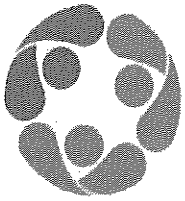
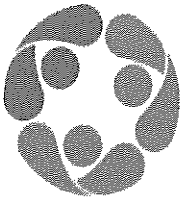


ABIVAX

Title: An Open-Label Study of the Safety, Pharmacokinetics, and Pharmacodynamics of ABX464 in HIV-1 Seronegative and Seropositive adult subjects.

NTC Number: NCT02990325

Protocol approve date: September 4th 2018



CLINICAL STUDY PROTOCOL
ABX464-005

Sponsor:	ABIVAX 5, rue de la Baume 75008 Paris FRANCE
Investigational product Name:	Not Available yet
Product code:	ABX464
Therapeutic indication:	An Open-Label Study of the Safety, Pharmacokinetics, and Pharmacodynamics of ABX464 in HIV-1 Seronegative and Seropositive Adults
EudraCT number:	2016-002797-12
Study code:	ABX464-005
Version number:	9.0
Release date:	September 4th 2018

CLINICAL STUDY PROTOCOL

Study code	ABX464-005
Investigational product code	ABX464
EudraCT number	2016-002797-12
Detailed Title	An Open-Label Study of the Safety, Pharmacokinetics, and Pharmacodynamics of ABX464 in HIV-1 Seronegative and Seropositive adult subjects.
Study Phase	Phase Ib
Investigator	**Ross D. Cranston MD FRCP** Hospital Germans Trias i Pujol, Carretera de Canyet s/n 08916 Barcelona / Spain E-mail: rdcranston@outlook.com **Bonaventura Clotet, MD, PhD.** Hospital Universitari Germans Trias i Pujol, Carretera de Canyet s/n 08916 Barcelona / Spain E-mail: bclotet@irsicaixa.es
Sponsor	**ABIVAX** 5 rue de la Baume 75008 Paris Tel: +33 (0) 1 53 83 08 41 Fax: +33 (0) 1 53 83 07 48
Date/Version	September 4th 2018 / V9.0

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Coordinating investigator	**Hospital Universitari Germans Trias i Pujol** **Dr. Ross D. Cranston** Carretera de Canyet s/n 08916 Barcelona / Spain E-mail: rdcranston@outlook.com
Statistical Analysis	**KEYRUS BIOPHARMA** 18/20 rue Clement Bayard 92300 Levallois-Perret France
Pharmacovigilance	**ABIVAX** **Dr Jean-Marc Steens** 5 rue de la Baume 75008 Paris - France
Immune & Reservoir Analyses	**irsiCaixa AIDS Research Institute** **Dr Javier Martinez-Picado** Hospital Universitari Germans Trias i Pujol Carretera de Canyet s/n 08916 Barcelona / Spain
Rectal explant infection	**irsiCaixa AIDS Research Institute** **Dr. Cecilia Cabrera** Hospital Germans Trias i Pujol, Carretera de Canyet s/n 08916 Barcelona / Spain
Rectal microbiota	**irsiCaixa AIDS Research Institute** **Dr. Roger Paredes** Hospital Germans Trias i Pujol, Carretera de Canyet s/n 08916 Barcelona / Spain
Pharmacokinetics	**ATLANBIO (Bioanalyses)** ZI de Brais 1 Rue Graham Bell BP 40309 44605 Saint-Nazaire - France
Transcriptomics and Proteomics	**ABIVAX** **Dr Sandrine Crabé** Campus CNRS Languedoc-Roussillon 1919, route de Mende 34293 MONTPELLIER CEDEX 5

INVESTIGATOR AGREEMENT PAGE

EudraCT number: 2016-002797-12

Detailed Title: An Open-Label Study of the Safety, Pharmacokinetics, and Pharmacodynamics of ABX464 in HIV-1 Seronegative and Seropositive

I have carefully read all the pages of this clinical study protocol and I agree to the following:

- To conduct the study as outlined in the protocol, any mutually agreed future protocol amendments and with all the terms and conditions set out by ABIVAX.
- Not to implement any changes in the procedures described in the protocol without the prior approval of the sponsor and prior to review and written approval by the Ethics Committee and/or Regulatory Authorities, unless instructed otherwise by the Regulatory Authorities or the wellbeing of subjects is jeopardized.
- To conduct the study in accordance with the ICH/GCP guidelines, US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations), the European Union Clinical Trials Directive 2001/20/EC, the provisions of the Helsinki Declaration, and relevant legislation in force.
- I am thoroughly aware of the study drug specifications and adverse events as described in the protocol and the current Investigator's Brochure and any other information provided by the Sponsor.
- To ensure that sub-investigator(s) and other relevant members of my staff involved in the study are fully aware of their responsibilities regarding this study and will conduct the study according to the protocol.

Investigator's Name: _____

Investigator's Signature: _____

Date: _____

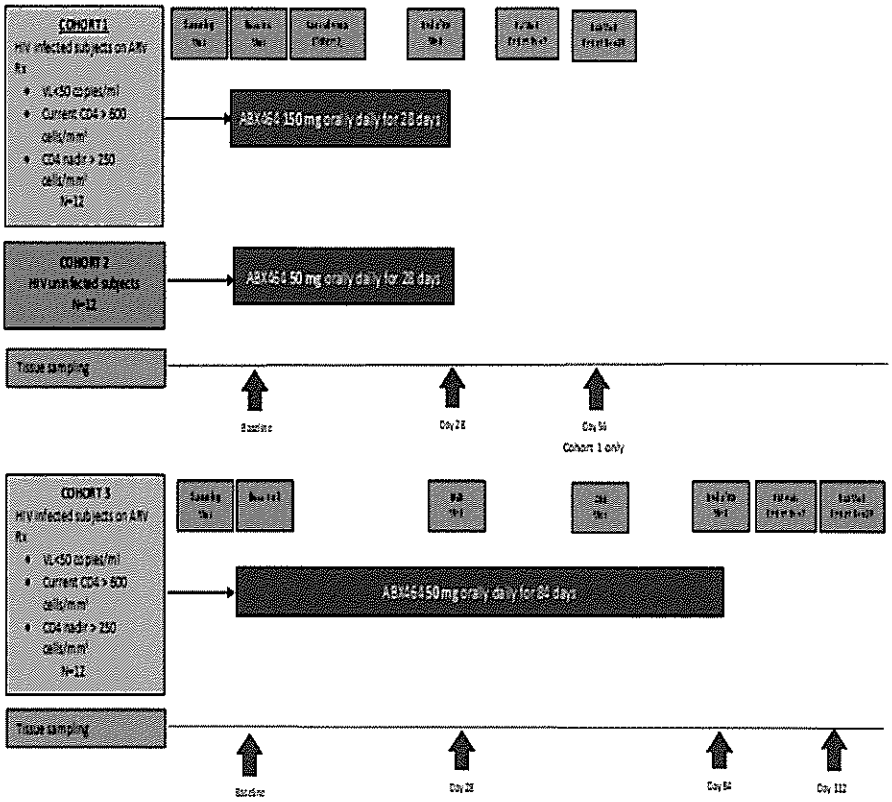
Table of Content

ABBREVIATIONS

Abbreviation or Term	Definition
AE	adverse event
ALT/SGPT	alanine aminotransferase/serum glutamic pyruvate transaminase
AST/SGOT	aspartate aminotransferase/serum glutamic oxaloacetic transaminase
AUC0-24	area under the plasma concentration-versus-time curve from zero to 24 hours
AUC0-∞	area under the plasma concentration-versus-time curve from zero to infinity
AUC0-	area under the plasma concentration-versus-time curve from time zero to the dosing interval
AUC0-t	area under the plasma concentration-versus-time curve from time zero to the time of the last quantifiable concentration
BMI	body mass index
CBC	Cap Binding Complex
CI	confidence interval
Cmax	peak plasma concentration
CRF	case report form
CTC-AE	Common Terminology Criteria for Adverse Events, version 4.0
COBI	Cobicistat
DBP	Diastolic Blood Pressure
DSMB	Data and Safety Monitoring Board
DRV	Darunavir
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
Frel	relative bioavailability
GCP	good clinical practice
GGT	gamma-glutamyl transferase
GM	geometric mean
H	hours
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human Immunodeficiency Virus
HR	heart rate
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IEC	Independent Ethics Committee
Max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
Min	minimum
mL	milliliter
mmHg	millimeters of mercury
NOAEL	No Observed Adverse Effect Level
o.d.	Once Daily
PD	pharmacodynamics
PK	pharmacokinetics
PT	preferred term
PCSA	potentially clinically significant abnormalities
QTc	heart-rate–corrected QT interval (time between the start of the Q wave and the end of the T wave in the heart's electrical cycle) using Bazett's formula
R	Accumulation ratio
RTV	Ritonavir
Rx	Treatment
SAE	serious adverse event
SBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
SOC	system organ class
TEAE	treatment emergent adverse event
tlag	interval between administration time and the sampling time preceding the first concentration above the limit of quantification
t1/2	terminal half-life
tmax	time to peak plasma concentration
Vd/F	volume of distribution
vs.	versus

SYNOPSIS

Study n°	ABX464-005	**Clinical Phase**	Ib
		Type of Study	PK/Safety Study
Study title	An Open-Label Study of the Safety, Pharmacokinetics, and Pharmacodynamics of ABX464 in HIV-1 Seronegative and Seropositive Adults		
Short title	Safety study of ABX464 in HIV-1 Seronegative and Seropositive adult subjects.		
Investigators and study centers	The study will be conducted at the Hospital Universitari Germans Trias i Pujol (Can Ruti), Badalona, Spain Collaborating centre: BCN Checkpoint, Barcelona, Spain		
Study Duration	Recruitment period: Q4 2016 – Q2 2018 Overall Study period: Q4 2016 – Q1 2019		
Investigational product			
Study Methodology	The purpose of the ABX464-005 study is to characterize the systemic and mucosal immunological sequelae associated with exposure to ABX464 and to explore selected immunological endpoints, compartmental pharmacokinetics, and pharmacodynamics. The site will screen and enroll 12 HIV-infected subjects who will receive 150 mg ABX464 orally once daily for 28 days (Cohort 1). Following completion of this cohort a further 24 subjects will be enrolled: 12 HIV-uninfected subjects will receive 50 mg ABX464 orally once daily for 28 days (Cohort 2) and 12 HIV-infected subjects (Cohort 3) who will 50 mg ABX464 orally once daily for 84 days. Following screening, eligible subjects inall cohorts will undergo a baseline visit during which blood and tissue samples will be collected. All subjects will have additional blood and tissue samples collected on Day 28 of dosing (and on Day 84 for subjects enrolled in cohort 3) and four weeks after completion of dosing (excluding cohort 2 for tissue samples). The HIV infected subjects should be receiving a stable combination antiretroviral regimen for 12 months and be fully virally suppressed (plasma viral load < 50 copies/mL) during the last 6 months prior to enrolment. Dose limiting toxicity (DLT) is defined as a grade 3 or higher adverse event as defined by the Division of AIDS table for grading the severity of adult and pediatric adverse events (including signs/symptoms, lab toxicities and/or clinical events) considered by the Data Safety Monitoring Board as probably or definitely related to study treatment. If more than 2 DLTs occur during the treatment period of the first four treated subjects, then the enrolment of additional subjects will be stopped. In addition, in case of a life threatening (grade 4) adverse reaction enrolment and treatment of ongoing subjects will be immediately discontinued. In both cases, enrolment will only be resumed upon the decisionof the sponsor if the Data Safety Monitoring Board can conclude that the causality of the event was unrelated or unlikely related to study treatment.		
Study Design			

Study n° ABX464-005	**Clinical Phase** Ib **Type of Study** PK/Safety Study
	
Study Objectives	**Primary objective:** ▪ To evaluate the distribution of ABX464 and its main metabolite (N-Glu) in various compartments in HIV-1 Seronegative and Seropositive adult subjects. **Secondary objectives:** ▪ To evaluate the safety of ABX464 administered once daily at one dose, alone in HIV-uninfected subjects and in combination with ARTs in HIV-1 infected adult subjects; ▪ To evaluate the systemic and mucosal immunological response to ABX464 in HIV-uninfected and or in HIV-infected adult subjects; ▪ To evaluate the effect of ABX464 on the HIV reservoir cells in HIV-infected adult subjects; ▪ To evaluate the effect of ABX464 on the control of the viral load and the CD4+/CD8+ in HIV-infected adult subjects; ▪ To evaluate the effect of ABX464 on the microbiome in HIV-1 Seronegative and Seropositive adult subjects; ▪ To evaluate the effect of ABX464 on the rectal transcriptome & proteome in HIV-1 Seronegative and Seropositive adult subjects.
Study Endpoints	**Primary endpoint:** Main pharmacokinetic parameters (Cmax, AUC) after first dose of ABX464 (Day 1): ▪ Cohort 1: at Day 28 and at Day 56 for plasma, PBMC and rectal tissue. ▪ Cohort 2: at Day 28 and at Day 56 for plasma and PBMC. Rectal tissue only at Day 28. ▪ Cohort 3: at Day 28, Day 84 and Day 112 for plasma, PBMC and rectal tissue. ▪

Study n° ABX464-005	**Clinical Phase** Ib **Type of Study** PK/Safety Study
	Secondary endpoints: ▪ Number and grade of treatment-emergent adverse events, including treatment-emergent serious adverse events, adverse events leading to investigational product discontinuation, during the study; ▪ Change in GALT HIV reservoirs in rectal tissue from Baseline to Day 28 and Day 56 in HIV-infected subjects (Cohort 1) and from Baseline to Day 28, Day 84 and Day 112 (Cohort 3); ▪ Change in total viral DNA in the reservoir cells from Baseline to Day 28 and Day 56 in HIV-infected subjects (Cohort 1) and from Baseline to Day 28, Day 84 and Day 112 (Cohort 3); ▪ Change in inflammatory markers in plasma from Baseline to Day 28 and Day 56 in subjects enrolled in Cohort 1 & 2 and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3; ▪ Change in immunephenotype and assessment of cell-activation markers from Baseline in PBMCs and MMC, using an 11-colours panel, including: CD3, CD8, CD4, CD45RA, CCR7, CD27, CD28, CD38, HLA-DR, PD-1, viability to Day 28 and Day 56 in subjects enrolled in Cohort 1 & 2 and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3; ▪ Change in HIV-1 RNA, CD4+ and CD8+ counts from Baseline to Day 28 and Day 56 in HIV-infected subjects (Cohort 1) and from Baseline to Day 28, Day 84 and Day 112 (Cohort 3); ▪ Change of rectal microbiota using taxonomic markers from Baseline to Day 28 and Day 56 in subjects enrolled in Cohort 1, change of rectal microbiota using taxonomic markers from Baseline to Day 28 in subjects enrolled in Cohort 2 and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3; ▪ Change in the transcriptome & proteome from Baseline to Day 28 and Day 56 in subjects enrolled in Cohort 1, change in the transcriptome & proteome from Baseline to Day 28 in Cohort 2 and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3.

Main Selection Criteria	**Inclusion criteria:**
	Subjects will be eligible for inclusion in this study only if ALL of the following criteria apply:
	• Males aged 18-65 years;
	• Subjects with hematological and biochemical laboratory parameters as follows and within 3 weeks of baseline:
	  • Hemoglobin > 9.0 g/dL;
	  • Absolute neutrophil count ≥ 750/mm^3;
	  • Platelets ≥ 100,000/mm^3;
	  • Total serum creatinine ≤ 1.3 x ULN (upper limit of normal);
	  • Creatinine clearance > 50 mL/min by the Cockcroft-Gault equation within 60 days of entry;
	  • Total serum bilirubin < 1.5 x ULN;
	  • Alkaline phosphatase, AST (SGOT) and ALT (SGPT) < 1.5 x ULN;
	  • Serum lipase less than or equal to 2.0 x ULN;
	• Subjects should be able and willing to comply with study visits and procedures as per protocol;
	• Subjects should understand, sign and date the written voluntary informed consent form at the screening visit prior to any protocol-specific procedures being performed;
	• Subjects must agree to use in addition to the condom, a second highly effective method (one for the subject and one for the partner) of contraception (defined as per the CTFG Guidance) during the study and for 3 months after end of study or early termination.

Study n° ABX464-005	**Clinical Phase** Ib **Type of Study** PK/Safety Study
	For HIV positive Subjects • Subjects with a positive HIV-1 serology at any time before the study entry. • Subjects treated for at least 12 months prior to screening with Dolutegravir or Raltegravir combined with either Tenofovir + Emtricitabine (TDF/FTC) or Abacavir + Lamivudine (ABC/3TC); • Subjects with HIV plasma viral load ≤ 50 copies/mL during the 6 months prior to screening with a maximum of 2 blips ≤ 1000 copies during this period; • Subjects' CD4+ T cells count ≥ 250 cells per mm^3 at any time since diagnosis; • Subjects with CD4+ T cells count ≥ 600 cells per mm^3 at screening.
Exclusion criteria	**The following criteria should be checked at the time of screening. If ANY exclusion criterion applies, the individual will not be included in the study:** • History of allergic disease, anaphylaxis or reactions likely to be triggered or exacerbated by any component of investigational products; • Acute or chronic infectious disease other than HIV infection (include but not limited to viral hepatitis such as hepatitis B, hepatitis C, active tuberculosis, active syphilis [i.e. currently treated]). • Acute, chronic or history of clinically relevant pulmonary, cardiovascular, gastrointestinal, hepatic, pancreatic or renal functional abnormality, encephalopathy, neuropathy or unstable CNS pathology, angina or cardiac arrhythmias, or any other clinically significant medical problems as determined by physical examination and/or laboratory screening tests and/or medical history; • Severe hepatic impairment; • Acute, chronic or history of immunodeficiency or autoimmune disease other than HIV infection; • Unstable asthma (defined as sudden acute attacks occurring in less than three hours without an obvious trigger, hospitalization for asthma in the last two years); food or wine induced asthma; • History of malignancy unless there has been surgical excision that is considered to have achieved cure; • Active malignancy that may require chemotherapy or radiation therapy; • Seizure disorder or any history of prior seizure; • Serious illness requiring systemic treatment and/or hospitalization within 7 days prior to baseline; • Active drug or alcohol abuse or dependence; • Subjects reporting a use of a prohibited medication; • History of significant gastrointestinal bleeding in the opinion of the investigator; • Abnormalities of the colorectal mucosa, or significant symptom(s), which in the opinion of the clinician represents a contraindication to protocol-required biopsies (including but not limited to presence of any unresolved injury, infectious or inflammatory condition; • Any condition, which in the opinion of the investigator, could compromise the subject's safety or adherence to the study protocol.
Medications	**Mandatory treatment:** • HIV infected Subjects must be on a stable antiretroviral regimen for 12 months and have a plasma viral load of < 50 copies/mL for at least 6 months prior to enrolment into the study and be willing to continue their antiretroviral regimen for the duration of the ABX464-005 study All subjects enrolled in Cohort 1 will receive ABX464 150 mg dose from Day 1 to Day 28 while subjects enrolled in Cohorts 2&3 will receive ABX464 50 mg dose respectively from Day 0 to Day 28 and from Day 0 to Day 84.

Study n° ABX464-005	**Clinical Phase** Ib **Type of Study** PK/Safety Study
	Prohibited prior or Concomitant treatments: Following medications are prohibited potentially prior to baseline and during the course of the study. • Medications prohibited within 3 months prior to baseline and during the whole course of the study o Immunomodulators or immunosuppressive drugs (such as IL-2, IL-7, intravenous immunoglobulin-IVIG, IFNα, or Imiquimod); o Any investigational or non-registered product. • Medications prohibited within 1 month prior to baseline and during the whole course of the study o HIV uninfected Subjects should not be taking antiretroviral pre-exposure prophylaxis (PrEP) or have completed a course of antiretroviral post exposure prophylaxis (PEP) within one month of the enrollment visit. • Medications prohibited within one week prior to baseline and during the whole course of the study. o Heparin, including Lovenox® (enoxaparin sodium); o Warfarin; o Plavix® (clopidogrel bisulfate); o Any other drugs that are associated with increased likelihood of bleeding following mucosal biopsy (e.g., daily high dose aspirin (> than 81 mg), NSAIDs, or Pradaxa®); o Rectally administered medications (including over-the-counter products); o Multiple dose dexamethasone. • Medications prohibited during the whole course of the study o Drugs that could interact either with ABX464 should be avoided especially the CYP1A2 substrates or inducers (cf. Appendix#2). The following CYP1A2 substrates or inducers with a narrow therapeutic margin are prohibited during the whole course of the study (clozapine, theophylline, ropinirole, warfarin and methadone).
Premature trial discontinuation	Subject's premature trial discontinuation could occur for the following reasons: • Investigator's decision; • An Adverse Event or an intercurrent condition that preclude continuation of treatment; o Specifically, an increase ≥ 2.0 x ULN in liver transaminases (AST/SGOT and/or ALT/SGPT), in alkaline phosphatase or in total bilirubin should be considered a treatment discontinuation criterion. • Major protocol violation; • Subject's decision; • Withdrawal of consent.
Data Safety Monitoring Board (DSMB)	A Data Safety Monitoring Board with expertise and experience in the management in HIV will review the safety of the trial, every 4 patients are recruited, in order to recommend, if appropriate, the continuation of the study.
Number of subjects planned	No formal sample size calculations were performed. 36 subjects (24 HIV infected and 12 HIV uninfected subjects) are deemed sufficient to fulfil the study objectives.
Statistical Methods	**Safety:** Analysis of safety will be performed on the safety data set consisting in all subjects who received at least one dose of ABX464 in the study. The assessment of safety will be based on the frequency of adverse events (with and without regard to causality) graded according to the Division of AIDS table for grading the severity of adult and pediatric adverse events (Version 2.0 November 2014) and also, the review of individual values for clinical laboratory data, vital signs and ECG focusing on the detection of abnormal values and PCSAs [potentially clinically significant abnormalities (PCSAs) determined upon investigator

Study n° ABX464-005	**Clinical Phase** Ib **Type of Study** PK/Safety Study

considerations].

Adverse events will be tabulated (counts and percents) by group and dose. All adverse events will be listed and the data will be tabulated by body system/organ class. Adverse event tabulations will include all treatment emergent adverse events, which will be further classified by severity, and relationship to treatment and dose level.

Clinical laboratory parameters, vital signs, ECG will be summarized by using descriptive statistics (n, mean, SD, SEM, median, minimum and maximum). Number of subjects with at least one abnormal values will be tabulated (counts and percents) for each parameter in summary shift tables, by group and dose.

Efficacy:

All efficacy endpoints will be summarized descriptively. Virological control, HIV reservoirs, etc. will be analyzed before versus after treatment

Interim analysis:

An interim analysis will be produced when the first 12 HIV-positive subjects (Cohort 1) reach day 56 of follow-up. The interim analysis will include the following data:

- Safety and clinical parameters;
- HIV-1 RNA, CD4+ and CD8+ counts;
- Hematology and biochemistry parameters;
- Pharmacokinetic parameters in plasma, PBMCs and rectal tissue;
- Total HIV-1 DNA reservoir in PBMC and rectal tissue.

Pharmacokinetics:

ABX464 has a very short $t_{1/2}$ (1 to 2 h) and generally no drug could be quantified after 10h post-dose, so steady-state is virtually reached at the second administration.

NGlcABX464 (main ABX464 metabolite) has a $t_{1/2}$ of 90 to 110 h, meaning that steady-state is reached after 19 to 22 days of administration.

Pharmacokinetic parameters will be assessed in plasma, in PBMC and in rectal mucosa.

Intensive Pharmacokinetic runs will be conducted after the first dose of ABX464 (Day 1), after the final dose (Day 28/Day 84) and at day 56/112 for plasma, PBMC and rectal tissue (Rectal tissue only cohorts 1 and 3 at day 56). Samples will be collected according to the schedule below:

	Day 1	Day 28 (Cohort 1&2) Day 28 & 84 (Cohort 3)	Day 35 (Cohort 1&2) Day 91 (Cohort 3)	Day 56 (Cohort 1&2) Day 112 (Cohort 3)
HIV infected Subjects				
Plasma	0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours	0h, 0.5h, 1h, 1.5h,2h, 4h, 6h and 8 hours	Trough level	Trough level
PBMC	0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours	0h, 0.5h, 1h, 1.5h,2h, 4h, 6h and 8 hours	Trough level	Trough level
Rectal tissue	Rectal biopsies	Rectal biopsies		Rectal biopsies
HIV non-infected Subjects				
Plasma	0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours	0h, 0.5h, 1h, 1.5h,2h, 4h, 6h and 8 hours	Trough level	Trough level
PBMC	0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours	0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours	Trough level	Trough level
Rectal tissue	Rectal biopsies	Rectal biopsies		

Immunological assays

Tissue Immunological evaluation:

Up to 20 rectal biopsies will be collected 15 cm from the anal verge and will be used to:

- Determine whether ABX464 alters gut associated lymphoid tissue (GALT) immunology;
- Assess whether exposure to ABX464 alters HIV GALT reservoirs in the HIV-positive subjects or reduce the incidence or magnitude of HIV infection in rectal tissue from HIV-negative subjects.

GALT HIV reservoirs (RNA and DNA) will be characterized using previously published techniques.

Study n° ABX464-005	**Clinical Phase** Ib **Type of Study** PK/Safety Study
	Ex vivo / in vitro rectal explant challenge will also be performed using previously published techniques in which rectal biopsies are challenged with high titers of HIV-1BaL and cumulative, weight adjusted HIV-1 p24 antigen is quantified in the explantsupernatant. **Effect on inflammation and/or activation:** Potential changes related to the investigational product on the inflammation and activation status of the subjects will be assessed throughout: • Quantification of systemic inflammatory markers in plasma Immunephenotype and assessment of cell-activation markers in PBMCs and MMC, using an 11-colours panel, including: CD3, CD8, CD4, CD45RA, CCR7, CD27, CD28, CD38, HLA-DR, PD-1, viability.
Microbiome, transcriptome & proteome	Additional studies of rectal microbiome, mucosal transcriptome and proteome will provide a comprehensive assessment of mucosal biology before and after exposure to ABX464 in HIV-1 Seronegative and Seropositive adult subjects.

1. INTRODUCTION AND STUDY RATIONALE

1.1. HIV Infection

1.1.1. Disease

1.1.2. Management of subjects

1.2. ABX464 rationale

1.2.1. Investigational treatment description

1.2.2. Investigational product description

1.2.3. Investigational product mode of action

1.2.4. Rationale for the development of ABX464

1.2.5. Preclinical data of ABX464

1.2.5.1. Non-clinical background information

1.2.6. Previous clinical experience with ABX464

1.3. Rationale for the clinical study and study design

1.3.1.

2. STUDY OBJECTIVES

2.1. Primary Objective

- To evaluate the distribution of ABX464 and its main metabolite (ABX464-N-glucuronide) in various compartments in HIV-1 Seronegative and Seropositive adult subjects.

2.2. Secondary Objective

- To evaluate the safety of ABX464 administered once daily at one dose, alone in HIV-uninfected subjects and in combination with ARTs in HIV-1 infected adult subjects;
- To evaluate the systemic and mucosal immunological response to ABX464 in HIV-uninfected and or in HIV-infected adult subjects;
- To evaluate the effect of ABX464 on the HIV reservoir cells in HIV-infected adult subjects;
- To evaluate the effect of ABX464 on the control of the viral load and the CD4+/CD8+ in HIV-infected adult subjects;
- To evaluate the effect of ABX464 on the microbiome in HIV-1 Seronegative and Seropositive adult subjects;
- To evaluate the effect of ABX464 on the rectal transcriptome & proteome in HIV-1 Seronegative and Seropositive adult subjects.

3. INVESTIGATIONAL PLAN

3.1. Study design

3.1.1. Design and methodology

The ABX464-005 study is a Phase Ib, sequential cohort open label trial designed to provide additional safety and compartmental PK data on ABX464.

The study will also generate exploratory immunological and virological data that combined with the compartmental PK data will help further characterize the mechanism of action of ABX464.

The study will enroll both HIV-infected (N=24) and HIV-uninfected (N=12) study subjects in three distinct cohorts:

- Cohort 1: 12 HIV-infected subjects treated with ABX464 150 mg o.d. for 28 days
- Cohort 2: 12 HIV-uninfected subjects treated with ABX464 50 mg o.d. for 28 days
- Cohort 3: 12 HIV-infected subjects treated with ABX464 50 mg o.d. for 84 days

HIV-infected subjects will add the investigational product to their current antiretroviral regimen whereas the HIV-uninfected subjects will only receive the investigational product.

Cohort 1 will consist of 12 HIV infected subjects. After full enrollment and an interim analysis (including PK and immunological markers) of Cohort 1, Cohort 2 and 3 will begin enrollment and will consist of both HIV infected (n=12) and HIV uninfected (n=12) subjects.

Subjects who successfully complete a screening visit (Visit 1) will advance to a Baseline visit (Visit 2; Day 1). During this visit all subjects will undergo a physical examination and have blood and rectal tissue samples collected.

The rectal tissue samples will be collected before initiating oral dosing with the investigational product (150 mg or 50 mg of ABX464).

After receiving the first dose of ABX464, additional blood PK samples will be collected at 0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours.

All subjects will receive the investigational product once daily for 28 days (Cohorts 1 &2) and for 84 days (Cohort 3).

At Day 28 for subjects enrolled in cohort 3 and at the end of the treatment period (End of Rx Visit; Day 28 for Cohorts 1&2 and Day 84 for Cohort 3) subjects will undergo a physical examination and have blood and rectal tissue collected. PK blood samples will be collected at 0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours.

HIV infected subjects will continue with their antiretroviral regimen i.e. there will be no antiretroviral treatment interruption in this study.

Additional follow-up visit will occur 1 week (Day 35 / Day 91) and 1 month (End of study visit; Day 56 / Day 112) after stopping study product. During these two visits the subjects will undergo a physical examination and have blood (through PK) and rectal tissue samples collected at End of Study (EoS) visit only (Rectal tissue only for Cohorts 1 and 3).

The blood samples collected in this study will be used to characterize general safety, and in HIV infected subjects to monitor viral load and CD4+ counts, to quantify PK exposure to ABX464 and its metabolites, and blood samples collected will be used to assess systemic immunological effect of ABX464 and alteration of HIV reservoir.

Rectal biopsies will be collected 15 cm from the anal verge and will be used to quantify PK exposure in rectal tissue, to determine whether ABX464 alters gut associated lymphoid tissue (GALT) immunology, and to assess whether exposure to ABX464 alters HIV GALT reservoirs in the HIV-infected subjects or reduce the incidence or magnitude of HIV infection in rectal tissue from HIV-uninfected subjects. GALT HIV reservoirs (RNA and DNA) will be characterized using previously published techniques (4). Ex vivo / in vitro rectal explant challenge will also be performed using previously published techniques in which rectal biopsies are challenged with high titers of HIV-1_{BaL} and cumulative, weight adjusted HIV-1 p24 antigen is quantified in the explant supernatant (2). Additional studies of mucosal proteome, transcriptome, and microbiome will provide a comprehensive assessment of mucosal biology before and after exposure to ABX464.

The ABX464-005 study design is summarized below.

3.1.2. Dose limiting toxicity (DLT)

A dose limiting toxicity (DLT) is defined as a grade 3 or higher adverse event as defined by the Division of AIDS Toxicity Table for Severe Adult and Pediatric Adverse Events (including signs/symptoms, lab toxicities and/or clinical events) considered by a safety review board as probably or definitely related to study treatment. The first four subjects will be enrolled first and followed up for at least 2 weeks of treatment. If more than 2 DLTs occur of the first four treated subjects during ongoing study participation, then the enrolment of additional subjects will be stopped.

In addition, in case of a life threatening (grade 4) adverse reaction enrolment and treatment of ongoing subjects will be immediatelydiscontinued.

In both cases, enrolment will only be resumed upon the decision of the sponsor if the Data Safety Monitoring Board can conclude that the causality of the event was unrelated or unlikely related to study treatment.

3.1.3. Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB), with expertise and experience in the pathology, and without direct involvement in the conduct of the trial, will be set up specifically to guarantee effective protection of subjects, insure the ethical conduct of the trial, benefit/risk ratio of the trial, and to ensure the independent review of the scientific results during the trial (every 4 patients recruited) and at the end of the trial.

The DSMB may recommend the early termination of the trial at any time if an unacceptable toxicity occurs.

The DSMB has only a consultative role; it will inform the sponsor who will decide whether the DSMB recommendation will be followed. A DSMB charter must be available upon submission of the trial (initial protocol) to the respective competent authorities.

3.2. Duration of study participation

From a subject standpoint, the study participation is defined as

- Two to four weeks of screening period;
- Four weeks (Cohorts 1 & 2) or twelve weeks (Cohort 3) as the treatment period (ABX464);
- Follow-up visits at 1 week and 4 weeks after cessation of study medication.

Overall, the minimal study duration will be approximately 70 days and up to 140 days depending on the subject Cohort.

4. STUDY POPULATION

4.1. Number of Subjects/Centers

36 Subjects will be enrolled in this study. These subjects will be enrolled at one site in Spain.

4.2. Eligibility Criteria

4.2.1. Inclusion Criteria

Subjects will be eligible for inclusion in this study only if ALL of the following criteria apply:

- Males aged 18-65 years;
- Subjects with hematological and biochemical laboratory parameters as follows and within 3 weeks of baseline:
  - Hemoglobin > 9.0 g/dL;
  - Absolute neutrophil count ≥ 750/mm^3;
  - Platelets ≥ 100,000/mm^3;
  - Total serum creatinine ≤ 1.3 x ULN (upper limit of normal);
  - Creatinine clearance > 50 mL/min by the Cockcroft-Gault equation within 60 days of entry;
  - Total serum bilirubin < 1.5 x ULN;
  - Alkaline phosphatase, AST (SGOT) and ALT (SGPT) < 1.5 x ULN;
  - Serum lipase less than or equal to 2.0 x ULN;
- Subjects should be able and willing to comply with study visits and procedures as per protocol;
- Subjects should understand, sign and date the written voluntary informed consent form at the screening visit prior to any protocol-specific procedures being performed;
- Subjects must agree to use in addition to the condom, a second highly effective method (one for the subject and one for the partner) of contraception (defined as per the CTFG Guidance) during the study and for 3 months after end of study or early termination.

For HIV infected Subjects

- Subjects with a positive HIV-1 serology at any time before the study entry.
- Subjects treated for at least 12 months prior to screening with Dolutegravir or Raltegravir combined with either Tenofovir + Emtricitabine (TDF/FTC) or Abacavir + Lamivudine (ABC/3TC);
- Subjects with HIV plasma viral load ≤ 50 copies/mL during the 6 months prior to screening with a maximum of 2 blips ≤ 1000 copies during this period;
- Subjects' CD4+ T cells count ≥ 250 cells per mm3 at any time since diagnosis;
- Subjects with CD4+ T cells count ≥ 600 cells per mm3 at screening.

4.2.2. Exclusion Criteria

The following criteria should be checked at the time of screening. If ANY exclusion criterion applies, the individual will not be included in the study:

- History of allergic disease, anaphylaxis or reactions likely to be triggered or exacerbated by any component of the vaccine such as lactose;
- Acute or chronic infectious disease other than HIV infection (include but not limited to viral hepatitis such as hepatitis B, hepatitis C, active tuberculosis, active syphilis [i.e. currently untreated]).
- Acute, chronic or history of clinically relevant pulmonary, cardiovascular, gastrointestinal, hepatic, pancreatic or renal functional abnormality, encephalopathy, neuropathy or unstable CNS pathology, angina or cardiac arrhythmias, or any other clinically significant medical problems as determined by physical examination and/or laboratory screening tests and/or medical history;

- Severe hepatic impairment;
- Acute, chronic or history of immunodeficiency or autoimmune disease other than HIV infection;
- Unstable asthma (defined as sudden acute attacks occurring in less than three hours without an obvious trigger, hospitalization for asthma in the last two years); food or wine induced asthma;
- History of malignancy unless there has been surgical excision that is considered to have achieved cure;
- Active malignancy that may require chemotherapy or radiation therapy;
- Seizure disorder or any history of prior seizure;
- Serious illness requiring systemic treatment and/or hospitalization within 7 days prior to baseline;
- Active drug or alcohol abuse or dependence;
- Subjects reporting a use of a prohibited medication;
- History of significant gastrointestinal bleeding in the opinion of the investigator;
- Abnormalities of the colorectal mucosa, or significant symptom(s), which in the opinion of the clinician represents a contraindication to protocol-required biopsies (including but not limited to presence of any unresolved injury, infectious or inflammatory condition;
- Any condition, which in the opinion of the investigator, could compromise the subject's safety or adherence to the study protocol.

5. STUDY ASSESSMENTS AND PROCEDURES

5.1. Study Flow Chart

A detailed study flow chart (with all assessments) is displayed below.

Study procedures	Screening Visit	Baseline Visit	Control Visits	Day 28/D56	End of Study treatment visit	FU visit	EoS visit Cohort 1&3	EoS visit Cohort 2
	Screening 21 ± 7 days	Baseline	Cohort 2 only ± 2 days	Cohort 3 only ± 2 days	28-Day (Cohort1&2), 84-Day (Cohort3) ± 2 days	FU visit 1-week post completion ± 2 days	FU visit 4 weeks post completion ± 7days	FU visit 4 weeks post completion ± 7days
		Day 1	**Day 7, 14, 21**	**Day 28/D56**	**Day 28 / Day 84**	**Day 35 / Day 91**	**Day 56 / Day 112**	**Day 56**
Informed Consent	X							
Inclusion/Exclusion Criteria		X						
ECG	X	X			X		X	X
Adverse Event reporting	X	X	X	X	X	X	X	X
Concomitant medication checks and compliance	X	X	X	X	X	X	X	X
Physical examination and vital signs	X	X	X	X	X	X	X	X
Hematology & Biochemistry	X	X		X	X	X	X	X
Syphilis, HBV/HCV serology	X							
HIV-1 antibody serology #	X				X		X	X
Urine and Rectal GC/CT (NAAT)	X						X	X
INR & PT	X	Δ		Δ	Δ	Δ	Δ	Δ
HIV-1 viral load $	X	X		X*	X	X	X	X
CD4+ and CD8+ counts $	X	X		X*	X	X	X	X
Pharmacokinetics: Plasma and PBMC		X 0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8h		X* 0h, 0.5, 1, 1.5, 2, 4, 6 and 8h	X 0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8h	X Trough	X Trough	X Trough
Flexible sigmoidoscopy for Rectal Biopsy		X		X*	X		X	
- *Explant infection #*		X		X*	X		X	
- *MMC flow cytometry*		X		X*	X		X	
- *Rectal microbiome*		X		X*	X		X	
- *Rectal proteomics*		X		X*	X		X	
- *Rectal transcriptomics*		X		X*	X		X	
- *Rectal tissue pharmacokinetics*		X		X*	X		X	
- *Rectal HIV Reservoir $*		X		X*	X		X	
Blood samples for Immunology & Molecular assays		X		X*	X		X	X
- *PBMC flow cytometry*		X		X*	X		X	X
- *Plasma SCA $*		X		X*	X		X	X
- *Plasma Inflammatory markers*		X		X*	X		X	X
Blood samples for HIV reservoir assays $		X		X	X		X	X
- *PBMC HIV Reservoir $*		X		X	X		X	X

CT; Chlamydia trachomatis, GC; Neisseria gonorrhea, HBV; hepatitis B virus, HCV; hepatitis C virus, INR; international normalized ratio, NAAT; nucleic acid amplification testing, MMC; mucosal mononuclear

cell, PBMC; peripheral blood mononuclear cell, PT; prothrombin time, SCA; single copy assay, Δ; if clinically indicated; $ HIV-1 infected subjects only; # HIV uninfected only; *only at D28 visit.

5.2. Study Endpoints

- Primary endpoint:
  Main pharmacokinetic parameters (Cmax, AUC) after first dose of ABX464 (Day 1):
  - Cohort 1: at Day 28 and at Day 56 for plasma, PBMC and rectal tissue.
  - Cohort 2: at Day 28 and at Day 56 for plasma and PBMC. Rectal tissue only at Day 28.
  - Cohort 3: at Day 28, Day 84 and Day 112 for plasma, PBMC and rectal tissue.
  -
- Secondary endpoints:
  - Number and grade of treatment-emergent adverse events, including treatment-emergent serious adverse events, adverse events leading to investigational product discontinuation, during the study;
  - Change in GALT HIV reservoirs in rectal tissue from Baseline to Day 28 and Day 56 in HIV-infected subjects (Cohort 1) and from Baseline to Day 28, Day 84 and Day 112 (Cohort 3);
  - Change in total viral DNA in the reservoir cells from Baseline to Day 28 and Day 56 in HIV-infected subjects (Cohort 1) and from Baseline to Day 28, Day 84 and Day 112 (Cohort 3);
  - Change in inflammatory markers in plasma from Baseline to Day 28 and Day 56 in subjects enrolled in Cohort 1 & 2 and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3;
  - Change in immunephenotype and assessment of cell-activation markers from Baseline in PBMCs and MMC, using an 11-colours panel, including: CD3, CD8, CD4, CD45RA, CCR7, CD27, CD28, CD38, HLA-DR, PD-1, viability to Day 28 and Day 56 in subjects enrolled in Cohort 1 & 2 and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3;
  - Change in HIV-1 RNA, CD4+ and CD8+ counts from Baseline to Day 28 and Day 56 in HIV-infected subjects (Cohort 1) and from Baseline to Day 28, Day 84 and Day 112 (Cohort 3);
  - Change of rectal microbiota using taxonomic markers from Baseline to Day 28 and Day 56 in subjects enrolled in Cohort 1, **change of rectal microbiota using taxonomic markers from Baseline to Day 28 in subjects enrolled in Cohort 2** and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3;
  - Change in the transcriptome & proteome from Baseline to Day 28 and Day 56 in subjects enrolled in Cohort 1, **change in the transcriptome & proteome from Baseline to Day 28 in Cohort 2** and to Day 28, Day 84 and Day 112 in subjects enrolled in Cohort 3.

5.3. Study conduct

It is the investigator's responsibility to ensure that all the assessments are carried out during each visit and that the intervals between visits/follow-ups are adhered to.

5.3.1. Screening Visit (21 ± 7 days prior tobaseline)

The subjects will be informed about the general aspects of the study and will sign the screening informed consent form. The subject's number will be allocated once the subjects will be created in the eCRF. Only when consent has been given may further study procedures be carried out. During the screening phase, the following assessments will be performed:

- Signed informed consent form;
- Demographic data: date of birth;ethnicity;
- Medical history
- Physical examination and vital signs;
- Hematology (incl. INR andPT);
- Biochemistry;

- Serology (HIV, HBV, HCV, and syphilis) & Urine and Rectal GC/CT NAAT;
- Viral Load, CD4/CD8 count (HIV infected Subjects);
- 12 lead ECG;
- Concomitant medications.

5.3.2. Baseline Visit (Day 1)

- Physical examination and vital signs;
- Microbiome specimen collection (swabs);
- Hematology (incl. INR and PT if clinically indicated);
- Biochemistry;
- 12 lead ECG;
- Viral Load, CD4/CD8 count (HIV infected Subjects);
- Blood collection for HIV reservoir assessment (HIV infected Subjects) and immuno-evaluation;
- Flexible sigmoidoscopy with collection of rectal biopsies;
- Dispense study treatment to subject and instruct how to take them
- Perform PK blood samples (0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours);
- Adverse event assessment.

5.3.3. Control Visits 3,4, & 5 (Days 7, 14, & 21)- Cohort 2

To be performed only in HIV-uninfected subjects:

- Physical examination and vital signs;
- Check Study treatment compliance;
- Adverse event assessment.

5.3.4. Day 28 Visit (± 2 days)- Cohort 3

To be performed only in subjects enrolled in the Cohort 3:

- Physical examination and vital signs;
- Microbiome specimen collection (swabs);
- Hematology (incl. INR and PT if clinically indicated);
- Biochemistry;
- Viral Load, CD4/CD8 count;
- Blood collection for HIV reservoir assessment and immuno-evaluation;
- Flexible sigmoidoscopy with collection of rectal biopsies;
- Perform PK blood samples (0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours);
- Check Study treatment compliance;
- Adverse event assessment.

5.3.5. Day 56 Visit (± 2 days)- Cohort 3

To be performed only in subjects enrolled in the Cohort 3:

- Physical examination and vital signs;
- Hematology and Biochemistry;

- Blood collection for HIV reservoir assessment and immuno-evaluation;
- Check Study treatment compliance;
- Adverse event assessment.

5.3.6. End of study treatment visit (D28 for cohort 1&2 or D84 for cohort 3; ±2 days)

- Physical examination and vital signs;
- Microbiome specimen collection (swabs);
- Hematology (incl. INR and PT if clinically indicated);
- Biochemistry;
- 12 lead ECG;
- Viral Load, CD4/CD8 count (HIV infected Subjects);
- Blood collection for HIV reservoir assessment (HIV infected Subjects) and immuno-evaluation;
- Flexible sigmoidoscopy with collection of rectal biopsies;
- Perform PK blood samples (0h, 0.5h, 1h, 1.5h, 2h, 4h, 6h and 8 hours);
- Check Study treatment compliance;
- Adverse event assessment.

5.3.7. Follow-up Visit (D35 for cohort 1&2 or D91 for cohort 3; ± 2 days)

- Physical examination and vital signs;
- Hematology (incl. INR and PT if clinically indicated);
- Biochemistry;
- Viral Load, CD4/CD8 count (HIV infected Subjects);
- Perform PK blood sample (at 9AM ± 1 hour);
- Adverse event assessment.

5.3.8. End of Study Visit (D56 for cohort 1&2 or D112 for cohort 3; ± 7 days)

- Physical examination and vital signs;
- Microbiome specimen collection (swabs);
- Hematology (incl. INR and PT if clinically indicated);
- Biochemistry;
- Urine GC/CT NAAT & Rectal GC/CT NAAT;
- HIV serology (HIV uninfected participants)
- 12 lead ECG;
- Viral Load, CD4/CD8 count (HIV infected Subjects);
- Blood collection for HIV reservoir assessment (HIV infected Subjects) and immuno-evaluation;
- Flexible sigmoidoscopy with collection of rectal biopsies (Cohort 1 and 3 only)
- Perform PK blood sample (at 9AM ± 1 hour);
- Adverse event assessment.

5.4. Detail of the study assessments

5.4.1. Physical Examination and Vital Signs

A routine physical examination (including body weight) will be done at each study visit. Physical examination at baseline will cover eyes, ears, nose, throat, lungs/thorax, heart/cardiovascular system, abdomen, skin and mucosae, nervous system, lymph nodes, musculoskeletal system, and, if applicable, others. At follow up, physical exams will be symptom directed. Any new clinically relevant finding compared to baseline must be documented as adverse event.

Measurements of vital signs will be done at each visit (Blood pressure, Heart Rate, Body temperature). The subject should rest for at least 15 minutes prior to measurements. The measurements can be performed either in sitting or supine position of the subject. The right or left arm may be used. However, the position and the arm used for measurement should be kept constant throughout the trial for an individual subject.

The investigator should ensure that each parameter outside the normal range is assessed for clinical significance. For any deviation assessed clinically significant, the investigator has to document the change as an AE in the CRF.

In addition, it is at the discretion of the investigator to document any change or trend over time in vital signs as an AE if he considers the change to be clinically significant, even if the absolute value is within the alert limit or reference range.

5.4.2. ECG

Electrocardiograms have to be done at all study visits. A 12-lead ECG with recordings of at least 6 action potentials in lead II (paper speed 25mm/s, amplitude 10mm/mV) has to be done in a resting position. Prior to the recording the subject should be at rest for at least five minutes. Resting ECG should be performed before any examinations.

The ECG printout will be reviewed by the investigator and a signed and dated copy of the ECG will be attached to the medical file. The original ECG printouts are considered as source data and should be stored at site. In case thermal paper is used, a copy of the original ECG must be kept as well. All abnormal findings must be documented in the CRF. Any clinically relevant findings compared to Visit V0 must be documented as adverse events.

5.4.3. Hematology and biochemistry

For hematology and biochemistry local laboratories will be used. All laboratory results and reference ranges for safety parameters must be entered in the eCRF. Each laboratory value that is outside of the institution's normal range will be identified. The investigator will be responsible for assessing the clinical significance of laboratory abnormalities. If the investigator is uncertain about the clinical significance of a laboratory abnormality, he/she will consult with the Sponsor medical monitor. The investigator should follow any clinically significant laboratory abnormalities until resolution.

Table 4 displays the clinical laboratory parameters that must be measured at every visit.

Table 4: Laboratory Tests

HEMATOLOGY	BIOCHEMISTRY
Hemoglobin	Sodium
Hematocrit	Potassium
WBC	Chloride
Neutrophils	Calcium
Lymphocytes	Phosphate
Monocytes	Glucose
Eosinophils	BUN or urea
Basophils	Creatinine
Platelet count	AST
INR	ALT
PT	Alkaline phosphatase
	Total bilirubin
	GLDH
	Total protein
	Lipase
	Albumin
	LDH
	gGT
	CRP

5.4.4. Viral load and HIV Reservoirs assessment

Regarding HIV-1 viral load, local laboratories will be used as this is done in routine for HIV infected subjects and as

method is standardized so that comparisons between laboratories can be performed.

Viral persistence will be assessed by quantification of total HIV DNA and cell-associated RNA in peripheral blood CD4 T cells and in rectal mucosal leukocytes. In addition, ultrasensitive viral load in plasma will be also assessed.

Experimental design:

Longitudinal analysis of proviral HIV DNA: CD4+ T cells will be isolated by positive selection from total PBMC. Pellets from all time points of each patient (pre- and post-treatment with ABX464) will be lysed, and proviral HIV DNA will be measured by digital droplet PCR (ddPCR, BioRad). Two pairs of primers located in different regions of the viral genome will be used, in order to avoid technical amplification problems due to HIV-1 variability and not due to a real small reservoir. The results will be normalized by the number of cells in each reaction, which will be quantified in parallel through primers targeting the cellular gene RPP30.

Longitudinal analysis of CA-HIV RNA: CD4+ T cells will be isolated by positive selection from total PBMC. Pellets from all time points of each patient (pre- and post-treatment with ABX464) will be lysed, and CA-HIV RNA will be measured by droplet digital PCR (ddPCR, BioRad). Two pairs of primers located in different regions of the viral genome will be used. The results will be normalized by the number of cells in each reaction, which will be quantified in parallel through primers targeting a house-keeping gene.

Longitudinal analysis of plasma VL: ultrasensitive analysis of the VL will be carried out by Single-Copy Assay (SCA) in plasma. Briefly, 10ml of plasma from all time points of each patient (pre- and post-treatment with ABX464) will be concentrated by ultracentrifugation and the determination of the VL will be then performed by the Abbott system. This technique allows detecting down to 1 copy/ml of HIV.

5.4.5. Immunological and molecular assays

Regarding immunological assays, inflammatory and specific response will be assessed in PBMC and in plasma at Visits 2 6, and 8.

General immune phenotype of cellular populations, including activation markers: PBMCs from visits 2, 6 and 8 will be analyzed by flow cytometry, using an 11-colours panels, including: CD3, CD8, CD4, CD45RA, CCR7, CD27, CD28, CD38, HLA-DR, PD-1, viability. In addition to activation and PD-1 longitudinal assessment, change in the percentage of naïve, memory and activated CD4+ and CD8+ T cell subsets changes in peripheral blood and in GALT (rectal tissue), will be analyzed in different time points in comparison to baseline (before treatment with ABX464).

Inflammatory response will be quantified using ELISA assay. This assay is based on the detection of cytokine production in the plasma.

5.4.6. Rectal Biopsy/ Flexible sigmoidoscopy

Flexible sigmoidoscopy is routinely utilized in gastrointestinal and HIV research clinical trials. Based on data from more than 1,000 procedures. Flexible sigmoidoscopy with up to 20 biopsies taken using jumbo forceps has shown a low adverse event rate, even when performed repeatedly in clinical trial participants.

At baseline (Day 1), at Day 28, at Day 56 (only for cohort 1&3) and at Day 84/Day 112 (only for cohort 3) a flexible sigmoidoscope will be inserted into the rectum of the subjects to 15 cm from the anal margin, where 15 to 20 biopsies will be collected for flow cytometry, ex vivo tissue infectivity assays, and PK.

5.4.7. Microbiome assays

The microbiome composition might affect the reservoir size and is key to mucosal immunity. Microbiome specimen will be collected using a rectal swab before each flexible sigmoidoscopy procedure.

The microbiota will be characterized using 16S rDNA Illumina (R) deep sequencing and analyzed using similar methods [5]. Briefly, to characterize alpha diversity in each dataset, species richness (Observed richness and the numeric richness estimators Chao1 and ACE) as well as diversity / evenness measurements (Shannon and Simpson indices) we will use Vegan [6] and Biodiversity-R R packages, respectively. Study ordination analyses will be performed using non- metric multidimensional scaling (NMDS) in a two-dimensional space based on ecological distance matrices calculated by Bray- Curtis dissimilarities as implemented in R/phyloseq wrappers. Findings will be further confirmed using different ecological distances implemented in the same package. The influence of different covariates (demographical, medical, biological) on microbiome structure similarity measures will be tested using permutational analysis of variance using Bray-Curtis distances as implemented in adonis function from R/Vegan package. To further explore structuring of our datasets in clusters, we will use the Partitioning Around Medoids (PAM) algorithm [7] as implemented in the R/cluster[8] package. Silhouette coefficients will be used to assess the goodness-

of-fit of the different possible partitions of each dataset. To assess genus abundance, OTU counts will be collapsed to the bacterial genus level and genus proportions will be calculated for each sample. The differential genus abundance by categories will be compared using the Kruskal- Wallis and Dunn's post-hoc test when required. Multiple comparisons will be corrected with the Benjamini-Hochberg method [9] The LEfSe algorithm [10] will be used to describe genus significantly enriched in different categories.

5.4.8. Pharmacokinetics

Plasma and tissue ABX464 and its metabolite concentrations will be determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) methods validated for all matrices by ATLANBIO.

ABX464

After single oral administration of 50 to 200 mg ABX464, ABX464 is relatively quickly absorbed and undergoes a very extensive biotransformation into its N-glucuronide metabolite, NGlc.

In most of the subjects, regardless of the dose, ABX464 plasma concentration is generally not measured after 8 to 10 h post-dose. Cmax is generally observed within the first 2 h post-dose and then ABX464 plasma concentrations decreased with a $t_{1/2}$ of about 1 to 2h.

ABX464-N-glucuronide's plasma concentrations were markedly higher than those of the parent drug with mean Cmax about 200 to 300-fold higher than ABX464 Cmax. Cmax was observed around 4 h post-dose with a very limited variability thereafter ABX464 NGlc was slowly eliminated and had a $t_{1/2}$ of 90 to 110 h.

Relationship between rate and extent of absorption of ABX464 and ABX464 dose is quite erratic with a reduced increase with the dose up to 150 mg and saturation like behavior at 200 mg.

ABX464-N-glucuronide's exposure-dose relationship is somehow comparable to that of ABX464 although increase of C_{max} and AUCs tend to be dose proportional between 50 and 150 mg.

Since ABX464 undergoes a high level of biotransformation, ratios between metabolite and parent drug appear to be unchanged across the studied dose range but inter-individual variability prevented from definite conclusions.

Therefore, ABX464 should rather be considered as a pro-drug with regard to its short $t_{1/2}$. Its metabolite, ABX464-N- glucuronide exhibits markedly higher blood concentrations and has a much longer $t_{1/2}$ which could be of pharmacological interest. Last, dose-exposure relationship appears erratic between 50 and 200 mg but tends to linearity up to 150 mg.

ABX464 was shown to have a very short $t_{1/2}$ (1 to 2 h), so steady-state is virtually reached at the second administration, while ABX464-N-glucuronide has a $t_{1/2}$ of 90 to 110 h, meaning that steady-state is reached after 19 to 22 days of administration. Intense PK blood collection is planned to be done on Day 1 (initiation of ABX464 administration) and Day 28 (final dose of ABX464) to characterize accumulation and decay of ABX464 and its metabolite (ABX464-N- glucuronide) in plasma and PBMC.

Additional blood PK samples will be collected on Days 35 and 56 to characterize the terminal decay of ABX464. In addition, ABX464 and ABX464-N-glucuronide will be quantified in rectal tissue biopsies collected during Visit 2, 6 and 8.

A total of 18 blood samples per HIV negative subjects and 18 blood samples per HIV positive subjects will be collected for PK assessment. These samples will be processed according to the appendix 3 in order to get Plasma and PBMC samples.

Blood samples for determination of ABX464 and ABX464-N-glucuronide, will be collected from all subjects using direct venipuncture or an indwelling catheter.

Then, the following PK parameters will be derived for ABX464 and ABX464-N-glucuronide

- C_{max}, t_{max}: the maximum plasma concentration (Cmax) and the time taken to reach Cmax (tmax) will be obtained directly from the concentration-time data.
- $AUC_{0-\tau}$: the area under the concentration-time curve from time zero to the time of dosing interval τ (24 h post-dose for ABX464 and ABX464-N-glucuronide). If no concentration can be measured at this time point, AUC0-last (from time zero to the last quantifiable concentration) will be calculated. Both parameters will be presented independently. For both parameters, a linear trapezoidal method will be used.

Moreover, evaluation of steady-state will be made by visual inspection of the pre-dose concentration measurement collected over the study duration.

In addition, five rectal biopsies at each time point will be sent to ALTANBIO for tissue PK determination.

5.4.9. Transcriptomics / Proteomics

Two rectal biopsies will be cryopreserved at -80ºC, one in RNA later and another one snap-frozen, to enable future transcriptomics and proteomics analyses. The decision of performing such proteomics and/or transcriptomics analyses will depend on the results obtained from the remaining study analyses.

5.5. Summary of blood samples

Table 5 below summarizes the volume of blood to be sampled at each study visit.

Table 5: Summary of Blood Volumes (mL)

	HIV positive (Cohort 1)	HIV positive (Cohort 3)	HIV negative subjects
Screening (Visit 1)	32.2	32.2	19.2
Day 1 (Baseline Visit)	167 – 169,7	167 – 169,7	124 – 126,7
Day 28 + Day 56 (Cohort 3)		167 – 169,7 + 38-40,7	
Day 28/84 (End of Rx Visit)	167 – 169,7	167 – 169,7	132, 5 – 135,2
Day 35/91 (FU Visit)	33 – 35, 7	33 – 35, 7	20 – 22,7
Day 56/112 (EoS Visit)	83 – 85,7	83 – 85,7	48,5 – 51,2
TOTAL	**482.2 – 493.0**	**687.2 – 703.4**	**345.2 – 354.0**

Laboratory testing	Tube	Volume (ml)
Biochemistry	SST II advance (5ml)	5
Hematology	K3E 5,4mg (3ml)	3
INR&PT	9NC 0.129M (2,7mL)	2.7
Viral load (HIV)	K2E 18mg (EDTA) (10ml)	10
CD4/CD8 (HIV)	K3E 5,4mg (3ml)	3
HBV, HCV, HIV, syphilis screen (HIV and non-HIV, screening)	SST II advance (8.5ml)	8.5
HIV serology (non-HIV, screening day 28 and day 56)	SST II advance (8.5ml)	8.5
PK (per each PK timepoint)	CPT (8mL) + Li-Hep (4mL)	12
Blood for reservoir, immunology and Mol. Assays (HIV)	K2E 18mg (EDTA) (10ml) * 5	50
Blood for immunology and Mol. Assays (non-HIV)	K2E 18mg (EDTA) (10ml) * 2	20

5.6. Summary of rectal mucosal biopsies

Approximately 20 rectal mucosal punches (2.7 mm diameter) will be obtained during each flexible sigmoidoscopy procedure. Table 6 below summarizes the number and use of such rectal punches by study analysis.

Table 6: Number of rectal punches per study analysis

Study analysis	HIV-positive subjects	HIV-negative subjects
Explant HIV-1 infection	none	8
Flow cytometry	5	6
HIV-1 Reservoir	7	none
PK	4	4
Transcriptomics	1	1
Proteomics	1	1
TOTAL	**18**	**20**

The remaining biopsies, if any, will be cryopreserved at irsiCaixa.

6. INVESTIGATIONAL PRODUCT(S)

All investigational products to be used in this study have been manufactured, packaged and labeled by contract manufacturers for ABIVAX, according to GMP standards and are supplied to investigators free of charge.

6.1. Description of investigational treatment

The study treatment that will be administrated to subjects enrolled in this Phase Ib study consists of capsules containing ABX464 given orally once daily for 28 days (Cohort 1&2) or for 84 days (Cohort 3).

6.2. Description of investigational Product

6.2.1. Active investigational product (ABX464)

The ABX464 investigational medicinal product (IMP) is a hard gelatin capsule intended for oral administration.

For the proposed clinical trial, the IMP consists of size 00 capsules containing 50 mg of ABX464 drug substance in the form of a granulate prepared with a number of common excipients (microcrystalline cellulose, polyvinylpyrrolidone, magnesium stearate and colloidal silica). It is supplied in high-density polyethylene bottles closed with high-density screw caps.

ABX464 will be manufactured by SEPS Pharma (Belgium).

SEPS Pharma CTM
Technologiepark 4
9052 Gent Belgium

Primary packaging labelling as well as Qualified Person release of the IMP are performed at the following site:

SODIA
Avenue Robert Schuman
51 000 REIMS

The study drug has to be stored at ambient temperature (< 25°C). The capsule or combination of capsules to be used depends on the dose-level to be administered as per the clinical study protocol.

6.3. Administration and Dosing

6.3.1. Administration of the investigational product

Subjects will be dosed with a daily dose of 150 mg of ABX464 (Cohort 1) or of 50 mg ABX464 (Cohorts 2&3). ABX464 will be administered using 50 mg capsules. Subjects will be orally dosed in fed condition (regular breakfast) with 240 mL of water.

A subject diary, in which the subject should report the number of capsules taken and the intake time, will be given to the subject at baseline. Moreover, this diary will enable the subject to report also potential discomfort or side effects s/he could experience.

6.3.2. Guidelines for treatment postponement and dose modifications

No intra-subject dose escalation/dose adjustment are allowed.

6.4. Method of Assigning Subjects to Treatment Arms

All subjects will be assigned a unique and incremental subject Identification (ID) number. Subject IDs will be unique (i.e. reallocation of the ID will not be permitted). The format will be a seven-digit number as follows: ABX-country/site number (4 digits) – subject number (3 digits). The latter 3-digit subject number will be assigned according to the subject's order of inclusion in the center.

Initial enrollment will occur into Cohort 1, and subsequently into Cohort 2 & 3.

Potential patients prematurely withdrawn from the study for any reason will be replaced. In all cases, subject should return his/her used and unused bottles at each study visit for a compliance check.

6.5. Packaging

The IMP consists in hard gelatin, powder-filled capsules (size 00) containing 50 mg of ABX464, supplied in high-density polyethylene bottles closed with high-density screw caps.

6.6. Storage

ABX464 capsules will be shipped to the investigational site at ambient temperature. ABX464 capsules should not be stored above 25°C. DO NOT FREEZE OR REFRIGERATE.

The IMP should not be used beyond the expiration date. Drug supplies are to be stored in a secure, limited- access location under the storage conditions required by GCP/GMP guidelines.

6.7. Product Accountability

An accurate and current accounting of the dispensing and return of IMP(s) will be maintained on an ongoing basis by the pharmacist and a member of the study site staff in the Accountability Log and case report form and will be verified by the study's monitor.

6.8. Prior and Concomitant Medication

6.8.1. Background and allowed concomitant treatment

All HIV positive Subjects must be receiving treatment with a combination antiretroviral regimen.

6.8.2. Prohibited prior or concurrent medications

Prohibited prior or concomitant treatments:

Following medications are prohibited potentially prior to baseline and during the course of the study.

- Medications prohibited within 3 months prior to baseline and during the whole course of the study
  - Immunomodulators or immunosuppressive drugs (such as IL-2, IL-7, intravenous immunoglobulin- IVIG, IFNγ, or Imiquimod, IVIG, IFNγ, or Imiquimod);
  - Any investigational or non-registered product.
- Medications prohibited within 1 month prior to baseline and during the whole course of the study
  - HIV negative Subjects should not be taking antiretroviral pre-exposure prophylaxis (PrEP) or have completed a course of antiretroviral post exposure prophylaxis (PEP) within one month of the enrollment visit.
- Medications prohibited within one week prior to baseline and during the whole course of the study.
  - Heparin, including Lovenox® (enoxaparin sodium);
  - Warfarin;
  - Plavix® (clopidogrel bisulfate);
  - Any other drugs that are associated with increased likelihood of bleeding following mucosal biopsy (e.g., daily high dose aspirin (> than 81 mg), NSAIDs, or Pradaxa®);
  - Rectally administered medications (including over-the-counter products);
  - Multiple dose dexamethasone.
- Medications prohibited during the whole course of the study
  - Drugs that could interact either with ABX464 should be avoided especially the CYP1A2 substrates or inducers (cf. Appendix#2). The following CYP1A2 substrates or inducers with a narrow therapeutic margin are prohibited during the whole course of the study (clozapine, theophylline, ropinirol, warfarin and methadone).

Potential concomitant medications should be kept at constant dose during the course of the study and properly reported in the medical file of the subject and the eCRF.

This information should include the name of the medication (international nonproprietary name), daily dosage, duration, indication and the time of last intake before all PK samplings.

7. SUBJECT COMPLETION AND WITHDRAWAL

7.1. Subject Completion

Treatment with ABX464 shall continue until Day 28 (Cohort 1&2) or Day 84 (Cohort 3), except if a subject fulfils a premature discontinuation criterion (defined below). After discontinuation of study product (End of Rx Visit; Day 28/84) subjects will return for two follow-up visits (FU Visit; Day 35/91 and EoS Visit; Day 56/112).

7.2. Premature trial discontinuation

A subject can be withdrawn at any time from the study for the following reasons:

- Investigator's decision;
- An Adverse Event or an intercurrent condition that preclude continuation of treatment;
    - Specifically, an increase ≥ 2.0 x ULN in liver transaminases (AST/SGOT and/or ALT/SGPT), in alkaline phosphatase or in total bilirubin should be considered a treatment discontinuation criterion
- Major protocol violation;
- Subject's decision;
- Withdrawal of consent.

A subject who prematurely exits the study before Day 28 (Cohort 1&2) or Day 84 (Cohort 3) will be replaced (i.e. an additional subject will be randomized and receive the next treatment allocation).

7.3. Study Discontinuation

All subjects, regardless of the completion or premature discontinuation, should perform the End of Study Visit (EoS) according to the study flow-chart. In case a premature discontinuation this EoS visit should occur within the next two weeks after the last dosing and should include but depending on the subject willingness the PK evaluation and the sigmoidoscopy.

7.4. Screen and Baseline Failures

A subject is considered to be a baseline failure if the subject signs the informed consent but withdraws before the screening visit. All potential subjects who are screened for enrolment in this study will be listed on the Subject Screening Log/Identification List. Reasons for exclusion will be recorded for potential subjects who do not enter the study.

A subject who does not fulfil the randomization criteria at the end of the screening will be considered as screen failure. All subject data should be entered in the eCRF including the screen failure data.

Re-screening of a subjects is allowed but limited to rectal and urinary tract infection. In such a case the time frame for a re-screening is 1 month.

8. ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE. During the study, in case of a safety evaluation, the investigator or site staff will be responsible for reporting AEs and SAEs, as detailed in this section of the protocol.

During the screening period, only adverse event related to the screening procedures will be collected. Adverse events related to the antiretroviral treatments will be reported directly to the concerned pharmaceutical company according to the local process.

Any disease progression will not be reported in the eCRF as an adverse event, but will be documented in the efficacy section.

8.1. Definition of an AE

Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to 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 (new or exacerbated) temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Note: The official definition also extends to AEs occurring in the placebo arm. Because of regulatory requirements, events occurring during pre-and post-treatment periods will also be designated as AEs. Therefore, reporting of such events, AEs and SAEs, will commence when the subject is enrolled into the study (date of signature of the informed consent) up until 4 weeks after the end of the treatment visits. The period after discontinuing study drug may be extended if there is a strong suspicion that the drug has not yet been eliminated.

8.2. Definition of a SAE

A serious adverse event (experience) or reaction is any untoward medical occurrence that, at any dose:

a) Results in death

NOTE: Death is an outcome of an AE, and not an AE in itself. Event which led to death should be recorded with fatal outcome.

b) Is life-threatening

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, which hypothetically might have caused death, if it were more severe.

c) Requires hospitalization or prolongation of existing hospitalization

NOTE: In general, hospitalization means that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-subject setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred, or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen after informed consent was given is not considered an AE.

d) Results in persistent or significant disability/incapacity,

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect

f) Is another medically important condition: This refers to an AE that may not be immediately life- threatening or results in death or hospitalization but may jeopardize the subject or may require intervention to prevent

one of the outcomes listed above. Based on medical and scientific judgment this should usually be considered serious.

If there is any doubt about whether or not an AE is serious, the investigator should contact the sponsor.

8.2.1. Events and/or Outcomes Not Qualifying as SAEs

Any hospitalization, or prolongation of hospitalization due to the circumstances listed below, will not be reported as SAE:

- Planned medical/surgicalprocedure;
- Planned medical/surgical admission (planned prior to entry into study, appropriate documentation required), for the disease understudy;
- Administrative or social reasons (e.g. lack of housing, economic inadequacy, care-giver respite, family circumstances).

8.3. Events or Outcomes Qualifying as AEs or SAEs

8.3.1. Clinical laboratory parameters

Abnormal laboratory findings (e.g., clinical chemistry, hematology) or other abnormal assessments (e.g. vital signs) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definitions of sections 8.1 and 8.2 respectively. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at informed consent and significantly worsen during the study will be reported as AEs or SAEs. Clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, and are present at the start of the study but do not worsen, will **not** be reported as AEs or SAEs. However, if these findings or assessments are judged by the investigator to be more severe than expected considering the subject's condition, then they may be reported as AEs or SAEs.

8.3.2. Pregnancy report

Partner of a subject who become pregnant at any time while the subject is taking part in the study must be reported.

The investigator will record pregnancy information and submit it to ABIVAX or its designee within 24 hours after knowledge of a partner's pregnancy. The partner will also be followed to determine the outcome of the pregnancy, be it full- term or prematurely terminated. Information on the status of the mother and child will be forwarded to ABIVAX or its designee. Follow-up will normally end 6 to 8 weeks following the estimated delivery date.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.

The time period for collecting pregnancy information is identical to the time period for collecting AEs, as stated in Section 8.4, Time Period, Frequency, and Method of Detecting AEs and SAEs. Pregnancy information is collected from the signing of informed consent to 4 weeks after the last dose.

8.4. Time Period, and Frequency of Detecting AEs and SAEs

All AEs and SAEs occurring from the time a subject consents to participate in the study until 4 weeks after he or she has completed or discontinued the investigational product must be recorded in the subject's eCRF. Importantly, SAEs will have to be reported to ABIVAX or its designee within 24 hours of awareness of an SAE.

Legislative guidance requires also reporting any **related** SAEs to be reported after the subject finished the study if the investigator becomes aware of them.

8.5. Recording AEs and SAEs

Severity of AEs will be assessed according to "Division of AIDS table for grading the severity of adult and pediatric adverse events", version 2.0 of November2014.

Subjects will be asked to report all AEs as part of the procedures performed at each study visit. The site personnel will document all AEs in the subject's medical record. All AEs subsequently must be recorded in the appropriate eCRF sections.

The following points must be recorded for each event:

- A description of the event in medical terms, not as reported by the subject;
- Date of onset (start date);
- Date of resolution (stop date);
- The time of onset with respect to administering the investigational product;
- The severity of the sign/symptom or clinically significant abnormal laboratory value according to CTC AE Classification;
- The causal relationship between the investigational product and the occurrence of each AE. This will be assessed by each investigator using clinical judgment. Alternative causes, such as natural history of the underlying diseases, concomitant medications, other risk factors and the temporal relationship of the event to the investigational product will have to be considered. The causality of all AEs should be assessed by the investigator with the following question: Is there a reasonable possibility that the AE may have been caused by the investigational product? And answered "NO" (if not related) and "YES" (if related);
- Action taken regarding the investigational product:
  - No action;
  - Temporary discontinuation;
  - Permanent discontinuation;
- Subject's outcome:
  - Recovered without sequelae / resolved without sequelae;
  - Recovered with sequelae / resolved with sequelae;
  - Recovering/Resolving;
  - On-going;
  - Fatal (for SAEs only).

If in any one subject, the same AE occurs on several occasions, the AE in question must be documented and assessed anew each time.

8.6. Reporting of SAEs to ABIVAX or its designee

Throughout the study, the reporting of SAEs to the Sponsor or its designee will be done through the SAE forms in the eCRF. In case the electronic SAE reporting system does not work, or after freezing of the subject's eCRF, paper SAE report forms should be used which will be faxed to ABIVAX or its designee.

It is the investigator's responsibility to ensure that the SAE report is submitted to ABIVAX or its designee, within **24 hours after knowledge of the event(s).** The SAE forms or paper report forms should be completed as thoroughly as possible, with all the available details of the event and signed by the investigator or designee. An assessment of causality should always be provided at the time of the initial report. If the investigator or designee does not have all information regarding the SAE, he/she should not wait to receive additional information before completing the form and notifying ABIVAX or its designee.

Additional or follow-up information relating to the initial SAE report, will be requested, if necessary. Again, this information is to be completed and submitted through the SAE forms within 24 hours of receipt of the information. If the paper SAE report form was used due to technical constraints (see above), the SAE forms in the eCRF should be updated once the system is functional again (and not later than 24 hrs. afterwards), prior to using it to report any additional information. Furthermore, when additional information is received on an SAE after freezing of the subject's eCRF, new or updated information is to be recorded on a paper SAE report form with all the changes signed and dated by the investigator.

In the rare occasion when the electronic system for reporting SAEs does not work and in the absence of facsimile equipment, the investigator should notify ABIVAX or its designee by telephone within the given timeframe, and send a copy of the SAE report form by mail or email.

8.7. Reporting of SAEs to Regulatory Authorities

ABIVAX has a legal responsibility to notify, as appropriate, both the local regulatory authorities and other regulatory agencies about the safety of the investigational product. It is therefore important that the investigator notifies promptly ABIVAX or designee of any SAEs, in order for legal obligations and ethical responsibilities towards other subjects to be met.

In addition, the investigator or designee, will comply with the local regulatory requirements (when applicable) in reporting of SAEs to the ethics committee and, if required, to the relevant government authority.

Safety reports on adverse events that are serious AND unexpected AND associated with the investigational product are prepared according to ABIVAX's policy and applicable regulations and are forwarded to the investigators. These reports are filed with the investigator brochure or other appropriate study documentation. It is the Sponsor or its designee and/or investigator's responsibility to notify the IRB or IEC of these reports, if applicable according to local requirements.

9. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

A summary of the principal features of the statistical analysis of the data will be described here, in the statistical section of the protocol. A more technical and detailed elaboration of the principal features stated in the protocol will be given in the first version of the statistical analysis plan (SAP).

Any amendments to the SAP will be clearly documented and signed prior to the final database lock including justifications and details of their potential impact on the interpretation of the study results.

9.1. Statistical and Analytical Plans

An interim analysis is planned once the first cohort has completed their last protocol specified visit.

9.1.1. Protocol deviations

Protocol deviations will be reviewed and classed as major or minor during the blind-review meeting. Major protocol deviations are defined as deviations liable to bias the evaluation of the main efficacy endpoint. The following deviations will be considered as major (non-exhaustive list):

- Noncompliance with the inclusion or exclusion criteria;
- Noncompliance with the study treatment;
- Intake of prohibited medication;
- Noncompliance with time window.

9.1.2. Definition of study analysis sets

The following datasets will be defined and used for the analyses:

- The **Safety dataset (SAF population)** is defined as those subjects included in the study, who have received at least one dose of the study treatment.
- The **Full Analysis dataset (FAS population)** is defined as those subjects included in the study, who have received at least one dose of the study treatment, and who have at least baseline data.
- The **Per Protocol dataset (PP population)** is defined as those subjects of the FAS population without any major protocol deviation.

9.1.3. Subjects/Subjects disposition

The number and the percentages of subjects enrolled and included in each of the populations will be tabulated. The reason for subject exclusions from each of the populations will also be listed. In addition, the number of discontinued subjects with their reason for discontinuation will be tabulated.

9.1.4. Demographic and other baseline characteristics

Demographics and other baseline characteristics will be summarized by treatment arm. This analysis will be conducted on the FAS population.

9.1.5. Treatment compliance

Treatment compliance/adherence will be summarized by treatment arm. This analysis will be conducted on the FAS population.

9.1.6. Interim analysis

An interim analysis will be produced when the first 12 HIV-positive subjects (Cohort 1) reach day 56 of follow-up. The interim analysis will include the following data: (a) Safety and clinical parameters; (b) HIV-1 RNA, CD4+ and CD8+ counts; (c) Hematology and biochemistry parameters; (d) Pharmacokinetic parameters in plasma, PBMCs and rectal tissue, and (e) Total HIV-1 DNA reservoir in PBMC and rectal tissue.

In addition, regarding the cohort 3, the screening and the D28 viral DNA results should be analyzed immediately once the 12 HIV-positive subjects reach the day 28 time point.

9.2. Efficacy Analysis

All efficacy analysis will be conducted on both FAS population and PP population except if otherwise specified. Descriptive statistics will be presented by treatment arm and include:

- Quantitative variables: mean, standard deviation, minimum and maximum, 95% confidence intervals, median and quartiles will be presented when considered relevant. Number of filled and missing values will also be presented.
- Qualitative variables: count, percentage for each modality and 95% confidence intervals when relevant. Number of missing values will also be presented.

9.2.1. Pharmacokinetics

Individual plasma, PBMC, and tissue concentrations of ABX464 and ABX464-N-glucuronide, will be presented by treatment and time points. Descriptive statistics for the concentrations will be presented as number of available data (N), mean, standard deviation (SD) and will be calculated if at least 2/3 of the values per time-point are above LOQ (i.e. N ≥ 10 if 15 subjects are available for a given group). For descriptive statistics calculations, BLQ concentrations below the limit of quantification will be set to zero (0) if they are reported before the first quantifiable sample or considered as missing data if they are reported after the first quantifiable data.

Then, the following PK parameters will be derived for ABX464 and ABX464-N-glucuronide for each Subjects:

C_{max}, t_{max}: the maximum plasma concentration (C_{max}) and the time taken to reach C_{max} (t_{max}) will be obtained directly from the concentration-time data.

$AUC_{0-\tau}$: the area under the concentration-time curve from time zero to the time of dosing interval τ (24 h post-dose for ABX464 and ABX464-N-glucuronide). If no concentration can be measured at this time point, AUC0-last (from time zero to the last quantifiable concentration) will be calculated. Both parameters will be presented independently. For both parameters, a linear trapezoidal method will be used.

Individual derived PK parameters will be presented by treatment and day of PK assessment when appropriate. Descriptive statistics of the PK parameters will be presented as N, mean, SD, coefficient of variation (CV %), median, minimum (Min), maximum (Max) values, and geometric mean (GM).

In the tables of individual PK parameters, all the deviations from planned analysis will be mentioned by flagging the abnormal results.

Possible exclusion of flagged PK parameters could be performed if, in the judgment of the pharmacokineticist, they are deemed not to be "pharmacokinetically relevant". Exclusion of PK parameters will be discussed in the PK results section. If data are excluded from the PK dataset, all subsequent statistical analyses will be performed twice, once using the complete available PK dataset and once using the final PK dataset as defined by the pharmacokineticist. Study conclusion will be based on the final dataset as defined by the pharmacokineticist.

For each compound, plasma concentration vs. time curves will be presented on a log-linear scale:

- Mean plots showing the data of both treatments (administration alone and co-administration with ABX464);
- By subject plots with the treatments on separate plots (spaghetti plots).

Formal PK Statistics

Evaluation of drug-drug interaction

The relative bioavailability (F_{rel}) after multiple dose administration with either co-administration of ABX464 will be evaluated on PK parameters determined on Day 28.

The comparison will be performed on Cmax and $AUC_{0-\tau}$ using a 1-way ANOVA model with treatment as main effect on logarithmically transformed data. For each parameter, a point estimate for the ratio of geometric means (ARTs with 150 mg doses of ABX464) will be obtained by calculating the difference of least square means on the logarithmic scale and subsequent back transformation with the exponential function. Likewise, 90% confidence interval for the ratios will be obtained by back transforming the 90% confidence intervals of least square mean differences on log-transformed PK parameters.

Moreover, evaluation of steady-state will be made by visual inspection of the pre-dose concentration measurement collected over the study duration.

9.3. Safety Analyses

Adverse events will be coded using the standard dictionary (MedDRA) down to the lower level term (LLT).

An overall summary table will be presented: any adverse event, any treatment emergent adverse event (TEAE), any serious adverse event (SAE), death, any grade 3 or higher adverse events from baseline to EoS.

Two periods will be defined for TEAE:

- Any adverse event which occurs or worsens from first dosing to Day 28;
- Any adverse event which occurs after Day 28.

Adverse events will be described by primary system organ class and preferred term. Numbers and percentage of subjects, and number of occurrence of adverse event will be presented for:

- TEAE;
- Serious TEAE;
- TEAE leading to drug discontinuation;
- TEAE of grade 3 or4;
- TEAE for which relationship with the study drug is recorded as possible or probable.

The assessment of safety will be based on the frequency of adverse events (with and without regard to causality) graded according to the Division of AIDS table for grading the severity of adult and pediatric adverse events (version 2.0, November 2014) and also, the review of individual values for clinical laboratory data, vital signs and ECG focusing on the detection of abnormal values and PCSAs [potentially clinically significant abnormalities (PCSAs) determined upon investigator considerations].

Adverse events will be tabulated (counts and percents) by group. All adverse events will be listed and the data will be tabulated by body system/organ class. Adverse event tabulations will include all treatment emergent adverse events, which will be further classified by severity, and relationship to treatment and dose level.

Clinical laboratory parameters, vital signs, ECG will be summarized by using descriptive statistics (n, mean, SD, SEM, median, minimum and maximum). Number of subjects with at least one abnormal values will be tabulated (counts and percents) for each parameter in summary shift tables, by group and dose.

9.3.1. Clinical laboratory evaluation

Descriptive statistics for laboratory parameters will be computed at each scheduled assessment. If relevant for some parameter, change from baseline will also be tabulated.

In addition, shift tables from baseline will be presented.

9.4. Determination of Sample Size

No formal sample size calculations were performed. 36 subjects (24 HIV infected and 12 HIV uninfected subjects) are deemed sufficient to fulfil the study objectives.

10. STUDY CONDUCT CONSIDERATION

10.1. Regulatory and Ethical Considerations

10.1.1. General Requirements

The study will be conducted in compliance with the study protocol, ABIVAX Standard Operating Procedures and in accordance with any local regulatory requirements, to ensure adherence to Good Clinical Practice (GCP) as described in the following documents:

- ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
- US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
- Directive 2001/20/EC on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical studies on medicinal products for human use and its guidance.
- Declaration of Helsinki and its amendments.

Upon signing the protocol, the investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

Written informed consents will be obtained for each subject before he or she can participate in the study.

ABIVAX will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agencies in accordance with any applicable country-specific regulatory requirements prior to a site initiating the study in that country.

10.1.2. Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the study protocol and amendments if applicable as well as other appropriate study related documents will be submitted to an independent Institutional Review Board (IRB) or independent Ethics Committee (IEC), respectively.

For each center, it will be individually specified, who (investigator or sponsor) will be responsible for informing the IRB or IEC, respectively of any protocol amendments or new relevant information that require an ethical reconsideration of the study protocol.

If the investigator is responsible for obtaining approval, he/she should also obtain a statement from the IRB or IEC, respectively that it is organized and operates according to GCP and applicable laws and regulations.

10.1.3. Subject Informed Consent

It is the responsibility of the investigator to give each subject full and adequate verbal and written information regarding the aims, methods, anticipated benefits and potential hazards. The subject must be informed that participation is voluntary, and that they are free to withdraw from the study at any time without any disadvantages for their subsequent care. Although a subject is not obliged to give her/his reason(s) for withdrawing prematurely from the trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights. Written consent (signed and dated by the subject and the investigator) must be obtained prior to admission. The subject must be provided with a copy of the subject information and informed consent.

The data collected in this study will be processed anonymously at ABIVAX. Subjects should be informed about the purpose of the planned computer data processing and the publication of the data (e.g. at scientific meetings). The subject must give consent to the computer processing and to the publishing of anonymous data.

The subject must be informed of and consent in writing that personal data relating to the trial may be subject to audits by Health Authorities and the sponsor. However, personal data will be kept strictly confidential and will not be made publicly available.

10.1.4. Compensation to Subjects

Insurance coverage will be provided for all subjects enrolled in the study from the time of the subject's inclusion in the study (i.e. date of signing the ICF). The insurance coverage will be provided by the Sponsor and will be in line

with GCP guidance and legal requirements, but also in accordance with local regulations. Depending on the local policies and the services/availabilities of the insurance providers, different such providers may be used in individual countries. A confirmation of insurance and corresponding insurance conditions should be archived in the Investigator File.

11. STUDY MANAGEMENT

11.1. Remote Data Entry

An electronic case report form (eCRF) will be used to record all data required by the protocol. Remote Data Entry (RDE) will be used for data collection, i.e. the subject's information pertaining to the study, will be entered into the eCRF via a computer at the investigational site.

Prior to the start of the study, the investigator will complete a "*Delegation of significant study-related duties*" form, showing the signatures and initials of any person who is authorized to make or change entries in the eCRF and any person authorized to electronically sign the eCRF.

The eCRF used for this study is validated and fulfils the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) requirements, European and FDA (21 CFR Part 11) regulations.

Training sessions will be held for all the Subjects who will use this tool (*e.g.* investigators, ABIVAX staff and contract research organization [CRO] staff, including project managers, CRAs and data managers).

Several supports are available to help all users with this tool including eCRF user manuals (CRA and site manuals) and five days a week / working hour's helpdesk (support line).

All of the information will be recorded through transcription from source documents into the eCRF by an authorized person.

The investigator is responsible for the management and accuracy of the information in the eCRF. At each monitoring visit, the subject medical files should be at the clinical research associate's (CRA) disposal for review.

11.2. Data management

Data management will be outsourced to a Contract Research Organization (CRO). The data managers will issue electronic edit checks via EDC, and modification of the data will be permitted by the investigator to achieve accuracy with source documents and eliminate all inconsistencies in the data.

The data will be reviewed for completeness and logical consistency. Automated validation programs will identify missing data, out of range data and other data inconsistencies at the time of entry.

All new/updated information will be reviewed and verified by the appointed monitor.

11.3. Data coding

Adverse events, concomitant diseases, medical/surgical histories will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medication will be coded using the WHO-DRUG dictionary.

11.4. Study Monitoring

The study will be conducted in accordance with the ICH Note for Guidance on GCP (ICH, Topic 6, 1996). The appointed monitor will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and ABIVAX requirements. Throughout the study, the monitor will arrange visits to the study center at appropriate intervals to assess the progress of the study and review the completed eCRFs.

During the monitoring visits, the monitor will:

- Ensure that the safety and the rights of subjects are being protected;
- Check that the data are authentic, accurate, and complete and discuss any inconsistencies;
- Ensure that all study materials are correctly stored and dispensed with particular emphasis to the investigational product;
- Verify that the site staff and facilities continue to be adequate for the proper conduct of the study;
- Ensure that the study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements;
- Help resolve any problems that may have arisen.

In line with ICH GCP guidelines, monitoring will include verification of data entered in the CRF against the original

subject records. Therefore, for the purpose of monitoring review, direct access to all study-related site and source documents is mandatory. Data items for which the eCRF will serve as the source document will be identified, agreed upon and documented. The investigator must also ensure provision of sufficient time, space and qualified personnel for the monitoring visits.

11.5. Records Retention

Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff.

ABIVAX will inform the investigator/institution of the required time period for retaining these records in order to be compliant with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that study site, as dictated by ICH GCP E6 Section 4.9, any institutional requirements or local laws and regulations, or ABIVAX standards/procedures; otherwise, by default the retention period will be 25 years.

The investigator must notify ABIVAX of any changes in the archival arrangements, including, but not limited to, the following: archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site. In addition, the investigator should seek the written approval of the Sponsor prior to disposing any of the archived records.

11.6. Quality Assurance and Inspection by Authorities

To ensure compliance with GCP and all applicable regulatory requirements, ABIVAX may conduct quality assurance audits. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. By signing the protocol agreement page, the investigator agrees to permit drug regulatory agencies and ABIVAX audits. If an audit or inspection occurs, the investigator and institution will allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues. Items of particular interest in case of an audit are, but not limited to, the following:

- IRB/IEC and regulatory authorityapprovals;
- Informed consent forms of the subjects;
- Approved study protocol and amendments andinvestigator brochure;
- Treatmentaccountability;
- Safetyreporting;
- Studyfile;
- Studypersonnel;
- Log of monitoring visits and monitoring process;
- Medical records and other source documents;
- Sitefacilities;
- Reports to the IRB/IEC and the sponsor;
- Record retention.

11.7. Study and Site Closure

If the study is terminated prematurely or suspended for any reason, the investigator/institution should promptly inform the study subjects and should assure appropriate therapy and follow-up for the subjects

ABIVAX reserves the right to temporarily suspend or prematurely discontinue this study, at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites. If such action is required, the Sponsor will discuss this with the investigator or the head of the medical institution (where applicable), including the reasons for taking such action, at that time. Advance notification will be provided to the site(s) when feasible, on the impending action prior to it taking effect.

All investigators and/or medical institutions conducting the study will be informed in writing should the Sponsor decide to suspend or prematurely discontinue the study for safety reasons. The regulatory authorities will also be informed of the suspension or premature discontinuation of the study and the reason(s) for the action. If required by local regulations, the investigator must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

Upon premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and ABIVAX procedures. All data must be returned to ABIVAX. Arrangements will be made for any unused investigational product based on the relevant ABIVAX procedures for the study.

11.8. Study report and Publication

Upon conclusion of the study, an integrated clinical and statistical study report will be written by the Sponsor in consultation with the Coordinating Investigator. This report will be based on the items detailed in this study protocol. When the clinical study report is completed, ABIVAX will provide the investigators with a full summary of the study results. The investigators are encouraged to share the summary results with the subjects, as appropriate.

The first resulting publication will be a full publication of all data from all participating sites, coordinated by ABIVAX. Any secondary publications by the investigators (abstracts in journals, oral presentations etc.) will reference the original publication and will require pre-submission review by the Sponsor. Note that the Sponsor is entitled to delay any proposed secondary publication, in order to obtain patent protection, if required.

The Coordinating Investigator as well as other members of the study committee will be authors on the first publication. The principal investigator of the trial will be the first author. Authorship for other investigators will be assigned on the basis of their recruitment contribution, as well as intellectual and administrative input. Ranking will be according to the number of subjects randomized as well as contribution to the study conduct and preparation of final manuscript.

11.9. Ownership and Confidentiality

All information provided by ABIVAX and all data and information generated by the sites, as parts of the study (excluding the subjects' medical records) are property of ABIVAX.

All potential investigators must be aware of and agree in writing (confidentiality agreement) to the confidential nature of the information pertaining to this study. Furthermore, all information provided by ABIVAX and all data and information generated by the sites during the study must be kept confidential by the investigator and other site staff, and may not be used for any purpose other than conducting this study.

12. REFERENCES

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13. APPENDICES

Appendix 1: Division of AIDS table for grading the severity of adult and pediatric adverse events

The severity of adverse events will be assessed using the division of AIDS table for grading the severity of adult and pediatric adverse events, version 2.0 of November 2014. A copy can be downloaded from the internet web site below or supplied upon request toABIVAX:

http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_Grading_Table_v2_NOV2014.pdf

Appendix 2: CYPIA2 substrates or inducers (in bold: prohibited concomitant medications)

Amitriptyline, Clomipramine, Imipramine, Agomelatine, Fluvoxamine, **Clozapine**, Olanzapine, Haloperidol, Ropivacaine, **Theophylline,** Zolmitriptan, Tamoxifen, Erlotinib, Cyclobenzaprine, Mexiletine, Naproxen, Ondansetron, Phenacetin, Paracetamol, Propranolol, Tacrine, Tizanidine, Verapamil, **Warfarin**, Zileuton, **Ropinirole, Methadone**

Appendix 3: Standard operating procedures for laboratory analyses

Cf. Lab Manual