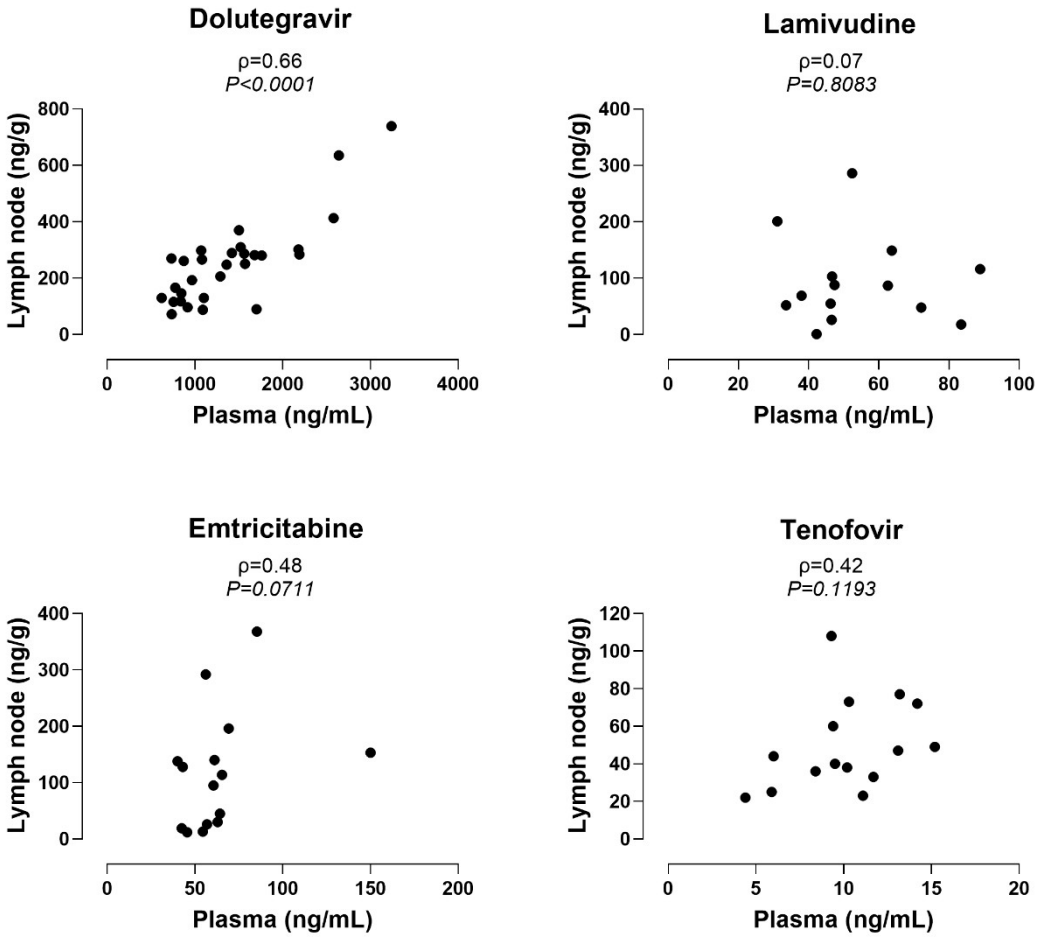
Supplementary Table 1. Demographic and clinical characteristics of study participants at study entry.

	DTG + 3TC (n=19)	DTG+FTC/TAF (n=20)	Total (n=39)
Age (years)	30.3 (27.0–37.4)	33.5 (27.9–36.0)	32.4 (27.0–36.7)
Sex at birth, Male (n, %)	19 (100.0)	20 (100.0)	39 (100.0)
Ethnicity (n, %)			
White	12 (63.2)	12 (60.0)	24 (61.5)
Hispanic/Latino	7 (36.8)	6 (30.0)	13 (33.3)
Other	0 (0.0)	2 (10.0)	2 (5.2)
BMI (kg/m^2)	24.4 (22.2–25.8)	23.6 (21.1–24.7)	23.9 (21.5–25.2)
pVL, copies/mL	23,820 (4,921–50,045)	38,264 (14,764–92,314)	29,579 (8,391–81,633)
pVL > 10^5 log copies/mL (n, %)	3 (15.8)	5 (25.0)	8 (20.5)
CD4+ T-cell count (cells/mm^3)	493 (335–607)	448 (392–535)	460 (364–568)
CD4/CD8 ratio	0.5 (0.3–0.8)	0.5 (0.4–0.8)	0.5 (0.4–0.8)
Total HIV-1 DNA (copies/10^6 CD4+ T cells)	1,348 (596–2,426)	1,457 (554–3,751)	1,457 (604–2,764)
Intact HIV-1 DNA (copies/10^6 CD4+ T cells)	500 (192–1,197)	775 (183–1,535)	559 (197–1,377)
HIV-1 ca-RNA (copies/10^3 copies TBP)	13.6 (6.2–67.9)	6.4 (0.9–42.7)	9,5 (2.1–38.9)
VIP-SPOT (HAP cells/10^6 CD4+ T cells)	16.9 (4.0–34.2)	17.2 (6.6–54.4)	17.1 (5.6–41.9)

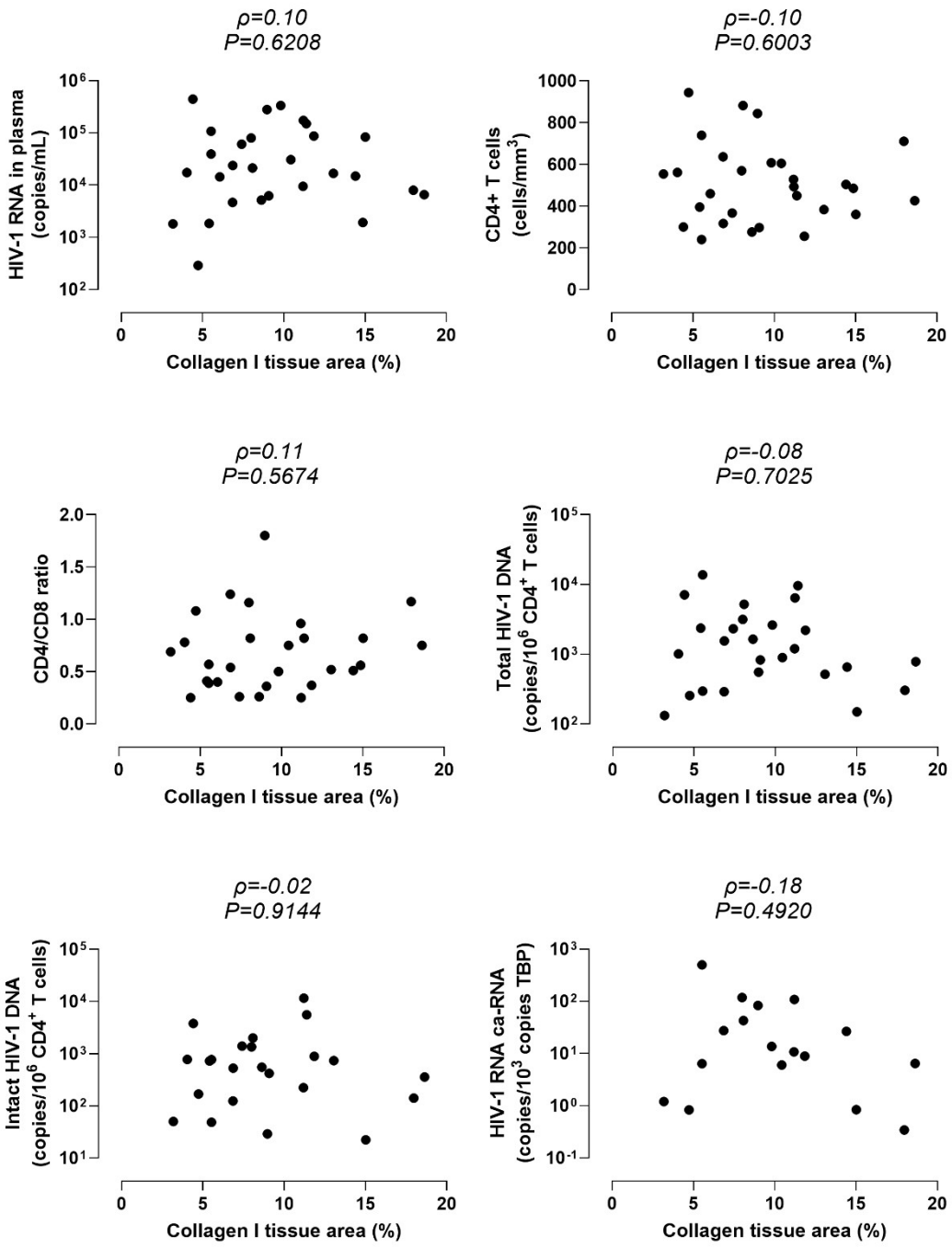
Data are expressed as median (IQR) unless otherwise indicated.

Abbreviations: BMI, body mass index; ca-RNA, cell-associated RNA; DTG + 3TC, dolutegravir plus lamivudine; DTG + FTC/TAF, dolutegravir plus emtricitabine/tenofovir alafenamide; HAP, human immunodeficiency virus antigen–producing; HIV-1, human immunodeficiency virus type 1; pVL, plasma viral load; TBP, TATA-binding protein; VIP-SPOT, viral protein spot assay.

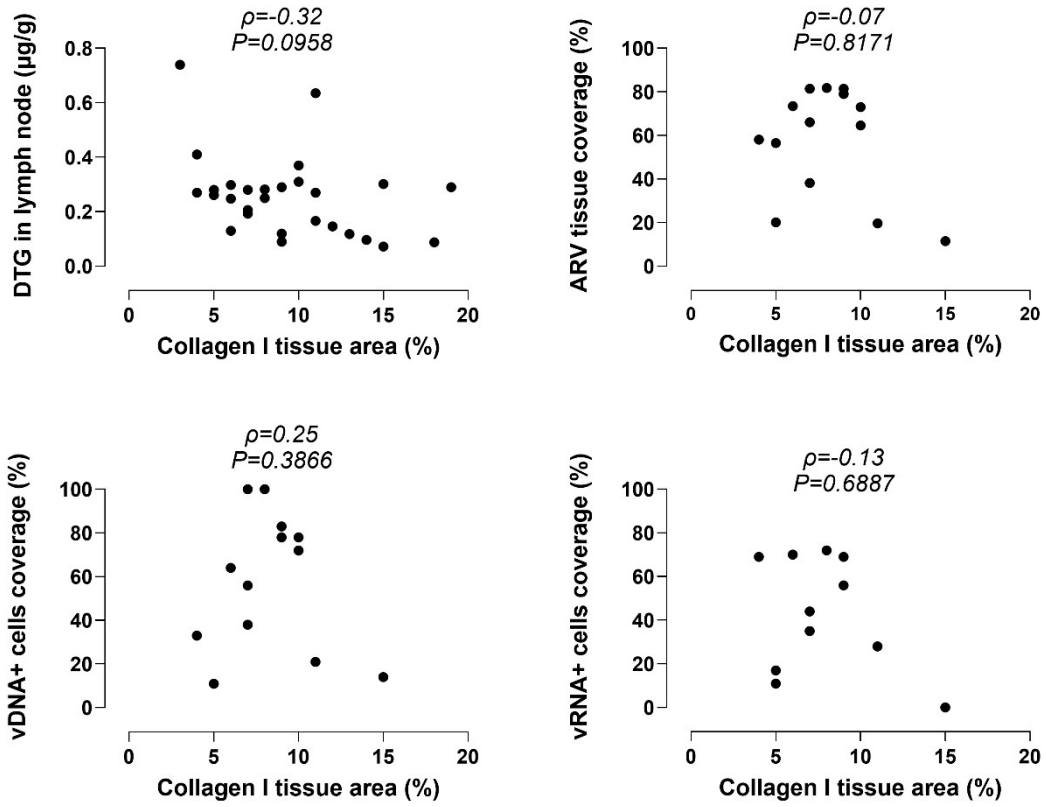
Supplementary Figure 1. Concentrations of antiretroviral drugs in plasma and in lymph nodes, measured by LC-MS/ MS. Spearman's ρ and p-values are shown.



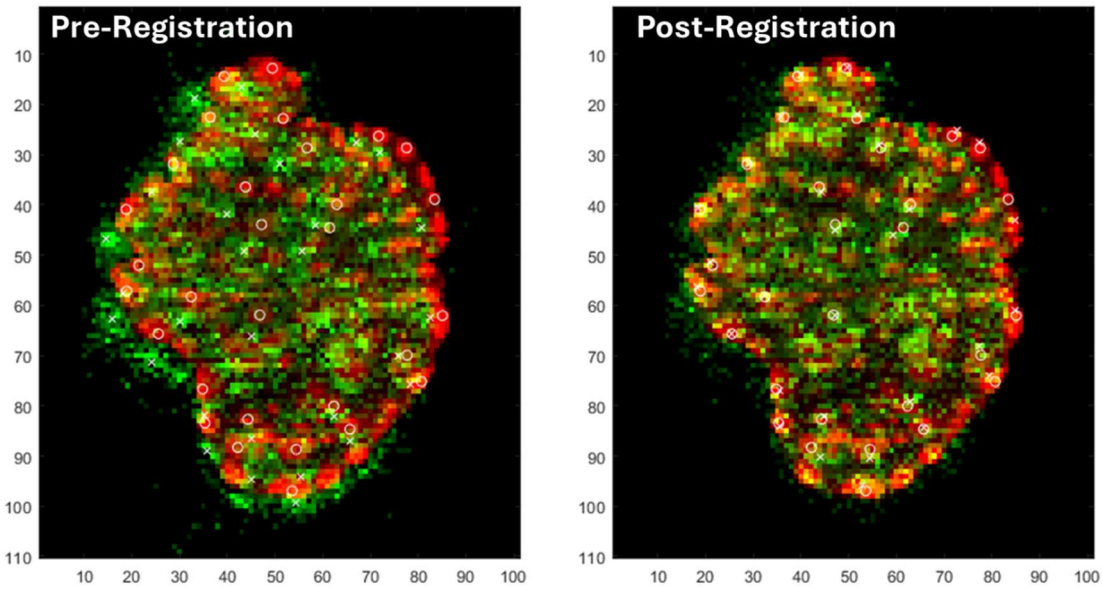
Supplementary Figure 2. Correlation between clinical characteristics at study entry and collagen I deposition in lymph nodes. Spearman's ρ and p-values are shown.



Supplementary Figure 3. Correlation between collagen I deposition and dolutegravir (DTG) concentration and antiretroviral (ARV) coverage by DTG/3TC within the lymph node. Spearman's ρ and p-values are shown.



Supplementary Figure 4. Image registration.



FIGHT AIDS FOUNDATION

EXPLORATORY, OPEN-LABEL, RANDOMIZED CLINICAL TRIAL TO ASSESS THE EFFICACY OF FIRST-LINE DUAL VS. TRIPLE ANTIRETROVIRAL THERAPY (ART) IN HIV-1 RESERVOIR AND IN PERIPHERAL COMPARTMENTS IN HIV-INFECTED PATIENTS.

Code: Dual-Triple-ART

Version 2.0, 09th July 2020

EudraCT: 2019-002733-10

Sponsor:

Fundació Lluita contra la SIDA
Hospital Universitari Germans Trias i Pujol
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Principal Investigator: José Moltó Marhuenda, MD, PhD

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SIGNATURES

The coordinating investigator and the sponsor of the study:

EXPLORATORY, OPEN-LABEL, RANDOMIZED CLINICAL TRIAL TO ASSESS THE EFFICACY OF FIRST-LINE DUAL VS. TRIPLE ANTIRETROVIRAL THERAPY (ART) IN HIV-1 RESERVOIR AND IN PERIPHERAL COMPARTMENTS IN HIV-INFECTED PATIENTS

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

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

Principal Investigator: José Moltó Marhuenda, MD, PhD

Signature and Date:

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

Signature and Date:

1. GENERAL INFORMATION

1.1 TITLE

Exploratory, open-label, randomized clinical trial to assess the efficacy of first-line dual vs. triple antiretroviral therapy (ART) in HIV-1 reservoir and in peripheral compartments in HIV-infected patients.

1.2 CODE

Dual-Triple-ART

1.3 PROTOCOL VERSION AND DATE

Version 2.0, 09th July2020.
Any modification of the protocol must also bear the amendment number and date.

1.4 SPONSOR

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

1.5 MONITOR

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1.6 PRINCIPAL / COORDINATING INVESTIGATOR

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This is a single-site trial.

1.7 SITES AND INVESTIGATORS

The trial will be performed at HIV Clinic of the Hospital Universitari Germans Trias i Pujol, Badalona.

1.8 TECHNICAL SERVICES INVOLVED

Biochemistry, hematology, plasma HIV-1 RNA levels and CD4 cell counts will be performed at local laboratory of the Hospital Universitari Germans Trias i Pujol.

The ultrasound guided lymph node biopsies will be performed in collaboration with Radiodiagnostic and Surgery Departments of the Hospital Universitari Germans Trias i Pujol.

The following determinations are going to be centrally performed at the IrsiCaixa Laboratory (Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain):

- Proviral HIV-1 DNA in peripheral CD4 T cells, plasma viral load, Ultrasensitive HIV-1 viral load in plasma, cell-associated HIV RNA in peripheral CD4 T cells and Cell-associated p24 Ag. Person in charge: Dr. Javier Martínez-Picado.
- Lymphocyte activation markers in cryopreserved PBMCs. Person in charge: Dr. Beatriz Mothe.
- Inflammatory markers in plasma. Person in charge: Dr. Julià Blanco.
- Resistance- associated mutations in those participants who develop virologic failure. Person in charge: Dr. Roger Paredes.

Drug concentrations in plasma and in lymph node, as well as HIV-1 RNA-scope in lymph node will be determined at the Pharmacotherapy and Experimental Therapeutics Department in the University of North Carolina at Chapel Hill, USA (persons of reference Dr. Angela Kashuba/Dr. Elias P Rosen), and at the Division of Pathobiology and Immunology (Oregon Health & Sciences University) at Beaverton, USA (person of reference Dr. Jake Estes).

Electronic Capture Data Form (eCRF) will be performed by REDCap (Research Electronic Data Capture).

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2. BACKGROUND INFORMATION

Current guidelines for the initial treatment of HIV-infected patients recommend combinations of 3 antiretroviral drugs including 2 nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus 1 third agent belonging to a different family, with preference for integrase strand transfer inhibitors.[1]

Widespread use of triple antiretroviral treatment (ART) has resulted in rates of virologic suppression exceeding 90% at 48 weeks, and in the life expectancy of adults with HIV infection approaching that of the general population.[2,3]

Despite effective long-term ART, residual plasma viremia persists below the limit of detection of standard methodologies. In addition, cell-associated HIV-1 DNA and RNA are detected in blood and tissues, replication-competent virus can be recovered from lymphocytes, immune activation and inflammation do not completely normalize, and ART cessation in patients maintaining undetectable plasma viral load results in almost universal viral rebound in blood.[4-6] Chronic immune activation is one of the leading causes of T-cell immune exhaustion and, consequently, impairment in T-cell functionality.[7-11] Such persistence of HIV-1 despite ART is likely to be the consequence of cryptic viral production (presumably in pharmacological sanctuaries), and reflects the inability of the standard ART in eliminating a viral reservoir which is formed by latently infected cells and in which the integrated provirus remains quiescent and very stable since very early stages of infection.[12-19] Some longitudinal studies have evaluated the evolution of the viral reservoir during standard effective ART.[20, 21] A polyexponential decrease in the viral reservoir (estimated as the amount of viral DNA in PBMCs) has been reported in such studies. A rapid decrease is seen during the first year after ART initiation, followed by a slower decay for up to 3-4 years, and a subsequent plateau phase.[22]

Persistence of viral replication, even at low levels, may be associated with persistent chronic immune activation, inflammation and immunesenescence.[23] On the other direction, recent data suggest that persistent immune-activation may be associated with greater fibrosis in the lymph nodes, which may limit drug penetration, and may facilitate viral persistence.[24-26] So, it is likely that both pathways are active, and that a `vicious cycle' might exist during treatment that results in maintenance of both immune activation and HIV persistence.[27] Chronic immune-activation, inflammation and immune-senescence have been associated with both disease progression and non-AIDS morbidity and mortality.[27-30] So, the effect of ART on these parameters is a crucial determinant of patients outcome that needs to be evaluated before implementing new ART strategies in clinical care.

Approaches to determine the HIV reservoir

Multiple techniques have been proposed to identify the HIV reservoir during recent years. The different approaches may be used differently, according to the issue in question, such as the overall level of HIV infection in the body, viral persistence, reservoir activity, or the role of the HIV reservoir in maintaining immune activation. Clearly, no single marker can answer all these questions, but each can provide part of the answer.[31, 32]

The reservoir may be defined with Quantitative Viral Outgrowth Assays (QVOA) for cells that release infectious virus after one round of T cell activation (replication competent reservoir).[33, 34] However, although QVOA is the gold standard for reservoir quantification, it is important to know that it may underestimate the reservoir size because one round of activation may not be able to induce all proviruses.[35, 36] Moreover, QVOA requires a large volume of blood or leukapheresis to obtain the necessary number of viable cells, and it is labor-intensive and expensive. These limitations make QVOA difficult to apply for frequent serial measurements in clinical studies.[31, 37]

Total HIV-1 DNA measurement is probably the simplest and most sensitive, reproducible, and standardized approach for HIV reservoir measurement, and it can be performed routinely in clinical practice.[38-42] It requires a relatively small amount of blood, allowing repeated determinations. It can be quantified in blood and other body fluids, is unaffected by freeze-thawing, and is the method most widely used to quantify the HIV reservoir

in tissue biopsy specimens. However, the measure of total and integrated HIV-1 DNA overestimates the size of the reservoir due to the high prevalence of integrated, but "defective" proviruses.[38, 43, 44] More than 90% of proviruses in ART-treated individuals have been shown to be defective at the sequence level for replication-competent virus production.[35, 36]

HIV-1 DNA, RNA, and proteins constitute pathogen-associated molecular patterns recognized by innate immune sensors and could impact activation, inflammation, and pathogenesis. This supports the utility of quantifying all forms of HIV-1 DNA, including defective and silent forms that can be transcribed or translated without producing infectious virus. Several studies have shown that unintegrated HIV-1 DNA can participate in HIV transcription and in the synthesis of viral proteins and infectious virus,[45-49] and Imamichi et al.[50] introduced the term "zombie proviruses" referring to defective proviruses can still cause harm despite being "dead". This transcription-competent and translation-competent cellular reservoir may contribute to continued antigen presence, either through the production of replication-competent virus or through viral protein production only. Indeed, a number of studies demonstrated a correlation between the CA-RNA levels and markers of immune activation and dysfunction in untreated patients, as well as in patients on ART and in natural controllers.[51-54]

Given the limitation inherent in measuring nucleic acids, there are increasing efforts aimed at directly quantifying viral proteins such as the p24 viral capsid protein within the infected cell. The logic here is straightforward; in contrast to HIV-1 messenger RNA that can still be readily generated by defective viral genomes, a stable, correctly folded protein is unlikely to be encoded by a heavily mutated, replication-incompetent genome. Exploratory studies demonstrate that ultrasensitive enzyme-linked immunosorbent assay (ELISA) can be used to measure p24 in infected cells.[55, 56]

Where to measure the HIV reservoir

Where HIV persists in the body is a central question for understanding factors contributing to persistent immune dysfunction. Regardless of the ability of any single assay to detect HIV nucleic acids, proteins, or replication-competent viruses, current assays only allow for detection of HIV from the cells directly surveyed. While cells from peripheral blood are easily accessed, it is important to consider that the circulating CD4+T cells comprise less than 2% of total-body CD4+T cell numbers.[57]

It is becoming increasingly evident that numerous compartments that have the capacity to harbor HIV are not accessible to routine sampling. In this regard, lymph nodes are a primary site for viral replication and contain massive number of infected cells and free virions captured on the follicular dendritic cell network.[58, 59] It has been shown that B-cell follicles are an important sanctuary for simian immunodeficiency virus (SIV)-infected CD4+ T lymphocytes, and that follicular helper cells (Tfh) are particularly enriched in cell-associated HIV-1 DNA and RNA, supporting high levels of viral replication at that level.[60-62] The virus that resides in these tissues may not freely circulate, suggesting that blood may ultimately prove to be a poor surrogate for what is happening systemically. Moreover, it has been shown that viral DNA and/or RNA in lymph node decreases much less than in plasma, and that, probably because of poor penetration of antiretroviral drugs in this compartment,26 HIV-RNA and DNA can still be detected in the lymph node after years of potent ART.[63-65]

The persistence of HIV replication within anatomic reservoirs needs the use of tissue pharmacology to inform the design of effective treatment strategies. This requires knowledge of tissue penetration to sites of action. Several groups have assessed antiretrovirals penetration in tissues by directly measuring drug concentrations by liquid chromatography-mass spectrometry (LC-MS) of homogenized whole tissue. Although these methods can provide useful quantitative data, they do not have the ability to spatially define the distribution of the drug within the tissue, as the entire sample is consumed in the homogenization process. This is a critical limitation of these methodologies, as preliminary data have shown that antiretroviral distribution across tissue may not be uniform.[66]

MS imaging (MSI) offers an alternative strategy for quantifying antiretroviral distribution in tissues that maintains the sensitivity and specificity of LC-MS while preserving the spatial distribution of analytes within the tissue. Through stepwise interrogation of discrete sample locations, MSI simultaneously collects information that can be concatenated into images of multiple molecules, which is an important advantage for ART due to

its combinatorial nature. One approach to MSI which has been shown to permit the detection of antiretrovirals in human tissue is infrared matrix assisted laser desorption-electrospray ionization (IR-MALDESI).[67, 68] In addition, application of in situ hybridization (ISH) techniques to localize and quantify HIV-1 RNA and DNA in various tissues with single-cell resolution is experiencing a scientific renaissance.[69, 70] Rosen et al. combining IR-MALDESI MSI and ISH to evaluate the biodistribution of antiretroviral drugs relative to viral and target cell expression in lymph nodes of Rhesus macaques. Interestingly, MSI revealed that antiretrovirals accumulated in the lymph node heterogeneously; and, combination of MSI and ISH showed up to half of vRNA expression was not co-localized with any antiretroviral. While vRNA was mainly expressed in secondary follicles of lymph nodes, antiretrovirals penetration in that localization was absent or poor.[71]

Less drug antiretroviral regimens

The requirement for life-long ART, together with the desire to further reduce drug toxicity, exposure and cost, has fueled interest in "NRTIreducing" regimens.[72, 73]
Efficacy of maintenance monotherapy with boosted protease inhibitors (PI/r) as a treatment simplification strategy was evaluated in different clinical trials as well as in cohort studies.[74, 75] Despite demonstrating high efficacy for maintaining virological suppression, the efficacy of PI/r monotherapy was found to be inferior to that of triple ART. Similarly, although early results from small studies using dolutegravir (DTG) monotherapy were promising, these studies were generally stopped due to the unpredictability of viral rebound in some participants, along with a high likelihood of development of cross-class integrase inhibitor drug resistance.[76, 77] Therefore, current ART guidelines do not recommend monotherapy with either PI/r or DTG for the treatment of patients with HIV infection.[1]
Combination of DTG plus lamivudine (3TC) may be an attractive dual antiretroviral regimen because both drugs are potent and well tolerated, because DTG has a high barrier to resistance, and because it should be possible to combine the two drugs in one single tablet. Efficacy of first line dual ART with DTG+3TC in naïve patients was first shown in the single-arm studies PADDLE and ACTG A5353;[78, 79] and these results were confirmed by large, fully-powered trials.80 The Gemini-1 and Gemini-2 were two randomized trials which compared the efficacy of dual therapy with DTG+3TC to a three-drug regimen of DTG plus TDF/FTC in more than 1,400 participants with baseline HIV RNA levels up to 500,000 copies/m. At week 48, DTG+3TC demonstrated noninferior virologic efficacy to triple ART in terms of virologic efficacy, and no treatment-emergent resistance was seen in either group.[80] However, information on the capacity of DTG+3TC as first-line dual ART to reduce the viral reservoir size, and its impact in chronic immune activation is lacking, and needs to be addressed before this strategy can be widely recommended for routine clinical care.

Through the development of this project, we aim at analysing the impact of dual vs triple first-line ART in the HIV-1 reservoir by studying one the most critical sanctuaries, the lymph node, and using the most novel techniques for that end.

HIPOTHESIS:

First-line dual ART with DTG + 3TC in HIV-infected patients without prior ART experience could result in similar decay of the HIV-1 reservoir, as well as in comparable levels of residual viral replication and immune activation compared with triple ART with DTG +Emtricitabine/Tenofovir Alafenamide Fumarate (FTC/TAF).

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3. TRIAL OBJECTIVE AND PURPOSE

The purpose of the present study is to compare the dynamic effect of first-line dual ART with DTG+3TC versus standard triple ART with DTG+FTC/TAF in HIV-1 persistence and immune activation in ART-naïve HIV-infected patients, in periphery and in lymph node tissue.

3.1 PRIMARY OBJECTIVE

To compare changes in the HIV-1 reservoir (proviral HIV-1 DNA in CD4+ T cells) from baseline to week 48 between first-line treatment with DTG+3TC versus DTG +FTC/TAF.

3.2 SECONDARY OBJECTIVES

- To compare the following variables during the study period between first-line treatment with DTG+3TC versus DTG +FTC/TAF.
  - Plasma viremia decay.
  - Proportion of participants with HIV-1 RNA in plasma below 50 copies/mL and below 1 copy/mL.
  - Changes in CD4/CD8.
  - Changes in cell-associated HIV-1 RNA in CD4+ T cells.
  - Changes in plasma and cell-associated p24 (single molecule assay) from baseline to week 48.
  - Changes in activation (HLA-DR/CD38) and exhaustion (PD-1/TIGIT) markers from baseline to week 48 indifferent lymphocyte subsets defined by CD3/CD4/CD8/CDR7/CD45RA and CD27 surface markers by multiparametric Flow cytometry.
  - Change in Inflammatory markers (sCD14, CRP, IL-6, IL-10, d-dimer, IP-10, sCD163, LFABP and TRAIL) in plasma.
- To evaluate DTG, TFV, FTC and 3TC penetration and distribution in peripheral lymph node.
- To evaluate the longitudinal and spatial association between drug distribution and cell-associated HIV-1 RNA and DNA expression within peripheral lymph node.
- To evaluate the resistance-associated mutations in those patients who develop virologic failure.

4. TRIAL DESIGN

4.1 TYPE OF TRIAL

This is an exploratory, single-center, randomized, open-label clinical trial in 44 ART-naïve HIV infected patients.

4.2 DESCRIPTION OF THE DESIGN

The study will include 44 adults infected by HIV-1 without prior ART experience.

After inclusion, participants will be randomized (1:1) to start receiving dual (n=22) or triple (n=22) ART.

Triple ART Group

Dolutegravir (Tivicay) 50 mg every 24 hours.
Emtricitabine/Tenofovir alafenamide fumarate (Descovy) 200/25 mg every 24 hours.
Patients will be advised to take antiretroviral medication orally.
Commercial medication will be used.

Dual ART Group
Dolutegravir (Tivicay) 50 mg every 24 hours.
Lamivudine (Lamivudine EFG) 300 mg every 24 hours.
Patients will be advised to take antiretroviral medication orally.
Commercial medication will be used.

4.3 ENDPOINTS

4.3.1 Primary endpoint(s)

Change in proviral HIV-1 DNA in CD4+ Tcells from baseline to week 48.

4.3.2 Secondary endpoints

- Comparative dynamics of plasma viremia decay during the study period.
- Proportion of participants with HIV-1 RNA in plasma below 50 copies/mL and below 1 copy/mL at 48 weeks.
- Changes in the ratio CD4/CD8.
- Change in cell-associated HIV-1 RNA in CD4+ T cells from baseline to week 48.
- Change in plasma and cell-associated p24 (single molecule assay) from baseline to week 48.
- Change in activation (HLA-DR/CD38) and exhaustion (PD-1/TIGIT) markers from baseline to week 48 indifferent lymphocyte subsets defined by CD3/CD4/CD8/CDR7/CD45RA and CD27 surface markers by multiparametric Flow cytometry.
- Change in inflammatory markers (sCD14, CRP, IL-6, IL-10, d-dimer, IP-10, sCD163, LFABP and TRAIL) from baseline to week 48.
- DTG, TFV, FTC and 3TC penetration and distribution in peripheral lymph node.
- Longitudinal and spatial association between drug distribution and cell-associated HIV-1 RNA and DNA expression within peripheral lymph node from baseline to week 48.
- Resistance-associated mutations in those patients who develop virologic failure during the study.

4.4 MEASURES TO AVOID BIAS

4.4.1 Stratification

Centralized randomization will be stratified considering the time from estimated infection to ART initiation (<3 months, >3 month) and HIV-1 RNA at the screening visit (</> 5 log10 copies/mL).

4.4.2 Blinding

Not applicable since it is an open clinical trial.

4.5 FORESEEN CALENDAR

First patient in: October 2019
Inclusion period ends: April 2021
Interim analysis: September 2021
Last patient last visit: April 2022
Final analysis: June 2022
Final communication

- Abstract submission: Q2-Q3 2022
- Manuscript sent for publication: Q4 2022-Q1 2023

4.6 END OF TRIAL

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

4.7 SOURCE DATA

The source data are the participant 's medical records, the results from blood test performed either through clinical routine of additional laboratory test performed on the same day.

The source data are the participant´s electronic health records including the results from blood test.
Study data will be collected through a study-specific Case Report Form (see Appendix I).

5. TRIAL INVESTIGATIONAL PRODUCT(S)

5.1 EXPERIMENTAL AND CONTROL TREATMENTS

Study treatments will include:

- Dolutegravir (Tivicay) 50 mg.
- Emtricitabine/Tenofovir alafenamide fumarate (Descovy) 200/25 mg.
- Lamivudine (Lamivudine EFG) 300 mg.

All of them will be administered by the Pharmacy Service of the Hospital Universitari Germans Trias i Pujol in their commercialized format. No conditioning is required.

5.2 SUPPLY, PACKAGING, LABELING AND STORAGE

All treatments will be stored at and dispensed by the Pharmacy Service of Hospital Universitari Germans Trias i Pujol.

All study medication will be stored in a safe place during the study. Storage shall be in accordance with the conservation conditions defined in the summary of products characteristics. Being marketed medication, specific temperature control for the study will not be performed, but the usual procedures will be followed in the Pharmacy for the custody and traceability of medication.

5.3 DOSE, INTERVAL, ROUTE AND METHOD OF ADMINISTRATION

After inclusion, participants will be randomized (1:1) to start receiving dual (n=22) or triple (n=22) ART.

Triple ART Group
Dolutegravir (Tivicay) 50 mg every 24 hours.
Emtricitabine/Tenofovir alafenamide fumarate (Descovy) 200/25 mg every 24 hours.

Dual ART Group
Dolutegravir (Tivicay) 50 mg every 24 hours.
Lamivudine (Lamivudine EFG) 300 mg every 24 hours.

All study medication will be administered orally. Participants will be instructed to take the medication in the morning.

No changes in the assigned treatment are foreseen during the study period.

5.4 DRUG ACCOUNTABILITY

Participants will return the study medication, and drug accountability will be performed by a study nurse during the study, at visits week 4, week 12, week 24, week 36 and week 48.

5.5 ARM DESCRIPTION

This is a randomized, open-label clinical trial consisting of 48 weeks of treatment period. During the study the participant will receive one of these treatments:

Triple ART Group

Dolutegravir (Tivicay) 50 mg every 24 hours.
Emtricitabine/Tenofovir alafenamide fumarate (Descovy) 200/25 mg every 24 hours.

Dual ART Group
Dolutegravir (Tivicay) 50 mg every 24 hours.
Lamivudine (Lamivudine EFG) 300 mg every 24 hours.

5.6 MODIFICATION OF THE TREATMENT REGIMEN

No changes in the assigned treatment are foreseen during the study period.

In case of adverse reactions related to the study medications, the investigator will consider the need of interrupting the related medication, based on their severity and persistence, and the participant will be withdrawn from the study.

5.7 CONCOMITANT TREATMENTS

Participants must be advised to notify their investigator of any current or proposed concomitant medication, whether prescribed or over-the-counter (including vitamins and/or herbal remedies and supplements). Concomitant medications (prescription and non-prescription) will be permitted during the course of the study at the investigator's discretion.
In case of use of concomitant medications, in the SmPC of each drug, detail on pharmacological interacions and dose recomendations with other drugs are specified.

All concomitant medications be documented in the study CRF. The minimum requirement is that the drug name, route, reason for use, and dates of administration are to be recorded.

5.8 COMPLIANCE

Treatment adherence will be self-reported by the patient by answering the adapted SERAD questionnaire at each study visit.
Drug accountability will be performed by a study nurse at weeks 4, 12, 24, 36 and 48, when the participant will return the study medication

6. SELECTION AND WITHDRAWAL OF PARTICPANTS

6.1 INCLUSION CRITERIA

- Age ≥18 years.
- Documented HIV-1 infection (confirmed by a NAT/PCR test).
- Naïve to cART (Pre-exposure prophylaxis with FTC/TDF or TAF/FTC or post-exposure prophylaxis will be allowed if more than 4 weeks before the screening visit).
- Willing and able to be adherent to antiretroviral therapy for the duration of the study.
- Willing to comply with the requirements of the protocol and available for follow-up for the planned duration of the study.
- In the opinion of the principal investigator or designee, the participant has understood the information provided and capable of giving written informed consent.
- If female in fertile age and heterosexually active; using an effective method of contraception (hormonal contraception, intra-uterine device (IUD), or anatomical sterility in self or partner*) from 14 days prior to the first study product administration until at least 12 weeks after the last study product administration; all female participants must be willing to undergo urine pregnancy tests at time points specified in the Schedule of Procedures.

** condom use nor diaphragm are considered as an additional method of contraception only and cannot be the only method of contraception used as not been considered an effective method by the Clinical Trial Facilitation Group (CTFG) guidelines.*

6.2 EXCLUSION CRITERIA

- Exposure to any antiretroviral drug within the 4 weeks prior to the screening visit.
- HIV-1 RNA in plasma >500,000 copies/mL.
- Active AIDS-defining illness within the prior 4 weeks.
- Chronic hepatitis B (positive HBsAg).
- Chronic hepatitis C (positive IgG HCV confirmed by positive HCV RNA in plasma).
- Estimated glomerular filtration rate (eGFR) <50 mL/min.
- Advance liver function impairment (Child-Pugh C).
- Use of systemic chemotherapy within the 12 months before the screening visit.
- Use of systemic corticosteroids during more than 7 consecutive days within the 3 months before the screening visit.
- Concomitant treatment with co-medications with known drug-drug interactions with study drugs.
- History or clinical manifestations of any physical or psychiatric disorder which could impair the subject's ability to complete the study.
- Any other current or prior therapy which, in the opinion of the investigators, would make the individual unsuitable for the study or influence the results of the study.
- In women, pregnancy or breastfeeding.

6.3 PARTICIPANT WITHDRAWAL CRITERIA

6.3.1 Early participant withdrawal

The participants will prematurely withdraw the clinical trial under the following circumstances:

- Resistance to the study drugs in the screening genotype.
- Virologic failure: defined as a
    - Less than 2 $\log_{10}$ drop in HIV-1 RNA **and** >50 c/ml at week 12, or
    - An HIV-1 RNA >50 copies/mL on two consecutive determinations at week 24 or later, or
    - An increase in HIV-1 RNA >50 copies/mL in two consecutive determinations after having reached HIV-1 RNA <50 copies/mL
- Interruption of study medication due to adverse events, intolerance or poor adherence during the study.
- Pregnancy.
- Concurrent process or illness which in the opinion of the investigator requires the withdrawal of the patient.
- The participant does not wish to continue in the study.

6.3.2 Medical approach to withdrawal

In case of early participant withdrawal, an End of Study visit will be performed. The reason for withdrawal must be recorded in the eCRF and in the subject's medical records.

Subsequent antiretroviral therapy will be prescribed by the treating physician in agreement with current Guidelines for the Management of Antiretroviral Therapy (http://gesida-seimc.org/wp-content/uploads/2019/02/Guia_Tar_Gesida_Ene_2019.pdf), considering specific conditions/circumstances of the patient.

6.3.3 Replacement of participants

Replacement of participants will be permitted only in case of patients withdrawing the study before receiving any IMP dose. (pre-baseline losses)

6.4 PRE-RANDOMIZATION / PRE-BASELINE LOSSES

Data from participants that do not meet the selection criteria after completing the screening visit and pre-baseline losses will be recorded in the screening log form, but will not be considered for the study.

7. TRIAL CONDUCTION AND RESPONSE EVALUATION

7.1 CRITERIA FOR RESPONSE EVALUATION

7.1.1 Primary parameter

Proviral HIV-1 DNA in CD4+ T cells.

7.1.2 Secondary parameters

- HIV-1 RNA in plasma.
- CD4/CD8+ T cells count.
- Cell-associated HIV-1 RNA
- Plasma and cell-associated p24 (single molecule assay).
- Proportion of HLA-DR+/CD38+ activated T-cells in different subsets.
- Proportion of PD-1+/TIGIT+ exhausted T-cells in different subsets.
- Inflammatory markers in plasma: sCD14, CRP, IL-6, IL-10, d-dimer, IP-10, sCD163, LFABP and TRAIL.
- DTG, TVF, FTC, and 3TC concentrations in plasma.
- DTG, TVF, FTC and 3TC concentrations and distribution in lymph node.
- Cell-associated HIV-1 DNA and RNA levels and distribution within the lymph node.
- Resistance-associated mutations in genotypic tests.

7.2 TRIAL DEVELOPMENT

After signing informed consent, participants will undergo a screening visit.
Participants fulfilling inclusion/exclusion criteria will be randomized (1:1) to start ART within one of the two study groups.
Study visits will be at entry (BL or week 0) and at weeks 1, 2, 4, 12, 24, 36 and 48.
Blood specimens will be obtained at specific time-points, according to the Study flow-chart (See 7.5. Assessments flow-chart)
An ultrasound-guided lymph node excisional biopsy will be performed at BL, and at weeks 2, 4, 12 and 48. One/two inguinal lymph nodes will be extracted from 4 different participants from each study group at each time-point (one single biopsy from 20 participants from each study group during the study). Paired plasma samples will be obtained from each participant.

7.3 CLINICAL RECORD AND PHYSICAL EXAM

Demographic, clinical variables and HIV infection-related data will be collected in order to characterize the study population (sex, age, time since HIV diagnosis, risk factor and history of opportunistic infections or tumors) and will be recorded at baseline.
Age, gender, time since estimated date of HIV infection, group of HIV transmission rout, prior use of PrEP/PEP, CD4+ T cell count, HIV-RNA load in plasma, other medical conditions, concomitant treatments)

Patients will be asked for all medication in previous or current use.

A complete physical examination will be performed at screening and baseline visits. Height and weight will be recorded at baseline visit.
At weeks 1, 2, 4, 12, 24, 36 and 48, aa symptom-guided physical exam will be done.

7.4 LABORATORY TESTS

Participants will fast for at least 8 hours prior to blood test, in the points specified in the flow chart of the study (section 7.5 Assessments Flow-chart). The following parameters will be quantified, as needed:

- **Blood count:**
  - Hematocrit
  - Red blood cell count
  - Hemoglobin
  - Leucocytes
  - Lymphocyte
  - Platelet count

- **Blood biochemistry:**
  - Glucose
  - Urea
  - Creatinine
  - CPK
  - Ionogramme: sodium, potassium
  - Total Bilirubin
  - Total proteins
  - Albumin
  - Liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-GT, alkaline phosphatase
  - Estimated glomerular filtration rate (CKD-EPI)

- **Microbiology:**
  - HIV-1 viral load
  - HBsAg
  - IgG HCV
  - HCV viral load (in participants with positive IgG HCV)

- **Immunology:**
  - CD4+T cells
  - CD8+T cells

- **Pregnancy test in women (urine test strip)**

- **Specific Procedures:**
  - Ultrasound guided lymph node excisional biopsy
  - Ultrasensitive plasma HIV-1 viral load
  - Proviral HIV-1 DNA in peripheral CD4 T cells

Ultrasensitive HIV-1 viral load in plasma (if viral load <40 copies/mL is found by conventional assay (Abbott)).
Cell-associated HIV RNA in peripheral CD4 T cells.
Plasma p24 Ag.
Cell-associated p24 Ag.
Lymphocyte activation markers in cryopreserved. Lineage and T cell subset markers will include: CD3/CD4/CD8 and CCR7/CD45RA/CD27.
Inflammatory markers in plasma (sCD14, CRP, IL-6, IL-10, d-dimer, IP-10, sCD163, LFABP and TRAIL).
Resistance- associated mutations.
DTG, TVF, FTC, 3TC concentrations in plasma.
DTG, TVF, FTC, 3TC, 3TC-TP, FTC-TP and TFV-DP concentrations and distribution within the lymph node.
HIV-1 RNA and DNA quantification and distribution within the lymph node.

Note: Before the beginning of the study, local lab from Hospital Universitari Germans Trias i Pujol will provide blood count and biochemistry reference normal ranges.

7.5 ASSESSMENTS FLOW-CHART

Parameter	SCR	BL	W1	W2	W4	W12	W24	W36	W48/EOS
Window (days)			±1	±2	±3	±3	±5	±5	±5
Informed consent	√								
Inclusion/ exclusion criteria		√							
Randomization		√							
Clinical visit	√	√	√	√	√	√	√	√	√
Complete physical exam[1]	√	√							
Symptom-guided physical exam[1]			√	√	√	√	√	√	√
Height and weight		√							
HBsAg, IgG HCV, HCV RNA [2]	√								
Genotype[2]	√			√	√	√	√		
Blood cell count and chemistry	√	√[3]			√	√	√	√	√
HIV-1 Viral load	√	√[3]	√	√	√	√	√	√	√
CD4/CD8+ T cell count	√	√[3]			√	√	√	√	√
Ultrasensitive pVL				√[4]	√[4]	√[4]			√[4]

Proviral DNA in CD4+ T cells		√				√			√
Cell-associated RNA in CD4+ T cells		√				√			√
Plasma p24 Ag		√	√	√	√	√	√	√	√
Cell-associated p24 Ag		√				√			√
Inflammatory markers		√				√			√
Immunophenotyping: activation, exhaustion and maduration		√				√			√
Lymph node biopsy[5]		√		√	√	√			√
Plasma and PBMCs storage		√		√	√	√	√		√
Pregnancy test in urine	√	√				√	√	√	√
Drug dispensing		√			√	√	√	√	
Drug accountability					√	√		√	√
SERAD questionnaire			√	√	√	√	√	√	√

[1]Including vital signs: Blood pressure, heart rate and body temperature.
[2]Not necessary if available results of laboratory tests performed within 3 months prior to study entry. In cases of positive IgG HCV, HCV RNA in plasma will be determined.
[3]Not necessary if tests performed in screening are within 1 month prior to baseline.

[4]Ultrasensitive RT-PCR assay (lower limit of quantification 1 copy/mL) will be performed in samples with HIV-1 RNA <40 copies/mL by commercial assays.
[5] One single biopsy from 20 participants from each study group during the study will be performed. One inguinal lymph node will be extracted from 4 participants from each study group at each time point (one single excisional biopsy from each participant during the study).

8. ADVERSE EVENTS

8.1 DEFINITION

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

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

- Causes participant death.
- Produces a life-threatening situation for the participant (Note: The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)

- Requires or prolongs in hospital admission.
- Produces important or persistent incapacitation/handicap or constitutes a congenital defect or anomaly.
- Needs action to prevent any of above situations.
- It is considered a medically important event or reaction: such events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes constituting SAEs. Medical and scientific judgment must be exercised to determine whether such an event is a reportable under expedited reporting rules. Examples of medically important events include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; and development of drug dependency or drug abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product will be considered a medically important event and subject to expedited reporting requirements.

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

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

8.2 DESCRIPTION OF THE IMPUTABILITY CRITERIA

The causal relation will be established according to the algorithm of the Spanish Pharmacovigilance System, which contemplates the following categories:

Definitive:

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

Probable:

- A reasonable time sequence exists in relation to administration of the drug.
- The observed manifestation coincides with the known adverse reactions profile of the implicated drug.
- The event is unlikely to be explained by the concurrent disease or by other drugs or chemical substances.
- Response to withdrawal is clinically plausible, i.e., the condition improves on discontinuing administration of the drug.
- No repeat exposure is required to complete this definition.

Possible:

- A reasonable time sequence exists in relation to administration of the drug.
- The observed manifestation coincides with the known adverse reactions profile of the implicated drug.

- The event might be attributable to the clinical condition of the patient or to other concomitantly administered drugs or chemical substances.
- Information concerning drug withdrawal may be unavailable or confusing.

Improbable:

- A clinical event, including anomalous laboratory test findings, with a time relation to administration of the drug which makes a causal association unlikely, and where other drugs, chemical substances or intercurrent disease afford plausible explanations for the observed event.

Unrelated:

- None of the above criteria are met.

8.3 ADVERSE EVENTS GRADING

Grading will be performed using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1. [March 2017].

Citation: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1. [March 2017]. Available from: https://rsc.tech-res.com/docs/default-source/safety/daids-ae-grading-table-mar2017.pdf

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

Grade 1 (mild):	Symptoms causing no or minimal interference with usual social & functional activities
Grade 2 (moderate):	Symptoms causing greater than minimal interference with usual social & functional activities
Grade 3 (severe):	Symptoms causing inability to perform usual social & functional activities
Grade 4 (potentially life-threatening):	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
Grade 5 (death):	Any AE where the outcome is death.

8.4 PROCEDURE FOR REPORTINGADVERSE EVENTS

8.4.1 Investigator

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

The report will be done during the first 24 hours after knowing about the serious adverse event. Notification will be made by means of the adverse events reporting form contained in Appendix V of this protocol.

Contact details for Sponsor
Fundació Lluita contra la SIDA
e-mail: afiguerola@fls-rs.com
Fax. +34 93 465 76 02

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

Depending on the nature of the condition, each adverse event is to be classified as:

- serious / not serious
- unexpected / expected

The recording of adverse events is the responsibility of the trial investigator team, which should indicate the time of appearance of the event (expressed in the shortest time unit possible), its serious / not serious status, and in case it is considered related to investigational products, whether it was expected or unexpected. The intensity of the event (grade 1 to 5) is to be specified, along with the measures adopted (none, treatment, temporal or permanent discontinuation of investigational product), course (complete remission, partial remission, persistence) and imputability based on the criteria indicated in section 8.2.

8.4.2 Sponsor

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

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

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

The deadlines to notify suspect adverse reaction are, from the moment the SUSAR is known by the Sponsor:

- 15 days
- 7 days if the SUSAR has resolved in death or has been life-threatening. The information will be completed in 8 further days.

If the notification is sent in electronic form, it is not necessary to notify the competent authorities of the autonomous region.

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

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

8.5 HOSPITALIZATION AND FORESEEN PROCEDURES HOSPITALIZATION AND FORESEEN PROCEDURES

If a participant has to be hospitalized or undergone a procedure (i.e, Elective surgery) scheduled before he/she was included in the trial (i.e, before the participant [or representative Legal thereof] signed informed consent) for any event / pre-trial disease, the hospitalization is considered a therapeutic intervention and not the result of a SAE. However, if the event / disease worsens during the test should be reported as an AE (SAE or if the event / disease ends in a serious situation such as hospitalization).

8.6 ABNORMAL LABORATORY PARAMETERS

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

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

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

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

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

8.7 DOCUMENTATION RELATED TO AE AND SAE

Each AE and SAE to take place during the study should be documented in the medical records of the patient in accordance with standard clinical practice of the investigator, and in the CRF. For each SAE, an independent set of SAE form will be used independently. Only if there are multiple SAE at the time of the initial report and these are temporary and / or clinically interrelated can be registered on the same set of SAE form.

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

SAE pages found in the investigator's file shall be completed as precisely as possible, printed and shall be signed by the investigator before being sent to the sponsor. It is very important that the initial page SAE investigator provide its opinion in regard to the relationship of the event to the investigational product.

The SAE reports that are subject to the above reporting provisions are those that occur following the first dose and through to 28 days after discontinuation of the study medication. The report will be done during the first 24 hours after knowing about the serious adverse event.

8.8 SAE FOLLOW-UP

The investigator must record all AE occurring from the moment the participant signs the informed consent until the last study visit.

In case of an ongoing AE at the end of study visits/w48, the participant will be followed until resolution or decrease to grade ≤2.

SAE will be followed preferably until:

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

Otherwise, they will continue until:

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

The investigator should ensure that follow-up reports include any additional information to enable a full assessment of the nature and/or the cause/effect of SAE, including any other laboratory test, pathology report and examination by a specialist.

8.9 PREGNANCY

Due to the potential risk from neural tube defect associated with DTG treatment during the first trimester of pregnancy, study staff must review the following information with all female participants of reproductive potential:

- Participants should be informed that in an early analysis of one observational study, women who were taking DTG when they became pregnant had an increased risk of having babies with serious brain and spine defects. These defects happen early in pregnancy, before many women even know they are pregnant.
- Women of reproductive potential should be counselled on the importance of avoiding pregnancy, safer sexual practices and the proper use of their chosen contraceptive methods in accordance with the applicable contraceptive product label, or, for non-product methods, as determined by the investigator.
- The need for the participant's chosen contraceptive method to be used for an adequate time period before dosing with the DTG is initiated.
- The need to continue the use of contraception throughout the treatment- and post-treatment-periods with DTG, until it is predicted that a clinically insignificant amount of DTG is present in the participant (i.e. 30 days or one month after stopping DTG).

Additionally, site staff should document the participant's chosen contraception methods in her medical records and study records/eCRF.

As an additional point in compliance with current legislation, the sponsor and investigators shall notify exposures during pregnancy that might have suffered a patient with Dolutegravir.

All the related information will be reported at the pregnancy notification during the first 24 hours after becoming aware of the pregnancy, following the same procedures as a SAE.

The cases of pregnancy shall be recorded in a pregnancy notification form (Appendix VII). This form must be completed by the subject or subject's partner who becomes pregnant during the study period.

Pregnancy is also a protocol deviation requiring premature termination of the patient. The investigator will provide medical support to the pregnant patient.

Reporting Period

The pregnancy reports that are subject to the above reporting provisions are those that occur following the first dose and through to 28 days after discontinuation of the study medication.

9. STATISTICS

9.1 DATA ANALYSIS

A general descriptive analysis of all the variables of the study, overall and separately by groups concerning baseline and demographic characteristics will be done by using mean, standard deviation, median, interquartile range, maximum and minimum values for the quantitative variables and absolute and relative frequencies of each category for categorical variables.
The response evaluation is going to be done per-protocol, based on available data from each patient from baseline to the last follow-up visit.
Parametric or non-parametric tests will be used to compare continuous variables, as appropriate. Proportions will be compared with the Chi-Squared test, with Fisher correction when needed. The statistical significance of the longitudinal changes in primary and secondary variables will be assessed by the calculation of the slope of decay, and compared between treatment groups using t-student, Wilcoxon or Mann-Whitney test, as appropriate.
Changes in the viral reservoir in the lymph node during the study will be assessed within each study group. Initially, it will be evaluated according to a naïve pooled-data analysis (Mahmood I. Am J Ther 2014;21:269-275). Mean/median values at each time point will be calculated, and they will be then combined as if they came from one single individual; and differences in the slope of decay between groups will be assessed by standard parametric/non-parametric tests.
Additionally, to account for variability between participants, a population non-lineal mixed effects model describing changes in the tissue reservoir during the study will be developed (NONMEM 7.4 software. Sheiner LB. Drug Metabolism Reviews 1984;15:153-171). Influence of treatment group and patient characteristics at baseline in parameters describing longitudinal changes in the reservoir will be explored by their introduction in the model through standard stepwise covariate model-building strategies.
An interim analysis of the primary endpoint as well as on dolutegravir pharmacokinetics in the lymph node is envisioned when the last participant reaches the week 12 visit.

9.2 SAMPLE SIZE DESCRIPTION

A total of 44 ART-naïve HIV-infected patients will be included in the study.
No formal sample size required since this is an exploratory study. However, based on the HIV-1 reservoir dynamics during the first year of antiretroviral treatment in a similar group of HIV-infected patients that has been described by Puertas et al[11], inclusion of 44 patients in the study would allow us to detect a minimum difference of 2-fold in HIV- 1 DNA in CD4+ T cells at week 48 between groups (potency 80%, alpha error 0.05).

9.3 DEVIATION OF STATISTICAL PLAN

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

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

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

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

11. QUALITY CONTROL AND QUALITY ASSURANCE

11.1 STUDY MONITORING

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

During these contacts, the monitor shall:

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

The goal of the monitoring activity is to verify that:

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

The investigator must agree to:

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

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

11.2 AUDITS AND INSPECTIONS

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

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

11.3 CASE REPORT FORM

Data collection will be done through an electronic CRF with a system of access by username and password. The application includes track changes monitoring (recording the changes that have been made and details of the user that has made these changes).

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

The content of the CRF is attached in Appendix I.

12. ETHICS

12.1 GENERAL CONSIDERATIONS

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

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

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

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

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

12.2 PATIENT INFORMATION SHEET AND INFORMED CONSENT

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

12.3 PAYMENT TO RESEARCH PARTICIPANTS

Participants will be reimbursed for their time, effort and time travels to study site due to study participation. Reimbursement amounts will be documented.
A net payment of 400€ will be paid for the entire protocol participation and will be set in 2 times:

- 50% after visit w12
- 50% after visit w48

An additional payment for the net amount of 300€ will be paid to those participants undergoing the ultrasound-guided lymph node excisional biopsy. This payment will be done after performing the biopsy.

13. DATA HANDLING AND RECORD KEEPING

13.1 DATA HANDLING

The processing of the data to be compiled by the study sponsor during the study will be participant to current legislation as regards data protection (LOPD, The Organic Law 3/2018 of 5 December on the Protection of Personal Data and the Guarantee of Digital Rights complementary to the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data).
The participant will be identified in the records by the corresponding unique code number. The participant is to be guaranteed anonymity and is to be informed that all communication will take place between him/her and the investigator and not the sponsor of the study.

It is not expected to transmit data to third parties.

13.2 RECORD KEEPING

13.2.1 Investigator file and document retention

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

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

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

The investigator should retain these documents at least twenty-five years, according to Royal Decree 1090/2015, provided that the sponsor does not express another period.

13.2.2 Source documents and basic data

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

14. FINANCING AND INSURANCE

14.1 SOURCE OF FINANCING

This independent research has received an unrestrictional grant from Viiv Healthcare. However, this contribution does not influence on the design of the study, its development or the data analysis.

14.2 INSURANCE POLICY

In accordance with the Royal Decree 1090/2015, of 4th December, the trial sponsor has a policy of liability insurance with Zurich Insurance Company PLC Branch in Spain established in Barcelona. The sponsor shall extend this policy or another with equivalent coverage until the end of the trial. The policy will cover the damages to the people that could be set as a result of the trial by an insured amount of € 600.000,00 per participant tested to a maximum of € 6.000.000,00 per year and clinical trial. This policy also covers the responsibilities of the sponsor, the principal and his/her collaborators, as well as the hospital or site where they carry out the clinical trial.

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

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

15. PUBLICATION POLICY

The publication of the trial results shall meet the requirements set out in Article 42 of Royal Decree 1090/2015.

APPENDIX I: CASE REPORT FORM (CRF)

APPENDIX II: INVESTIGATOR'S BROCHURE

APPENDIX III: PARTICIPANT INFORMATION AND WRITTEN INFORMED CONSENT

APPENDIX IV: INSURANCE

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

APPENDIX VI: PREGNANCY NOTIFICATION FORM