



Clinical Study Protocol

Sponsor:

GlaxoSmithKline Biologicals SA

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B-1330 Rixensart - Belgium

Primary Study vaccine/product and number	<ul style="list-style-type: none"> GSK Biologicals candidate HIV prophylactic vaccine (gp120/ NefTat) adjuvanted to AS01B (GSK SB732461)
Other Study vaccine/product	<ul style="list-style-type: none"> Not applicable
eTrack study number and Abbreviated Title	201606 (PRO-HIV-013 EXT:002)
Date of protocol Title	Final Version 1: 12 July 2017 Long-term immunogenicity of the HIV gp120-NefTat/AS01B vaccine (GSK SB732461)
Detailed Title	A Phase I, open-label study to evaluate the long-term immunogenicity of the gp120-NefTat/AS01B vaccine administered in HIV-1 uninfected subjects
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Protocol Sponsor Signatory Approval

eTrack study number and Abbreviated Title	201606 (PRO-HIV-013 EXT:002)
Date of protocol	Final Version 1: 12 July 2017
Detailed Title	A Phase I, open-label study to evaluate the long-term immunogenicity of the gp120-NefTat/AS01B vaccine administered in HIV-1 uninfected subjects
Sponsor signatory	François Roman, Clinical and Epidemiology Project Lead

Signature	_____

Date	_____

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Protocol Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' study vaccine/product and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccine(s)/product(s), and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

**eTrack study number and
Abbreviated Title**

201606 (PRO-HIV-013 EXT:002)

Date of protocol

Final Version 1: 12 July 2017

Detailed Title

A Phase I, open-label study to evaluate the long-term immunogenicity of the gp120-NefTat/AS01B vaccine administered in HIV-1 uninfected subjects

Investigator name

Prof. Dr. I. Leroux-Roels, University of Ghent,
Belgium

Signature

Date

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals

Rue de l'Institut, 89

B-1330 Rixensart – Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section [8.3.2](#).

SYNOPSIS

Detailed Title	A Phase I, open-label study to evaluate the long-term immunogenicity of the gp120-NefTat/AS01B vaccine administered in HIV-1 uninfected subjects
Rationale for the study and study design	<p>Study PRO HIV-002 was a phase I/II, double-blind, randomized controlled study conducted in HIV uninfected volunteers, to compare the safety and immunogenicity of the gp120-NefTat (20 µg/20 µg) vaccine, adjuvanted with either AS02A, AS02V or AS01B. The vaccine was administered in a 0, 1, 3 and 6 months schedule and subjects were followed up to 18 months after the last vaccination. The study was conducted from February 2003 until February 2005.</p> <p>The study vaccines were overall well tolerated and induced strong and long-lasting HIV-specific CD4+ T cell responses, with strongest responses in the gp120-NefTat/AS01B group. Strong binding antibody responses were detected against gp120, Nef and Tat in all vaccine groups that persisted up to 18 months after the last vaccination.</p> <p>The objective of the present study is to evaluate the long-term persistence of binding antibody responses against V1V2 and gp120 in subjects who were vaccinated with the gp120-NefTat/AS01B vaccine candidate. Other immune parameters like the HIV-specific CD4+ T cell and CD8+ T cell responses will also be evaluated.</p>
Objectives	<p>Primary</p> <ul style="list-style-type: none">• To assess the persistence of immune responses approximately 14 years after administration of the gp120-NefTat/AS01B vaccine candidate, in terms of anti-V1V2 binding antibody response by Binding Antibody Multiplex Assay (BAMA) versus historical time points in study PRO-HIV-002. <p>Secondary</p> <ul style="list-style-type: none">• To assess the persistence of immune responses approximately 14 years after administration of the gp120-NefTat/AS01B vaccine candidate, in terms of HIV-1 specific CD4+ T cell and CD8+ T cell responses versus historical time points in study PRO-HIV-002.• To assess the persistence of immune responses approximately 14 years after administration of the gp120-NefTat/AS01B vaccine candidate, in terms of anti-gp120 antibody response by BAMA versus historical time points

in study PRO-HIV-002.

Study design

- Experimental design: Phase I, open-label, descriptive mono-centric, and uncontrolled study with one single group. This study will consist of a single sample collection visit.
- Duration of the study: 1 day (single study visit) for each subject
 - Epoch 001: Prospective data collection at single study visit that will occur approximately 14 years post vaccination
- Primary completion Date (PCD): Year 14
- End of Study (EoS): Last reading results released for all subjects from Year 14 single study visit
- Study group:

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min – Max)	Epochs
			Epoch 001
Group 1	Approximately 30	30 years– 65 years	x

- Control: uncontrolled
- Vaccination schedule: Not applicable
- Treatment allocation: Not applicable
- Blinding: Not applicable

Synopsis Table 2 Blinding of study epochs

Study Epochs	Study Groups	Blinding
Epoch 001	Group 1	Open

- Sampling schedule:
 - Blood samples will be collected from all subjects during the single study visit to assess humoral immunity and cell-mediated immunity (CMI).
 - Retrospective serum samples from subjects in study PRO HIV-002 at Day (D)0, D182, and D672 time points will be re-tested for humoral immunity using exploratory humoral assays.
 - Retrospective PBMC samples from subjects in study PRO HIV-002 at D0, D98, and D672 time points will be re-tested for CMI responses using intracellular cytokine staining (ICS) assays.

- Type of study: extension of protocol PRO HIV-002 (732461)
- Data collection: Electronic Case Report Form (eCRF)
- Safety monitoring: Not applicable

Number of subjects

Planned target number of subjects is approximately 30 subjects who received the gp120-NefTat/AS01B vaccine in GSK Biologicals-sponsored PRO HIV-002 study

Endpoints

Primary

- Seropositivity status and anti-V1V2 (IgG, IgG1, IgG2, IgG3, and IgG4) binding antibody concentration as measured by BAMA at Y14, and at historical time points of study PRO HIV-002 (D0, D182, and D672).

Testing of samples for PRO HIV-002 time points will depend on availability of samples.

Secondary

- Magnitude, responder status, cytokine co-expression profile of HIV-1 specific CD4+ T cell and CD8+ T cell responses as assessed by ICS assay at Y14, and at historical time points of study PRO HIV-002 (D0, D98, and D672).
- Seropositivity status and anti-gp120 (IgG, IgG1, IgG2, IgG3, and IgG4) binding antibody concentration as measured by BAMA at Y14, and at historical time points of study PRO HIV-002 (D0, D182, and D672).

Testing of samples for PRO HIV-002 time points will depend on availability of samples.

Additional investigations, such as additional CMI parameters, memory B cell phenotypes and antibody function may be performed based on study results and availability of samples.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AS01B	Adjuvant System 01B
BAMA	Binding Antibody Multiplex Assay
CD	Cluster of Differentiation
CFC	Cell Flow Cytometry (= ICS)
CI	Confidence Interval
CLS	Clinical Laboratory Sciences
CMI	Cell-Mediated Immunity
eCRF	electronic Case Report Form
EoS	End of Study
GCP	Good Clinical Practice
GM(C)	Geometric mean (concentration)
gp	Envelope glycoprotein
GSK	GlaxoSmithKline
HIV	Human immunodeficiency virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining (= CFC)
IEC	Independent Ethics Committee
LTFU	Long-Term Follow-Up
LOQ	Limit Of Quantification
MPL	3-O-desacyl-4'-monophosphoryl lipid A
PBMC	Peripheral Blood Mononuclear Cell
PCD	Primary Completion Date
PP	Per-protocol
QS21	Quillaja saponaria Molina, fraction 21
RT	Reverse Transcriptase
SAE	Serious Adverse Event
SDV	Source Document Verification
SPM	Study Procedures Manual

GLOSSARY OF TERMS

Adverse event:	<p>Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.</p> <p>An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.</p>
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study (Synonym of End of Trial)	<p>For studies without collection of human biological samples or imaging data EoS is the Last Subject Last Visit (LSLV).</p> <p>For studies with collection of human biological samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV</p>
Epoch:	<p>An epoch is a set of consecutive time points or a single time point from a single protocol. Epochs are defined to support a main purpose which either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the time points included in an epoch must be sufficient to fulfil the purpose of the epoch.</p>
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the per-protocol analysis (see Section 10.4 for details on criteria for evaluability).
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.

Investigational vaccine/product:	A pharmaceutical form of an active ingredient being tested in a clinical trial, including a product with a marketing authorisation when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
(Synonym of Investigational Medicinal Product)	
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: ‘A written description of a change(s) to or formal clarification of a protocol.’ GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study.
Study vaccine/product	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/ product /placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s)/product(s) or as a control.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.

1. INTRODUCTION

1.1. Rationale for the study and study design

The RV144 trial provided the first evidence of protective efficacy against HIV-1 acquisition after vaccination in adults [Rerks-Ngarm, 2009]. This protective effect peaked at more than 60% one year after first vaccination but rapidly declined thereafter, suggesting the importance of persistent antibody responses to sustain protection over time [Robb, 2012]. An immune-correlates analysis of the RV144 trial identified vaccine-induced anti-V1V2 IgG responses as being inversely correlated with the rate of HIV-1 infection [Haynes, 2012].

Study PRO HIV-002 was a phase I/II, double-blind, randomized controlled study conducted in HIV uninfected volunteers, to compare the safety and immunogenicity of the gp120-NefTat (20 µg/20 µg) vaccine, adjuvanted with either AS02_A, AS02_V or AS01_B. The vaccine was administered in a 0, 1, 3 and 6 months schedule and subjects were followed up to 18 months after the last vaccination. The study was conducted from February 2003 until February 2005 [Leroux-Roels, 2010].

The study vaccines were overall well tolerated and induced strong and long-lasting HIV-specific CD4+ T cell responses, with strongest responses in the gp120-NefTat/AS01_B group. Strong binding antibody responses were detected against gp120, Nef and Tat in all vaccine groups that persisted up to 18 months after the last vaccination [Leroux-Roels, 2010].

A post-hoc analysis was performed on PRO HIV-002 samples from 30 subjects who were administered the gp120-NefTat/AS01_B vaccine candidate in PRO HIV-002 to determine the level of anti-V1V2 IgG responses. An ample and persistent anti-V1V2 response was observed after vaccination, with 100% of response rate two weeks after the third and fourth vaccination and 87% of response rate at 18 months after the last vaccine dose. These observations and the potential correlate of protection associated with anti-V1V2 response as described in RV144 study [Yates, 2011; Yates, 2014] support further assessment of long-term persistence of anti-V1V2 response.

GSK contributes to the public-private pox-protein partnership (P5) which is currently assessing the safety, immunogenicity and clinical efficacy of a prime-boost regimen aimed at preventing HIV transmission (<http://www.hvtn.org/en.html>). The booster component of the candidate vaccine used in this program consists of two gp120 clade C proteins administered with an adjuvant. In order to inform the selection of the adjuvant in future studies, data on long-term persistence of immunity after vaccination with gp120/AS01 would prove useful. The present study was designed to address this question using a cohort of volunteers vaccinated several years ago with a candidate vaccine containing gp120 and AS01.

The objective of the present study is to evaluate the long-term persistence of binding antibody responses against V1V2 and gp120 in subjects who were vaccinated with the gp120-NefTat/AS01_B vaccine candidate. Other immune parameters like the HIV-specific CD4+ T cell and CD8+ T cell responses will also be evaluated.

1.2. Benefit : Risk Assessment

The following section outlines the risk assessment and mitigation strategy for this study protocol:

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Risk from blood sampling	Blood sampling-associated risk of discomfort, syncope, dizziness, infection at the site during or after venepuncture.	<p>Blood samples will be obtained by a trained professional and medical assistance will be available.</p> <p>The potential risk of feeling faint, or experiencing mild local pain, bruising, irritation or redness at the site where blood was taken, will be mentioned in the ICF. The amount of blood to be taken for sampling will not be harmful to the subject's health.</p>

2. OBJECTIVES

2.1. Primary objective

- To assess the persistence of immune responses approximately 14 years after administration of the gp120-NefTat/AS01B vaccine candidate, in terms of anti-V1V2 binding antibody response by Binding Antibody Multiplex Assay (BAMA) versus historical time points in study PRO-HIV-002.

Refer to Section 10.1 for the definition of the primary endpoint.

2.2. Secondary objectives

- To assess the persistence of immune responses approximately 14 years after administration of the gp120-NefTat/AS01B vaccine candidate, in terms of HIV-1 specific CD4+ T cell and CD8+ T cell responses versus historical time points in study PRO-HIV-002.
- To assess the persistence of immune responses approximately 14 years after administration of the gp120-NefTat/AS01B vaccine candidate, in terms of anti-gp120 antibody response by BAMA versus historical time points in study PRO-HIV-002.

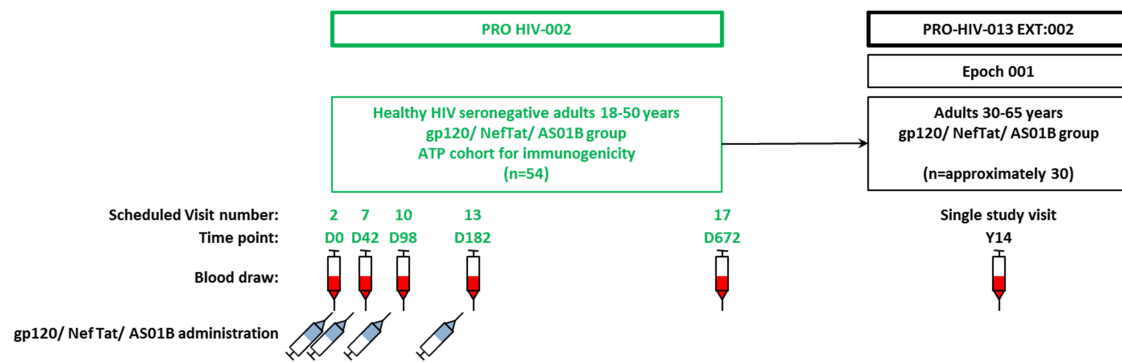
Based on the outcome of the primary responses, other exploratory investigations may be performed on stored samples (sera and peripheral blood mononuclear cells [PBMCs]) to better characterize the effect of vaccination on other immunologic parameters, such as

antibody function, memory B cells, or other read-outs that might be available at the time of the analysis.

Refer to Section 10.2 for the definition of the secondary endpoint(s).

3. STUDY DESIGN OVERVIEW

Figure 1 Schematic representation of the study design



D: Day, Y: Year

This study will only invite subjects from PRO HIV-002 Group 3 who received 4 doses of gp120/NefTat/AS01B at PRO HIV-002 D0, D28, D84, and D168.

Y14 biological samples will be tested in addition to historical PRO-HIV-002 biological samples of selected time points

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.5), are essential and required for study conduct.

- Experimental design: Phase I, open-label, descriptive, mono-centric, and uncontrolled study with one single group. This study will consist of a single sample collection visit.
- Duration of the study: 1 day (single study visit) for each subject
 - Epoch 001: Prospective data collection at single study visit that will occur approximately 14 years post vaccination
- Primary completion Date (PCD): Year 14

Refer to [glossary of terms](#) for the definition of PCD.

- End of Study (EoS): Last reading results released for all subjects from Year 14 single study visit

Refer to [glossary of terms](#) for the definition of EoS.

- Study group:

Table 1 Study group and epoch foreseen in the study

Study Groups	Number of subjects	Age (Min - Max)	Epochs
			Epoch 001
Group 1	Approximately 30	30 years - 65 years	x

- Control: uncontrolled
- Vaccination schedule: Not applicable
- Treatment allocation: Not applicable
- Blinding: Not applicable

Table 2 Blinding of study epochs

Study Epochs	Study Groups	Blinding
Epoch 001	Group 1	Open

- Sampling schedule:
 - Blood samples will be collected from all subjects during the single study visit to assess humoral immunity and cell-mediated immunity (CMI).
 - Retrospective serum samples from subjects in study PRO HIV-002 at Day (D)0, D182, and D672 time points will be re-tested for humoral immunity using exploratory humoral assays.
 - Retrospective PBMC samples from subjects in study PRO HIV-002 at D0, D98, and D672 time points will be re-tested for CMI responses using intracellular cytokine staining (ICS) assays.
- Type of study: extension of protocol PRO HIV-002 (732461)
- Data collection: Electronic Case Report Form (eCRF)
- Safety monitoring: Not applicable

4. STUDY COHORT

4.1. Number of subjects/centres

Planned target number of subjects is approximately 30 subjects who received the gp120-NefTat/AS01_B vaccine in GSK Biologicals-sponsored [PRO HIV-002](#) study.

Overview of the recruitment plan

This study will occur in only one medical centre. The recruitment is estimated to be completed in approximately 5 weeks.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- **Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol.**
- **Written informed consent obtained from the subject prior to performing any study specific procedure.**
- A subject who has received at least three doses of the gp120-NefTat/AS01B vaccine candidate in GSK Biologicals-sponsored PRO HIV-002 study.

4.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Use of any investigational or non-registered product (drug or vaccine) during 30 days prior to study enrolment.
- History of HIV-1 or HIV-2 infection.
- Participation to another clinical trial of an investigational HIV vaccine between study PRO HIV-002 and the present study.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting one month preceding this study. For corticosteroids, this will mean prednisone ≥ 20 mg/day (or equivalent). Inhaled and topical steroids are allowed.
- Administration of cytotoxic medication within six months preceding this study.
- History of daily, long-term (more than one year) immunosuppressive medication between study PRO HIV-002 and the present study.
- Administration of immunoglobulins and/or any blood products during the period starting 3 months before enrolment.
- Any confirmed or suspected immunosuppressive or immunodeficient condition other than HIV disease, based on medical history and physical examination (no laboratory testing required) between study PRO HIV-002 and the present study.
- Past administration of an investigational vaccine containing AS01 other than the gp120-NefTat/AS01B vaccine administered in PRO HIV-002 study.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for GCP, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

GSK will obtain favourable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written informed consent must be obtained from each subject prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

5.2. Subject identification and randomisation

5.2.1. Subject identification

Subjects will be assigned the identification number they received in [PRO HIV-002](#) study.

5.2.2. Randomisation of treatment

Not applicable.

5.3. Method of blinding

Not applicable.

5.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying Study Procedures Manual (SPM). The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

5.5. Outline of study procedures

Table 3 List of study procedures

Epoch	<i>Epoch 001</i>
Type of contact	Single Study Visit
Timepoint (s)	Year 14
Informed consent	●
Check inclusion/exclusion criteria	●
Collect demographic data	●
Record medical history	●
Record vaccination history	●
Laboratory Assays	
Blood sampling for HIV test (9 mL)	●
Blood sampling for antibody determination and exploratory characterisation (9 mL)	●
Blood sampling for CMI responses and exploratory characterisation (27 mL)	●
Safety Assessments	
Recording of SAEs related to study participation or to a concurrent GSK medication/product	●
Study conclusion	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.

CMI: cell-mediated immunity; SAE: serious adverse event.

All study procedures will occur during the Single Study Visit.

5.6. Detailed description of study procedures

5.6.1. Informed consent

The signed informed consent of the subject must be obtained before study participation. Refer to Section 5.1 for the requirements on how to obtain informed consent.

5.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.6.3. Collect demographic data

Record demographic data such as sex and race in the subject's eCRF.

5.6.4. Medical and vaccination history

Obtain the subject's medical and vaccination history by interview and/or review of the subject's medical records and record in the eCRF:

- any pre-existing conditions or signs and/or symptoms present in a subject within 30 days before the start of the study,
- past receipt of any vaccine administered within 30 days before the start of the study.
- past receipt of any experimental HIV vaccine (other than in [PRO HIV-002](#) study).

5.6.5. Blood sampling

Blood samples will be taken during the Single Study Visit (as specified in Section 5.5 List of Study Procedures) for the assessment of.

- HIV test,
- antibody determination,
- CMI responses, and
- exploratory characterisation.

An overall volume of 45 mL will be collected during the entire study period.

Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

5.6.6. Study Vaccine/product administration

Not applicable.

5.6.7. Recording of SAEs

- Refer to Section 8.2 for procedures for the investigator to record SAEs. Refer to Section 8.3 for guidelines and how to report SAE to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

5.6.8. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness
- complete the Study Conclusion screen in the eCRF.

5.7. Biological sample handling and analysis

Please refer to the SPM for details on biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject (subject number).

- **Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.**
- **It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in Belgium and will only be performed once an independent Ethics Committee or Review Board has approved this research.**

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject.

Refer also to the [Investigator Agreement](#), where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

If additional testing is performed, the marker priority ranking given in Section 5.7.4 may be changed.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the per-protocol analysis (See Section 10.4 for the definition of cohorts to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator’s site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

5.7.2. Biological samples

Table 4 Biological samples

Sample type	Quantity	Unit	Timepoint
Blood sample for HIV test	9	ml	Single Study Visit
Blood sample for serology	9	ml	Single Study Visit
Blood sample for CMI	27	ml	Single Study Visit

5.7.3. Laboratory assays

Please refer to **APPENDIX A** for a detailed description of the assays performed in the study. Please refer to **APPENDIX B** for the address of the clinical laboratories used for sample analysis.

Test for the determination of HIV infection (to exclude subjects with HIV infection) will be performed at the Investigator’s laboratory using standardized and validated procedures. The diagnostic algorithm used in UZ Gent is: 4th generation screening test that detects antibodies and p24 antigen (VIDAS DUO). In case of reactivity, an Inno Lia confirmation test (Fujirebio) is performed. This test will allow to distinguish between a real seropositivity and a vaccine-induced seropositivity (if any). The result will be encoded in eCRF as a qualitative result (HIV infected: yes/no) and raw results will be uploaded into the study database.

Serological assays ([Table 5](#)) for the determination of antibodies against gp120 and V1V2 to check the subjects’ response to the vaccine they received in study [PRO HIV-002](#) will be performed at Duke Human Vaccine Institute using standardized procedures.

Table 5 Humoral immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit	Cut-off *	Laboratory ***
SER	Human Immunodeficiency Virus Ab Anti-gp120** (IgG, IgG1, IgG2, IgG3, IgG4, and Total IgG Avidity)	BAMA	In-house	MFI	95 th percentile of the MFI-background-Neg from all baseline [†]	Duke Human Vaccine Institute
SER	Human Immunodeficiency Virus Ab Anti-V1V2** (IgG, IgG1, IgG2, IgG3, IgG4, and Total IgG Avidity)	BAMA	In-house	MFI	95 th percentile of the MFI-background-Neg from all baseline [†]	Duke Human Vaccine Institute

SER: serum; BAMA: Binding Antibody Multiplex Assay; MFI: mean fluorescence intensity

[†] Baseline is defined as Day 0 in [PRO HIV-002](#) study.

* Assay cut-off and unit might be subject to change during the course of the study (e.g., in case of requalification, revalidation or standardization). In this case, this will be documented in the clinical report.

** Performed on antigen corresponding to the vaccine strain (other HIV strains or HIV clades).

*** Refer to [APPENDIX B](#) for the laboratory addresses.

ICS (CFC) assays ([Table 6](#)) on HIV-1 specific CD4+ T cells and CD8+ T cells to check the subjects’ response to the vaccine they received in study [PRO HIV-002](#) will be performed at CEVAC using standardized procedures.

Table 6 Cell-mediated Immunity (CMI)

System	Component Family	Challenge	Method	Laboratory *
PBMC	Core CFC read-out specific CD4+ T cells and CD8+ T cells Cells CD4.CD40L(+)+Interleukin-2(-)+Tumor Necrosis Factor alpha(-)+Interferon gamma(-)	gp120	CFC	CEVAC

PBMC: peripheral blood mononuclear cells; CFC: cell flow cytometry

* Refer to [APPENDIX B](#) for the laboratory addresses.

Other assays may be performed on sample repositories taken for this purpose or leftover samples, with the aim to explore tertiary objectives of the study. The research may include, but is not limited to:

- Serological responses to Nef, Tat, or other HIV antigens.
- Evaluation of antibody quality by Surface Plasmon Resonance (SPR), Bio-Layer Interferometry (BLI) or other methods.
- Evaluation of antibody function by antibody profiling and systems serology.
- Characterisation of the impact of vaccination on possible new virological markers.
- Host transcriptome signature: evaluation of mRNA and/or microRNA signatures by microarray and/or RNA sequencing.
- Translational research using next generation technologies.

Additional exploratory testing on the vaccine and and/or the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

The GSK Biologicals’ clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals’ clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

5.7.4. Biological samples evaluation

5.7.4.1. Humoral immunity

Table 7 Humoral immunity read-outs

Blood sampling time point		No. subjects	Component	Components priority rank
Type of contact and time point	Sampling time point			
Single Study Visit + Historical PRO HIV-002 time points	Y14 (PIV)	Approximately 30 *	Anti-V1V2 antibodies (BAMA)	1
	PRO HIV-002 D0 (PRE), D182 (PIV), and D672 (PIV)		Anti-gp120 antibodies (BAMA)	2
			Total IgG Avidity	3

* to be determined based on sample availability.

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analysed according to priority ranking provided in [Table 7](#).

5.7.4.2. Cellular immunity

Table 8 Cellular immunity read-outs

Blood sampling time point		No. subjects	Component
Type of contact and time point	Sampling time point		
Single Study Visit + Historical PRO HIV-002 time points	Y14 (PIV) + PRO HIV-002 D0 (PRE), D98 (PIII), and D672 (PIV)	Approximately 30 *	HIV-1 specific CD4+ T cells and CD8+ T cells

* to be determined based on sample availability.

5.7.4.3. Molecular biology

Table 9 Molecular biology read-outs

Blood sampling time point		No. subjects	Component
Type of contact and time point	Sampling time point		
Single Study Visit	Y14 (PIV)	Approximately 30 *	Human Immunodeficiency virus 1 RNA (QPCR)

* to be determined based on sample availability.

5.7.5. Immunological correlates of protection

No generally accepted immunological correlate of protection has been demonstrated so far for the antigens used in the candidate vaccine/ product.

6. STUDY VACCINE/PRODUCT AND ADMINISTRATION

Not applicable.

7. HEALTH ECONOMICS

Not applicable.

8. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

Each subject will be instructed to contact the investigator immediately should they/the subject manifest any signs or symptoms they perceive as serious.

8.1. Safety definitions

8.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- **Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).**

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- **Pre-existing conditions or signs and/or symptoms present in a subject prior to the study vaccination. These events will be recorded in the medical history section of the eCRF.**

8.1.2. Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

- a. Results in death,**
- 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, had it been more severe.

c. Requires hospitalisation or prolongation of existing hospitalisation,

Note: In general, hospitalisation signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting. Complications that occur during hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity, OR

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, diarrhoea, influenza like illness, 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 in the offspring of a study subject.

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation.

8.1.3. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g., severe fluctuations in blood pressure) that are judged by the investigator to be clinically significant will be recorded as SAE if they meet the definition of an SAE (refer to Section 8.1.1). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

8.2. Detecting and recording serious adverse events

8.2.1. Time period for detecting and recording serious adverse events

SAEs related to study participation or to a concurrent GSK medication/product will be collected and recorded from the time the subject consents to participate in the study (i.e., ICF signature) until the subject is discharged from the study.

8.2.2. Post-Study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study procedure, the investigator will promptly notify the Study Contact for Reporting SAEs.

8.2.3. Evaluation of adverse events and serious adverse events

8.2.3.1. Active questioning to detect adverse events and serious adverse events

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

8.2.3.2. Assessment of adverse events

8.2.3.2.1. Assessment of intensity

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgement.

The intensity should be assigned to one of the following categories:

- 1 (mild) = An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- 2 (moderate) = An AE which is sufficiently discomforting to interfere with normal everyday activities.
- 3 (severe) = An AE which prevents normal, everyday activities

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as ‘serious’ when it meets one of the pre-defined outcomes as described in Section 8.1.1.

8.2.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between study procedure and the occurrence of each AE/SAE using clinical judgement.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

If an event meets the criteria to be determined as ‘serious’ (see Section 8.1.1), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Erroneous administration
- Other cause (specify).

8.2.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

8.3. Reporting of serious adverse events

8.3.1. Prompt reporting of serious adverse events to GSK Biologicals

SAEs that occur in the time period defined in Section 8.2 will be reported promptly to GSK within the timeframes described in Table 10, once the investigator determines that the event meets the protocol definition of a SAE.

Table 10 Timeframes for submitting serious adverse event to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*†	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

* Timeframe allowed after receipt or awareness of the information.

† The investigator will be required to confirm review of the SAE causality by ticking the ‘reviewed’ box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

8.3.2. Contact information for reporting serious adverse events

Study Contact for Reporting SAEs
Refer to the local study contact information document. Email address: PPD [redacted]
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD [redacted] or PPD [redacted] Email address: PPD [redacted]

8.3.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report **WITHIN 24 HOURS**. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated **WITHIN 24 HOURS**.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

8.3.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the Study Contact for Reporting SAEs (refer to the [Sponsor Information](#)) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and **NOT** if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

8.3.4. Updating of SAE information after removal of write access to the subject's eCRF

When additional SAE information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the [Sponsor Information](#)) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in [Table 10](#).

8.3.5. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section [8.3.1](#).

8.4. Follow-up of serious adverse events

8.4.1. Follow-up during the study

After the initial SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to [Table 10](#)).

All SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

8.4.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- with SAEs or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper/electronic Expedited Adverse Events Report and/or pregnancy report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognised follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

8.5. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of a SAE should be recorded in Expedited Adverse Event Report of the subject's eCRF.

8.6. Subject card

Study subjects must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject. In an emergency situation this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects must be instructed to keep subject cards in their possession at all times during the study duration.

9. SUBJECT COMPLETION AND WITHDRAWAL

Not applicable.

10. STATISTICAL METHODS

10.1. Primary endpoint

- Seropositivity status and anti-V1V2 (IgG, IgG1, IgG2, IgG3, and IgG4) binding antibody concentration as measured by BAMA at Y14, and at historical time points of study PRO HIV-002 (D0, D182, and D672).

Testing of samples for [PRO HIV-002](#) time points will depend on availability of samples.

10.2. Secondary endpoints

- Magnitude, responder status, cytokine co-expression profile of HIV-1 specific CD4+ T cell and CD8+ T cell responses as assessed by ICS assay at Y14, and at historical time points of study PRO HIV-002 (D0, D98, and D672).
- Seropositivity status and anti-gp120 (IgG, IgG1, IgG2, IgG3, and IgG4) binding antibody concentration as measured by BAMA at Y14, and at historical time points of study PRO HIV-002 (D0, D182, and D672).

Testing of samples for [PRO HIV-002](#) time points will depend on availability of samples.

Additional investigations, such as additional CMI parameters, memory B cell phenotypes and antibody function may be performed based on study results and availability of samples.

10.3. Determination of sample size

The sample size in this study is driven by the sample size of the primary [PRO HIV-002](#) study, in which 60 subjects were vaccinated with the gp120-NefTat/AS01_B vaccine candidate, of which 54 were included in the Per-protocol (PP) cohort for immunogenicity. Based on preliminary assessment by the study site, it is expected that approximately 30 to 40 subjects will be eligible to enter this long-term follow-up (LTFU) study.

10.4. Cohorts for Analyses

10.4.1. Total vaccinated cohort

The Total cohort will include all subjects who signed the informed consent and provided a blood sample.

10.4.2. Per-protocol cohort for analysis of immunogenicity

The PP cohort for immunogenicity will include all subjects from the Total cohort:

- who meet all eligibility criteria,
- who present a negative HIV-RNA test at Y14.

Subjects eliminated from the PP cohort for immunogenicity will have their results reported as line listings.

10.5. Derived and transformed data

Demography

- Age at vaccination will be computed as the difference between the date of dose 1 administration in primary [PRO HIV-002](#) study and the 15 JUNE of the year of birth (only the year of birth will be collected). Age at Y14 visit in the study will be computed as the difference between the date of Y14 visit and the 15 JUNE of the year of birth. The ages will be expressed in years.
- For a given subject and a given demographic variable, missing measurements will not be replaced.

Safety

- For analysis of SAEs and for the analysis of concomitant medications, all subjects will be considered. Subjects who did not report an event or a concomitant GSK medication will be considered as subjects without the event or without the concomitant medication respectively.

Immunogenicity

- Any missing or non-evaluable immunogenicity measurement will not be replaced. The descriptive analysis performed for each assay at each time point will exclude subjects with a missing or non-evaluable measurement.
- The geometric mean titers (GMTs)/geometric mean concentrations (GMCs) will be computed by taking the anti-logarithm of the arithmetic mean of the log₁₀ transformed titers/concentrations.
- A seronegative subject will be defined as a subject whose antibody titer/concentration is below the cut-off value of the assay. A seropositive subject is a subject whose antibody titer/concentration is greater than or equal to the cut-off value of the assay.

- Vaccine response rates for HIV-1- specific CD4+ T-cells expressing **at least two markers** among CD40L, IL-2, TNF- α and IFN- γ with responders will be defined as subjects with:
 - a 2-fold increase as compared to the cut-off (limit of quantification [LOQ] of the assay), for subjects with pre-vaccination frequency below the cut-off;
 - at least 2-fold increase as compared to pre-vaccination frequency, for subjects with pre-vaccination frequency above the cut-off.

Other definitions of the CMI vaccine response might be explored during the analysis. They will be described in the SAP.

10.6. Analysis of demographics

For the Total cohort and for the PP cohort for immunogenicity, demographic data (age at vaccination in years, age at Y14 visit of this current LTFU study in years, gender and race) will be summarized using descriptive statistics:

- Frequency tables for gender and race.
- Mean, median, standard error for age.

10.7. Analysis of immunogenicity

The primary analysis will be performed on the PP cohort for immunogenicity and, if the percentage of vaccinated subjects with serological results excluded from the PP cohort for analysis of immunogenicity is at least 10%, a second analysis will be performed on the Total cohort.

For the humoral immune response

For anti-V1V2 and anti-gp120 (IgG, IgG1, IgG2, IgG3, and IgG4) antibody concentrations (by BAMA), at each time point [PRE(D0), PIV(D182), PIV(D672), PIV(Y14)] where such information is available:

- Seropositivity rates with 95% CIs will be tabulated.
- Geometric mean concentrations (GMCs) with 95% CIs will be tabulated and presented graphically.
- Geometric mean of ratios (GMR) of antibody concentrations at each post-vaccination time point [PIV(D182), PIV(D672), PIV(Y14)] over pre-vaccination [PRE(D0)] will be tabulated with 95% CI.
- Antibody concentrations post-vaccination will also be presented using reverse cumulative curves.

For the cell-mediated immune response

The following parameters will be summarised using descriptive statistics (N, GM, min, Q1, median, Q3, max) at each time point [PRE(D0), PIII(D98), PIV(D672), PIV(Y14)] where CMI results are available:

- Frequency of HIV-1 specific CD4+/CD8+ T-cells expressing **at least two markers** among CD40L, IL-2, TNF- α and IFN- γ as measured by ICS using PBMCs.
- Frequency of HIV-1 specific CD4+/CD8+ T-cells expressing **at least one marker** among CD40L, IL-2, TNF- α and IFN- γ as measured by ICS using PBMCs.
- Geometric mean ratios (GMR) of frequency of HIV-1 specific CD4+/CD8+ T-cells expressing **at least two markers** among CD40L, IL-2, TNF- α and IFN- γ at each post-vaccination time point [PIII(D98), PIV(D672), PIV(Y14)] over pre-vaccination (D0) will be tabulated with 95% CI.
- Vaccine response rates for HIV-1-specific CD4+ T-cells expressing **at least two markers** among CD40L, IL-2, TNF- α and IFN- γ .

These analyses will be further detailed in the SAP document.

10.8. Analysis of safety

For the Total cohort, a detailed description of SAEs considered by the investigator to be related to study participation or concurrent GSK medication/product will be provided.

10.9. Interpretation of analyses

The analyses will be descriptive with the aim to characterise the gp120-NefTat/AS01B group in the endpoint(s) related to the objective(s). These descriptive analyses should not be interpreted.

10.10. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

10.10.1. Sequence of analyses

All pre-planned analyses will be performed when all data of the primary and secondary objectives for all enrolled subjects are available and cleaned.

An integrated clinical study report containing all data will be written and made available to the investigators.

If additional exploratory analyses are performed, they may be reported in subsequent annex reports, depending on data availability.

10.10.2. Statistical considerations for interim analyses

No interim analysis is planned for this descriptive LTFU study.

11. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements and publications must be fulfilled.

11.1. Electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

11.2. Study Monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by

comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or 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 GSK procedures.

11.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures, otherwise, the minimum retention period will default to 20 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.4. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. 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. If an audit or inspection occurs, the investigator and institution agree to 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.

11.5. Posting of information on publicly available clinical trial registers and publication policy

Interventional studies that do not evaluate vaccines/products are progressed for publication in the scientific literature when the results provide important scientific or medical knowledge or are relevant for patient care, and will be considered for disclosure on the GSK website and in publicly accessible regulatory registry(ies).

11.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

11.7. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non- GSK studies, to investigate further about the study product(s) and other product(s), and /or the disease/condition under investigation and related diseases and conditions.

12. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

13. REFERENCES

GlaxoSmithKline Biologicals Clinical Study Report 732461/002 (PRO HIV-002). A Phase I-II, double-blind, randomised study to compare the safety and immunogenicity of GSK Biologicals' candidate HIV vaccine gp120/NefTat (20 µg/20 µg) adjuvanted to AS02_A, AS02_V or AS01_B administered intramuscularly to healthy HIV seronegative volunteers.

Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *NEJM*. 2012; 366(14): 1275-86.

Leroux-Roels I, Koutsoukos M, Clement F, et al. Strong and persistent CD4+ T-cell response in healthy adults immunized with a candidate HIV-1 vaccine containing gp120, Nef and Tat antigens formulated in three Adjuvant Systems. *Vaccine*. 2010; 28(?): 7016-24.

Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *NEJM*. 2009; 361(23): 2209-20.

Robb ML, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Kunasol P, et al. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. *Lancet Infect Dis*. 2012;12(7):531-7. doi: 10.1016/S1473-3099(12)70088-9.

Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci Transl Med*. 2014;6(228):228ra39. doi: 10.1126/scitranslmed.3007730.

Yates NL, Lucas JT, Nolen TL, Vandergrift NA, Soderberg KA, Seaton KE, et al. Multiple HIV-1-specific IgG3 responses decline during acute HIV-1: implications for detection of incident HIV infection. *AIDS*. 2011 Nov 13;25(17):2089-97. doi: 10.1097/QAD.0b013e32834b348e.

APPENDIX A LABORATORY ASSAYS

Humoral immunity

Evaluation of antibody quality by BAMA avidity index or BLI

To evaluate avidity index using the BAMA protocol, samples will be incubated with beads in either the assay diluent above (PBS) or a sodium citrate solution at pH 4.0 (CIT) and then washed. Binding magnitude response will be evaluated for each, and the avidity index will be calculated as $(\text{MFI (CIT)}/\text{MFI (PBS)}) * 100$.

To evaluate antibody quality of total serum by BLI, HIV antigens will be immobilized onto Streptavidin (SA) or amine reactive (AR2G) using procedures and coupling reagents supplied by the manufacturer. The binding time courses will be monitored by dipping the antigen immobilized sensors directly into 1:50 diluted (in PBS buffer, pH 7.4) serum samples to monitor association for 300 seconds and then into PBS buffer to record dissociation for 300 seconds. Appropriate control peptide/protein immobilized sensors will be used as parallel reference sensors to record non-specific binding and will be subtracted to obtain antigen-specific binding curves. The specific binding curves will be analysed to obtain antigen-specific binding responses and dissociation rates of total antibodies. ForteBio Octet Red 384 instruments will be used for measurements.

HIV antigen-specific total IgG avidity assays will be carried out by capturing total IgG antibodies from serum onto anti-human IgG Fc immobilized sensors. These sensors will be dipped into HIV antigen constructs and PBS buffer to read binding and dissociation, respectively. Appropriate control sensors will be used in parallel as reference to collect non-specific binding and will be subtracted to get total IgG binding of HIV antigens. The subtracted binding curves will be processed to obtain antigen-specific binding response and dissociation rates.

The BLI determined antigen-specific binding response, which is a reflective of the magnitude of antigen-specific antibodies in a sample, will be compared to gauge the difference in magnitude of antibody responses in participants' serum samples. The antigen-specific dissociation rate is indicative of the quality of the antibodies present in a sample. The antigen-specific binding response and dissociation rate data together can be used to rank the serum samples of study participants from different groups with a score that includes both the magnitude of the antibody response and the dissociation rate of the antibody-antigen complex as measured by BLI. The binding response, dissociation rate and avidity rank for each antigen will be used in statistical analysis as described in Analytical Plan section.

Cell-mediated immunity (CMI)

The role of CMI in the protective response to HIV is thought to be important for protection. The CMI response will be assessed on thawed PBMCs at CEVAC.

Detection of gp120, Nef and Tat, intracellular cytokine staining (ICS) technique

Intracellular cytokine staining (ICS) is a method providing information on the frequency of CD4+ and CD8+ T cells responding to the antigen and secreting molecules involved in immunity such as IFN- γ , IL-2, TNF-a and CD40-L. The assay will be performed on

thawed PBMCs. Briefly, PBMCs of the subjects are stimulated for 2 hours by the vaccine antigen and/or derived peptides. Then, intracellular block (Brefeldin A) is added to inhibit cytokine secretion for a subsequent overnight stimulation. The cells are then harvested and stained for cell surface markers (e.g. CD4 and CD8), to identify specific sub-populations of T cells. Subsequently, the cells are fixed and then treated with a detergent such as saponin to permeabilise the cell membrane. The cells are then incubated with fluorescently labelled, cytokine-specific or activation marker-specific antibody that forms complexes with the cytokine in the cytoplasm. The cytokine producing cells can then be detected by cytofluorometry. Results are expressed as a frequency of antigen-specific CD4+ or CD8+ T cells identified as expressing cytokine/marker upon the in vitro stimulation.

APPENDIX B CLINICAL LABORATORIES

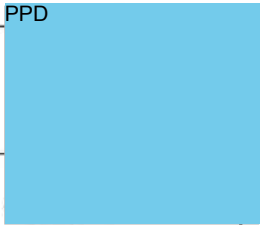
Table 11 GSK Biologicals' laboratories

Laboratory	Address
GSK Biological's Clinical Laboratory Sciences, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart – Belgium
GSK Biological's Clinical Laboratory Sciences, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 12 Outsourced laboratories

Laboratory	Address
Duke Human Vaccine Institute	Tomaras Laboratory Duke Human Vaccine Institute MSRB2 Bldg, Room 4116 / 2 Genome Ct. Durham, NC 27710 USA Email: PPD [REDACTED] PPD [REDACTED] Ph.D. PPD [REDACTED] Lab: PPD [REDACTED]
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium

Protocol Sponsor Signatory Approval

eTrack study number and Abbreviated Title	201606 (PRO-HIV-013 EXT:002)
Date of protocol	Final Version 1: 12 July 2017
Detailed Title	A Phase I, open-label study to evaluate the long-term immunogenicity of the gp120-NefTat/AS01B vaccine administered in HIV-1 uninfected subjects
Sponsor signatory	François Roman, Clinical and Epidemiology Project Lead
Signature	PPD 
Date	27 Nov 2017

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