZB, UMC Saint-Pierre). Potential participants will be informed on the study either by the local study team or during the consultation by the treating physician according to local customs.

### **4.2 Inclusion and exclusion criteria**

In order to be eligible, study participants **must meet all the following inclusion criteria:**

- Able and willing to provide written informed consent.
- Men and women age  $\geq 18$  and  $< 65$  years.
- Confirmed HIV-1 infection at any time prior to study entry.
- Infected with HIV-1 subtype A, B, C, D, CRF01\_AE and CRF02\_AG
- Participant should take ART for at least 2 years before baseline with no changes in the ART regimen for at least 90 days prior to study entry. ART regimen is defined as a mono- or bitherapy or a combination of three or more active antiretroviral drugs
- CD4 T-cell count is  $\geq 500/\mu\text{l}$  for a period of at least 3 months prior study entry

- Nadir CD4<sup>+</sup> T-cell count is  $\geq 300/\mu\text{l}$ . A lower nadir CD4<sup>+</sup> T-cell count will be allowed if measured at time of acute infection as far as the relative CD4<sup>+</sup> count remains above 20%. An acute infection is defined as an association of a clinical picture of retroviral syndrome together with a seroconversion in HIV serology or an incomplete confirmation test.
- Plasma viral load < 50 copies/ml for at least 2 years before baseline. (Occasional “blips” will be permitted if it happened more than six months before study entry. An occasional blip is defined as an intermittent viremic episode with a viral load above detection level but below 200 copies/ml and a return to an undetectable level in a next control).
- Willingness to complete scheduled assessments and participant visits.
- All female participants of childbearing potential should have a negative pregnancy test. These women and their partner should use double barrier contraception during the study.  
Females of reproductive potential will need a negative serum or urine pregnancy test at screening. They are defined as those who have not reached menopause or been post-menopausal for at least 24 consecutive months, who have had menses within the preceding 24 months, or women who have not undergone surgical sterilization, specifically hysterectomy, or bilateral oophorectomy or tubal ligation) .  
NOTE: Acceptable documentation of hysterectomy and bilateral oophorectomy, bilateral salpingectomy, tubal micro-inserts, partner who has undergone vasectomy, and menopause is participant-reported history.  
All participants must agree not to participate in a conception process (e.g., active attempt to become pregnant, sperm donation, or in vitro fertilization).
- Adequate peripheral vein access to perform leukapheresis

Participants meeting any of the following exclusion criteria **will not be enrolled in the study**:

- Previous or current history of AIDS defining event as defined in category C of the CDC clinical classification[39] .
- Any acute infection or serious medical illness within 60 days prior to study entry. Participants will be excluded from this study for a serious illness (requiring systemic treatment and/or admission) until the subject either completes therapy or is clinically stable on therapy, in the opinion of the Investigator, for at least 14 days prior to study entry
- History of resistance to antiretroviral drugs, documented by genotyping.
- Active hepatitis B or C virus infection: as defined with a positive serology for either disease with signs of active viral replication?
- Significant risk of HIV transmission during treatment interruption in the opinion of the investigator. This includes evidence for unsafe sexual contacts.
- Current or past history of cardiomyopathy or significant ischemic or cerebrovascular disease.
- History of HIV-related thrombocytopenia.
- Active renal disease (defined as a glomerular filtration rate (calculated by MDRD equation) below 50 ml/min or the presence of HIV associated nephropathy in the past medical history.

- Current or known history of cancer (with the exception of in situ cervix carcinoma or squamous cell carcinoma of the skin) within five years prior to screening.
- Pregnancy and breastfeeding.
- Any condition, including psychiatric and psychological disorders that might interfere with adherence to study requirements or safety of the participant.
- Prior use of any HIV vaccine and/or non-established experimental therapy
- Any of the following laboratory test results at screening:
  1. Confirmed hemoglobin <11g/dl for women and <12 g//dl for men
  2. Confirmed platelet count < 90,000/ $\mu$ l
  3. Confirmed neutrophil count <1200/ $\mu$ l
  4. Confirmed AST and/or ALT > 5 x upper limit on normal range (ULN).One retest within 14 days is allowed.
- Receipt of any immune modulator or suppressor within 30 days prior study entry, including, but not limited to drugs such as corticosteroids (with the exception of corticosteroids used for topical use), granulocyte-macrophage colony-stimulating factor, interleukin (IL)-2, IL-7 and IL-15.
- Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.
- Participation in other interventional studies involving an investigational drug.

### **4.3 Study feasibility and sample size**

In 2012, the number of HIV-infected patients in medical follow-up was estimated at 13,352 for Belgium and 5,241 for Flanders.

A thorough review of the research databases and clinical files of the four participating centers was conducted in order to estimate the sample size of the study population and the feasibility of the study.

#### **Step 1: Number of HIV-1 positive patients actively followed up in the participating centers.**

At the end of 2013 a total of 7,212 HIV-1 positive patients were actively followed up in the four participating centers (ITG 2,447; UZG 1,225; VUB 650 and UMC St-Pieter 2,890). A patient in active follow-up was defined as having received at least one consultation and viral load measurement during the year 2013.

#### **Step 2: Number of patients currently treated with cART**

The number of HIV-1 positive patients treated with cART within the research consortium was 6,320 in 2013 (ITG 2,118, UZG 1,027, VUB 5,45, UMC St-Pieter 2,630).

#### **Step 3: Inclusion and exclusion criteria**

According to the above described inclusion and exclusion criteria, a total of 643 potential participants can be selected for trial participation and measurement of viral reservoir during the screening period of 9 months (ITG 228, UZG 125, VUB 64, UMC St Pieter 226). This corresponds to approximately 10% of the total number of patients currently being under anti-retroviral treatment.

#### **Step 4: Proportion of participants that will qualify for a treatment interruption.**

The proportion of screened patient who may qualify for treatment interruption is based on preliminary research data generated in UZ Gent. It is evaluated at 7% of the screened patients corresponding to 45 potential patients to be included in the trial.

#### **Step 5: Proportion of patients that will accept to undergo a treatment interruption.**

Although many patients have manifested their interest in participating into HIV trials, a

conservative and rational drop-out rate of 30% has to be taken into account in this project. Not all participants will be eager to interrupt a longstanding and most probably well supported treatment. On the other hand, the participants at this stage will be very motivated persons as they already underwent a stringent selection procedure (among others interview with psychologist) and heavy procedure (leukapheresis). The estimated number of participants would therefore be reduced to 32.

The proportion of post-treatment controllers is conservatively estimated at 5 to 15%, i.e. 2 to 5 out of the 32 study participants. (Of note this estimate is derived from data of PTC that were not selected based on a low HIV reservoir in contrast to the present study).

#### **4.4 Randomization**

There will be no randomization as this is a single arm study.

#### **4.5 Withdrawal and termination of the study**

In accordance with the Declaration of Helsinki, ICH Good Clinical Practice Guidelines and the Belgian Law of 2004, a participant has the right to withdraw from the study at any time for any reason without prejudice to his/her future medical care by the physician at the institution. The Investigator also has the right to withdraw patients from the study if one or more of the following events occur:

- Consent withdrawal,
- Significant protocol violation or noncompliance on the part of the participant
- Refusal of the participant to continue observations
- Unacceptable adverse events (AEs)
- Unrelated serious medical illness or complication
- Criteria for cART re-initiation met based on viral rebound (see section 5.1.5 below),
- Pregnancy
- Decision by the Investigator that termination is in the patient's best medical interest. This includes, but is not limited to, new clinical events, even if not related to the study procedure, ongoing risk of HIV transmission (e.g. new sexually transmitted infections) and unprotected sexual contact with seronegative partner(s).
- Lost to follow-up, as defined further in this section.

##### *Handling of Withdrawals*

A complete final evaluation should be made at the time of the patient's withdrawal. The Study Status Outcome form in the case report form should be completed with an explanation of why the patient is withdrawing. An attempt should be made to perform a follow-up evaluation.

Participants withdrawn from study will continue to receive standard of care for their condition. This includes, if needed, timely re-initiation of the best possible antiretroviral treatment as well as care of any adverse event or complication, whether related or not to the study procedures. The Principal Investigator (PI) will assure this standard of care is provided and (s)he will discuss specific cases when needed with the Coordinating Investigator and other PIs.

Participants will be considered lost to follow-up at study closure if (s)he discontinued study visits without informing the study staff and could not be traced. Participants informing the study staff about their withdrawal from trial will be considered as early withdrawals.

Participants discontinuing study visits without informing the study staff during but presenting themselves again after a few months, will be re-scheduled according to the study protocol with documentation of the reason(s) for temporarily discontinuation of the study.

A participant has the right to withdraw his/her consent at any time and to ask for retrospective withdrawal of all personal data/samples related to the trial. These participants will be considered consent withdrawals.

#### *Termination of Study*

The study may be prematurely closed or interrupted by the sponsor in case of futility or adverse health outcomes for the study participants. The decision to interrupt the study will be taken by the study steering committee after consultation of the advisory board.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the concerned investigators/institutions, IRBs and IECs about the termination or suspension of the study and the reason(s) justifying this decision, as specified by the applicable regulatory requirement(s). Finally, the funding organisms will be alerted of the study termination.

## **5. STUDY PROCEDURES**

Before including participants into the trial, a written Institutional Review Board and Ethics Committee approval of the protocol and informed consent form will be obtained.

### **5.1 Study/visit schedule**

The study schedules are summarized below. A window of +/- 7 days will be allowed around the planned study visit.

#### **5.1.1 FIRST ELIGIBILITY SCREENING AND INCLUSION IN THE FIRST PART OF THE STUDY (RESERVOIR MEASUREMENT STUDY)**

Study participants will be selected from the four participating clinical centers. Screening evaluations to determine eligibility must be completed within 7 days prior to study entry.

The patient will be invited to participate in the trial and provided information about the aims and risks of the project. After obtaining written informed consent (first written informed consent), clinical eligibility will be assessed. Patients will be eligible for enrollment if they fulfill all of the inclusion criteria described in section 4.2. Patients will not be eligible for the study if they meet any of the above described exclusion criteria.

3x9ml blood will be drawn in order to evaluate the magnitude of the viral reservoir. This sample will be sent to UZ Gent for quantification of total HIV DNA and unspliced cell-associated RNA. More information on the method used is to be found in section 5.3 of the protocol.

The following will be assessed at this visit:

- Assessment of subject eligibility according to the inclusion and exclusion criteria
- Demographic and epidemiological data
- Medical history (past and current), including HIV-associated conditions
- Full antiretroviral history (including result of resistance assays if available)
- Review of non-antiretroviral medication within the last 30 days
- Physical examination including vital parameters, height and weight
- HIV viral load and CD4 T cell count.



- Laboratory test (non-fasting): hemoglobin, absolute neutrophil count (ANC), platelets, creatinine, liver enzymes AST & ALT. Hepatitis HCV & HBV serology if necessary.
- Pregnancy test among women.

### **5.1.2 PSYCHOLOGICAL INTERVIEW**

Before enrolment in the second part of the study, an appointment with the psychologist of the Aids Reference Centre (ARC) will be scheduled, for the selected participants, in order to assess the patient's motivation for interrupting antiretroviral treatment. If in the opinion of the psychologist a patient is not suitable for further inclusion, this will be reported in the participant's file (source document) and to the local investigator. Both psychologist and investigator will decide whether the concerned participant will be included or not. Participants will be offered facultative follow-up at no cost in the form of additional consultation with the psychologist of the ARC during or after the course of the study. The psychologists will be provided with a "Guide for the Interview" (see Annex), in order to ensure that the same elements are consistently considered and addressed across patients and across sites.

The psychological interview will be digitally audiotaped if the participant agrees. To this end, the psychologist will ask the participant whether he/she would agree with the audiotaping of the interview. The participant will be informed that he/she may interrupt or rewind the tape at any time. If the patient agree, the psychologist will start taping by stating his/her name, the starting time and the date. Then he/she will proceed with stating that this record concerns the audiotape of the psychological interview of participant [Isala study code]. In this way there will be no personal identifier recorded. The psychologist will end the tape by recording "end of recording of the psychological interview for participant [Isala study code] and the end time. If the participant deny audiotaping, the psychologist will tape the following text: "name of the psychologist, hour and date, "no record for [Isala study code], end of tape". This will serve as a record that interview took place.

Each Interview will be saved as audio file with as name "Isala\_PsychologicalInterview\_ISALASTUDYCODE\_ddmmyyyy.fileextension". The audio file will be kept in a content protected folder dedicated to the Isala study on a secured research server at each site. The Isala folder will only be available for the local study team. The audio files will be deleted at the end of the study.

### **5.1.3 SECOND SCREENING AND ENROLMENT IN THE SECOND PART OF THE STUDY (TREATMENT INTERRUPTION STUDY)**

Study participants meeting the criteria for TI will be contacted preferably within one to three months after the first screening visit. Treatment interruption will be proposed to these participants with the lowest viral reservoir and consenting with the second phase of the study (second written informed consent). The criteria allowing treatment interruption are:

- Undetectable level of total HIV DNA (i.e. < 66 copies/ 10<sup>6</sup> PBMC).
- Undetectable level of unspliced cell-associated RNA (i.e. <10 copies/10<sup>6</sup> PBMC).

Those criteria are based on previous research from the HIV Translational Research Group in UZ Gent [40].

### **5.1.4 Leukapheresis**

A leukapheresis will be scheduled between the enrolment visit and the baseline visit.

The necessary sample will be transferred to the Virology Laboratory of ITM in order to perform a Viral Outgrowth Assay (VOA) at later time. More information on the VOA is to be found in section 5.3 of the protocol.

When patients consent, all remaining samples will be kept locally at each site according to the procedure for the sample storage described in the laboratory analytical plan. These samples will be used later for sub studies as described in relevant amendments and in section 2.2 of this protocol.

If a site doesn't have the capacity to store samples locally, these may be transferred to the FAGG accredited biobank of BIMETRA at the UZ Gent. Samples will be kept for a maximum of twenty years after study completion. Remaining samples will be destroyed at the end of the storage period.

**TABLE 1:FLOW CHART SCREENING PROCEDURES**

Procedure	Screening 1	Screening 2	Leukapheresis	comments
<b>Eligibility</b>				
Informed Consent 1	X			
Informed Consent 2		X		
Incl./Excl. Criteria	X	X		
Medical History	X			
ARV history	X			
Plasma Viral load	X			
Resistance Test	X			
CD4-cell count	X			
Concom.medications	X			
Psych. Interview		X		
<b>Phys. Examination</b>	X			Full PE, incl. vital signs
<b>Laboratory Tests</b>				
Hemoglobin	X			
ANC	X			
Platelets	X			
Ureum/Creatinin	X			
AST	X			
ALT	X			
HBs Ab	X			
HBc Ab	X			
HBs Ag	X			
HCv Ab	X			
Pregnancy Test	X			
<b>Reservoir Assessment</b>				
Tot. HIV DNA	X			
Us RNA	X			if tot hiv DNA negative
VOA			X	
<b>Sample storage</b>	X		X	

### 5.1.5 BASELINE VISIT

Upon successful completion of all screening activities and second written informed consent, patients will be invited for a baseline visit, preferably within four months of the first screening visit.

The following will be assessed:

- Control of the inclusion and exclusion criteria
- Notification of concomitant medication use
- Medical history check
- Targeted physical examination including vital parameters measurement
- HIV viral load and CD4 T cell count
- Laboratory test (non-fasting): hemoglobin, ANC, platelets, creatinine, AST & ALT
- 12 lead ECG
- Pregnancy test for women
- Proviral DNA and cell-associated RNA measurement

Participants will then be asked to interrupt their treatment.

In general, the participants will be advised to stop their treatment all at once. In case the participant is taking an antiretroviral treatment containing a non-nucleoside analogue a “tailing” of the nucleoside analogues will be organized. Participants will take another cART during 7 days to avoid the risk of developing resistance to non-nucleoside analogues known to have a very long half-life. The choice of cART will be left at the discretion of the investigator but will have to comply with existing guidelines on HIV treatment.

**TABLE 2: FLOW CHART ON-STUDY PROCEDURES**

Procedures	Bl	W2	W4	W6	W8	W10	W12	W16	W20	W24	W32	W40	W48
<b>Eligibility</b>													
Incl./Excl. Criteria	X												
<b>Safety</b>													
Targeted Phys Exam.	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X
Concom. Medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X												
SAE assesement		X	X	X	X	X	X	X	X	X	X	X	X
AE assesement		X	X	X	X	X	X	X	X	X	X	X	X
ECG, 12 leads	X												
<b>Laboratory Tests</b>													
Plasma Viral Load	X	X	X	X	X	X	X	X	X	X	X	X	X
CD4 Cell count	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemoglobin	X		X				X		X		X		X
ANC	X		X				X		X		X		X
Platelet	X		X				X		X		X		X
Ureum/Creatinin	X		X				X		X		X		X
AST/ALT	X		X				X		X		X		X
Pregnancy test	X												X
<b>Reservoir Assessment</b>													
Tot. HIV DNA	X	X	X	X	X	X	X	X	X	X	X	X	X <sup>1</sup>
Us RNA	X	X	X	X	X	X	X	X	X	X	X	X	X <sup>1</sup>

Proviral DNA	X	X	X	X	X	X	X	X	X	X	X	X	X <sup>1</sup>
Sample Storage	X	X	X	X	X	X	X	X	X	X	X	X	X <sup>1</sup>

1. at Week 48 for post-treatment controllers. For pts with viral rebound, Us RNA will be performed on the last frozen sample before viral rebound.

**5.1.6 STUDY VISITS (48 WEEKS)**

Following treatment interruption, study visits will be planned at week 2, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 48.

The evaluations performed at each study visit are detailed in the table 2 above. Beside the described information thorough counselling on the risk of transmitting HIV will be provided at each study visit.

At each time point PBMC from an extra sample (Three EDTA tubes of 9ml) will be stored according to the sample storage procedure. These samples will be used to measure the dynamic of usRNA during and after treatment interruption. The last study visit (either at week 48 or at viral rebound) will coincide with the first visit of the post-intervention phase.

**5.1.7 STUDY DISCONTINUATION**

The study will be discontinued and cART will be restarted whenever:

- A pVL increase above 1,000 copies/ml at two consecutive time points during ATI, at least 3 days apart
- A single viral load increase > 10.000 copies
- CD4<sup>+</sup> T-cell count drops below 350/µl at two consecutive measurements at least 2 weeks apart or a drop of more than 50% compared to baseline
- Any new significant clinical condition (linked to HIV or not) is documented
- A new sexually transmitted infection is diagnosed
- A woman would become pregnant despite the recommended anti-conception
- A participant decides to withdraw from trial

As soon as such event is noticed, the participant will be contacted urgently by phone to come back earlier in order to perform an End Of Study visit and restart cART without delay. If a participant was in mono-or bitherapy before treatment interruption, it will be strongly advised to restart with tritherapy upon viral rebound.

**5.1.8 POST-INTERVENTION PHASE (FROM WEEK 48 OR UPON VIRAL REBOUND FOR A DURATION OF 12 WEEKS).**

The post-intervention phase will start either at week 48 for the post-treatment controllers (undetectable viral load without treatment) or at time of cART re-initiation for the patients with viral rebound. During this period of 12 weeks, additional evaluation of the viral reservoir will be performed as well as an assessment of the efficacy of the cART restarted among participant with viral rebound.

The assessments performed during the post-intervention phase are resumed in the flowchart below.

**Table 3: Flowchart of the post-intervention phase**

Procedure	EOS*	W4	W12	Comments
<b>Safety</b>				
Targeted Phys Exam.	X	X	X	
Vital Signs	X	X	X	
Concom. Medications	X	X	X	
SAE assesement	X	X	X	
AE assesement	X	X	X	
cART history	X	X	X	
<b>Laboratory Tests</b>				
Plasma Viral Load	X	X	X	
CD4 Cell count	X		X	
Resistance Test	X	(X)	(X)	(if indicated)
Hemoglobin	X	(X)	(X)	(if indicated)
ANC	X	(X)	(X)	(if indicated)
Platelet	X	(X)	(X)	(if indicated)
Ureum/Creatinin	X	(X)	(X)	(if indicated)
AST/ALT	X	(X)	(X)	(if indicated)
Pregnancy test	X		X	
<b>Reservoir Assessment</b>				
Tot. HIV DNA	X		X	
Us RNA	X		X	
Proviral DNA	X		X	
Sample storage	X		X	

EOS: end Of Study

### 5.1.9 POST-STUDY PERIOD

For study participants with sustained viral suppression , the maximal duration of the clinical trial will be 60 weeks post TI . Thereafter, they will be monitored conform the standard of care (i.e. every 3-4 months) and will come back within regular medical care. Participants will continue to be sensitized by their treating physician about the risk of new HIV infection as well as onwards HIV transmission in case of viral rebound. The study team will review the clinical chart of these PTC participants every three to four months in order to advise the treating physician if necessary. cART will be restarted whenever any of the criteria mentioned in chapter 5.1.5 is met.

### 5.1.10 UNSCHEDULED VISITS

At any time during trial, participants and study physician may request interim contacts or unscheduled visits whenever deemed necessary. All unscheduled visits will be documented in the participant's clinical record and CRF.

### 5.1.11 MISSED VISITS

At study entry, clinic staff will obtain contact information of all participants. Whenever a participant would miss a scheduled appointment, study staff will try to establish

communication, with insurance of confidentiality. All attempts to contact participants will be documented in the clinical record.

The importance of maximal adherence to the scheduled visits will be emphasized at every visit.

## **5.2 OBTAINING INFORMED CONSENT**

Patients invited to participate into the trial will be offered to enter or not the trial after receiving the information about the aims and risks of the project. At any times, a participant has the right to withdraw from the study for any reason without prejudice to his/her future medical care by the physician or at the institution.

Inclusion will occur step-wise. The first inclusion step (part I of the study) allows to verify the inclusion and exclusion criteria and to measure the viral reservoir among selected participants. During the second inclusion step (part II of the study), a treatment interruption will be proposed to participants with the lowest viral reservoir. Both inclusion steps will be preceded by an informed consent interview during which potential participants will be given oral and written comprehensive information about the purpose and procedures of the trial, the potential risks and benefits of participation to the trial, the notion of voluntary participation, etc. This will be repeated whenever considered necessary by the patient or study team. Patients eligible for the trial will have no time restraint for deciding whether they want to participate to the trial. In addition to an informed consent interview, the second inclusion step will be preceded by a structured interview conducted by a psychologist at the local ARC. The purpose of this interview will be to document the motivation of the participant and his/her good understanding of the concept of HIV cure and the potential risks and benefits of treatment interruption strategies. During both informed consent procedures participants will be evaluated for their ability and willingness to have blood samples collected, stored and used for various substudies related to this project.

The Informed Consent Form (ICF) documents will be designed in accordance with the requirements of the Helsinki Declaration (2013), the E6 ICH GCP Guidelines (1996) and the Belgian Law on Experiment on the Human Person (2004). The interviews will be conducted either in Dutch, French or English, according to the participant's preference. Upon agreement on participation, the consent form will be signed in two copies, namely by the participant and by the person administering the consent. The participant will receive one copy of the ICF, while the other copy will remain in the Investigator file.

## **5.3 SPECIFIC PROCEDURES AND ACTIVITIES**

To assess the size, dynamics and transcriptional activity of the viral reservoir, three viral markers will be measured on PBMC, i.e. total HIV DNA, integrated proviral HIV DNA and unspliced cell associated HIV RNA (usRNA), respectively. This work will be performed at the HIV Translational Research Group in UZ Gent. The decision to interrupt treatment and move forward with the study will be based on the results of these assays (cf first and second strategic goals in rationale above).

The fourth marker, based on a viral outgrowth assay (VOA) will measure the amount of virus that is replication competent upon *in vitro* stimulation. This activity will be performed at the Virology Laboratory at ITG. The result of the latter test will not be used to prospectively propose treatment interruption, but will retrospectively be evaluated as a potential predictive factor for functional cure (cf third strategic goal in rationale above)

Total HIV DNA and HIV usRNA will be quantified using the droplet digital PCR (QX100, Bio-Rad). This digital PCR platform was recently implemented at Ghent University and outperforms standard quantitative PCRs in terms of quantitative accuracy of low abundant templates [41,42]. Total DNA, isolated from peripheral mononuclear blood cells (PBMCs) will be *EcoRI* digested to ensure a random distribution of DNA in the picoliter droplets. Crude digest will be added to the ddPCR master mix, containing PCR mix as well as a probe and primers specific for HIV[42]. After droplet generation, PCR will be performed. The fluorescence of the droplets will be assessed with the droplet reader in order to quantify the template HIV DNA using Poisson statistics[43]. By performing an additional ddPCR for the ribonuclease P subunit 30 (RPP30) gene to quantify the number of cellular genomes in each sample, normalization to HIV DNA copies/million PBMCs will be obtained. Patients. Participants with <66 copies total HIV DNA/10<sup>6</sup> PBMC's will be considered as having a low reservoir[44].

For HIV usRNA quantification, the mRNA isolated from PBMCs will be reverse transcribed to cDNA. Subsequently ddPCR will be performed with specific sequences designed in the Gag-Pol region of the HIV sequence[41]. The cDNA copy numbers will first be normalized to RNA copy numbers by assessing the transcription efficiency of the RT reaction using a validated RNA standard. Subsequently, normalization to sample input will be performed by using multiple pre-validated reference genes as described earlier[45].

Integrated proviral HIV DNA will be assessed using a digital PCR based Alu-HIV PCR that we recently optimized at Ghent University and that proved to be a more optimal quantitative tool compared to alternative integrated copy PCR assays[46]. For this method, total DNA derived from PBMCs will be isolated and distributed over 70 replicate PCR reactions, 40 containing Alu-HIV primers for logarithmic amplification of integrated HIV DNA and 30 containing only an HIV primer for linear amplification of all HIV DNAs. In the subsequent nested HIV specific quantitative PCR Alu-HIV replicates with higher fluorescence than the background signal will be counted as positive. The proportion of positive to negative replicates will be assessed by Poisson statistics to obtain absolute quantities of integrated proviral DNA[46]. Normalization to proviral HIV DNA copy numbers/million PBMCs will be performed in analogy with total HIV DNA normalization, as described above.

The viral outgrowth assay will be performed according to Laird et al[47]. Briefly, resting CD4<sup>+</sup> T cells are isolated from the mononuclear fraction of the participants' blood taken at baseline. A triplicate 5-fold dilution series of those cells, starting at 10 million, is first fully activated with irradiated PHA-stimulated healthy donor PBMCs and IL-2 to maximally break latency. Afterwards fresh healthy donor CD4<sup>+</sup> T blasts are added weekly to expand any induce HIV replication/transcription. Viral outgrowth is evaluated by measuring HIV p24 antigen in the supernatant. Negative results at the highest cell concentration will be confirmed by RT-PCR.

#### **5.4 Laboratory procedures**

All analysis of clinical trial samples will be carried out in compliance with Good Clinical Laboratory Practice (GCLP). A laboratory analytical plan will be made available to all investigators before study start in order to harmonize procedures across participating centers. Beside prioritizing sub-studies, the laboratory committee will take the lead on administering the laboratory procedures and drafting the laboratory analytical plan.

## **6. STUDY INVESTIGATIONAL PRODUCT**

No investigational product will be assessed during this study. The intervention consist of interrupting antiretroviral therapy in HIV positive participants identified to have low viral reservoir.

## **7. SAFETY ASSESSMENT**

### **7.1 Adverse events**

Safety of the intervention will be evaluated by recording Adverse Events (AE's) and grading laboratory and vital signs evaluations.

#### *Definition of an Adverse Event*

An Adverse Event (AE) is generally described as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

However, this definition does not entirely fit the purpose of this study, because we will not assess a pharmaceutical product. Therefore, we will consider the intervention (=interruption antiviral treatment) as the pharmaceutical product under investigation and will adapt the definition accordingly:

An Adverse Event (AE) is generally described as any untoward medical occurrence in a subject or clinical investigation subject with treatment interruption and that does not necessarily have a causal relationship with the treatment interruption. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the treatment interruption, whether or not related to the treatment interruption.

Once treatment interruption has started, until the end of study, clinic staff will enquire about common possible side effects based on the “division of AIDS table for grading the severity of adverse events (see annex)” and will ascertain the occurrence of any adverse events since the last visit. All of them, including clinically significant laboratory abnormalities, will be recorded in the electronic Case Record Form (eCRF) and categorized in terms of severity, relationship to ARV interruption, outcome and seriousness, throughout the study duration. Episodes of viral acute syndrome, possible events of special interest, all serious adverse events (SAEs) and all the events leading to participants' withdrawal from the study will be monthly communicated to the Scientific Advisory Board.

All the events will be followed up till resolution, except those at the final end of study (=end of follow-up period).

#### *Severity, relationship of event to ARV interruption and outcome*

1. **Mild:** events require minimal or no treatment and do not interfere with the subject's daily activities.
2. **Moderate:** events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning.



3. **Severe:** events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events may be temporarily incapacitating.
4. **Life-threatening:** Subject at risk for death at the time of the event

#### Assessment of causality

The investigator will assess the relationship between the ARV interruption and the occurrence of each AE/SAE. A list of expected adverse event is given. In addition, the investigator will use clinical judgement to determine the possible relationship of other (not expected) events.

The relationship of an adverse event to ARV interruption is to be assessed according to the following definitions and can only be done by the study physician:

1. **Definitely unrelated:** Reserved for those events which cannot be even remotely related to ARV interruption (e.g. injury caused by a third party).
2. **Unlikely:** There is no reasonable temporal association between the ARV interruption and the AE, the AE is not expected (according to a pre-defined list) and the event could have been linked to other known reasons.
3. **Possible:** The suspected AE may or may not follow a reasonable temporal sequence from ARV interruption but It is not fully unexpected but it could be linked to other known reasons.
4. **Likely:** The suspected adverse event follows a reasonable temporal sequence from ARV interruption. Either it is expected (according to based on the "division of AIDS table for grading the severity of adverse events) or it is likely caused by ARV interruption in the investigator's judgment.
5. **Definitely related:** Reserved for those events which have no uncertainty in their relationship to ARVs interruption.

#### Outcome

The outcome of each AE must be assessed at the same visit and according to the following classification:

Completely recovered :	The participant has fully recovered with no observable residual effects
Not yet completely recovered :	Improvement in the participant's condition has occurred, but the participant still has some residual effects
Deterioration :	The participant's overall condition has worsened
Permanent damage :	The AE has resulted in a permanent impairment
Death :	The participant died due to the AE
Ongoing :	The AE has not resolved and remains the same as at onset
Unknown :	The outcome of the AE is not known because the participant did not return for follow-up (lost to follow-up)

#### Serious Adverse Events

A Serious Adverse Event (SAE) is any adverse event/experience that results in any of the following outcomes:

- Death;
- Life threatening (subject at immediate risk of death);
- Requires participant hospitalization or prolongation of existing hospitalization;
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant disability or incapacity;
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

### Fast-track reporting

All SAEs will be compiled by the clinic supervisor on the paper report form for the corresponding visit. The Investigator will systematically check and validate the paper records on SAE and other points, and correct / complete as required before entering the data in the electronic CRF. The SAE will be registered as soon as treatment has been interrupted and up to the end of the study.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to the pharmacovigilance. However, it is very important that the investigator always makes an assessment of causality for every event prior to transmission of the report to the pharmacovigilance. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE-report form accordingly.

All SAE's whether or not deemed related or expected must be reported immediately or within 24 hours (one working day) using the SAE Notification Form by telefax or email to:

**Clinical Trials Unit**  
**Institute of Tropical Medicine**  
**Nationalestraat 155**  
**B-2000 Antwerp – Belgium**  
**Fax: +32 3 247 66 47**  
**Email: [pharmacovigilance@itg.be](mailto:pharmacovigilance@itg.be)**

The title of the e-mail/fax should mention the name of the study code, the patient code and the wording "SAE – Initial/follow up report".

Line listings of all reported SAE's will be sent at a monthly base to the joint SAB/CAB, IRB and local ECs. Due to no investigational medicinal product, there was agreed upon no SUSAR reporting (ref. Kristof Bonnarens – FAGG).

## **7.2 Scientific Advisory Board and Community Advisory Board**

A merged scientific and community board will be composed of scientists with broad experience in the field of HIV clinical trials as well as members from patient organizations.

The main task of this board will be to advise the study steering committee on the onwards conduct of the study. The communication will preferably take place electronically.

Members will meet, preferably by means of teleconference, at least three times a year in order to be informed about the evolution of the study.

### **7.3 Independent Safety Monitor**

A Data Safety and Monitoring Boards is not mandatory for this type of project, where there is not a pharmacological ‘intervention’. However, to ensure an extra layer of independent supervision on safety issues, the Sponsor will appoint an independent data safety monitor, who is fully independent from the study team. He/she will receive and review all SAE reports on an ongoing basis. A Charter will be set up to describe the Safety Monitor role. The independent data safety monitor will be experienced in HIV medicine and clinical research. In case of major safety concerns, this independent safety data monitor may advise the sponsor to halt recruitment of the trial and/or request to organize a formal meeting with the TMG members with a complete overview of all available safety data.”

Every three months, the CTU will provide safety and efficacy reports to the steering committee, relevant IEC’s and members of the advisory board.

## **8. STATISTICAL METHODS**

The statistical analysis of the clinical trial will be performed according to a Statistical Analysis Plan which will be approved before database lock.

### **8.1 Study hypothesis**

A small proportion, estimated at 5 to 15%, of participants identified to have a low proviral DNA in PBMC while on long-term ART will have a sustained viral suppression 48 weeks after analytical treatment interruption.

### **8.2 Variables of interest**

The primary endpoint is the proportion of participants with undetectable viral load (<50 HIV RNA copies/ml plasma) 48 weeks after interruption of their antiretroviral treatment.

### **8.3 Statistical methods**

#### ***8.3.1 Population to be analyzed***

The statistical analyses will be performed on all enrolled participants.

#### ***8.3.2 Baseline characteristics***

The number of participants screened, enrolled and excluded will be presented. Excluded participants will be summarized by reason for exclusion. For the participants enrolled in the study, the number discontinued or lost to follow-up will be tabulated by reason. This information will be summarized in a CONSORT flow diagram. Participants will be described with respect to baseline characteristics. The description will be done in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical characteristics.

#### ***8.3.3 Primary objective/endpoint***

The proportion of participants with undetectable viral load (<50 HIV RNA copies/ml plasma) 48 weeks after interruption of ART will be estimated together with a 95% Wilson confidence interval.

#### **8.3.4 Secondary endpoints**

Safety of interrupting ART will be evaluated based on AEs and SAEs percentages together with a 95% Wilson confidence interval.

Reservoir replenishment and HIV viral load kinetics following ART interruption will be summarized in terms of medians and interquartile ranges. If present, pVL rebound will be represented graphically with a spaghetti plot.

Potential predictors of post-treatment control will be assessed by Fisher's exact test at 5% significance level.

#### **8.3.5 Subgroup analyses**

No subgroup analyses will be performed.

#### **8.3.6 Multiplicity and Missing Data**

Complete-case analyses will be performed.

### **8.4 Sample size and power**

It is estimated (section 4.3) that from all patients screened, 32 patients will finally enter the study and stop ART. Assuming a proportion of post-treatment control ranging between 5% and 15%, the width of the 95% Wilson confidence interval will be between 18 and 25 percentage points.

## **9. MONITORING AND QUALITY ASSURANCE**

This study will be monitored according to the Monitoring SOP of the ITM Clinical Trials Unit, as agreed with the Sponsor. The PIs and research staff of the involved sites will allocate adequate time and resources for such monitoring activities. The investigators will also ensure that the monitor is given access to all the above noted study-related documents, the Case Report Forms and study-related facilities (e.g. diagnostic laboratory, etc.) and has adequate space, time and resources to conduct the monitoring.

The Principal Investigator at each site declares, by signing this protocol, that he/she will permit trial-related monitoring, audits either conducted by the Sponsor or externally requested, Independent Ethic Committee review and regulatory inspections providing direct access to source data/documents, relevant electronic systems and trial facilities.

The Principal Investigators and co-Investigators agree to conduct the present study in full agreement with the Declaration of Helsinki (October 2013)(see Appendix I) and the ICH Good Clinical Practice guidelines (1996).

## **10. DATA MANAGEMENT**

Data Management will be performed by the data manager at the CTU (ITM) and in collaboration with the study staff assigned for data management at the 4 sites (PIs, data entry clerks).

### **10.1. Confidentiality and security of trial participant data**

Private information on trial participants will be handled confidential (see also section 11.5). Information such as the participant name or any other data which could lead to the identification of the participant will not be included on the electronic Case Report Forms (eCRFs), nor on any other paper documents or electronic files used for data management. Name and contact data for each participant will be kept separate and limited to the clinical staff at the respective sites only.

All paper documents and electronic files needed for data management will be restricted to authorized study staff, both at the CTU and at the sites. The study computers and eCRFs are only accessible via a LogIn with personal username and password. A list of authorized users of the eCRFs (and database) will be kept at the CTU and updated during the study.

### **10.2. Data Collection**

For each trial participant, a file is provided at the sites to store all original or source data for that person. Only data defined by the study protocol will be collected.

### **10.3. Data entry**

The collected source data will be recorded via electronic Case Report Forms (eCRFs) into the clinical trial database, using MACRO. MACRO is a clinical trials management software for electronic data capture (EDC) and clinical data management (CDM). MACRO supports ICH-GCP quality standards and guidelines and requirements such as CFR 21 part 11 (e.g. electronic signature, electronic audit trail). The software features amongst others an integrated CRF tracking and query management system and capabilities for importing and exporting data in various forms.

The eCRFs and database will be designed by the CTU data manager. Edit checks, programmed onto the eCRFs will validate data at the point of data entry. Extensive system validation of the eCRFs will be done according to the CTU's internal procedures, in particular on the edit checks. It is only after final approval of the testing phase and the Validation Report that the actual data entry may start.

Trial participants will be identified by a study specific participant code and initials.

Data entry and subsequent data handling will be done at each of the 4 sites by trained study staff.

The database will consist of data from all screened participants.

### **10.4. Data quality and checking**

Ensuring data quality and data integrity is an essential task throughout the trial, with the CTU data manager in a coordinating role. Good practices during the design of the eCRFs, such as use of coding, check boxes, question input masks and drop down menus will enhance the data quality. Aside the automatic edit checks, also manual checks will be performed to identify out of range data, missing data and inconsistent data.

A system will be put in place to ensure timely data entry, data checking, resolving of queries and lastly database lock. The CTU data manager and the concerned study staff will regularly hold conference calls on Data Management throughout the study.

### **10.5. Records keeping and Archiving (see also section 13)**

Electronic documents for purpose of data management, including txt and pdf formats, Excel files, Access databases, mdb and ldf data files, eCRF generated files (especially study designs), programmes and emails are stored and organized on an access controlled study

specific folder on the ITM server, in particular in a specific data management subfolder. Paper documents for purpose of data management, such as mail prints, data listings, data management documentation will be organized at the CTU in specific study binders.

Specific details on the minimum retention time for paper documents and electronic files, for CTU and sites, can be found in section 13.

## **11. ETHICAL ISSUES**

The study will be carried out according to the principles stated in the Declaration of Helsinki 2013 (see annex 2), the applicable Belgian law on experiments in human subjects (2004) and according to ICH-GCP guidelines (1996).

### **11.1 Specific ethical aspects related to this study**

By interrupting ART, this project introduces a clear change in treatment paradigm of HIV-infected patients. In fact, large-scale controlled studies such as the SMART trial [15], previously showed that repeated TI until CD4<sup>+</sup> T cells dropped to < 250/μL were associated with increased morbidity and mortality due to opportunistic infections as well as cardiovascular, hepatic and renal diseases. Inflammatory and coagulation markers during STI, including IL-6 and D-dimers, were predictive of mortality [48]. Treatment interruption strategies are therefore no longer recommended in routine care. However, based on a re-analysis of subset data from SMART, Routy *et al.* concluded that analytical treatment interruption in the context of clinical trials can safely be done, provided that patients are carefully selected (i.e. high nadir and actual CD4<sup>+</sup> T-cell counts) and that ART is restarted upon viral rebound [30]. Other research groups have demonstrated that although most, if not all patients with high nadir and actual CD4<sup>+</sup> T-cell counts interrupting ART showed a rebound in pVL, the incidence of serious adverse events was very low. Moreover, viral load returned to an undetectable level upon restart of the ARV treatment[49] (Ruxandra Calin personal communication on the Ultrastop trial in France).

Based on literature data and on the inclusion/exclusion criteria that will be used in this trial, we believe the risk of AIDS and non-AIDS defining events in patients interrupting ART in this project will be low. Moreover, strict criteria on lymphocyte T-cell count will be used regarding treatment re-initiation.

A matter of concern is the risk of developing an episode of acute HIV seroconversion syndrome upon interruption of ART. Acute seroconversion has not been described in the 14 patients included in the Visconti trial [13]. On the contrary, in another research project in Boston two patients who were declared functionally cured after receiving a bone marrow transplantation developed clinical signs respectively three and eight months after treatment interruption[49]. However, following cART resumption, complaints progressively disappeared along with the decrease of the pVL back to undetectable levels.

In this project, it is expected that viral rebound will appear in a majority of the included participants and some of them will probably suffer from mild to moderate clinical signs. The clinical complaints are most likely to disappear once the treatment will be restarted. In this highly selected group, there is no evidence to suspect that the treatment will not function correctly when restarted. The two Boston cases also teach us that the follow-up of the patients who interrupted treatment will have to be very strict and intensive, as described in the present protocol. Clear procedures with regard to follow-up or reinstatement of antiretroviral treatment have also been defined for the post-study period.

Before inclusion in the trial, potential study participants will be counseled thoroughly about the pros and cons of treatment interruption.

A last important ethical conundrum lays in the false perception that potential participants may have with regards to this project. Participants may overestimate the very theoretical advantage of being cured of HIV and underestimate the risk of adverse events. Therefore, much effort will be put to inform potential participants correctly and to perform a well-balanced informed consent procedure. Participants will need to be aware that the potential risks related to the study (namely viral rebound post TI, whether or not accompanied by clinical symptoms) by far outweigh the chances of being functionally cured from HIV.

According to an ethical framework in clinical research[50], seven requirements need to be considered and fulfilled during trial conductance:

1. Social and scientific value.

This project will promote the field of HIV cure research. Results from this study will have an important translation in patient care.

2. Scientific validity

This study uses state of the art techniques developed in the different laboratories of the research consortium. Several proofs-of-concept about post treatment control are available, both within and outside the research consortium. Statistical power is important for the feasibility of this study. Sample size calculations have been performed according to the best estimate available in each participating center. We therefore think that this project serve a legitimate scientific purpose..

3. Fair subject selection

Most of the partners involved in HIV care in Flanders are involved in order to propose trial participation to a high number of eligible patients. The selection procedure is clearly described with objective criteria and should therefore take place in fair manner.

4. Favorable risk-benefit ratio

As argued above, at individual level, the potential risks related to trial participation outweigh the potential benefits. However, potential risks in the long term will be minimal as viral load will most likely return to pre-interruption levels following re-initiation of cART. At study population level, this trial will undoubtedly set interesting landmarks in the path of HIV cure research and give interesting insights in the understanding of HIV reservoirs. During the first six months of the study an estimated amount of 360 ml blood will be collected from the participants both to assess the efficacy and safety endpoints and to allow some substudies. This remains within acceptable limits.

5. Independent review

The present project is subjected to ethical approval in each of the participating centers.

6. Informed consent

As stated above, the redaction of the informed consent and conduct of the informed consent discussion will be crucial for the understanding of the study. The study aims, methods, risks and benefits will be described very clearly in the informed consent forms. These forms will be available in various languages: English, Dutch and French. The alternative to the study (continuing ART) will be clearly mentioned. A two-step informed consent procedure has been developed to allow patients to first obtain a measurement of their viral reservoir and to make an independent and well-informed decision later on about whether they agree to interrupt antiviral treatment or not.

7. Respect for potential and included subjects

As in any other research project the participant's privacy is protected as much as possible, withdrawing from the study will be possible at any time and participants will be informed of the results during and after the end of the trial.

### **11.2 Ethical and regulatory review**

This clinical trial will be submitted for formal review and approval to the Institutional Review Board of the ITM, the Independent Ethics Committee (IEC) of the University Hospital of Antwerp which will act as the Ethics Committee providing the single opinion, and the IECs of UZ Gent, UZ Brussel & Hospital Saint-Pierre. No study-specific subject related activities will be performed before formal approval from all bodies is obtained.

No formal approval from the Belgian's Regulatory Authority is required (written communication in annex).

### **11.3 Protocol amendments**

Once the final clinical study protocol has been issued and signed by the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the appropriate steps before being implemented. Any substantial change must be approved by all the bodies and IEC's that have approved the initial protocol, prior to being implemented, unless it is due to participant's safety concerns (in which case the immediate implementation can be necessary for the sake of the participant's protection).

### **11.4 Informed consent**

No subject may be included into the study until the Investigator or designee has obtained the written informed consent form from the participant. All participants will be asked to give their informed consent to participate in the study, before undergoing any study-specific procedures. More details are given in 5.2.

### **11.5 Confidentiality**

All the participant data will be coded in the CRF and database by means of a unique subject assigned participant study number. The documents which can identify the participants, e.g. the ICF's, laboratory print-outs and medical record, will only be accessible to the relevant study staff, study monitors, auditors and inspectors under confidentiality agreements.

The Sponsor provides all study documents to the Investigators and his/her appointed staff in confidence. Materials may not be disclosed to any party not involved in the study, unless written permission from the Sponsor.

### **11.6 Risks and benefits**

The personal benefit of participating in this study is very hypothetical. There is little chance that a participant will be cured of HIV upon completion of the trial. The benefit for the HIV-infected population of should not be underestimated as the findings of this study will deliver important insights in the viral reservoir measurements and dynamics. This may lead to improvement in clinical care in the future. As previously written the study findings will allow to improve the research on HIV cure.

The personal risks of participating in this project are well known and are related to the viral rebound that will happen in most participants short of late after interruption of ART. This rebound may be associated with clinical signs comparable to those seen in acute HIV infection. There is also a higher risk of transmitting the virus either through sexual intercourse



or during a pregnancy when the viral load increase. Evidence from published literature show however that the plasma viral load will decrease once the treatment is restarted in this highly selected group.

### **11.7 Compensation for participation**

There will be no compensation for study participation.

### **11.8 Insurance**

The Sponsor has taken a no-fault liability insurance agreement by AMLIN Europe, to cover for any harms, losses or damages to study participants which may be caused directly or indirectly by their participation in this trial.

## **12 DISSEMINATION OF RESULTS, INTELLECTUAL PROPERTY**

The Sponsor provides all study documents to the Investigators and his/her appointed staff in confidence. Materials may not be disclosed to any party not directly involved in the study, unless written permission from the Sponsor.

An intellectual property (IP) Board will be created upon start of the study. It will be composed of one person from each participating centers. This body will monitor the IP property and patenting issues. They will produce ad hoc reports to the Sponsor and the study steering committee.

## **13. ARCHIVING**

The relevant study documents are those permitting individual and collective assessment of the trial conduct, the quality of data produced and the compliance with GCP standards and applicable regulatory requirements. The PI's file on site should at least contain all the (essential) documents as listed in the sponsor's procedure "Set up and maintenance of the Investigator Trial File". A copy of all source data and Case Report Forms must be kept at each site at all time.

The sites' PIs are ensuring a secure and appropriate location for his Investigator's File and any other trial related documentation present at site. They also ensure that only site staff competent and delegated to work for the trial have access to the files.

All the relevant study documentation (including the investigator file) present at all partners involved should be retained and will remain available for audits and inspections for a minimum of twenty (20) years after completion of the study, as set out by the current Belgian law. The Sponsor will be informed prior to destruction of the files.

## **14. STUDY ORGANIZATION**

The following bodies will oversee the general organization of the study (see study organigram in attachment):

1. Steering committee
2. Laboratory committee
3. IP board
4. Advisory board
5. Clinical Trial Unit of ITM
6. Coordinating Investigator
7. Independent safety monitor

The steering committee is composed of the four investigators. Its main task will be to overview the general management of the trial and will delegate tasks to the various bodies as described in the study organigram. The steering committee will review the safety and efficacy study endpoints and will report to the relevant Ethics committees.

The laboratory committee is composed of one investigator from each of the participating center and will advise the steering committee on the prioritization of ancillary projects, such as substudies (see section 2.2). They will design the laboratory analytical plan (see section 5.4).

The IP board will monitor the IP property and patenting issues. They will produce ad hoc reports to the Sponsor and the study steering committee (section 12).

The advisory board will be composed of scientists with broad experience in the field of HIV clinical trials as well as members from patient organizations. The main task of this board will be to advise the study steering committee on the onwards conduct of the study (see section 7.2).

The clinical trial unit of the ITM is in charge of overseeing the practical aspects of the trial: regulatory issues, safety assessment (section 7), statistical analysis (section 8), monitoring and quality assurance (section 9) and data management (section 10). They report to the coordinating investigator and the study steering committee.

The independent safety monitor is fully independent from the study team. The independent data safety monitor will be experienced in HIV medicine and clinical research and will receive and review all SAE reports on an ongoing basis (see section 7.3).

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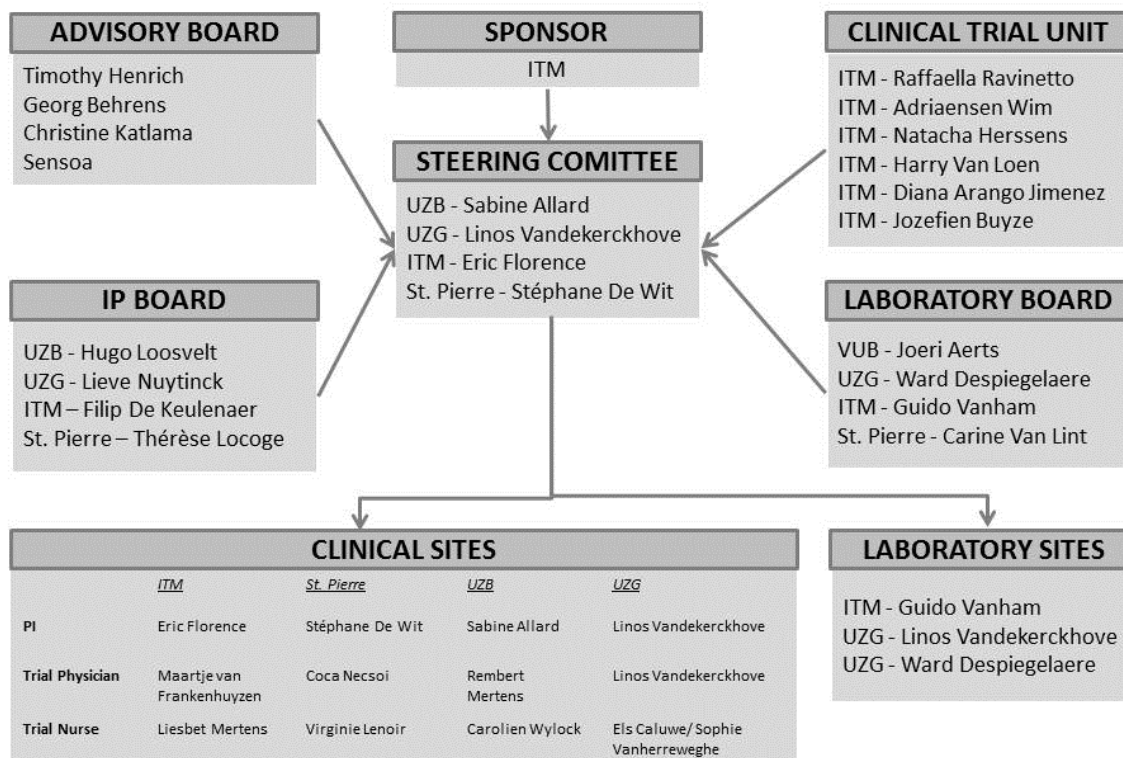
## 16. LIST OF ABBREVIATIONS

ANC	Absolute Neutrophils Count
ARC	AIDS Reference Center
cART	Combined antiretroviral treatment
ATI	Analytical Treatment Interruption
CA	Competent Authorities
CI	Coordinating Investigator
CTU	Clinical Trial Unit
DSMB	Data and Safety Monitoring Board
(e-)CRF	(electronic) Case Report Form
EC	Elite Controllers
ECG	Electrocardiogram
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
HIVAN	HIV associated nephropathy
IC(F)	Informed Consent (Form)
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITM	Institute of Tropical Medicine
MDRD	Modification of Diet in Renal Disease (equation to estimate kidney function)
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PTC	Post Treatment control(ler)
QA	Quality Assurance
QC	Quality Control
(S)AE	(Serious) Adverse Event
SC	Steering Committee
SDV	Source Data Verification
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infections
TI	Treatment Interruption
TMG	Trial Management Group
usRNA	Cell-associated unspliced HIV RNA
UZA	Universitair Ziekenhuis Antwerpen (~ University Hospital Antwerp)
VL	Viral Load
VOA	Viral Outgrowth Assay

## 17. ANNEXES

### Annex 1: Organigram

## Organigram ISALA trial



### Annex 2: World Medical Association Declaration of Helsinki

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964  
 and amended by the:

- 29th WMA General Assembly, Tokyo, Japan, October 1975
- 35th WMA General Assembly, Venice, Italy, October 1983
- 41st WMA General Assembly, Hong Kong, September 1989
- 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
- 52nd WMA General Assembly, Edinburgh, Scotland, October 2000
- 53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
- 55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
- 59th WMA General Assembly, Seoul, Republic of Korea, October 2008
- 64th WMA General Assembly, Fortaleza, Brazil, October 2013

### Preamble



1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

### **General Principles**

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

#### **Risks, Burdens and Benefits**

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

#### **Vulnerable Groups and Individuals**

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

#### **Scientific Requirements and Research Protocols**

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

#### **Research Ethics Committees**

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

#### **Privacy and Confidentiality**

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

#### **Informed Consent**

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

### **Use of Placebo**

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

### **Post-Trial Provisions**

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

### **Research Registration and Publication and Dissemination of Results**

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

### **Unproven Interventions in Clinical Practice**

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

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### **Annex 3: Division of AIDS table for grading the severity of adverse events**

[http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/daids\\_aegradingtable.pdf](http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/daids_aegradingtable.pdf)

### **Annex 4: Communication with FAGG**

*From: Bonnarens Kristof [<mailto:kristof.bonnarens@fagg-afmps.be>]  
Sent: thursday 26 march 2015 11:38  
To: Celine Schurmans  
Subject: RE: trial ART stop – not to be submitted to the FAMHP?*

*Dear Celine,*

*Sorry for the late reply. I confirm that, when there is no medication used for the research, the experiment does not need to be submitted to the FAMHP.*

*With kind regards,*

*Federaal agentschap voor geneesmiddelen en gezondheidsproducten  
Federal agency for medicines and health products*



*Kristof Bonnarens  
Afdelingshoofd – Head of  
Division*

*DG Pre Vergunning/Afdeling  
Onderzoek en Ontwikkeling  
DG Pre Authorisation/Division  
Research and Development*

*Victor Hortaplein 40/40  
1060 Brussel - Brussels*

*T +32 2 524 80 00*

*[kristof.bonnarens@fagg-afmps.be](mailto:kristof.bonnarens@fagg-afmps.be)*

*From: Celine Schurmans [mailto:cschurmans@itg.be]  
Sent: thursday 26 march 2015 11:37  
To: Bonnarens Kristof  
Subject: RE: trial ART stop – not to be submitted to the FAMHP?*

*Dear Kristof,*

*Did you have time to look at the below message?*

*Thank you,  
Céline*

*From: Celine Schurmans  
Sent: friday 20 march 2015 16:22  
To: 'kristof.bonnarens@fagg-afmps.be'  
Subject: trial ART stop – not to be submitted to the FAMHP?*

*Dear Kristof,*

*Soon we will start a trial in Belgium in which the ART for HIV-1 adult patients with a good immunity (CD4 count > 500/ $\mu$ l) and with an undetectable viral load (< 50 copies RNA/ml) will be stopped. These patients will be followed-up closely during 48 weeks.*

*Since we will not use any study medication, it was agreed that this trial did not need to be submitted to the FAMHP. Could you confirm that this is still the case so we don't miss any important step in the approval process?*

*With kind regards,  
Céline*

---

*Céline Schurmans  
Clinical Research Scientist*

*Institute of Tropical Medicine  
Nationalestraat 155  
B-2000 Antwerpen, Belgium  
phone: +32 3 2470778  
fax: +32 3 2476647  
email: [cschurmans@itg.be](mailto:cschurmans@itg.be)  
Website: [www.itg.be](http://www.itg.be)*

 *Please consider the environment before printing this e-mail*

*Disclaimer*

*Directions to our location(s)*

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Revision history: Template version 2.0
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