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Study vaccine	GSK Biologicals candidate HIV prophylactic vaccine F4co (p24-RT-Nef-p17) adjuvanted with AS01B
Study vaccine number	732461
eTrack study number and abbreviated title	108706 (PRO HIV-005)
EudraCT number	2006-003796-12
Date of approval	December 5, 2006 (final)
Date of amendment 1	September 24, 2007
Date of amendment 2	October 08, 2007
Title	Dose-ranging study to evaluate the safety and immunogenicity of GSK Biologicals' HIV vaccine 732461, adjuvanted or not, when administered as a ~~3~~ ***2***-dose schedule to healthy adult HIV seronegative volunteers, aged 18 to 40 years. **(Amended September 24, 2007)**
Detailed title	A Phase I-II partially-blinded, randomized, dose-ranging study (10-30-90 µg) to compare the safety and immunogenicity of GSK Biologicals' candidate HIV vaccine F4co (p24-RT-Nef-p17), adjuvanted or not with AS01B, administered intramuscularly according to a vaccination schedule of 0, ~~1~~, ~~6 m~~onths to healthy adult HIV seronegative volunteers, aged 18 to 40 years. **(Amended September 24, 2007)**
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eTrack study number 108706

eTrack abbreviated title 108706 (PRO HIV-005)

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Synopsis

Title	A Phase I-II partially-blinded, randomized, dose- ranging study (10-30-90 μg) to compare the safety and immunogenicity of GSK Biologicals' candidate HIV vaccine F4co (p24-RT-Nef-p17), adjuvanted or not with AS01B, administered intramuscularly according to a vaccination schedule of 0, ~~1~~, ~~6 m~~onths to healthy adult HIV seronegative volunteers, aged 18 to 40 years. **(Amended September 24, 2007)**
Indication/Study population	Healthy HIV seronegative adults, 18--40 years of age
Rationale	The most prominent immune effector mechanisms directed against HIV are neutralizing antibodies and CD8+ T cells. In addition, accumulating evidence suggests that CD4+ T cells, which provide essential help to the other compartments of an adaptive immune response, are equally crucial for the induction of effective HIV immunity. In particular, the analysis of immune responses in HIV-infected individuals suggests a crucial role for CD4+ T cells. It appears that CD4+ cells provide essential help to CD8+ effector cells in long-term non-progressors. In contrast, the absence of HIV-specific CD4+ T cells in chronically infected individuals seems to be related to an impairment of CD8+ T cell maturation into effector cells. Thus, it appears that in order to maintain functional CD8+ T cell immunity, strong and broad CD4+ T cell responses are required and that these responses should be induced by an effective HIV vaccine. Probably the most potent approach to induction of CD4+ T cell responses is the use of adjuvanted protein vaccines. Indeed, GSK's previous candidate HIV vaccines consisted of two recombinant proteins, gp120 and NefTat, which were formulated in potent proprietary adjuvants (AS01B, AS02A and AS02V). These vaccines were shown to induce very potent CD4+ T cell responses in two independent clinical trials (HVTN-041 and HIV-002). The gp120 antigen was the most immunogenic vaccine component, and the Nef and Tat-directed responses were lower in comparison. AS01B (liposome-based formulation containing the immunostimulants 3D-MPL [3-deacylated monophosphoryl lipid A] and QS21 [a triterpene glycoside purified from the bark of Quillaja saponaria]) was chosen among other potent proprietary adjuvants, from several clinical studies (for exhaustive details please refer to the Investigator's Brochure)

for its propensity to induce a stronger Th1 cellular-mediated immune response and its acceptable safety profile.

Since such CD4+ cell responses need to cover the broadest possible spectrum of circulating HIV strains, an HIV vaccine should contain many different CD4 epitopes from different viral proteins. It was therefore desirable to include well-conserved and highly immunogenic HIV antigens in an improved vaccine formulation. The viral antigens that contain the highest number of conserved T cell epitopes are Gag, Pol, and Nef. Consequently, GSK chose to include these antigens as a single fusion protein (F4co) into an adjuvanted (AS01B) vaccine formulation.

Objectives

Primary

- To evaluate the reactogenicity and safety of the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B adjuvant at three different doses (10-30-90 μg).
- To evaluate the CD4+ T-cell response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) in terms of proportion of responders to at least 1, 2, 3 antigens and to all 4 antigens determined two weeks after the second vaccination.

Secondary

- To evaluate the CD4+ T-cell immune response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) after two ~~and three~~ vaccinations.
- To evaluate the serological response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) after two ~~and three~~ vaccinations. **(Amended September 24, 2007)**
- To evaluate the persistence of cell-mediated and serological responses to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg).

Exploratory

- To evaluate the cross clades reactivity determined by ICS 2 weeks and/or 1 month after the second vaccination.
- To evaluate the CD8+ T-cells immune response to the candidate vaccine F4co (p24-RT-Nef-p17) with or

without AS01B at three different doses (10-30-90 μg).

Study design

- Experimental design: Single center, dose-escalating, staggered, parallel group study
- Treatment allocation: Randomized for the adjuvantation (AS01B or water for injection)
- Blinding: Partially blind: observer-blind for adjuvantation, open for antigen content
- Treatment Groups:

  3 groups of 50 subjects receiving the candidate vaccine F4co (p24-RT-Nef-p17), adjuvanted with AS01B, respectively at 10, 30 or 90 μg

  3 groups of 10 subjects receiving the candidate vaccine F4co (p24-RT-Nef-p17), non-adjuvanted, in water for injection, respectively at 10, 30 or 90 μg
- Vaccination schedule: ~~3~~ ***2*** vaccinations administered intramuscularly according to a 0, ~~1~~, ~~6~~ month schedule **(Amended September 24, 2007)**
- Controls: None
- Type of study: Self-contained
- Data collection: Remote data entry (RDE)
- Duration of the study: The intended duration of the study, per subject will be approximately 14 months

Number of subjects

Target number of enrolled subjects = 180 (6 groups: 3 groups of 50 subjects and 3 groups of 10 subjects).

Primary endpoints

Reactogenicity and safety

- Occurrence, intensity and relationship to vaccination of solicited local and general symptoms during a 7-day (Day 0 to Day 6) follow-up period after each vaccination.
- Occurrence, intensity and relationship to vaccination of unsolicited symptoms during a 30-day (Day 0 to Day 29) follow-up period after each vaccination.
- Occurrence and relationship to vaccination of serious adverse events during the whole study period.
- Haematological and biochemical levels at months 0, 1, 2, 6, ~~7~~ **9**, 12 and at Day 44 (two weeks after the second vaccination) in all subjects. **(Amended September 24, 2007)**

Immunogenicity

- Frequency of CD4+ T cells expressing at least two cytokines including IL-2 equal or above the cut-off to at least 1, 2, 3 antigens and to all 4 antigens at Day 44 (two weeks after the second vaccination).

Secondary endpoints

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing at least 2 cytokines including IL-2 equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Antibody titers to p17, p24, Nef, RT and F4co as measured by ELISA at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**

Exploratory endpoints

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells to other HIV clades expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Day 44 (two weeks after the second vaccination) and/or at Month 2.
- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing at least 2 cytokines (IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L) equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**

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µg	Microgram
3D-MPL	3 Deacylated Monophosphoryl Lipid A
ADCC	Antibody-Dependent Cell-mediated Cytotoxicity
AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
AS0x	GlaxoSmithKline Adjuvant System x
AST	Aspartate Aminotransferase
ATP	According To Protocol
CD4 (8, 40L)	Immune cells that carry a marker on its surface known as Cluster of Differentiation 4 (8, 40L)
CDM	Clinical Development Manager
CEVAC	Center for Vaccinology, Ghent University, Belgium
CI	Confidence Interval
CMI	Cell-Mediated Immunity
CSC	Central Study Coordinator
CTL	Cytotoxic T Lymphocyte
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration
GMT	Geometric Mean Titer
GSB	Global Safety Board
GSK	GlaxoSmithKline

HCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICS	Intracellular Cytokine Staining
IDMS	Internal Data Monitoring Committee
IEC	Independent Ethics Committee
IFN-γ	Interferon gamma
IL-2	Interleukin 2
IN	Integrase
IRB	Institutional Review Board
ISCOMS	Immunostimulating Complexes
LTR	Long Terminal Repeat
MedDRA	Medical Dictionary for Regulatory Activities
mL	Millilitre
mm^3	Cubic millimetre
MVA	Modified Vaccinia virus Ankara
PBMC	Peripheral Blood Mononuclear Cell
PCV	Packed Cell Volume
PID	Subject Identification number
PR	Protease
QS21	Quillaja Saponaria 21: a triterpene glycoside purified from the bark of Quillaja saponaria
RBC	Red Blood Cell
RDE	Remote Data Entry
RNA	Ribonucleic Acid

RT	Reverse Transcriptase
SAE	Serious Adverse Event
SBIR	Central randomization call-in on internet
SHIV	A chimeric virus composed of an HIV envelope and SIV nucleocaspid and replication machinery
SIV	Simian Immunodeficiency Virus
SOP	Standard Operating Procedure
SRT	Safety Review Team
STD	Sexually Transmitted Disease
TNF-α	Tumour necrosis factor alpha
UNAIDS	Joint United Nations Programme on HIV1AIDS
VRC	Vaccine Research Center
VSMB	GSK Vaccine Safety Monitoring Board
WBC	White Blood Cell
WFI	Water For Injection
WHO	World Health Organization

Glossary of Terms

Adverse event:	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. 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.
Azoospermia	Documented azoospermia refers to the outcome of the investigator's/designee's medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records.
Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. In a single-blind trial, the investigator and/or his staff are aware of the treatment assignment but the subject is not. In an observer-blind study, the subject and the study personnel involved in the clinical evaluation of the subjects are blinded while other study personnel may be aware of the treatment allocation. When the investigator and sponsor staff who are involved in the treatment or clinical evaluation of the subjects and review/analysis of data are also unaware of the treatment assignments, the study is double blind. Partially blind is to be used for study designs with different blinding levels between different groups, e.g. double blinded consistency lots which are open with respect to the control group. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event.
Central Study Co-ordinator:	An individual assigned by and centrally located at GSK Biologicals at Rixensart who is responsible for assuring proper conduct of a clinical study.

Contraception	Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly (when applicable, as mentioned in the product label) for example abstinence, combined or progestogen oral contraceptives, injectable progestogen, implants of levonorgestrel, oestrogenic vaginal ring, percutaneous contraceptive patches or intrauterine device (IUD) or intrauterine system (IUS), vasectomy with documented azoospermia of the sole male partner or double barrier method (condom or occlusive cap plus spermicidal agent).
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
eTrack	GSK's clinical trials tracking tool
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Sections 4 and 10.5 for details on criteria for evaluability).
Investigational product:	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization 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.
Medical Monitor:	An individual medically qualified to assume the responsibilities of the sponsor (GSK Biologicals) especially in regards to the ethics, clinical safety of a study and the assessment of adverse events.
Post-menopause	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after one year without menses with an appropriate clinical profile at the appropriate age, e.g. > 45 years.

Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study. N.B. ny change that falls under the definition of a protocol amendment (e.g. a change that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study) MUST be prepared as an amendment to the protocol.
Protocol amendment:	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.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	Adverse events (AEs) to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Study Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of a clinical study.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Subject:	Term used throughout the protocol to denote an individual that has been contacted in order to participate or participates in the clinical study, either as a recipient of the investigational product(s) or as a control.
Treatment number:	A unique number identifying a treatment to a subject, according to the study randomization or treatment allocation.
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, identified by a unique number, according to the study randomization or treatment allocation.

Unsolicited adverse event:	Any adverse event (AE) reported in addition to those solicited during the clinical study. Also any "solicited" symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

1. INTRODUCTION

1.1. Background

1.1.1. Epidemiology and disease manifestation

Since the first clinical evidence was reported in 1981, HIV has continued its relentless spread and today it is estimated that around 40 million people are living with HIV/AIDS in the world, and that more than 20 million people have already died from the disease [WHO, 2005]. Of the estimated 40 million people living with HIV/AIDS in the world, 95% of them lives in developing countries, especially in Africa, which is home to 26 million people already infected with HIV. In at least six Sub-Saharan African countries, one in five adults are already infected with HIV, and in another ten countries, 10% of adults are infected. HIV continues to spread relentlessly at a rate of 14,000 new HIV infections every day. The Joint United Nations Programme on HIV/AIDS (UNAIDS) has forecasted that from now until 2010, we could be adding some 5 million new infections every year [WHO, 2005; Esparza, 2005; Excler, 2005].

1.1.2. The Human Immunodeficiency Virus-1

The Human Immunodeficiency Virus-1 (HIV-1), causative agent of AIDS, is a member of the lentivirus group of retroviruses. Retroviruses have the unique ability to transcribe their RNA to DNA, and are capable of integrating their genome into the host cell DNA early in infection. They achieve this feat with the aid of the reverse transcriptase enzyme they encode. The retroviruses also show strong cell-cycle dependence for productive infection requiring therefore proliferating cells for infection. However, the lentivirus HIV is not dependent on host cell mitosis for establishment of provirus. Thus, non-dividing cells such as primary macrophages, follicular dendritic cells and microglial cells sustain a productive replication cycle in the absence of cell division, with the exception of quiescent T lymphocytes which are refractory to HIV replication [Stevenson, 1995].

The HIV-1 virion is spherical consisting essentially of an inner electron-dense core and an outer envelope [Gelderblom, 1987; Hockley, 1988]. Three major genes are found in the viral genome, *gag*, *pol* and *env*. They are flanked on both sides by identical sequences, long terminal repeats (LTR). The LTRs contain *cis*-acting elements with signals for viral integration, transcription and RNA synthesis. Additional open reading frames that flank the *env* gene encode several regulatory proteins including Vif, Vpr, Tat, Rev, Vpu and Nef. They have important functions during the early stages of the viral life cycle and are essential for efficient viral replication.

The *env* gene encodes the outer membrane protein gp120, and the transmembrane glycoprotein gp41. Gp41 is responsible for virus attachment to the host cell during infection. Gp120 binds to the major binding receptor, CD4, on CD4+ cells; chemokine receptors CCR5 and CXCR4 act as coreceptors. Gp120 contains five variable and five conserved regions; one variable region, the V3 loop, contains the principal neutralizing determinant of HIV-1. Gp120 contains other variable and conserved neutralizing epitopes

and cytotoxic T-lymphocyte (CTL) epitopes. Gp120 and gp 41 form trimers on the surface of the mature virion.

The *gag* gene encodes the 4 Gag proteins, MA or p17, a matrix protein, CA or p24, the viral capsid and NC or p6 and p7, the nucleocapsid proteins. These structural proteins make up the core of the virion.

The *pol* gene encodes the viral enzymes, the most important of which are reverse transcriptase (RT), protease (PR) and integrase (IN). RT transcribes the viral RNA into double-stranded DNA. IN integrates the DNA produced by RT into the host's genome. PR is an enzyme that cuts proteins, issued from *gag* and *pol*, into separate functional units.

The Nef regulatory protein accumulates before Tat in newly infected cells and acts as a crucial determinant of HIV virulence. The Nef protein is believed to be involved in the modulation of CD4 expression by triggering the rapid endocytosis and lysosomal degradation of this main virus receptor. The Nef protein may disrupt T-lymphocyte function and signalling as a result of its interaction with critical signal transduction components.

Analyses of nucleic acid from the HIV-1 have revealed large amounts of variation both within and between patients. The extensive genetic variability of HIV is primarily due to the high error rates of the viral reverse transcriptase, with deletions and insertions occurring in addition to substitutions. Viral genetic variability occurs between geographical regions, mainly due to differential geographic distribution of the multiple genetic subtypes of HIV-1, with documented genetic variability in the *env* gene in excess of 30% [Myers, 1994; WHO, 1994; Delwart, 1994; Weniger, 1994]. Furthermore, genomic recombination between two different HIV-1 populations frequently occurs in vivo resulting in genetic shifts [Peeters, 2000]. With the continuous evolution and mixing of genetic subtypes and the reality of recombination, new vaccine strategies capable of coping with the possibility of antigenic shift will be needed.

Genetic variability is a major characteristic of HIV-1 [Thomson, 2005]. Different HIV-1 strains fall into 3 groups: group M (major), group O (for 'outliers'), and group N (non-M/non-O). Group M viruses are the most common globally and are further subdivided into different genetic subtypes or clades. Ten clades have been identified to date, designated A--E, F--H, J, and K. The genetic subtypes E is the predominant group of viruses involved in heterosexual transmission in Thailand [Alaeus, 2000; Thomson, 2002]. Genetic subtype B is the dominant subtype in North America, Brazil, The Caribbean, Australia and Europe, and is the most studied of all genetic subtypes [Alaeus, 2000; Osmanov, 2002] representing 12.3% of all HIV-1 infections worldwide. Clade C is the most prevalent clade worldwide, responsible for more than 47% of infections. South East Asia is dominated by clade C, but the clade E is also important, especially in Thailand. In South East and South Africa, subtype C predominates [Osmanov, 2002; Esparza, 2000]. In East Africa subtypes A and D dominates. In West Africa, CRF AG is dominant with a low frequency of group O viruses [Alaeus, 2000].

1.1.3. Pathogenicity

There are a number of immunological features of HIV pathogenicity that makes HIV vaccine development a challenging task. There is a high probability that antibodies and T cells select successive immunological variants of the virus which continue to evade the host response. There is evidence for the existence of specific immunity following natural infection, and the virus is held in check by the host immune response, often for many years, until the immune system is finally incapable of containing the virus. During the course of infection with HIV, cellular immune responses (helper and specific cytotoxic T cells) as well as binding antibodies, virus neutralization, and antibody-dependent cell mediated cytotoxicity (ADCC) can be detected [Letvin, 1993; Weiss, 1993].

1.1.4. Background to existing vaccine technology

The main scientific obstacles to the development of an efficacious AIDS vaccine can be summarized as follows [Excler, 2005]:

- Antigenic diversity and hypervariability of the virus
- Transmission of disease by mucosal route
- Transmission of the virus by infected cells
- Resistance of wild type virus to seroneutralization
- Integration of the virus genome into the host cell chromosomes
- Latency of the virus in resting memory T-cells
- Rapid emergence of viral escape mutants in the host
- Downregulation of MHC class I antigens

As mentioned above, the development of an AIDS vaccine remains an important goal in the global effort to control the HIV epidemic. Unfortunately, even more than 20 years after the discovery of HIV-1, there are very few promising candidate vaccines in late stages of development, and the first phase III trials based on VaxGen's AIDSVAX have failed to demonstrate significant efficacy against virus infection [McCarthy, 2003]. There is one ongoing phase III trial in Thailand that combines the AIDSVAX product with an ALVAC canarypox vector, but the chances of demonstrating vaccine efficacy are considered low [Joseph, 2005].

The most desirable goal for novel HIV vaccine approaches is the prevention of virus infection through induction of neutralising antibodies. The principal target for such antibodies is the envelope glycoprotein of HIV. In fact, it has been demonstrated in the past that immunization with monomeric gp120 elicited neutralizing antibodies that were able to protect non-human primates from homologous virus infection after challenge with HIV or chimeric SHIV. However, since the principal neutralizing domain on gp120 is highly variable (V3 loop), this protection is generally narrow and strain-specific. Therefore, a broadly effective vaccine based on a conventional gp120 immunogen is not considered promising and recent data from VaxGen's phase III trials with gp120 have reinforced this view. Several strategies are being pursued to generate improved envelope

immunogens that would induce more meaningful antibodies, but to date none of these approaches has generated entirely convincing results and/or is being investigated in clinical trials.

A different and potentially more feasible aim is the control of virus load in the infected host using an HIV vaccine that induces cell-mediated immunity against conserved epitopes. CD8-positive cells are the main immune effector mechanism for the elimination of virus-infected cells. It has been demonstrated in the SIV monkey model that experimental depletion of CD8 cells leads to the loss of control of an established virus infection. However, it has also been demonstrated that the CD8 effector cells are subject to immune escape. Therefore, CD8 responses should be directed against as many conserved epitopes as possible.

Several new technologies are being developed for the induction of HIV-specific CD8 T cells. These technologies include numerous plasmid DNA-based approaches and a number of different viral and bacterial vectors. Phase I clinical immunogenicity data are available for DNA vaccines from the NIH Vaccine Research Center (VRC) and the Oxford University group [Cebere, 2006; Estcourt, 2004], demonstrating only marginal immunogenicity. The best DNA vaccine to date has been developed by Merck, but the phase I data indicate inferiority to the adenovirus 5 (Ad5) technology. Phase I data also exist for three novel poxvirus vectors. NYVAC from the EuroVacc consortium, Fowlpox from Virax, and a modified vaccinia virus Ankara (MVA) from the McMichael group have yielded unconvincing results in terms of CD8 T cell induction. A different MVA from Bavarian Nordic appears more potent in preliminary phase I trials in uninfected volunteers and in HIV-infected individuals. The most convincing CD8 T cell-inducing technology seems to be Ad5 (Merck), which has been evaluated alone and in combination with DNA. It represents the first adenovirus-based vaccine that is being tested in large phase IIb proof-of-concept trials.

A fair number of additional vector technologies have reached or will reach the clinic in the coming years. The first alphavirus-based vector (VEE from Alphavax) is currently being evaluated in a phase I trial in the US and South Africa. Other alphavirus-based approaches are at the pre-clinical stage (SFV from Bioption, Sindbis from Chiron). Alternative adenovirus vectors are at the pre-clinical stage (notably human Ad11 and Ad35, Ad5 chimeras, as well as nonhuman primate adenos). A negative strand RNA virus vector (VSV) has been developed by Wyeth, and a recombinant measles vector is being pursued by the Pasteur Institute and GSK. Several DNA vaccine approaches are at the pre-clinical level [Spearman, 2006].

1.1.4.1. Role of anti-HIV antibodies

Antibodies against viral pathogens usually play a critical role in preventing infection, and this is also true for HIV. The principal antibody responses comprise neutralizing antibodies and antibodies that mediate ADCC. Both types of antibodies are directed against the viral Env protein.

Vaccine studies in primate models have revealed that neutralizing antibodies correlated with protection from cell-free intravenous infection with homologous virus strains [Mooij, 2002]. Recently passive immunization studies with a synergistic combination of

three broadly neutralizing antibodies in *Rhesus* monkeys have demonstrated that N Ab alone can protect from heterologous and pathogenic virus challenge by different mucosal routes [Mooij, 2002]. However, it has not been possible to generate similar broad responses by active immunization with envelope protein. This has been a major drawback for *env*-based HIV vaccines, since they afford only very narrow protection against virus strains that resemble closely the vaccine antigen.

The difficulty in inducing effective neutralizing antibodies to HIV-1 is predominantly a reflection of the enormous variation in the envelope protein and the failure of most antibodies that recognize this protein to neutralize the virus. It is therefore generally accepted that a combination of both humoral and cell mediated immune responses may be needed for effective protection, with potentially further improvement on addition of a mucosal immune component [Joseph, 2005].

1.1.4.2. Role of Cell Mediated Immunity in HIV vaccines

The role of CD8+ T cell responses in prophylactic HIV vaccine protection has become more evident in recent years. It has been demonstrated in the simian immunodeficiency virus (SIV) model that CD8+ cytotoxic T lymphocytes (CTLs) play a role in protection from high virus load and disease. These observations have been extended to the SHIV model using $SHIV_{89.6p}$ [Mooij, 2002; Reimann, 1996].

The beneficial effect of both HIV-specific CD4+ and CD8+ T-cell responses in seropositive individuals has been demonstrated in a number of studies [Johnson, 1998]. In addition to direct cytotoxic activity, chemokines and suppressor factors are released from CD8+ T-cells with subsequent inhibition of viral replication in vitro. CD8+ CTL responses following primary infection are associated with the control of viral replication during acute infection. Furthermore, strong CTL responses are observed in long-term non-progressors [Rinaldo, 1995] and in highly-exposed, seronegative African sex workers [Rowland-Jones, 1995]. In chronically infected individuals, a significant inverse correlation was established between HIV-specific CTL frequency and plasma RNA level [Ogg, 1998]. The protective role of CTL was further substantiated by the characterisation of virus mutants escaping CTL recognition [Borrow, 1997].

Furthermore, polyclonal, persistent, vigorous CD4+ T-cell proliferative responses have been found to be associated with the control of viremia and proliferative responses to recombinant p24 were found to be inversely related to viral load [Rosenberg, 1997]. In addition to providing help to B-cell and CD8+ T-cells, these CD4+ T-cells are necessary to maintain HIV-specific CTL memory. Following antigenic stimulation CD4+ T-cells may also provide viral interference by secretion of cytokines and chemokines.

Despite accumulating evidence that CD8+ T cells contribute significantly to the control of the virus, viral escape following a single point mutation in a CTL epitope has resulted in the death of a monkey that was protected by a DNA vaccine expressing *gag* and *env* [Barouch, 2002].

1.1.4.3. Role of mucosal immune responses

Although still in its infancy, preclinical studies with vaccines designed to elicit mucosal immunity and block sexual transmission have demonstrated the induction of protective mucosal immunity in the macaque and chimpanzee [Lehner, 1996; Benson, 1998; Girard, 1996].

1.1.4.4. Role of adjuvants

Adjuvants can enhance the immune response to HIV vaccines by improving delivery of the antigen, selecting the immune response and decreasing the number of immunizations.

Due to the modulation by adjuvants of antigen presentation, vaccines are able to elicit both T-helper cells and CTLs. The major histocompatibility complex (MHC) class I restricted T cell immune response usually occurring in response to intracellular pathogens, such as viruses, leading to CTL activity is, however, not generally observed with protein or peptide antigens. There is evidence that presence of immunostimulating complexes (ISCOMS) or QS-21 can elicit CTL activity with protein, peptides or inactivated viruses [Le, 2001; Vandepapeliere, 2005]. Addition of such immunostimulants is therefore an important step in the development of a protein based HIV-1 vaccine in order to induce or augment the activation of cell-mediated immune response.

Seven clinical studies have been performed with the AS01B adjuvant (liposome-based formulation containing the immunostimulants 3D-MPL [3-deacylated monophosphoryl lipid A] and QS21 [a triterpene glycoside purified from the bark of Quillaja saponaria]) formulated with various antigens. A total of 450 adult or elderly subjects have received between 1 and 4 doses of vaccines containing AS01B adjuvant. Fifty children and 75 elderly subjects have received 1 dose of the AS01E adjuvant (contains half the concentrations of MPL and QS-21 but in the same volume as AS01B). Table 1 summarizes the clinical trials in which AS01B or AS01E has been used. For more details, please refer to the Investigator's Brochure.

Table 1 Summary of the clinical trials conducted with the AS01B or the AS01E adjuvant

Study No. (Status)	Study Design	Age	Number subjects	Number injections	Adjuvant	Antigens
Th-Adj-005, Concluded	Double-blind, randomized, safety/immuno	18-40 years	50	3	AS01B	HBsAg
HIV-002, Concluded	Double-blind, randomized, safety/immuno	18-50 years	60	4	AS01B	gp120/NefTat
EXPLO-CRD-004, Concluded	Open, randomized, safety/immuno	18-30 50-70 years	20 90	2	AS01B	gE
Malaria-027, Completed	Double-blind, randomized, efficacy, safety/immuno	18-45 years	50	3	AS01B	RTS,S
Malaria-044, Concluded	Double-blind, randomized, safety/immuno	18-35 years	85	3	AS01B	RTS,S
Malaria-046, Concluded	Double-blind, randomized, safety/immuno	18 months -4 years	50	3	AS01E	RTS,S
Malaria-051, Ongoing	Double-blind, randomized, efficacy	18- 50 years	20	3	AS01B	LSA-1
FluAS25-003, Completed	Open, randomized, safety/immuno	≥ 65 years	75 75	1 1	AS01B AS01E	A/New Caledonia/20/99 A/New York/55/2004 B/Jiangsu/10/2003

HBsAg : Hepatitis B surface antigen
RTS,S: CS antigen with HBsAg
gp120/NefTat: HIV antigens
gE: glycoprotein E - Varicella Zoster Virus (VZV) antigen
Concluded -study completed but analysis not completed

1.2. Rationale for the study

The most prominent immune effector mechanisms directed against HIV are neutralizing antibodies and CD8+ T cells. In addition, accumulating evidence suggests that CD4+ T cells, which provide essential help to the other compartments of an adaptive immune response, are equally crucial for the induction of effective HIV immunity. In particular, the analysis of immune responses in HIV-infected individuals suggests a crucial role for CD4+ T cells. It appears that CD4+ cells provide essential help to CD8+ effector cells in long-term non-progressors. In contrast, the absence of HIV-specific CD4+ T cells in chronically infected individuals seems to be related to an impairment of CD8+ T cell maturation into effector cells. Thus, it appears that in order to maintain functional CD8+ T cell immunity, strong and broad CD4+ T cell responses are required and that these responses should be induced by an effective HIV vaccine.

Probably the most potent approach to induction of CD4+ T cell responses is the use of adjuvanted protein vaccines. Indeed, GSK's previous candidate HIV vaccines consisted

of two recombinant proteins, gp120 and NefTat, which were formulated in potent proprietary adjuvants (AS01B, AS02A and AS02V). These vaccines were shown to induce very potent CD4+ T cell responses in two independent clinical trials (HVTN-041 and HIV-002). The gp120 antigen was the most immunogenic vaccine component, and the Nef and Tat-directed responses were lower in comparison.

AS01B (liposome-based formulation containing the immunostimulants 3D-MPL [3-deacylated monophosphoryl lipid A] and QS21 [a triterpene glycoside purified from the bark of Quillaja saponaria]) was chosen among other potent proprietary adjuvants, from several clinical studies (for exhaustive details please refer to the Investigator's Brochure) for its propensity to induce a stronger Th1 cellular-mediated immune response and its acceptable safety profile.

Since such CD4+ cell responses need to cover the broadest possible spectrum of circulating HIV strains, an HIV vaccine should contain many different CD4 epitopes from different viral proteins. It was therefore desirable to include well-conserved and highly immunogenic HIV antigens in an improved vaccine formulation. The viral antigens that contain the highest number of conserved T cell epitopes are Gag, Pol, and Nef. Consequently, GSK chose to include these antigens as a single fusion protein (F4co) into an adjuvanted (AS01B) vaccine formulation.

Please refer to the Investigator's Brochure for a review of the pre-clinical studies of the F4co (p24-RT-Nef-p17) candidate vaccine and for exhaustive details concerning clinical studies conducted with gp1201NefTat (HVTN-041 and HIV-002) and1or AS01B (HIV-002 and TH-ADJ-005).

2. OBJECTIVES

2.1. Primaryobjectives

- To evaluate the reactogenicity and safety of the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B adjuvant at three different doses (10-30-90 μg).
- To evaluate the CD4+ T-cell response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) in terms of proportion of responders to at least 1, 2, 3 antigens and to all 4 antigens determined two weeks after the second vaccination.

Refer to Section 10.1 for definition of primary endpoints.

2.2. Secondary objectives

- To evaluate the CD4+ T-cell immune response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) after two ~~and three~~ vaccinations. **(Amended September 24, 2007)**

- To evaluate the serological response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) after two ~~and three~~ vaccinations.**(Amended September 24, 2007)**
- To evaluate the persistence of cell-mediated and serological responses to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg).

Refer to Section 10.2 for definitions of secondary endpoints.

2.3. Exploratory objectives

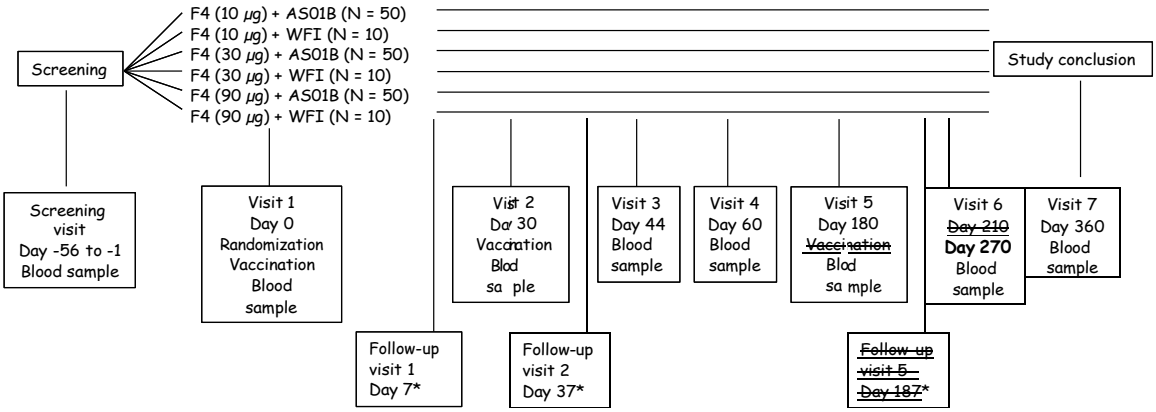
- To evaluate the cross clades reactivity determined by ICS 2 weeks and/or 1 month after the second vaccination.
- To evaluate the CD8+ T-cells immune response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg).

Refer to Section 10.3 for definitions of exploratory endpoints.

3. STUDY DESIGN OVERVIEW

Treatment	Dose (μg)	Adjuvant	
		AS01B (Number of subjects)	Water for injection (WFI) (Number of subjects)
F4co (p24-RT-Nef-p17)	10	50	10
F4co (p24-RT-Nef-p17)	30	50	10
F4co (p24-RT-Nef-p17)	90	50	10

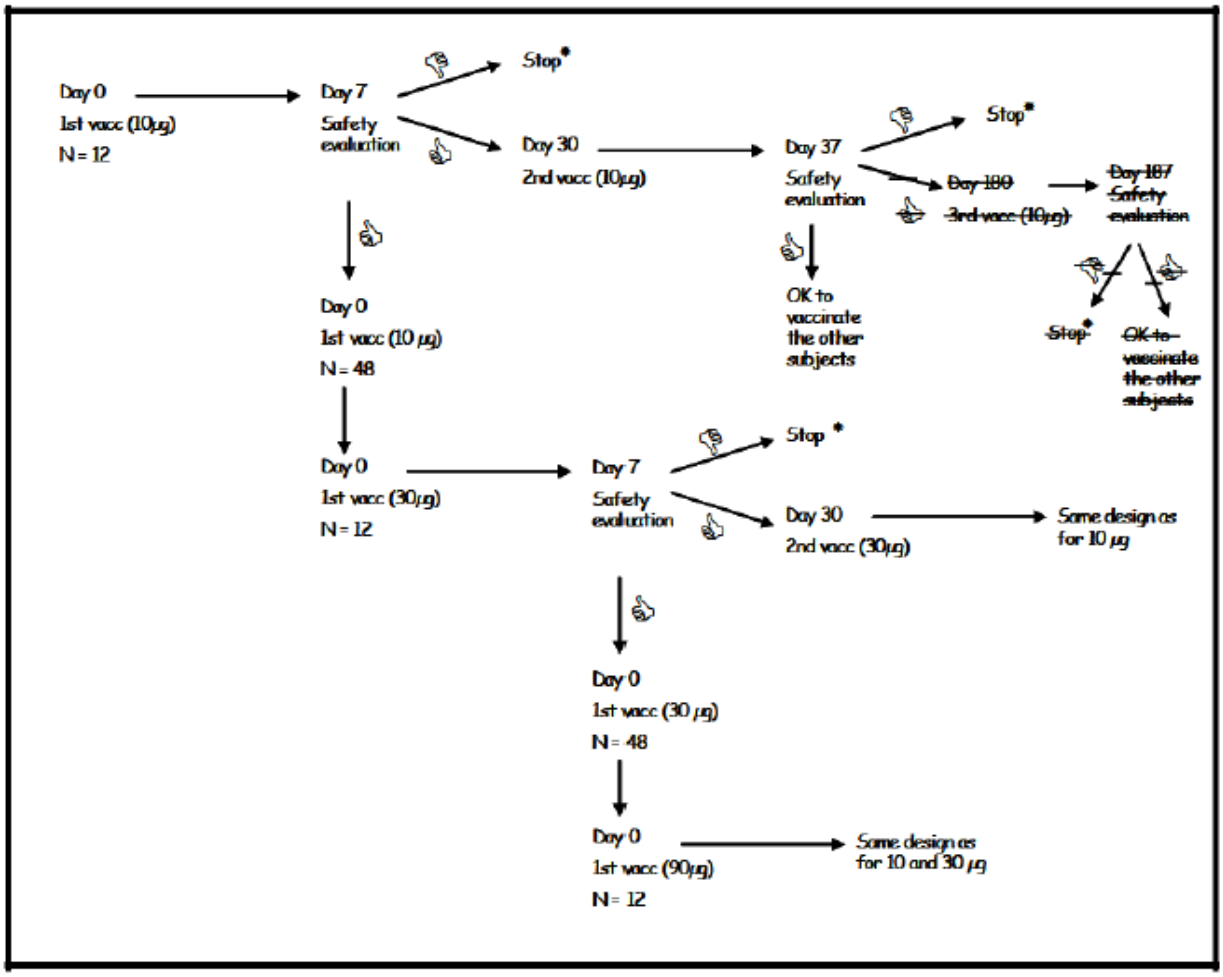
Figure 1 Overall study design overview



* For the 12 first vaccinated subjects only at each of the 3 vaccine doses (10, 30 and 90 µg)

(Amended September 24, 2007)

Figure 2 Staggered design overview



(Amended September 24, 2007)

Each vaccination in each dose group will be administered to the first 12 subjects at the rate of maximum 1 subject per day during the first 5 working days. After that period, provided stopping rules do not apply, the rate of vaccination will be unlimited.

** Holding rules = vaccinations will be put on hold if at least 3 out of the 12 subjects develop a Grade 3 general solicited AE, during the 7-day follow-up period after vaccination which persists at Grade 3 for more than 48 hours and which is considered to be related to vaccination* ***or*** *if at least 4 out of the 12 subjects develop any other Grade 3 unsolicited AE considered to be related to vaccination during the 7-day follow-up period after vaccination* ***or*** *if at least 4 out of the 12 subjects develop any Grade 3 general solicited (persisting at Grade 3 for more than 48 hours and considered related to vaccination) or unsolicited AE (considered related to vaccination) during the 7-day follow-up period after vaccination* ***or*** *at least one life threatening vaccine related SAE occurs* ***or*** *at least one subject shows ulceration (necrosis of the dermis) at the injection site following vaccination. The same approach will be repeated with each of the three vaccine doses (10, 30 and 90 μg) and for each of the* ~~*three*~~ ***two*** *vaccinations (see Section 5.6.3).***(Amended September 24, 2007)**

- Experimental design: Single center, dose-escalating, staggered, parallel-group study with 6 groups (Figure 1 and Figure 2):

  3 groups of 50 subjects receiving the candidate vaccine F4co (p24-RT-Nef-p17), adjuvanted with AS01B, respectively at 10, 30 or 90 μg.

  3 groups of 10 subjects receiving the candidate vaccine F4co (p24-RT-Nef-p17), non-adjuvanted, in water for injection, respectively at 10, 30 or 90 μg.

- Control groups: None.

- Vaccination process: A staggered dose-escalation design will be used for safety reasons. A first group of 12 subjects (10 receiving the adjuvanted vaccine and 2 receiving the vaccine in WFI) will be enrolled to receive their first vaccination at the lowest dose (10 μg), at a rate of maximum 1 subject per day during the first 5 working days. Provided no safety issues leading to clinical hold or stop occurred (Figure 2), the 12 subjects will be allowed to receive their second vaccination on Day 30, according to the same rate (1 subject/day during the first 5 working days). The remaining subjects of the same dose group (N=48) will receive their first vaccination. Then, a new group of 12 subjects will be enrolled to receive their first vaccination at the medium dose (30 μg) at a rate of maximum 1 subject per day during the first 5 working days. Provided no safety issues leading to clinical hold or stop occurred, subjects will be allowed to receive their second vaccination on Day 30 at the same rate, the remaining subjects (N=48) of the same dose group will receive their first vaccination. Then, a new group of 12 subjects will be enrolled to receive their first vaccination at the highest dose (90 μg) at a rate of maximum 1 subject per day during the first 5 working days. Provided no safety issues leading to clinical hold or stop occurred, subjects will be allowed to receive their second vaccination on Day 30 at the rate of maximum 1 subject per day during the first 5 working days and the remaining subjects (N=48) of the same dose group will receive their first ~~v~~vaccination. ~~Exactly the same methodological approach will be replicated for the~~

~~third vaccination in each dose group~~ (see Section 5.6.3).**(Amended September 24, 2007)**

- Blinding: Partially blind: observer-blind for adjuvantation, open for antigen content
- Treatment allocation: Randomized for the adjuvantation (AS01B or water for injection) (see Section 6.4 for a detailed description of the randomization method).
- ~~Three~~***Two***-vaccination course given at 0~~,~~ ***and*** 1 ~~and 6~~ months. All vaccinations will be administered by the intramuscular route in the deltoid of the non-dominant arm. **(Amended September 24, 2007)**
- Duration of the study: The intended duration per subject will be approximately 14 months
- 7-day follow-up visits after each vaccination for the 12 first vaccinated subjects and 30-day follow-up visits after each vaccination for all subjects.
- Blood samples will be collected at 8 visits
- Diary cards will be provided to the subjects to record temperature and local (at the injection site) or general adverse events occurring on the day of vaccination and during the 6 subsequent days (Day 0 to Day 6) (see Section 5.2.1.3)
- One-month (Day 0 to Day 29) follow-up for unsolicited symptoms after each injection of the vaccine
- Recording of serious adverse events (SAEs) in a prospective manner throughout the study period, i.e., the period beginning with the first vaccination and ending ~~6~~ ***11*** months after the ~~third~~ ***second*** vaccination. **(Amended September 24, 2007)**
- Recording of SAEs related to study procedures during screening.
- ~~Three~~ ***Two*** separate analyses will be performed on cleaned data:

  A first analysis will be performed when all data (immunogenicity and reactogenicity) up to and including 1 month post-vaccination II are available.

  ~~A second analysis will be performed when all data (immunogenicity and reactogenicity) up to and including 1 month post-vaccination III are available.~~

  A ~~third~~ ***second*** analysis ~~on the immunogenicity persistence data and SAEs data~~ will be performed at the completion of the study (Month 12).**(Amended September 24, 2007)**
- Data collection: Remote Data Entry (RDE)
- All details concerning the recruitment plan can be found in Appendix C.

4. STUDY COHORT

4.1. Number of subjects / centers

This study will be conducted at a single center in Belgium. The target number of eligible subjects will be 180 (3 groups of 50 subjects and 3 groups of 10 subjects).

Refer to Section 10.3 for a description of the criteria used in the estimation of sample size.

Please refer to Appendix C of this document for details of recruitment at the center.

4.2. Inclusion criteria

All subjects must satisfy the following criteria at study entry:

- A male or female between and including 18--40 years at the time of first vaccination.
- Written informed consent obtained from the subject prior to any study procedure.
- Subjects who the investigator believes that they can and will comply with the requirements of the protocol (e.g., completion of the diary cards and return for follow-up visits).
- Good general health without significant medical history, physical examination findings, or clinically significant abnormal laboratory results.
- If the subject is female, she must be of non-childbearing potential, i.e. have a current tubal ligation, hysterectomy, ovariectomy or be post-menopausal, or if she is of childbearing potential, she must practice adequate contraception for 30 days prior to vaccination, have a negative pregnancy test and continue such precautions for 2 months after completion of the vaccination series. For a definition of adequate contraception, azoospermia and post-menopause please refer to the Glossary of Terms or to Section 5.5.
- Negative for antibodies against HIV-1, HIV-2 and negative for HIV p24 antigen, using Abbott AxSYM autoanalyser within 56 days (8 weeks) prior to enrolment.

  Note: Subjects must be willing to accept HIV test results. Individuals who elect not to receive test results will not be enrolled.
- Negative for anti-HBc Ab, HBsAg and anti-HCV Ab.

4.3. Exclusion criteria for enrolment

The following criteria should be checked at the time of study entry. If any apply, the subject must not be included in the study:

- Women who are pregnant or breast-feeding.
- Subjects with a history of, or current, alcohol or substance abuse.
- The subject is at high risk of acquiring HIV according to the behavioural risk assessment questionnaire.
- Morbid obesity
- Previous inclusion in a HIV vaccines trial.
- Receipt of live attenuated vaccines within 30 days of enrolment.

- Receipt of medically indicated subunit or killed vaccines (e.g., influenza, pneumococcal) or allergy treatment with antigen injections (including a tuberculin skin test) within 14 days of study vaccine administration.
- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines within 30 days preceding the first dose of study vaccine, or planned use during the study period.
- Prior receipt of HIV-1 vaccines or placebo in a previous HIV vaccine trial.
- Receipt of blood products 120 days prior to HIV screening.
- Receipt of immunoglobulin 120 days prior to HIV screening.
- History of serious adverse reactions including anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema and abdominal pain to vaccines.
- History of serious allergic reaction to any substance requiring hospitalization or emergency medical care (e.g., Steven-Johnson syndrome, bronchospasm, or hypotension).
- History of immunodeficiency or autoimmune disease.
- History of malignancy (unless there has been surgical excision followed by a sufficient observation period, of at least 5 years, to give a reasonable assurance of sustained cure and which, in the estimate of the investigator, is not likely to recur during the study period).
- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within six months prior to the first vaccination. (For corticosteroids, this will mean prednisone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed.)
- History of type I or type II diabetes mellitus including cases controlled with diet alone.

  Note: A subject with past gestational diabetes is eligible.
- Thyroid disease including history of thyroidectomy and diagnoses requiring medication.

  Note: A subject not requiring thyroid medicine within the past 12 months is eligible.
- Acute disease at the time of enrolment. Acute disease is defined as the presence of a moderate or severe illness with or without fever.
- Asthma requiring daily steroid or long acting β agonist prevention.
- 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
- Known sensitivity to sulfites or aspirin

- Bleeding disorder that was diagnosed by a physician; e.g., factor deficiency, coagulopathy or platelet disorder that requires special precautions.

  Note: A subject who states that he or she has easy bruising or bleeding, but does not carry a formal diagnosis and has intramuscular (IM) injections and blood draws without any adverse experience is eligible.

- History of any serious neurologic disorder or seizure
- History of major congenital defect
- History of chronic fatigue syndrome or fibromyalgia
- Splenectomy
- Hypertension.

  Note: A subject with hypertension is eligible if he or she is controlled on medication and the documented blood pressure is less than 150/100.

- Any medical, psychiatric or social condition, or occupational or other responsibility that, in the judgement of the investigator, would interfere with or serve as a contradiction to adherence to the study protocol or ability to give informed consent.

4.4. Elimination criteria during the study

The following criteria should be checked at each visit subsequent to the first visit. If any become applicable during the study, it will not require withdrawal of the subject from the study but may determine a subject's evaluability in the according-to-protocol (ATP) analysis. See Section 10.5 for definition of study cohorts to be evaluated.

- Positive for antibodies against HIV-1, HIV-2 and positive for HIV p24 antigen, using Abbott AxSYM autoanalyser.
- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines during the study period.
- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs during the study period. For corticosteroids, this will mean prednisone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed.
- Administration of a vaccine not foreseen by the study protocol during the period starting from 30 days (14 days in the case of subunit or killed vaccines [e.g., influenza, pneumococcal] or allergy treatment with antigen injections) before each administration of vaccine(s) and ending 30 days after.
- Administration of immunoglobulins and/or any blood products during the study period.
- Pregnancy
- Intravenous drug use

See Section 10.5 for definition of study cohorts to be evaluated.

4.5. Contraindications to subsequent vaccination

The following adverse events (AEs) constitute absolute contraindications to further administration of GSK Biologicals' candidate vaccines; if any of these AEs occur during the study, the subject must not receive additional injections of vaccine but may continue other study procedures at the discretion of the investigator (see Section 9). The subject must be followed until resolution of the event, as with any AE (see Section 8.6):

- Early or delayed onset reactions after vaccine administration such as anaphylaxis, bronchospasm, laryngeal oedema, generalized collapse, convulsions, encephalopathy or severe Arthus reaction.
- Any event which is considered by the investigator and the sponsor to be serious or severe which would suggest any significant hazard that may be associated with the use of the study vaccine.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including human immunodeficiency virus (HIV) infection.
- Pregnancy (see Section 8.10).

The following AEs constitute contraindications to administration of GSK Biologicals' candidate vaccines at that point in time; if any one of these AEs occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 5.4), or withdrawn at the discretion of the investigator (see Section 9). The subject must be followed until resolution of the event, as with any AE (see Section 8.5.3).

- Acute disease at the time of vaccination. Acute disease is defined as the presence of a moderate or severe illness with or without fever. All vaccines can be administered to persons with a minor illness such as diarrhea, mild upper respiratory infection with or without low-grade febrile illness, i.e., oral temperature < 37.5°C/axillary temperature < 37.5°C.
- Any vaccination less than 14 days (30 days in the case of live attenuated vaccines) before vaccine administration.
- Oral temperature ≥ 37.5°C / axillary temperature ≥ 37.5°C.

5. CONDUCT OF STUDY

5.1. Ethics and regulatory considerations

The study will be conducted according to Good Clinical Practice (GCP), the 1996 Declaration of Helsinki (Appendix A) and local rules and regulations of the country. Submission of the protocol and any protocol amendments to regulatory agencies will occur in accordance with local regulatory requirements. For some countries, submission to the local regulatory authority may not be required. When submission to the local regulatory authority is required, the timing of the submission relative to IEC/IRB submission or approval and whether or not the authority will provide their approval of or favourable opinion on the protocol or amendment before it can be implemented will depend on local regulatory requirements.

5.1.1. Institutional Review Board/Independent Ethics Committee (IRB/IEC)

The IRB/IEC must be constituted according to the local laws/customs of each participating country. The ICH Harmonized Tripartite Guideline for Good Clinical Practice recommends that the IRB/IEC should include:

a. At least five members.

b. At least one member whose primary area of interest is in a non-scientific area.

c. At least one member who is independent of the institution/ study site.

Only those IRB/IEC members who are independent of the investigator and the sponsor of the study should provide opinion on a study-related matter.

A list of IRB/IEC members and their qualifications should be obtained by the Central Study Coordinator (CSC) and the Principal Investigator. This protocol and any other documents that the IRB/IEC may need to fulfil its responsibilities, including subject recruitment procedures and information about payments and compensation available to subjects, will be submitted to the IRB/IEC by the Principal Investigator. Written and dated unconditional approval/favourable opinion from the IRB/IEC of the protocol and amendment (if any and applicable), written informed consent form, consent form updates (if any), subject recruitment procedure(s) (e.g. advertisements), and any other written information to be provided to subjects must be in the possession of the investigator and GSK before commencement of the study. This approval/favourable opinion must refer to the study by study title and number with exact protocol version and date, and should identify the documents reviewed and state the date of review. Relevant GSK Biologicals' data will be supplied by the Principal Investigator to the hospital/ university/ independent IRB/IEC for review and approval of the protocol. Verification of the unconditional approval/favourable opinion of the IRB/IEC will be transmitted by the Principal Investigator to the CSC prior to shipment of vaccine supplies and CRFs to the site.

No deviations from, or changes to, the protocol should be initiated without prior written sponsor and IRB/IEC approval/ favourable opinion of an appropriate amendment, except when necessary to eliminate immediate hazards to the subjects or where permitted by all applicable regulatory requirements or when the change(s) involves only logistical or administrative aspects of the study (e.g., change of monitor[s], telephone number[s].) Administrative changes and amendments not submitted for approval are submitted to the IRB/IEC for information only. However, written verification that such documents were submitted should be obtained. Approvals/ verifications must be transmitted in writing to the CSC by the Principal Investigator.

The IRB/IEC must be informed by the Principal Investigator of:

- all subsequent protocol amendments, informed consent changes or revisions of other documents originally submitted for review,
- serious and/or unexpected adverse events occurring during the study, where required,

- all subsequent protocol administrative changes (for information, except for US studies),
- new information that may affect adversely the safety of the subjects or the conduct of the study,
- an annual update and/or request for re-approval, where required,
- when the study has been completed, where required.

If a trial is prematurely terminated or suspended for reasons including, but not limited to, safety or ethical issues or severe non-compliance, the sponsor will promptly inform the regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. If required by applicable regulations, the investigator must inform the IEC/IRB promptly and provide the reason for the suspension or termination (see Appendix B for further details).

5.1.2. Informed consent

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the 1996 Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favourable opinion of the written informed consent form and any other written information to be provided to the subjects.

Freely given informed consent should be obtained from every subject prior to clinical trial participation.

Information should be given in both oral and written form whenever possible and as deemed appropriate by the IRB/IEC.

An investigator or designate will describe the protocol to potential subjects face to face. The Informed Consent Form may be read to the subjects, but, in any event, the investigator or designate shall give the subjects ample opportunity to inquire about details of the study and ask any questions before dating and signing the Informed Consent Form.

While informed consent information can be presented to groups at an initial information session, each subject must be given the opportunity to individually pose questions to the investigator or designate prior to the subject dating and signing the Informed Consent Form.

Informed Consent Form must be in a language fully comprehensible to the prospective subjects. Informed consent shall be documented by the use of a written consent form approved by the IRB/IEC and signed and dated by the subjects and by the person who conducted the informed consent discussion. The signature confirms the consent is based on information that has been understood. All illiterate individuals will have the study, the Informed Consent Form explained to them point by point by the interviewer in the presence of an impartial witness. The witness will personally sign and date the consent form. Oral witnessed consent will replace written consent only in countries where the

local custom is contrary or if the subject's incapacity precludes this and provided that the local legal obligations are fulfilled.

Each subject's signed informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or GSK Biologicals' professional and Regulatory Compliance persons. The subjects should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects, and should receive copies of any signed and dated consent form updates. Any amendments to the written information will be provided to subjects.

Both the informed consent discussion and the written informed consent form and any other written information to be provided to the subjects should include explanations of the following:

a. That the trial involves research.

b. The purpose of the trial.

c. The trial treatments and the probability for random assignment to each treatment.

d. The trial procedures to be followed, including all invasive procedures.

e. The subject's responsibilities.

f. Those aspects of the trial that are experimental.

g. The reasonably foreseeable risks or inconveniences to the subjects and, when applicable, to an embryo, fetus or nursing infant.

h. The reasonable expected benefits. When there is no intended clinical benefit to subjects, the subjects should be made aware of this.

i. The alternative procedure(s) or course(s) of treatment/ methods of prevention that may be available to subjects, and their important potential benefits and risks.

j. The compensation and/or treatment available to subjects in the event of trial-related injury.

k. The anticipated prorated payment, if any, to subjects for participating in the trial.

l. The anticipated expenses, if any, to subjects for participating in the trial.

m. That the subjects' participation in the trial is voluntary and subjects may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which subjects are otherwise entitled.

n. That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of subjects, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent, the subject is authorizing such access.

o. That records identifying subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, subjects' identity will remain confidential.

p. That the subjects will be informed in a timely manner if information becomes available that may be relevant to the subjects' willingness for continued participation in the trial.

q. The person(s) to contact for further information regarding the trial and the rights of trial subjects, and who to contact in the event of trial-related injury.

r. The foreseeable circumstances and/or reasons under which a subject's participation in the trial may be terminated.

s. The expected duration of a subject's participation in the trial.

t. The approximate number of subjects involved in the trial.

GSK Biologicals will prepare a model Informed Consent Form which will embody all the elements described above. While it is strongly recommended that this model document 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 document.

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

5.2. General study aspects

5.2.1. Study evaluations

Assessment of the product will include a behavioural risk assessment questionnaire, clinical observations and monitoring of haematological, biochemical and immunologic parameters.

5.2.1.1. Behavioural risk assessment questionnaire

The behavioural risk assessment questionnaire is designed to determine the risk of infection based on sexual behaviour, sexually transmitted diseases (STD) and injection drug use. The questionnaire (hard copy) will be completed by the subjects in full confidentiality at screening and at visits 5 and 7. The subject's number will be used. The subject's classification (high risk or low risk) will be recorded in the electronic CRF (eCRF).

At screening, high risk subjects will be excluded. During the study, subjects found at increased risk will not be eliminated but will receive accentuated HIV prevention counselling.

The behavioural risk assessment questionnaire is attached.

5.2.1.2. Clinical observations

Safety will be evaluated by monitoring of subjects in the clinic for 30 minutes after each vaccination.

A 7-day follow-up (Day 0 to 6) of solicited local and general reactions will be performed by the subjects after each vaccination. Local reactions, e.g., pain, redness, swelling, and general reactions, e.g., temperature (fever), fatigue, headache, sweating, myalgia and GI symptoms, will be recorded by the subjects on diary cards. The intensity of solicited symptoms will be recorded. The relationship to vaccination of general solicited symptoms will be assessed by the investigator. All local solicited symptoms will be considered related to the vaccination.

The occurrence, intensity and relationship to vaccination of unsolicited symptoms during a 30-day follow-up period (Day 0 to 29) after each vaccination and the occurrence and relationship of SAEs at any time during the whole study period will be recorded.

5.2.1.3. Diary Cards

To collect safety data about the vaccine, diary cards will be used for day-by-day recording of the solicited symptoms from Day 0 to Day 6 following each vaccination and collection of information on unsolicited symptoms and concomitant medication from Day 0 to Day 29 after each injection of the vaccine. Three kinds of diary cards will be used in this study (Table 2):

- A first type of diary card will record information from Day 0 to Day 6 after each vaccination in the 12 first vaccinated subjects.
- A second type of diary card will record information from Day 7 to Day 29 after each vaccination in the 12 first vaccinated subjects.
- A third type of diary card will record information from Day 0 to Day 29 after each vaccination in all the other subjects.

Subjects will be instructed to bring the completed diary card with them at the next visit and the investigator (or designee) will transcribe all information collected on the diary cards promptly in the eCRF as this information is needed for the scheduled safety evaluations.

Table 2 Overview of diary cards

Type of diary card	Subjects	Distribution	Return
Day 0 - Day 6	12 first subjects of each dose group 12 first subjects of each dose group ~~12 first subjects of each dose group~~	Visit 1 (Day 0) Visit 2 (Day 30) ~~Visit 5 (Day 180)~~	Follow-up Visit 1 (Day 7) Follow-up Visit 2 (Day 37) ~~Follow-up Visit 5 (Day 187)~~
Day 7 - Day 29	12 first subjects of each dose group 12 first subjects of each dose group ~~12 first subjects of each dose group~~	Follow-up Visit 1 (Day 7) Follow-up Visit 2 (Day 37) ~~Follow-up Visit 5 (Day 187)~~	Visit 2 (Day 30) Visit 4 (Day 60) ~~Visit 6 (Day 210)~~
Day 0 - Day 29	48 other subjects of each dose group 48 other subjects of each dose group ~~48 other subjects of each dose group~~	Visit 1 (Day 0) Visit 2 (Day 30) ~~Visit 5 (Day 180)~~	Visit 2 (Day 30) Visit 4 (Day 60) ~~Visit 6 (Day 210)~~

(Amended September 24, 2007)

5.2.1.4. Laboratory parameters

Effects on the haematological and biochemical parameters will be monitored at the Screening Visit, Visit 1 (Day 0), Visit 2 (Day 30), Visit 3 (Day 44), Visit 4 (Day 60), Visit 5 (Day 180), Visit 6 (Day ~~210~~ ***270***) and Visit 7 (Day 360). **(Amended September 24, 2007)**

5.2.1.5. Immunogenicity parameters

All subjects will be monitored longitudinally for HIV-specific serologic and cellular immune responses (refer to Section 5.4 flowsheet and Section 5.5 for details on immunogenicity determinations and timepoints).

5.2.2. Detection of intercurrent HIV infection

The study vaccine contains multiple antigens of the HIV-1 virus, therefore, vaccine-induced immune responses in study subjects detected by screening serologic assays potentially could be confused with natural infection. However, the risk for serological cross-reaction by 4th generation ELISAs including the Axysm test used in the study is extremely low. Nevertheless, several precautionary measures will be taken to clarify this distinction.

- Frequent counselling on avoidance of HIV infection and pregnancy.
  The investigator or his delegate will ensure that the privacy and confidentiality of the subject is kept during these informative sessions.

  HIV Infection: subjects will receive information regarding HIV prevention and transmission. The following points will be explained to the subjects.

  - Knowledge: what is HIV, HIV modes of transmission, factors that increase the risk of becoming infected.
  - Risk reduction: never have unsafe/ unprotected sex (always use condoms and the correct use of condoms, provide condoms), have safer sex, have fewer or

less risky partners, avoid needle-sharing, avoidance of sex while high on drugs or alcohol.

- HIV Testing: Why and when the subject should seek additional counselling and testing. Signs and symptoms of primary HIV infection (see below).
- Vaccine: Remind the subjects that the effectiveness of these vaccines is not known. Therefore, it is very important that the subjects avoid any behaviour that would put them at risk for getting infected with HIV.
- Answer any relevant questions from the subject.

Additional counselling will be delivered for special situations:

- Subjects with newly identified or suspected HIV infection
- Subjects with a single, recent non-occupational HIV exposure
- Subjects with a recent occupational exposure
- Subjects with indeterminate HIV test results
- Subjects seeking repeat HIV testing

Pregnancy (female subjects only): notes on the following will be documented in the subject's file.

- - The subject's contraceptive status will be assessed.
- - The subject will be reminded of the importance of using contraception.

- Signs and symptoms of HIV-1 infection:

  Signs or symptoms of acute HIV infection syndrome (e.g., sore throat, diarrhea, fever, chills, night sweats, neurologic syndromes and rash).

  and

  Signs and symptoms of an opportunisitic infection.

- Laboratory assessment of potential intercurrent HIV-1 infection

  HIV Ag /Ab ELISA testing will be routinely performed at screening, vaccine dose 1, ~~1 month post-vaccination III~~ ***Month 9*** and ~~Month~~ 12. **(Amended September 24, 2007)**

  If intercurrent HIV-1 infection is suspected, further diagnostic work-up will be performed (see Appendix H).

  In addition to routine testing for HIV-1 infection planned during the course of the study, HIV tests may be requested by the investigator or the subject (free of charge) at any time during the study.

  The flowchart for the standard algorithm and the full procedure are presented in Appendix H of the protocol and will also be included in the study file.

- Infected subjects will be withdrawn from the study and will be referred to the HIV referral unit at the University Hospital of Ghent for treatment and management of the HIV-1 infection. Neither the study sites, study investigators, nor GSK Biologicals

will be responsible for providing ongoing medical care or anti-retroviral medications in the event of HIV-1 infection.

- At the final study visit (Visit 7, Month 12, Day 360) or following a subject's withdrawal from the study, a blood sample will be taken and stored. At the end of the study, these samples will be analysed using a panel of different HIV tests commonly used in Europe.

5.3. Subject identification

Subject numbers will be assigned sequentially to subjects consenting to participate in the study, according to the range of subject numbers allocated to the study center.

5.4. Outline of study procedures

The schedule of study procedures is summarized in Table 3.

Table 3 List of study procedures

Visit	Screening	1	Follow-up visit 1[1]	2	Follow-up visit 2[1]	3	4	5	~~Follow-up visit 5[1]~~	6	7
Timing (Months) Timing (Days)	Screening -56 to -1	0 0	7	1 30	37	44	2 60	6 180	~~187~~	~~7~~ ~~9~~ ~~210~~ *270*	12 360
Sampling time point		PRE (M0)		P I (M1)		P II (M1.5)	P II (M2)	P II (M6)		~~P III~~ *PII* ~~(M7)~~ *(M9)*	~~P III~~ *PII* (M12)
Vaccination		I		II				~~III~~			
Examination and Procedure:											
Signed Consent Form	•										
***Signed Addendum to Consent Form* (Amended October 08, 2007)**								~~•~~		•	
Randomization		•									
Physical examination and vital signs	•	•		•				•			•
Demographic data	•										
General medical history	•										
Prevention Counselling (pregnancy and HIV)	•	•		•				•			
Inclusion/exclusion criteria	•	○									
Elimination criteria			•[1]	•	•[1]	•	•	•	~~•~~[1]	•	
Contraindications		•		•				~~•~~			
Behavioural risk assessment [questionnaire to be filled in by the subject and high or low risk to be recorded on CRF]	•							•			•
Pre-vaccination body temperature		•		•				~~•~~			
Distribution of diary card		○	○[1]	○	○[1]			~~○~~	~~○~~[1]		
Return of diary card			○[1]	○	○[1]		○		~~○~~[1]	~~○~~	
Transcription of solicited symptoms recorded by the subjects (Day 0 to Day 6)			•[1]	•	•[1]		•		~~•~~[1]	~~•~~	
Recording of non-serious unsolicited adverse events occurring within one month post-vaccination by Investigator (Day 0 to Day 29)			•[1]	•	•[1]		•		~~•~~[1]	~~•~~	
Reporting of serious adverse events	•	•	•[1]	•	•[1]	•	•	•	~~•~~[1]	•	•
Record any concomitant medications		•	•[1]	•	•[1]	•	•	*•[4]*	~~•~~[1]	*•[4]*	*•[4]*
Study conclusion							•			~~•~~	•

Visit	Screening	1	Follow-up visit 1[1]	2	Follow-up visit 2[1]	3	4	5	~~Follow-up visit 5[1]~~	6	7
Timing (Months) Timing (Days)	Screening -56 to -1	0 0	7	1 30	37	44	2 60	6 180	~~187~~	~~7~~ *9* ~~210~~ *270*	12 360
Sampling time point		PRE (M0)		P I (M1)		P II (M1.5)	P II (M2)	P II (M6)		~~P III~~ *PII* ~~(M7)~~ *(M9)*	~~P III~~ *PII* (M12)
Vaccination		I		II				~~III~~			
Blood Sample (ml):	11	101		6.5		96.5	96.5	56.5		~~61~~ *11*	69
Virology(4.5 ml)											
Hep B/C markers: anti HBc Ab, HBsAg, *Anti HCV Ab*	•										
HIV Ag/Ab[t]	•	•								•	•
Panel of HIV tests (8 ml)*											•
Biochemistry/Haematology (6.5 ml)	•	•		•		•	•	•		•	•
Serology (10 ml)											
Anti-p17,-24,-RT,-Nef, -F4co antibodies (ELISA)		•				•	•	•		~~•~~	•
Cellular-mediated immunity (40 or 80 ml)**											
p17-,24-,RT-,Nef- Specific effector T-cells (ICS)		•				•	•	•		~~•~~	•
Urine Sample:											
Pregnancy test (HCG), if applicable	•	•		•				~~•~~			

Note: The double-line border following Month 2 ~~and Month 7~~ indicates the ~~2~~ overall interim analyses that will be performed on all serology and immunogenicity data obtained up to PII (M2) ~~and PIII (M7)~~.

•: Is used to indicate a study procedure which requires documentation in the individual eCRF; O is used to indicate a study procedure that does not require documentation in the individual eCRF; •[1]: In the 12 first vaccinated subjects of each dose group only; • : Only those SAEs that are considered related to study procedures need to be recorded (see Section 8.4); • : To be recorded only if the subject is not a screening failure. •[4] ***Recording of concomitant medications till 30 days after vaccination. Please refer to Section 6.9 for description of concomitant/contraindicated medication to be recorded.***

[t] Abbott AxSYM HIV Ag/Ab ELISA assay, which allows detection of Absto HIV-1 group "M" gp41, HIV-2 gp36 and HIV group "O" gp41, and detection of the HIV p24 Ag.

* At the final study visit or following a subject's withdrawal from the study.

** 80 mL of blood are needed for cellular-mediated immunity when cross-reactivity is determined pre-vaccination (Day 0), PII (M 1.5) (Day 44) and PII (M2) (Day 60). Otherwise, 40 mL of blood are enough for classical cellular-mediated immunity assessment at the other time points.

(Amended September 24, 2007)

It is the investigator's responsibility to ensure that the intervals between visits are strictly followed (Table 4).

Table 4 Intervals between study visits

Interval	length of interval (Days)
1 (Screening → Visit 1)	1 to 56 (8 weeks)
2 (Visit 1 → Follow-up Visit 1)*	7- 8*
3 (Visit 1 → Visit 2)	30 - 34
4 (Visit 2 → Follow-up Visit 2)*	7 - 8*
5 (Visit 2 → Visit 3)	14 ± 2
6 (Visit 2 → Visit 4)	30 - 34
7 (Visit 1 → Visit 5)	180 ± 14
~~8 (Visit 5 → Follow-up Visit 5)*~~	~~7 - 8*~~
9 (Visit ~~5~~ ***1*** → Visit 6)	~~30 - 34~~ ***270*** ± ***21***
10 (Visit 5 → Visit 7)	180 ± 14

* For the 12 first vaccinated subjects only

(Amended September 24, 2007)

5.5. Detailed description of study stages/visits

Evaluations and blood draws will be performed on all subjects at the timepoints indicated in the Flowsheet (Section 5.4), unless otherwise noted.

When considering the enrollment of females of childbearing potential (see Section 4.2), please take note of the following definitions:

- Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly (when applicable, as mentioned in the product label) for example abstinence, combined or progestogen oral contraceptives, injectable progestogen, implants of levonorgestrel, oestrogenic vaginal ring, percutaneous contraceptive patches or intrauterine device (IUD) or intrauterine system (IUS), vasectomy with documented azoospermia of the sole male partner or double barrier method (condom or occlusive cap plus spermicidal agent).
- For azoospermia, "documented" refers to the outcome of the investigator's or designee's medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records.
- Post-menopause: Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after one year without menses with an appropriate clinical profile at the appropriate age e.g. >45 years.

When materials are provided by GSK Biologicals, it is **MANDATORY** that all clinical samples (including serum samples) will be collected and stored using exclusively those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 10.5 for definition of study cohorts to be evaluated). 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,

then appropriate materials from the investigator's site are to be used. Refer to Appendix D and Appendix E.

The vaccinees will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

5.5.1. Screening Visit (Day -56 to Day -1)

- Signed informed consent form prior to initiation of subject screening.
- Complete physical examination, including vital signs (supine blood pressure and heart rate).
- Weight and height
- Demographic data of the subjects: date of birth, gender and race.
- Medical history and current medical status of the subjects.
- Counselling on avoidance of HIV infection and pregnancy.
  The investigator or his/her delegate will ensure that the privacy and confidentiality of the subject is kept during these informative sessions.
- Screening tests and evaluations will be used to determine the eligibility of each subject for the study. All inclusion and exclusion criteria must be assessed within 8 weeks (56 days) prior to study entry.
- Behavioural risk questionnaire (hard copy) to be filled in by the subject.
- SAEs that are considered related to study participation or are related to GSK concomitant medication need to be recorded (see Section 8.4).
- Collection of blood (11 mL) for virology, biochemistry and haematology.
- HCG (Human Chorionic Gonadotropin) urine pregnancy test will be performed in female subjects of childbearing potential.
- Screening failures will also be recorded in the eCRF (demography, eligibility criteria and study conclusion) (see Section 9.3).

5.5.2. Study Visit 1: First vaccination (Day 0 - Month 0)

Prior to Vaccination

Note: All test results and assessments required for establishing eligibility must be completed.

- Check inclusion and exclusion criteria
- The subject will be randomized (Internet randomization will be used).

- History-directed physical examination and vital signs.
- Pre-vaccination assessment of body temperature
- Counselling on avoidance of HIV infection and pregnancy.
- Check of contraindications to vaccination.
- Recording of any concomitant medication/treatment/vaccination taken since the last visit (see Section 6.9).
- Collection of blood (101 mL) for virology, biochemistry, haematology, serology and cellular-mediated immunity.
- HCG (Human Chorionic Gonadotropin) urine pregnancy test will be performed in female subjects of childbearing potential.
- Evaluations and blood draws must be performed prior to vaccination, but it is not necessary to have results (except for pregnancy test) prior to the administration of the vaccine.

Vaccination

- Randomization and vaccine number: upon providing the subject number, the randomization system will determine the treatment number to be used for the subject.
- Vaccination: intramuscular administration in the deltoid muscle of the non-dominant arm. The vaccinees will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.
- Reporting of serious adverse events. The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
- Diary cards will be provided for the subjects to record temperature and any local (at the injection site) or general adverse events occurring on the day of vaccination and the 6 following days.
- The first 12 vaccinated subjects will be instructed to return their completed Day 0 to Day 6 diary cards on Day 7 to the investigator. All the other subjects will be instructed to return their Day 0 to Day 29 diary cards on Day 30 to the investigator.

5.5.3. Follow-up Visit 1 (Day 7; for the 12 first vaccinated subjects only)

- Check elimination criteria
- The subjects will return their completed (Day 0 to Day 6) diary cards to the investigator, who will transcribe them into the eCRF.
- Reporting of any unsolicited adverse events which might have occurred since vaccination.
- Reporting of any serious adverse events which might have occurred since vaccination.

- Recording of any concomitant medication/treatment/vaccination taken since the last visit (see Section 6.9).
- Subjects will be distributed Day 7 to Day 29 diary cards and will be instructed to return them completed on Day 30 to the investigator.

5.5.4. Study Visit 2: Second vaccination (Day 30 - Month 1)

Prior to Vaccination

- The subjects will return their completed diary cards (Day 7 to Day 29 diary cards for the 12 first vaccinated subjects and Day 0 to Day 29 diary cards for all the other subjects) to the investigator, who will transcribe them into the eCRF.
- The investigator will record any non-serious unsolicited adverse events which might have occurred within one month (Day 0 to Day 29) post-vaccination.
- Reporting of serious adverse events which might have occurred since the last visit.
- Recording of any concomitant medication/treatment/vaccination taken since the last visit (see Section 6.9).
- History-directed physical examination and vital signs.
- Pre-vaccination assessment of body temperature
- Counselling on avoidance of HIV infection and pregnancy.
- Check of elimination criteria.
- Check of contraindications to vaccination.
- A urine pregnancy test for females of reproductive potential will be performed.
- Collection of blood (6.5 mL) for biochemistry and haematology.
- Evaluations and blood draws must be performed prior to vaccination, but it is not necessary to have results (except for pregnancy test) prior to the administration of the vaccine.

Vaccination

- Vaccination: intramuscular administration in the deltoid muscle of the non-dominant arm. The vaccinees will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.
- Reporting of serious adverse events. The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
- Diary cards will be provided for the subjects to record temperature and any local (at the injection site) or general adverse events occurring on the day of vaccination and the 6 following days.

- The first 12 vaccinated subjects will be instructed to return their completed Day 0 to Day 6 diary cards on Day 37 to the investigator. All the other subjects will be instructed to return their Day 0 to Day 29 diary cards on Day 60 to the investigator.

5.5.5. Follow-up Visit 2 (Day 37; for the 12 first vaccinated subjects only)

- Check elimination criteria
- The subjects will return their completed (Day 0 to Day 6) diary cards to the investigator, who will transcribe them into the eCRF.
- Reporting of any unsolicited adverse events which might have occurred since vaccination.
- Reporting of any serious adverse events which might have occurred since vaccination.
- Recording of any concomitant medication1treatment1vaccination taken since the last visit (see Section 6.9).
- Subjects will be distributed Day 7 to Day 29 diary cards and will be instructed to return them completed on Day 60 to the investigator.

5.5.6. Study Visit 3 (Day 44 - 2 weeks after second vaccination)

- Check elimination criteria
- Reporting of serious adverse events which might have occurred since the last visit.
- Recording of any concomitant medication/treatment/vaccination taken since the last visit.
- Collection of blood (96.5 mL) for biochemistry, haematology, serology and cellular-mediated immunity.

5.5.7. Study Visit 4 (Day 60 - Month 2)

- Check elimination criteria
- The subjects will return their completed diary cards (Day 7 to Day 29 diary cards for the 12 first vaccinated subjects and Day 0 to Day 29 diary cards for all the other subjects) to the investigator, who will transcribe them into the eCRF.
- The investigator will record any non-serious unsolicited adverse events which might have occurred within one month (Day 0 to Day 29) post-vaccination.
- Reporting of serious adverse events which might have occurred since the last visit.
- Recording of any concomitant medication/treatment/vaccination taken since the last visit (see Section 6.9).
- Collection of blood (96.5 mL) for biochemistry, haematology, serology and cellular-mediated immunity.

- Study conclusion (for interim analysis).

5.5.8. Study Visit 5: ~~Third vaccination~~ (Day 180 - Month 6)

~~**Prior to Vaccination**~~

- ~~***Signed addendum to informed consent form prior to study continuation.***~~ **(Amended October 08, 2007)**
- History-directed physical examination and vital signs.
- ~~Pre-vaccination assessment of body temperature~~
- Counselling on avoidance of HIV infection and pregnancy.
- Check of elimination criteria.
- ~~Check of contraindications to vaccination.~~
- Behavioural risk assessment questionnaire (hard copy) to be filled in by the subject.
- Reporting of any serious adverse events which might have occurred since vaccination. ***The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.***
- Recording of any ***contraindicated*** concomitant medication/treatme~~n~~t/va~~ccination~~ taken since the last visit (see Section 6.9).
- ~~A urine pregnancy test for females of reproductive potential will be performed.~~
- Collection of blood (56.5 mL) for biochemistry, haematology, serology and cellular-mediated immunity.
- Evaluations and blood draws must be performed prior to vaccination, but it is not necessary to have results (except for pregnancy test) prior to the administration of the vaccine.

~~**Vaccination**~~

- ~~Vaccination: intramuscular administration in the deltoid muscle of the non-dominant arm. The vaccinees will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.~~
- ~~Reporting of serious adverse events. The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.~~
- ~~Diary cards will be provided for the subjects to record temperature and any local (at the injection site) or general adverse events occurring on the day of vaccination and the 6 following days.~~
- ~~The first 12 vaccinated subjects will be instructed to return their completed Day 0 to Day 6 diary cards on Day 187 to the investigator. All the other subjects will be instructed to return their Day 0 to Day 29 diary cards on Day 210 to the investigator.~~ **(Amended September 24, 2007)**

~~5.5.9. Follow-up Visit 5 (Day 187; in the first 12 vaccinated subjects only)~~

- ~~Check elimination criteria~~
- ~~The subjects will return their completed (Day 0 to Day 6) diary cards to the investigator, who will transcribe them into the eCRF.~~
- ~~Reporting of any unsolicited adverse events which might have occurred since vaccination.~~
- ~~Reporting of any serious adverse events which might have occurred since vaccination.~~
- ~~Recording of any concomitant medication/treatment/vaccination taken since the last visit (see Section 6.9).~~
- ~~Subjects will be distributed Day 7 to Day 29 diary cards and will be instructed to return them completed on Day 210 to the investigator.~~ **(Amended September 24, 2007)**

5.5.10. Study Visit 6 (Day ~~210~~ *270*- Month ~~7~~ *9*)

- ***Signed addendum to informed consent form prior to study continuation.*** **(Amended October 08, 2007)**
- Check elimination criteria
- ~~The subjects will return their completed diary cards (Day 7 to Day 29 diary cards for the 12 first vaccinated subjects and Day 0 to Day 29 diary cards for all the other subjects) to the investigator, who will transcribe them into the eCRF.~~
- ~~The investigator will record any non-serious unsolicited adverse events which might have occurred within one month (Day 0 to Day 29) post-vaccination.~~
- Reporting of serious adverse events which might have occurred since the last visit.
- Recording of any ***contraindicated*** concomitant medication/treatme~~n~~t/va~~ccination~~ taken since the last visit (see Section 6.9).
- Collection of blood (~~6~~1 ***11*** mL) for virology, biochemistry, ***and*** haematology, ~~-serology and cellular-mediated immunity~~.
- ~~Study conclusion (for interim analysis).~~ **(Amended September 24, 2007)**

5.5.11. Study Visit 7 (Day 360 - Month 12)

- Physical examination and vital signs
- Behavioural risk assessment questionnaire (hard copy) to be filled in by the subject.
- Reporting of any serious adverse events which might have occurred since the last visit

- Recording of any contraindicated concomitant medication/treatment taken since the last visit (see Section 6.9).
- Collection of blood (69 mL) for virology, HIV tests panel, biochemistry, haematology, serology and cellular-mediated immunity.
- Study conclusion (final analysis).

5.6. Safety evaluation and processes for holding-on, stopping and/or restarting the trial

5.6.1. Staggered design

A staggered dose-escalation design will be used for safety reasons.

A first group of 12 subjects (10 receiving the adjuvanted vaccine and 2 receiving the vaccine in WFI) will be enrolled to receive their first vaccination at the lowest dose (10 μg), at a rate of maximum 1 subject per day during the first 5 working days. Provided no safety issues leading to clinical hold or stop occurred (Figure 2), the 12 subjects will be allowed to receive their second vaccination on Day 30 according to the same rate (maximum 1 subject/day during the first 5 working days) and the remaining subjects of the same dose group (N=48) will receive their first vaccination. Then, a new group of 12 subjects will be enrolled to receive their first vaccination at the medium dose (30 μg) at the rate of maximum 1 subject per day during the first 5 working days. Provided no safety issues leading to clinical hold or stop occurred, subjects will be allowed to receive their second vaccination on Day 30 at the same rate and the remaining subjects (N=48) of the same dose group will receive their first vaccination. Then, a new group of 12 subjects will be enrolled to receive their first vaccination at the highest dose (90 μg) at a rate of maximum 1 subject per day during the first 5 working days. Provided no safety issues leading to clinical hold or stop occurred, subjects will be allowed to receive their second vaccination on Day 30 at the same rate and the remaining subjects (N=48) of the same dose group will receive their first vaccination. ~~Exactly the same methodological approach will be replicated for the third vaccination in each dose group~~**(Amended September 24, 2007)**

5.6.2. Review of safety data

A safety review team (SRT), including as core members the GSK Biologicals' Central Safety Physician, the Clinical Development Manager and Biostatistician of the project, will be responsible for reviewing the safety data 'in-stream' (i.e. throughout the course of the study), as appropriate. In case a safety signal is observed or the predefined holding rules are met (see Section 5.6.3), the SRT leader is responsible for the urgent communication and escalation to the GSK Vaccine Safety Monitoring Board (VSMB), who will decide at an ad hoc meeting suspending or not the study. Whenever unblinding would be needed in the in-stream review of the safety data, an Internal Data Monitoring Committee (IDMC) will be constituted and designated by the Vice President - World Wide Regulatory, Epidemiology and Safety (VP-WWRES) and Vice President -World Wide Clinical Development (VP-WWCD), including three GSK Physicians from

Biologicals Clinical Safety and Pharmacovigilance (BCSP) and WW Clinical Development (WWCD) that are not otherwise involved in the conduct of the study, in order to ensure that the study related personnel will remain blinded to all investigational product treatment codes (if applicable) until the study has been completed per protocol or is terminated for any cause.

Vaccinations are scheduled on Days 0, ***and*** 30 ~~and 180~~ and additional safety evaluations 7 days after each vaccination. The following events must occur within the specified timeframe to ensure proceeding to the next vaccination and to the next dose as per schedule. **(Amended September 24, 2007)**

- The investigative site must complete eCRFs promptly.
- The SRT must review the demographic and safety data for each subject within 2 business days of receiving the listings/tables and, if warranted, instruct the site to withdraw individual subjects and/or suspend further vaccination at least 1 business day prior to the next vaccination.

5.6.3. Holding rules

Holding one or stopping rules are defined according to the following criteria:

- At least 3 out of the 12 subjects develop a Grade 3 general solicited AE, during the 7-day follow-up period after vaccination which persists at Grade 3 for more than 48 hours and which is considered to be related to vaccination

or

- At least 4 out of the 12 subjects develop any other Grade 3 unsolicited AE considered to be related to vaccination during the 7-day follow-up period after vaccination

or

- At least 4 out of the 12 subjects develop any Grade 3 general solicited (persisting at Grade 3 for more than 48 hours and considered related to vaccination) or unsolicited AE (considered related to vaccination) during the 7-day follow-up period after vaccination

or

- At least one life threatening vaccine related SAE occurs.

or

- At least one subject shows ulceration (necrosis of the dermis) at the injection site following vaccination.

These holding or stopping rules will be applied to each of the three vaccine doses (10, 30 and 90 μg) and to each of the ~~three~~ ***two*** vaccinations. **(Amended September 24, 2007)**

In addition to the above stated holding rules, the withdrawal of an individual subject from vaccination can be at the investigator's discretion for serious local and adverse events or for any serious laboratory adverse event considered to be associated with vaccination. For all such withdrawals, GSK Biological's CDM has to be informed promptly (see Section 9).

5.6.4. Process for restarting vaccination/trial

Although the vaccination may be put on hold temporarily, further vaccination will restart only if the VSMB agrees by consensus. In the absence of consensus, the issue will be brought back to VSMB for decision when further information has been obtained to address the concerns of individual members or will be escalated directly to the GSK Global Safety Board (GSB) for decision.

5.6.5. Process for stopping of trial

In the event that the trial is stopped, GSK Biologicals will inform both the IEC through the investigator and competent authorities. A report will be written detailing the rationale used for reaching the decision.

5.7. Sample handling and analysis

5.7.1. Treatment and storage of biological samples

See Appendix D of the protocol for details of treatment and storage of biological samples.

See Appendix E for instructions for shipment of biological samples.

5.7.2. Laboratory assays

The following measurements will be performed when indicated in the Flowsheet (Section 5.4):

5.7.2.1. Virology

The following virology assessments will be performed in the Investigator's laboratory.

- Abbott kits will be used for the analysis of: anti-HBc Ab (+1--): CORE Abbott AxSYM, HBsAg (+1--): HBsAg (V2) Abbott AxSYM and anti-HCV Ab (+1--): HCV version 3.0 Abbott AxSYM.
- An Abbott kit will be used for the analysis of:
  HIV Ag/Ab ELISA test; Abbott AxSYM.
  If this test is positive, confirm using INNO-LIA™ Immunoblot Assay and proceed with algorithm to detect intercurrent HIV Infection as explained in Section 5.2.2 and Appendix H.
- Panel of HIV tests commonly used in Europe

5.7.2.2. Biochemistry

These tests will be performed in the Investigator's laboratory.

- Sodium
- Potassium
- Urea
- Creatinine
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

5.7.2.3. Haematology

These tests will be performed in the Investigator's laboratory.

- Red blood cell count (RBC)
- White blood cell count (WBC)
- Differential count
- Platelets count
- Haemoglobin
- Haematocrit

5.7.2.4. Urinalysis

- Human Chorionic Gonadotropin (HCG) will be determined using a urine pregnancy test in female subjects of childbearing potential.

The GSK Biologicals' laboratory at Rixensart has established Quality Control Procedures and an established Quality System. Both are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department to document the competency of the facility to perform the required tests and support the reliability of the results. Methods and equipment are validated, where required.

5.7.3. Immunological read-outs

The following immunological read-outs (Table 5) will be performed when indicated in the Flowsheet (Section 5.4) and in Table 6:

Table 5 Immunological read-outs (characteristics of the assays)

Assay	Assay method	Test kit/ Manufacturer	Assay unit	Assay cut-off or limit of detection	Laboratory
Anti-p17,-24,-RT,-Nef, -F4co antibodies	ELISA	In-house ELISA	EU/mL	To be determined	CEVAC, Belgium
p17-,24-,RT-,Nef-specific effector T-cells	Flow Cytometry, ICS	In-house ICS	Number of cytokine(s) positive cells per 10^6 cells	To be determined	*CEVAC, Belgium

* Or GSK Biologicals,

Table 6 Immunological read-outs (timings, markers, numbers of subjects and laboratory)

Blood sampling timepoint			Marker	No. subjects	Laboratory
Timing	Timepoint	Visit			
Pre	Day 0	1	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium
Post II	Day 44	3	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium
Post II	Day 60	4	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium
Post II	Day 180	5	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium
~~Post III~~	~~Day 210~~	~~6~~	~~Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA)~~ ~~p17-,p24-,RT-, Nef-Specific effector T cells (ICS)~~	~~180~~ ~~180~~	~~CEVAC, Belgium~~ ~~*CEVAC, Belgium~~
~~Post III~~ ***Post II***	Day 360	7	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium

* Or GSK Biologicals

(Amended September 24, 2007)

- Samples will not be labelled with information that directly identifies the subjects but will be coded with the identification number of the subject.

- Collected samples may be used for purposes related to the quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these current tests, the maintenance or improvement of these current tests, the development of new test methods for the markers described in this protocol, as well as making sure that new tests are comparable to previous methods and work reliably.
- It may be that any findings in the present or in other studies necessitate further investigation by GSK Biologicals into the efficacy or immunogenicity of the vaccines and their constituents under study or further research in the disease under study. Under these circumstances, additional testing on the samples may be performed by GSK Biologicals outside the scope of this protocol.
- A GSK Biologicals Research & Development Position Paper is available (upon request) which describes the rationale for and some examples of what these further investigations might include.
- Any sample testing will be done in line with the consent of the individual subject.
- Collected samples will be stored for up to 15 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.

5.7.3.1. Serology: antibody responses

Antibody level will be determined by evaluating antibody (IgG) responses to p17, p24, RT, Nef and F4co using standard ELISA techniques. The ELISA utilizes antibody and antigen interactions to test for the presence of specific antibodies in the serum sample. The antigens are chosen from the evaluated vaccine based on what is likely to be an immunogenic component.

Partially purified antigen is pre-coated onto a 96-well plate. Serum samples are added to the plate followed by a secondary anti-human IgG antibody conjugated with an enzyme. The addition of substrate provides a means of detecting the serum antibody that is specific for the antigens. Positive control, calibrators are run on each plate in order to assess the relative titer of each test sample. Negative controls are also run on each plate to ensure specificity.

5.7.3.2. Cell-mediated immune responses

Flow cytometry, using Intracellular Cytokine Staining (ICS) provides information on the frequency of CD4+ and CD8+ T cells responding to the antigen. It provides simultaneously information on the frequency of CD4+ 1 CD8+ T-lymphocytes expressing molecules involved in immunity such as IFN-γ, IL-2, TNF-α and/or CD40L. Other markers may be used for exploratory analysis and further definition of the quality of the induced immune response.

PBMCs are stimulated in vitro with pools of peptides covering the sequences of p17, p24, RT or Nef antigens for 2 hours; then intracellular block is added (to inhibit cytokine secretion) for an additional overnight incubation. The cells will be stained for surface markers (e.g. CD4+ or CD8+ for T cells), than fixed and permeabilized, and subsequently stained with fluorescent antibodies to detect cytokines. The cells will then be analysed by flow cytometry.

6. INVESTIGATIONAL PRODUCT AND ADMINISTRATION

6.1. Study vaccine

The HIV candidate vaccine, F4co/AS01B, consists of two fractions to be used for reconstitution just prior to injection:

- The freeze-dried fraction containing the F4co antigen, which is presented in a single-dose 3 mL clear glass vial.
- The liquid fraction consisting of AS01B adjuvant system, which is presented in a single-dose 3 mL glass vial.
- The HIV candidate vaccine contains 10, 30 or 90 μg per dose of F4co recombinant protein as active ingredient, adjuvanted with AS01B adjuvant system. Three groups of 50 subjects will receive F4co antigen (10, 30 or 90 μg dose) reconstituted with the adjuvant AS01B and three groups of 10 subjects will receive F4co antigen (10, 30 or 90 μg dose) reconstituted with water for injection (WFI). The reconstitution of antigen and adjuvant or WFI occurs just prior to injection.
- The vaccine contains sodium sulfite which can rarely cause severe allergic reactions. Please see the Investigator's Brochure for further details.

F4co is a fusion protein that comprises 4 HIV-1 derived antigens arranged as follows: p24-RT-Nef-p17. Each of the four antigens entering into the F4co fusion protein are:

- p24, a viral capsid protein coded by the *gag* gene
- RT (reverse transcriptase), a viral enzyme responsible for transcribing the viral RNA into double-stranded DNA. This enzyme was mutated in one amino acid (Tryptophan 229 substituted by Lysine) to remove the RT polymerase activity. This protein is coded by the *pol* gene
- Nef, a regulatory protein coded by an open-reading-frame (ORF) that flanks the *env* gene
- p17, a viral matrix protein coded by the *gag* gene.

AS01B is a liposome-based adjuvant containing the immunostimulants 3-D-MPL and QS21. The monophosphoryl lipid A (3-D-MPL) molecule consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the Gram negative bacterium *Salmonella minnesota*. The form used for the vaccine, 3-D-MPL® (GSK Biologicals North America) is the 3-O-deacylated form of the monophosphoryl lipid A.

QS21 is a natural saponine molecule purified from the bark of the South American tree, *Quillaja saponaria Molina*.

Water for injection (Ph. Eur. 0169) used for reconstitution of lyophilized F4co is produced by GSK Biologicals. Lots of WFI intended for clinical trials are commercial lots used for reconstitution of GSK Biologicals marketed vaccines.

The candidate vaccine to be used has been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for the candidate vaccine are described in separate release protocols and the required approvals have been obtained.

Table 7 Composition of the study vaccines

Vaccine	Formulation (approximately per injection)	Presentation	Volume
F4co (p24-RT-Nef-p17)	10 μg of p24-RT-Nef-p17 20 mg sucrose 630 μg sodium sulfite	Lyophilized pellet in vial	N/A
F4co (p24-RT-Nef-p17)	30 μg of p24-RT-Nef-p17 20 mg sucrose 630 μg sodium sulfite	Lyophilized pellet in vial	N/A
F4co (p24-RT-Nef-p17)	90 μg of p24-RT-Nef-p17 20 mg sucrose 630 μg sodium sulfite	Lyophilized pellet in vial	N/A
AS01B	50 μg of MPL® 50 μg QS21 in a suspension of liposomes in phosphate buffered saline per 0.5 mL	Liquid in monodose vial	0.5 mL/dose
Water for injection (WFI)	Ph.Eur. 0169	Liquid in single-dose syringe	0.5 mL/dose

Refer to the Investigator's Brochure for further information about the study product and its components.

Details concerning vaccine supplies can be found in Appendix G.

The following doses of antigens will be used:

Treatment	Dose (μg)	Adjuvant	
		AS01B (Number of subjects)	Water for injection (WFI) (Number of subjects)
F4co (p24-RT-Nef-p17)	10	50	10
F4co (p24-RT-Nef-p17)	30	50	10
F4co (p24-RT-Nef-p17)	90	50	10

6.2. Dosage and administration

6.2.1. Reconstitution of the F4co antigen with AS01B adjuvant

Disinfect the top of the AS01B and F4co antigen vials with alcohol swabs and let dry. Aspirate the contents of the AS01B vial in a syringe and inject adjuvant into the vial of lyophilized antigen. Remove and discard the syringe and needle under appropriate safety precautions. Dissolve the pellet by **gently shaking (by hands)** the vial. Wait for approximately 1 minute to ensure complete dissolution of vial contents before withdrawing 0.5 mL of the reconstituted vaccine solution using a fresh needle and syringe.

When reconstituted, the vaccine should not be kept for more than 4 hours at room temperature.

6.2.2. Reconstitution of the F4co antigen with water for injection (WFI)

Disinfect the top of the F4co antigen vial with alcohol swabs and let dry. Inject the complete contents of one pre-filled syringe of WFI into the vial of lyophilized antigen. Remove and discard the syringe and needle under appropriate safety precautions. Dissolve the pellet by **gently shaking (by hands)** the vial. Wait for approximately 1 minute to ensure complete dissolution of vial contents before withdrawing 0.5 mL of the reconstituted vaccine solution using a fresh needle and syringe.

When reconstituted, the vaccine should not be kept for more than 4 hours at room temperature.

6.2.3. Administration of reconstituted vaccine

Each 0.5 mL dose of the reconstituted vaccine can be injected after very gentle manual shaking and slight warm-up at room temperature. It cannot be kept for more than 4 hours at room temperature before being administered.

The needles should be suitable for intramuscular administration**.**

IMPORTANT:

(1) The vaccine will be administered slowly intramuscularly in the deltoid muscle of the non-dominant arm by the physician or delegate, with drugs and material readily available to treat emergencies.

(2) Any unused (not reconstituted) vaccine will be sent back to GSK Biologicals to be destroyed.

The vaccinees will be observed closely for at least 30 minutes following the administration of vaccines, with appropriate medical treatment readily available in case of a rare anaphylactic reaction.

6.3. Storage

All investigational products to be administered to subjects must be stored in a safe and locked place with no access by unauthorized personnel.

Vaccines will be stored at the defined temperature range (i.e. +2 to +8°C1 36°F to 46°F).

The storage temperature of vaccines will be monitored and recorded daily during working days, preferably at the same time of the day (e.g. at the beginning of the day).

At a minimum, a calibrated thermometer will be placed close to the vaccines and will be used to monitor and record the daily temperature (actual, min and max temperatures will be logged). Additionally, a continuous temperature recording system (e.g. 90-day Cox Recorder) will be used as a back up device and it will be opened in case of any temperature deviation (temperature outside the defined range, i.e. +2 to +8°C/ 36°F to 46°F) during weekends or holidays. Alternatively, the temperature monitoring system of the storage facility can be used (as a replacement of the GSK continuous temperature recording system), if:

- proper functioning was demonstrated during the monitor's site evaluation,
- if the system continues to work in case of a power failure, and
- if the system is maintained regularly (e.g. once/year) as documented in the site files.

It is also permitted to monitor the storage temperature using a validated temperature continuous recording device, provided it can read the daily actual and min/max temperatures, and that it keeps working after the alarm is activated.

It is also required to place a validated freezing point indicator (e.g. Freeze Tag®) close to the vaccines as a back-up device.

Any temperature deviation, i.e. temperature outside the defined range (i.e. +2 to +8°C/ 36°F to 46°F), must be reported within 24 hours to the sponsor's CSC.

Following exposure to a temperature deviation, vaccines will not be used until written approval is given by the sponsor.

Storage conditions for transport of vaccines from country medical department or dispatch center to study sites or between sites are described in Appendix G.

6.4. Treatment allocation and randomization

The target sample size is 180 subjects (50 subjects per adjuvanted groups and 10 subjects per non-adjuvanted groups).

6.4.1. Randomization of supplies

Three randomization lists (one for each antigen dose: 10, 30 and 90 μg) will be generated at GSK Biologicals, Rixensart, using a standard SAS® (Statistical Analysis System) program and will be used to number the vaccines. A randomization blocking scheme (5:1) will be used to ensure that balance between treatments is maintained: a treatment number will identify uniquely the vaccine doses to be administered to the same subject.

6.4.2. Randomization of subjects

The treatment allocation at the investigator site will be performed using a central randomization system on Internet (SBIR). The randomization algorithm will use a minimization procedure accounting for centre and vaccine dose (10, 30 or 90 μg). Center and dose minimization factors will have equal weight in the minimization algorithm.

After having checked that a subject is eligible, and after informed consent has been obtained, the person in charge of the vaccination will access the randomization system on Internet to receive a treatment number. Upon providing a subject number and a vaccine dose (10, 30 or 90 μg), the randomization system will use the minimization algorithm to determine the treatment number to be used for the subject.

The actual treatment number used for the first vaccination of the subject must be recorded by the investigator in the eCRF (Rando/Treatment Allocation Section).

When the first target number of 12 subjects has been reached for a given dose (10, 30 or 90 μg), the enrolment will be frozen for the safety evaluation. When a go decision has been taken, the enrolment will be re-opened for that dose until the total target number of 60 subjects has been reached.

The enrolment for each vaccine dose (adjuvanted or not) will be open in a staggered way. The enrolment for 30 μg vaccine dose will be open after completion of enrolment for 10 μg vaccine dose and the enrolment for 90 μg vaccine dose will be open after completion of enrolment for 30 μg vaccine dose.

6.5. Method of blinding and breaking the study blind

The study will be blinded for the randomized allocation to the adjuvanted vaccine or to the vaccine without adjuvant but not for the dose (partially-blind design). Because of the differences between the adjuvanted and non-adjuvanted vaccines, the following steps will be taken to maintain the blinding:

- The vaccine recipient and those responsible for the evaluation of safety and reactogenicity study parameters will all be unaware of which vaccine preparation was administered (observer-blind design).
- The Principal Investigator and or the co-investigators will be responsible only for assessing safety and post-vaccination adverse events.

The immunological data, which will lead to the unblinding of the treatment groups, will not be available during the course of the trial to any investigator or any person involved in the clinical conduct of the study (including data cleaning).

The investigator, or person designated by the investigator, should contact GSK Biologicals' Central Safety physician directly or via the local safety contact (see below and Study Contact for Emergency Code Break in Sponsor Information page) to discuss the need for emergency unblinding. No set of individual codes will be held at the GSK Biologicals' Central Safety Office. The GSK Biologicals' Central Safety Office will be allowed to access the individual randomization code. The code will be broken by the Central Safety physician (Study Contact for Emergency Code Break in Sponsor Information page) only in the case of medical events that the investigator1physician in charge of the subject feels cannot be treated without knowing the identity of the study vaccine(s).

GSK Biologicals Clinical Safety Physician
(Study contact for Emergency Code Break)

Phone: PPD

Fax: PPD

Mobile phone for 7/7 day availability:
.PPD

Back-up mobile phone contact:
.PPD

GSK Biologicals' policy (incorporating ICH E2A guidance, EU Clinical Trial Directive and Federal Regulations) is to unblind any SAE report associated with the use of the investigational product, which is unexpected and attributable/suspected, prior to regulatory reporting. The Central Safety physician is responsible for unblinding the treatment assignment in accordance with specified time frames for expedited reporting of SAEs (Refer to Section 8.8).

The independent statistician who will perform the analysis will have access to the randomisation codes at the time of analysis and will be able to break the codes to perform it.

6.6. Replacement of unusable vaccine

Additional vaccines will be provided to replace those that are unusable (see Appendix G for details of supplies).

In addition to the vaccines provided for the planned number of subjects (including over-randomization when applicable), at least 5% additional vaccines will be supplied. In case a vaccine is broken or unusable, the investigator should replace it with a replacement vaccine. Although the sponsor need not be notified immediately in these cases (except in the case of cold-chain failure), documentation of the use of the replacement vaccine must be recorded by the investigator on the vaccine administration page of the eCRF and on the vaccine accountability form.

The investigator will use the central randomization system (SBIR) to obtain the replacement vial number. The system will ensure, in a blinded manner, that the replacement vial is of the same formulation as the randomized vaccine.

6.7. Packaging

See Appendix G.

6.8. Vaccine accountability

See Appendix G.

6.9. Concomitant medication/treatment

At each study visit, the investigator should question the subject about any medication(s) taken.

All concomitant medication, with the exception of vitamins and/or dietary supplements, administered at ANY time during the period starting with administration of each vaccine and ending 30 days after each injection of study vaccine are to be recorded with generic name of the medication (trade names are allowed for combination drugs, i.e. multi-component drugs), medical indication, total daily dose, route of administration, start and end dates of treatment.

Any treatments and/or medications specifically contraindicated, e.g., any immunoglobulins, other blood products and any immune modifying drugs administered within 4 months before the study or at any time during the study period are to be recorded with generic name of the medication (trade names are allowed for combination drugs only), medical indication, total daily dose, route of administration, start and end dates of treatment (see Sections 4.3 and 4.4).

Any vaccine not foreseen in the study protocol administered in the period beginning 30 days (14 days in the case of subunit or killed vaccines [e.g., influenza, pneumococcal] or allergy treatment with antigen injections) preceding each injection of study vaccine and ending 30 days after each injection of study vaccine is to be recorded with trade name, route of administration and date(s) of administration (see Sections 4.3 and 4.4).

A prophylactic medication is a medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination (e.g. an anti-pyretic is considered to be prophylactic when it is given in the absence of fever [oral / axillary temperature < 37.5°C] and any other symptom, to prevent fever from occurring). Any concomitant medication administered prophylactically in anticipation of reaction to the vaccination must be recorded in the eCRF with generic name of the medication (trade names are allowed for combination drugs only), total daily dose, route of administration, start and end dates of treatment and coded as 'Prophylactic'.

Concomitant medication administered for the treatment of an AE or SAE within the follow-up period for adverse events must be recorded in the eCRF with generic name of

the medication (trade names are allowed for combination drugs only), medical indication (including which AE/SAE), total daily dose, route of administration, start and end dates of treatment. Similarly, concomitant medication administered for the treatment of an SAE, at any time, must be recorded on the SAE Report Form. Refer to Section 8.2 for definition of SAE.

7. HEALTH ECONOMICS

7.1. Outcomes measurement and analysis

Not applicable

7.2. Economic data collection and analyses

Not applicable

8. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

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

8.1. 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:

- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

- Signs, symptoms temporally associated with vaccine administration.

Examples of an AE DO NOT include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- 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.

AEs may include 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).

N.B. AEs to be recorded as endpoints (solicited events) are described in Section 8.4.1. All other AEs will be recorded as UNSOLICITED AEs.

Example of events to be recorded in the medical history section of the eCRF:

- Pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e. prior to the first study vaccination).

8.2. 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, if it were more severe.

c. requires hospitalization or prolongation of existing hospitalization,

NOTE: In general, hospitalization signifies 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-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils 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 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, 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.

f. 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 hospitalization but may jeopardize 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 hospitalization.

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

Abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g., vital signs) that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE, as defined in Section 8.1 or SAE, as defined in Section 8.2. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will 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.

8.4. Time period, frequency, and method of detecting adverse events and serious adverse events

All AEs occurring within 30 days following administration of each vaccine must be recorded on the AE form in the subject's eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

The standard time period for collecting and recording SAEs will begin at the first receipt of vaccine and will end ~~6~~ ***11*** months following injection of the last dose of vaccine for each subject. See Section 8.7 for instructions for reporting and recording SAEs.
(Amended September 24, 2007)

Additionally, in order to fulfil international reporting obligations, SAEs that are related to study participation (e.g. procedures, invasive tests, a change from existing therapy) or are related to a GSK concurrent medication will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged.

The investigator will inquire about the occurrence of AEs/SAEs at every visit/contact during the study.

All AEs either observed by the investigator or one of his clinical collaborators or reported by the subject spontaneously or in response to a direct question will be evaluated by the investigator. AEs not previously documented in the study will be recorded in the AE form within the subject's eCRF. The nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to vaccination should be established. Details of any corrective treatment should be recorded on the appropriate page of the eCRF. Refer to Section 6.9.

As a consistent method of soliciting AEs, the subject should be asked a non-leading question such as:

"Have you felt different in any way since receiving the vaccine or since the previous visit?"

N.B. The investigator should record only those AEs having occurred within the time frame defined above.

AEs already documented in the eCRF, i.e. at a previous assessment, and designated as "not recovered/not resolved" or "recovering/resolving" should be reviewed at subsequent visits, as necessary. If these have resolved, the documentation in the eCRF should be completed.

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 or designate will record all relevant information regarding an AE on the Adverse Event screen within the subject's eCRF. The investigator or designate will also record all relevant information regarding the SAE on the SAE screens in the eCRF.

The electronic system using SAE screens in the eCRF will be the primary mode for reporting SAEs to GSK Biologicals during the study period. In case this electronic system for reporting SAEs does not work or after clinical database freezing, paper SAE Report Forms and the facsimile (Fax) system should be used to report SAEs.

The investigator will attempt to establish a diagnosis of 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.

8.4.1. Solicited adverse events

Solicited symptoms are the symptoms that are most likely to occur. A 7-day follow-up (Day 0 to 6) of solicited adverse events will be performed by the subjects after each vaccination. Data concerning the following adverse events will be solicited using diary cards provided by the sponsor.

Solicited local (injection site) AEs

Table 8 Solicited local adverse events

Pain at injection site
Redness at injection site
Swelling at injection site

Solicited general AEs

Table 9 Solicited general adverse events

Fever
Fatigue
Headache
Sweating
Myalgia
Gastro-intestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain)

N.B. Temperature will be recorded in the evening. Should additional temperature measurements be performed at other times of day, the highest temperature will be recorded.

8.5. Evaluating adverse events and serious adverse events

8.5.1. Assessment of intensity

Intensity of the following AEs will be assessed as described:

Table 10 Intensity scales for solicited symptoms

Adverse Event	Intensity grade	Parameter
Pain at injection site	0	Absent
	1	Painful on touch
	2	Painful when limb is moved
	3	Pain that prevents normal activity
Redness at injection site		Record greatest surface diameter in mm
Swelling at injection site		Record greatest surface diameter in mm
Fever*		Record temperature in °C
Headache	0	Normal
	1	Headache that is easily tolerated
	2	Headache that interferes with normal activity
	3	Headache that prevents normal activity
Fatigue	0	Normal
	1	Fatigue that is easily tolerated
	2	Fatigue that interferes with normal activity
	3	Fatigue that prevents normal activity
Sweating	0	Normal
	1	Sweating that is easily tolerated
	2	Sweating that interferes with normal activity
	3	Sweating that prevents normal activity
Myalgia	0	None
	1	Myalgia that is easily tolerated
	2	Myalgia that interferes with normal activity
	3	Myalgia that prevents normal activity
Gastrointestinal symptoms (nausea, vomiting, diarrhoea and/or abdominal pain)	0	Gastrointestinal symptoms normal
	1	Gastrointestinal symptoms that are easily tolerated
	2	Gastrointestinal symptoms that interfere with normal activity
	3	Gastrointestinal symptoms that prevent normal activity

*Fever is defined as axillary or oral temperature ≥37.5°C.

The maximum intensity of local injection site redness/swelling will be scored at GSK Biologicals as follows:

0 : 0 mm
1 : > 0 to ≤ 20 mm
2 : > 20 to ≤ 50 mm
3 : > 50 mm

The investigator will make an assessment of the maximum intensity that occurred over the duration of the event for all other AEs, i.e. unsolicited symptoms, including SAEs reported during the study. The assessment will be based on the investigator's clinical judgement. The intensity of each AE and SAE recorded in the Adverse Event screen within the subject's eCRF, SAE screens in the eCRF or SAE Report Form (if back-up reporting system is used) 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. Such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category utilized 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 8.2.

8.5.2. Assessment of causality

The investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the Investigator's Brochure in the determination of his/her assessment.

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 completion and data submission of the SAE screens in eCRF (or transmission of the SAE Report Form to GSK Biologicals if back-up reporting is used). The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE screens in eCRF (or SAE Report Form if back-up reporting system is used). The causality assessment is one of the criteria used when determining regulatory reporting requirements.

In case of concomitant administration of multiple vaccines, it may not be possible to determine the causal relationship of general AEs to the individual vaccines administered. The investigator should, therefore, assess whether the AE could be causally related to vaccination rather than to the individual vaccines.

All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other AEs should be assessed by the investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the investigational product ?

NO	:	The AE is not causally related to administration of the study vaccine(s). There are other, more likely causes and administration of the study vaccine(s) is not suspected to have contributed to the AE.
YES	:	There is a reasonable possibility that the vaccine(s) contributed to the AE.

Non-serious and serious AEs will be evaluated as two distinct events. If an event meets the criteria to be determined "serious" (see Section 8.2 for definition of SAE), it will be examined by the investigator to the extent to be able to determine ALL contributing factors applicable to each SAE.

Other possible contributors include:

- Medical history
- Other medication
- Protocol required procedure
- Other procedure not required by the protocol
- Lack of efficacy of the vaccine(s), if applicable
- Erroneous administration
- Other cause (specify).

8.5.3. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if they received medical attention defined as hospitalization, an emergency room visit or a visit to or from medical personnel (medical doctor) for any reason and this information will be recorded in the eCRF.

8.6. Follow-up of adverse events and serious adverse events and assessment of outcome

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information to GSK Biologicals on the subject's condition.

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

Investigators will follow-up subjects:

- with SAEs or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, the event is otherwise explained, or the subject is lost to follow-up;
- or, in the case of other non-serious AEs, until they complete the study or they are lost to follow-up.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relative to the subsequent course of such an abnormality noted for any subject must be made available to the Study Monitor.

GSK Biologicals may request that the investigator perform or arrange for the conduct of supplemental measurements 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 recognized follow-up period, GSK Biologicals will be provided with a copy of any available post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed SAE screens in eCRF. The update and submission of SAE screens in eCRF should be done within 24 hours of receipt of the follow-up information as outlined in Section 8.7.1.

When paper SAE Report Form is used as back-up system during the study period, if electronic SAE reporting system does not work, the investigator or designate should update the SAE screens in eCRF once the electronic system is working again before using it to report additional information.

When paper SAE Report Form is used as back-up system (after clinical database freezing), new or updated information is to be recorded on the originally completed SAE Report Form, with all changes signed and dated by the investigator. The updated SAE Report Form should be resent to GSK Biologicals within 24 hours of receipt of the follow-up information as outlined in Section 8.7.1. In case paper SAE Report Forms are used, it is not acceptable for the investigator to send photocopies of the subject's medical records to GSK Biologicals in lieu of the appropriate completed AE/SAE pages. 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.

Outcome of any non-serious AE occurring within 30 days post-vaccination (i.e. unsolicited AE) or any SAE reported during the entire study will be assessed as:

- Recovered/resolved
- Not recovered/not resolved
- Recovering/resolving

- Recovered with sequelae/resolved with sequelae
- Fatal (SAEs only).

8.7. Prompt reporting of serious adverse events to GSK Biologicals

The electronic system using SAE screens in the eCRF will be the primary mode for reporting SAEs to GSK Biologicals during the study period. In case this electronic system for reporting SAEs does not work or after clinical database freezing, paper SAE Report Forms and the facsimile (Fax) system should be used to report SAEs.

8.7.1. Time frames for submitting serious adverse event reports to GSK Biologicals

SAEs will be reported promptly to GSK once the investigator determines that the event meets the protocol definition of an SAE.

The investigator or designate will encode and submit relevant information on the SAEs in the SAE screens in eCRF **WITHIN 24 HOURS OF HIS/HER BECOMING AWARE OF THESE EVENTS**. Additional or follow-up information relating to the initial SAE report is also to be encoded and submitted in the SAE screens in eCRF within 24 hours of receipt of such information.

When paper SAE Report Form is used as back-up system (if electronic SAE reporting system does not work or after clinical database freezing), the investigator or designate will fax the SAE reports to GSK Biologicals'Central Safety for Serious Adverse Event Reporting. Additional or follow-up information relating to the initial SAE report is also to be reported to the GSK Biologicals'Central Safety for Serious Adverse Event Reporting within 24 hours of receipt of such information.

8.7.2. Completion and transmission of serious adverse event reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator or designate will encode and submit the information in the SAE screens in eCRF within 24 hours as outlined in Section 8.7.1. The SAE screens in eCRF will always be completed as thoroughly as possible with all available details of the event and submitted by the investigator or designate. If the investigator or designate does not have all information regarding an SAE, he1she will not wait to receive additional information before notifying GSK of the event and completing the SAE screens in eCRF. The SAE screens in eCRF should be updated when additional information is received **WITHIN 24 HOURS** as outlined in Section 8.7.1.

When paper SAE Report Form is used as back-up system (if electronic SAE reporting system does not work or after clinical database freezing), the investigator or designate will report relevant information on SAEs to GSK Biologicals within the 24 hours as outlined in Section 8.7.1. The SAE Report Form will always be completed as thoroughly

as possible with all available details of the event, signed by the investigator or designate, and forwarded to GSK Biologicals within the designated time frames. If the investigator or designate does not have all information regarding a SAE, he/she will not wait to receive additional information before notifying GSK Biologicals of the event and completing the form. When additional information is received on a SAE reported to GSK Biologicals using the back-up paper SAE Report Form during the study period, the electronic system should be used to report the additional information within 24 hours if the electronic system is working again and only after updating the SAE screens in eCRF once the electronic system was working again. When additional information is received on a SAE after clinical database freezing, the paper SAE Report Form should be updated and forwarded to GSK Biologicals within 24 hours as outlined in Section 8.7.1.

The investigator will always provide an assessment of causality at the time of the initial report as described in Section 8.5.2.

In rare circumstances, if the electronic system for reporting SAEs does not work and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE Report Form sent by email or by mail. Initial notification via the telephone does not replace the need for the investigator or designate to complete and submit SAE screens in the eCRF (or complete and sign the SAE Report Form if back-up system need to be used) as outlined in Section 8.7.1.

In the event of a death determined by the investigator to be related to vaccination, completion of SAE screens in the eCRF 1 sending of the fax (if electronic SAE reporting system does not work or after clinical database freezing) must be accompanied by telephone call to the Study Contact for Reporting SAEs.

~~**Study Contact for Reporting SAEs**~~
~~PPD ,Central Study Coordinator, Clinical R & D GlaxoSmithKline Biologicals~~ ~~Tel:~~ PPD ~~Fax~~: PPD ~~E-mail address:~~ PPD
~~**Back-up** **Study Contact for Reporting SAEs**~~
GSK Biologicals Clinical Safety Physician Tel: PPD Fax: PPD Mobile phones for 717 day availability: PPD Back-up mobile phone contact: PPD **24/24 hour and 7/7 day availability**

(Amended September 24, 2007)

8.8. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 8.7. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to the IRB/IEC and, if required, to the applicable government authority.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to investigational product and unexpected. The purpose of the report is to fulfil specific regulatory and Good Clinical Practice (GCP) requirements, regarding the product under investigation.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK Biologicals will file it with the Investigator's Brochure or other appropriate study documentation and will notify the IRB or IEC, if appropriate according to local requirements.

8.9. 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 detection period defined in Section 8.4. Investigators are not obligated to actively seek AEs or SAEs in former study participants.

However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the investigator will promptly notify the Study Contact for Reporting SAEs.

After clinical database freezing, if SAE follow-ups or new SAEs have to be reported, the investigator or designate should use paper SAE Report Forms and the facsimile (Fax) system.

8.10. Pregnancy

Subjects who become pregnant during the study (up to maximum 30 days after receiving the last vaccine injection) must not receive additional injections of study vaccine but may continue other study procedures at the discretion of the investigator.

The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his/her

designee, will record pregnancy information on the Pregnancy Report Form and submit it to GSK within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether that be full-term or prematurely, information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than six to eight weeks following the estimated delivery date.

While pregnancy itself is not considered an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or a SAE, as described in Section 8.1 and 8.2, and will be followed as described in Section 8.5.3.

A spontaneous abortion is always considered to be a SAE and will be reported as described in Section 8.7. Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered reasonably related in time to receipt of the investigational product by the investigator, will be reported to GSK Biologicals as described in Section 8.9. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

Information on pregnancies identified during the screening phase/prior to vaccine administration does not need to be collected; this information need not be communicated to safety.

8.11. Treatment of adverse events

Treatment of any adverse event is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the subject's eCRF. Refer to Section 6.9.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

9.2. Subject withdrawal

Subjects who are withdrawn because of AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn as result of a SAE/AE until resolution of the event (see Section 8.5.3).

Withdrawals will not be replaced.

9.2.1. Subject withdrawal from the study

From an analysis perspective, a 'withdrawal' from the study is any subject who did not come back for the concluding visit foreseen in the protocol.

A subject qualifies as a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented on the Study Conclusion page of the eCRF. The investigator will document whether the decision to withdraw from the study was made by the subject or the investigator and which of the following possible reasons was responsible for withdrawal:

- serious adverse event
- non-serious adverse event
- protocol violation (specify)
- consent withdrawal, not due to an adverse event
- moved from the study area
- lost to follow-up
- other (specify).

9.2.2. Subject withdrawal from investigational product

A 'withdrawal' from the investigational product is any subject who does not receive the complete treatment, i.e. when no further planned vaccine is administered from the date of withdrawal. A subject withdrawn from the investigational product may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the investigational product will be documented on the Vaccine Administration page of the eCRF. The investigator will document whether the decision to discontinue further vaccination was made by the subject or the investigator and which of the following possible reasons was responsible for withdrawal:

- serious adverse event,
- non-serious adverse event,
- other (specify).

9.3. Screen and baseline failures

Screening tests and evaluations (as described in Sections 5.4 and 5.5) will be used to determine the eligibility of each subject for the study. All inclusion and exclusion criteria must be assessed within 56 days (8 weeks) prior to study entry. Subjects who do not fulfil the conditions for being included in the study will be considered as screen failures and will be replaced. Screening failure subjects will be recorded with demographic data and reason for failure.

10. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

10.1. Primary endpoints

Reactogenicity and safety

- Occurrence, intensity and relationship to vaccination of solicited local and general symptoms during a 7-day (Day 0 to Day 6) follow-up period after each vaccination.
- Occurrence, intensity and relationship to vaccination of unsolicited symptoms during a 30-day (Day 0 to Day 29) follow-up period after each vaccination.
- Occurrence and relationship to vaccination of serious adverse events during the whole study period.
- Haematological and biochemical levels at months 0, 1, 2, 6, ~~7~~ **9**, 12 and at Day 44 (two weeks after the second vaccination) in all subjects.**(Amended September 24, 2007)**

Immunogenicity

- Frequency of CD4+ T cells expressing at least two cytokines including IL-2 equal or above the cut-off to at least 1, 2, 3 antigens and to all 4 antigens at Day 44 (two weeks after the second vaccination).

10.2. Secondary endpoints

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**
- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing at least 2 cytokines including IL-2 equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**
- Antibody titers to p17, p24, Nef, RT and F4co as measured by ELISA at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**

10.3. Exploratory endpoints

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells to other HIV clades expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Day 44 (two weeks after the second vaccination) and/or at Month 2.
- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**
- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing at least 2 cytokines (IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L) equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**

10.4. Estimated sample size

As this study is a first evaluation of safety and immunogenicity of the HIV candidate vaccine F4co, no formal sample size determination has been performed.

However, Table 11 presents the precision achieved for estimation of the primary parameters.

Table 11 Precision achieved for different estimated proportions and for the two sample sizes of 10 and 50 subjects [by PASS 2005, 95% confidence interval of a proportion].

Estimated proportion	Precision N=50	Precision N=10
0.20 or 0.80	0.10	0.20
0.40 or 0.60	0.14	0.30

If we want to compare the three adjuvanted groups in terms of proportion of subjects with a given adverse event or in terms of proportion of responders to a vaccine antigen, the sample size of 50 subjects per group would achieve 80% power to detect around 20-30% difference in proportions with a two-sided alpha level of 0.0167 (Bonferroni's adjustment for 3 comparisons) (see Table 12).

Table 12 Difference between 2 groups of 50 subjects that can be detected with a power of 80% (two-sided alpha of 0.0167 for multiple comparisons between three groups) (by PASS 2005, two independent proportions, Fisher's Exact test)

Proportion in group 1	Proportion in group 2	Difference in proportions
0.10	0.38	0.28
0.20	0.52	0.32
0.30	0.63	0.33
0.40	0.73	0.33
0.50	0.82	0.32
0.60	0.89	0.29
0.70	0.95	0.25
0.80	0.99	0.19

10.5. Study cohorts to be evaluated

Total Vaccinated cohort

The Total Vaccinated cohort will include all vaccinated subjects.

Thus, the Total Vaccinated cohort for analysis of safety will include all subjects with at least one vaccine administration documented.

The Total Vaccinated cohort for analysis of immunogenicity will include vaccinated subjects for whom data concerning immunogenicity endpoint measures are available.

The Total Vaccinated cohort analysis will be performed per treatment actually administered.

According-To-Protocol (ATP) cohort for analysis of safety

The ATP cohort will include all vaccinated subjects

- who have received at least one dose of study vaccine according to their random assignment
- with sufficient data to perform an analysis of safety (at least one dose with safety follow-up)
- for whom administration route of study vaccines is known
- who have not received a vaccine not specified or forbidden in the protocol
- for whom the randomization code has not been broken.

According To Protocol (ATP) cohort for analysis of immunogenicity

The ATP cohort for analysis of immunogenicity will include all evaluable subjects, (i.e., those meeting all eligibility criteria, complying with the procedures and intervals defined in the protocol, with no elimination criteria during the study) for whom data concerning immunogenicity endpoint measures are available.

10.6. Derived and transformed data

Serology data

- The cut-off values for antibody titers will be defined by the laboratory before the analysis.
- A seronegative subject is a subject whose titer is below the cut-off value.
- A seropositive subject is a subject whose titer is greater than or equal to the cut-off value.
- The seropositivity rate is defined as the percentage of seropositive subjects.
- The Geometric Mean Titers (GMTs) calculations will be performed by taking the anti-log of the mean of the log titer transformations. Antibody titers below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation.

JCS data

- ICS data will be expressed as antigen-specific CD4/CD8 T-cells per million respectively of CD4 or CD8+ T-cells.
- The cut-off will be calculated by taking the 95 percentile of the pre-vaccination data of all subjects.
- A responder is a subject with antigen-specific CD4/CD8 T-cells response greater than or equal to the cut-off value.
- Frequency of CD4/CD8 T-cells expressing cytokines to the fusion protein F4co (all antigens) will be estimated by adding individual frequencies of CD4/CD8 T-cells to each 4 antigens (p17, p24, Nef, RT).

Handling of missing

- For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced.
- For the calculation of percentage of responders to 1, 2, 3 and all 4 antigens, subjects not responding to at least one of the concerned antigen(s) will not be taken into account.
- For the analysis of solicited symptom, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the total

vaccinated cohort will include only injections with documented safety data (i.e. symptom screen/sheet completed).

- For the analysis of unsolicited adverse events/serious adverse event/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

10.7. Final analyses

10.7.1. Analysis of demographics/baseline characteristics

Demographic characteristics (age, gender, race) of each study cohort will be tabulated.

The mean age (plus range and standard deviation) by gender of the enrolled subjects, as a whole, and per group, will be calculated.

10.7.2. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for analysis of immunogenicity. If the percent of enrolled subjects excluded from this ATP cohort is more than 5%, a second analysis based on the Total Vaccinated cohort will be performed to complement the ATP analysis.

Cell-mediated immune response

For cell-mediated immune response, the following parameters will be tabulated by vaccine groups at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination)**(Amended September 24, 2007)**:

- descriptive statistics of the frequency of CD4/CD8 T cell expressing at least two different cytokines (IFN-γ, IL-2, TNF-α,CD40L) to each antigen and to all antigens;
- descriptive statistics of the frequency of CD4/CD8 T cell expressing at least IFN-γ and another cytokine (IL-2, TNF-α,CD40L) to each antigen and to all antigens;
- descriptive statistics of the frequency of CD4/CD8 T cell expressing at least IL-2 and another cytokine (IFN-γ, TNF-α,CD40L) to each antigen and to all antigens;
- descriptive statistics of the frequency of CD4/CD8 T cell expressing at least TNF-α and another cytokine (IFN-γ, IL-2,CD40L) to each antigen and to all antigens;
- descriptive statistics of the frequency of CD4 T cell expressing at least CD40L and another cytokine (IFN-γ, IL-2, TNF-α) to each antigen and to all antigens.

CD4+ T-cells expressing JL-2 and another cytokine

For CD4+ T-cells expressing IL-2 and another cytokine response, the following parameters will be tabulated by vaccine groups at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination) **(Amended September 24, 2007)**:

- percentage of responders to each antigen (p17, p24, Nef, RT);

- percentage of responders to at least one antigen (p17, p24, Nef or RT);
- percentage of responders to at least two antigens;
- percentage of responders to at least three antigens;
- percentage of responders to all four antigens (p17, p24, Nef and RT).

CD8+ T-cells expressing at least 2 cytokines (IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L)

For CD8+ T-cells expressing at least 2 cytokines, percentage of responders to each antigen (p17, p24, Nef, RT) will be tabulated by vaccine groups at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination). **(Amended September 24, 2007)**

Humoral immune response

For humoral immune response, the following parameters will be tabulated by vaccine groups for each antigen (p17, p24, Nef, RT and F4co) at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination) **(Amended September 24, 2007)**:

- Geometric mean titers (GMTs) with 95% confidence intervals (CIs);
- Seropositivity rates with exact 95% CIs.

10.7.3. Analysis of safety

The primary analysis will be based on the Total Vaccinated cohort. If the percent of enrolled subjects excluded from the ATP cohort for analysis is more than 5%, a second analysis based on this ATP cohort will be performed to complement the total analysis.

Adverse events

The following parameters will be tabulated per treatment group:

- The percentage of subjects with at least one local AE (solicited and unsolicited), with at least one general AE (solicited and unsolicited) and with any AE during the solicited follow-up period will be tabulated with exact 95% confidence interval (CI) after each vaccine injection and overall.
- The percentage of vaccine injections followed by at least one local AE (solicited and unsolicited), by at least one general AE (solicited and unsolicited) and by any AE will be tabulated, overall vaccination course, with exact 95% CI.
- The percentage of subjects reporting each individual solicited local and general AE during the solicited follow-up period will be tabulated with exact 95% CI.
- The percentage of vaccine injections followed by each individual solicited local and general AE will be tabulated, overall vaccination course, with exact 95% CI.
- The proportion of subjects with at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported up to 30 days after vaccination will be tabulated with exact 95% CI.

The same tabulations will be done for grade 3 adverse events and/or for adverse events with relationship to vaccination.

Fever will be reported per 0.5°C cumulative increments.

Serious AEs and withdrawal due to AEs will be described in detail.

Haematological and biochemical parameters

The frequency distribution of values below, within and above normal ranges will be tabulated per treatment group at each scheduled time point.

10.8. Planned interim analyses

Safety interim analyses

~~Nine~~ ***Six*** interim analyses are planned for safety evaluation (see Section 5.6).

These analyses will be performed at the end of the 7-day follow-up period (Days 7, ***and*** 37 ~~and 187~~) after each vaccine injection in a subset of 12 subjects and for each vaccine dose (10, 30 and 90 μg).**(Amended September 24, 2007)**

Analysis will be done on uncleaned and blinded data. Individual data listings of the demographic and safety data (solicited/unsolicited AE, SAE, haematology and biochemistry values) will be provided to the SRT. Tables with the number of subjects responding to each stopping rules will be also provided.

Analyses will be performed by an external statistician if unblinding of data is required. Individual data listings and tables will then be provided to an internal data monitoring committee.

No clinical report will be written.

Final analyses

Final analyses will be performed by groups on cleaned data and in ~~three~~ ***two*** steps.

- A first analysis will be performed when all data up to and including month 2 (immogenicity and safety) will be available.
- ~~A second analysis will be performed when all data up to and including month 7 (immogenicity and safety) will be available.~~
- A ~~third~~ ***second*** analysis will be performed and a clinical report will be written at the completion of the study. **(Amended September 24, 2007)**

11. ADMINISTRATIVE MATTERS

To comply with Good Clinical Practice important administrative obligations relating to investigator responsibilities, monitoring, archiving data, audits, confidentiality and publications must be fulfilled. See Appendix B for details.

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Appendix A World Medical Association Declaration of Helsinki

Recommendations guiding physicians in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964

and amended by the
29th World Medical Assembly
Tokyo, Japan, October 1975
35th World Medical Assembly
Venice, Italy, October 1983
41st World Medical Assembly
Hong Kong, September 1989
and the
48th General Assembly
Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the etiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.
3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.
    Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

4. The refusal of the patient to participate in a study must never interfere with the physician--patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The Physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The investigator or the investigating team should discontinue the research if in his1her or their judgement it may, if continued, be harmful to the individual.

In research on man, the interest of science and society should never take precedence over considerations related to the well being of the subject.

Appendix B Administrative Matters

I. Responsibilities of the Investigator

- To ensure that he/she has sufficient time to conduct and complete the study and has adequate staff and appropriate facilities and equipment which are available for the duration of the study and to ensure that other studies do not divert essential subjects or facilities away from the study at hand.
- To submit an up-to-date curriculum vitae or Investigator Biography and other credentials (e.g., medical license number in the United States) to GSK and-where required-to relevant authorities. It is recommended that this documentation indicates any previous clinical research experience and history of training in GCP.
- 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 on site or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally authorized representative.
- To perform no other biological assays at the investigator site except those described in the protocol or its amendment(s).
- To prepare and maintain adequate subject source data or raw data designed to record observations, and other data pertinent to the study.
- To conduct the study in compliance with the protocol any amendment and "Good Clinical Practice" (GCP) and all applicable regulatory requirements.
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- To permit drug regulatory agencies and GSK audits.

II. Protocol Amendments and Administrative changes

- No changes to the study protocol will be allowed unless discussed in detail with the GSK Biologicals' Clinical Development Manager/Medical Monitor and filed as an amendment/administrative change to this protocol.
- Any amendment/administrative change to the protocol will be adhered to by the participating center(s) and will apply to all subjects. Written IRB/IEC approval of protocol amendments is required prior to implementation, except where permitted by all applicable regulatory requirements; administrative changes and amendments not submitted for approval are submitted to IRBs/IECs for information only.
- Submission of protocol amendments to regulatory agencies will occur in accordance with local regulatory requirements. For some countries, submission to the local regulatory authority may not be required. When submission to the local regulatory authority is required, the timing of the submission relative to IEC/IRB submission or approval and whether or not the authority will provide their approval of or

favourable opinion on the amendment before it can be implemented will depend on local regulatory requirements.

III. Sponsor's Termination of Study

GSK Biologicals reserves the right to temporarily suspend or prematurely discontinue this study either at a single site or at all sites at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. Reasons for suspension or early termination will be documented in the study file at GSK Biologicals.

If GSK Biologicals determines that suspension or early termination is needed, GSK Biologicals will discuss this with the Investigator (including the reasons for taking such action). When feasible, GSK Biologicals will provide advance notification to the investigator of the impending action prior to it taking effect.

GSK Biologicals will promptly inform, via written communication, all investigators and/or institutions conducting the study, if the study is suspended or terminated for safety reasons, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IEC/IRB promptly and provide the reason for the suspension or termination.

If the study is prematurely discontinued, all study data must be returned to GSK. In addition, arrangements will be made for all unused investigational product(s) in accordance with the applicable GSK procedures for the study. Financial compensation to investigators and/or institutions will be in accordance with the agreement established between the investigator and/or institutions and GSK.

IV. Case Report Form Instructions / Remote Data Entry Instructions

Remote Data Entry (RDE) will be used as the method for data collection, which means that subject information will be entered into a computer at the investigational site. The site will be capable of modifying the data to assure accuracy with source documentation. All new/updated information will be reviewed and verified by a GSK Biologicals' representative. This information will finally be stored in a central database maintained by GSK Biologicals. At the conclusion of the study, GSK Biologicals will archive the study data in accordance with internal procedures. In addition, the investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site.

Specific instructions for use of RDE will be included in the training material provided to the investigational site.

V. Monitoring by GSK Biologicals

To ensure compliance with the protocol, monitoring visits by a professional representative of the sponsor will be scheduled to take place early in the study, during the study at appropriate intervals and after the last subject has completed the study. It is anticipated that monitoring visits will occur at a frequency defined and communicated to the investigator before study start.

These visits are for the purpose of confirming that GSK Biologicals' sponsored studies are being conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with Good Clinical practice (GCP) and the applicable regulatory requirement(s) (verifying continuing compliance with the protocol, amendment(s), reviewing the investigational product accountability records, verifying that the site staff and facilities continue to be adequate to conduct the study. Direct access to all study-related site and source data/ documents is mandatory for the purpose of monitoring review. The monitor will perform a eCRF review and a Source Document verification (verifying eCRF/ RDE entries by comparing them with the source data/documents that will be made available by the investigator for this purpose: any data item for which the eCRF will serve as the source must be identified, agreed and documented. Data to be recorded directly into the eCRF pages/RDE screens will be specified in writing preferably in the source documentation agreement form that is contained in both the monitor's and investigator's study file. For RDE, the monitor will mark completed and approved screens at each visit. The investigator must ensure provision of reasonable time, space and adequate qualified personnel for monitoring visits. Source data verification (SDV) must be conducted using a GSK standard SDV sampling strategy (as defined at the study start in the study specific monitoring guidelines) in which monitors will perform partial SDV for all subjects and full SDV for selected subjects.

VI. Archiving of Data

Following closure of the study, the investigator must maintain all site study records 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. 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 for studies with an eCRF, for example); however, caution needs to be exercised before such action is taken. The investigator must assure 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 there is an acceptable back-up of these reproductions and that an acceptable quality control process exists 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. However, the investigator/ institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by ICH GCP E6 Section 4.9, any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/ institution must notify GSK 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.

VII. Audits

For the purpose of compliance with Good Clinical Practice and Regulatory Agency Guidelines it may be necessary for GSK or a Drug Regulatory Agency to conduct a site audit. This may occur at any time from start to after conclusion of the study.

When an investigator signs the protocol, he agrees to permit drug regulatory agencies and GSK audits, providing direct access to source data/ documents. Furthermore, if an investigator refuses an inspection, his data will not be accepted in support of a New Drug Registration and/or Application, Biologics Licensing Application.

Having the highest quality data and studies are essential aspects of vaccine development. GSK has a Regulatory Compliance staff who audit investigational sites. Regulatory Compliance assesses the quality of data with regard to accuracy, adequacy and consistency. In addition, Regulatory Compliance assures that GSK Biologicals' sponsored studies are in accordance with GCP and that relevant regulations/guidelines are being followed.

To accomplish these functions, Regulatory Compliance selects investigational sites to audit. These audits usually take 1 to 2 days. GSK's audits entail review of source documents supporting the adequacy and accuracy of eCRFs, review of documentation required to be maintained, and checks on vaccine accountability. GSK's audit therefore helps prepare an investigator for a possible regulatory agency inspection as well as assuring GSK Biologicals of the validity of the database across investigational sites.

The Inspector will be especially interested in the following items:

- Log of visits from the sponsor's representatives
- Study personnel
- Study file
- Safety reporting
- IRB/IEC and regulatory authority approvals
- Facilities
- monitoring
- Vaccine accountability
- Approved study protocol and amendments and Investigator's Brochure
- Informed consent of the subjects (written consent [or witnessed oral if applicable])
- Medical records and other source documents supportive of eCRF data
- Reports to the IRB/IEC and the sponsor
- Record retention.

GSK Biologicals will gladly help investigators prepare for an inspection.

VIII. Ownership, Confidentiality and Publication

Ownership:
All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) are the sole property of GSK.

All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of GSK, and are hereby assigned to GSK.

If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed between GSK and the study site, that contract's ownership provisions shall apply rather than this statement.

Confidentiality:
Documented evidence that a potential investigator is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the investigator or site staff; (2) information which it is necessary to disclose in confidence to an IEC or IRB solely for the evaluation of the study; (3) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

Publication:
For multicenter studies, the first publication or disclosure of study results shall be a complete, joint multicenter publication or disclosure coordinated by GSK. Thereafter, any secondary publications will reference the original publication(s).

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a "Publication"), the investigator shall provide GSK with a copy of the proposed Publication and allow GSK a period to review the proposed Publication (at least 21 (twenty-one) days [or at least 15 (fifteen) working days for abstracts/posters/presentations]. Proposed Publications shall not include either GSK confidential information other than the study results or personal data on any subject, such as name or initials.

At GSK's request, the submission or other disclosure of a proposed Publication will be delayed a sufficient time to allow GSK to seek patent or similar protection of any

inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract's publication provisions shall apply rather than this statement.

Appendix C Overview of the Recruitment Plan

The investigator may use one of the following strategies for recruiting volunteers:

- Advertising / posters inside Ghent University Hospital buildings
- Group meetings
- University and Hospital personnel and students
- E-mails to the existing volunteers
- Recruitment via the center's website http://www.cevac.be
- If needed: advertisement (via an interview with an investigator) in the regional/Flemish written and spoken media.

At the study center, subjects will be screened for admission criteria and the first 180 subjects who meet all inclusion/exclusion criteria will be enrolled and allocated a randomization number according to the order in which they are enrolled.

The enrollment period, i.e., the period between the study start and the last enrolled subject, is maximum 20 weeks. This will be followed by the study monitor.

The recruitment plan must be discussed and defined with the Investigator by the Site Monitor.

Appendix D Handling of Biological Samples Collected by the Investigator

Instructions for Handling of Serum Samples

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) will be collected and stored using exclusively those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis. 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, then appropriate materials from the investigator's site are to be used.

1. Collection

The whole blood (by capillary or venous route) should be collected observing appropriate aseptic conditions. It is recommended that Vacutainer® tubes WITH integrated serum separator (e.g. Becton-Dickinson Vacutainer® SST or Corvac® Sherwood Medical) be used to minimize the risk of haemolysis and to avoid blood cell contamination of the serum when transferring to standard serum tubes.

2. Serum separation

These guidelines aim to ensure high quality serum by minimizing the risk of haemolysis, blood cell contamination of the serum or serum adverse cell toxicity at testing.

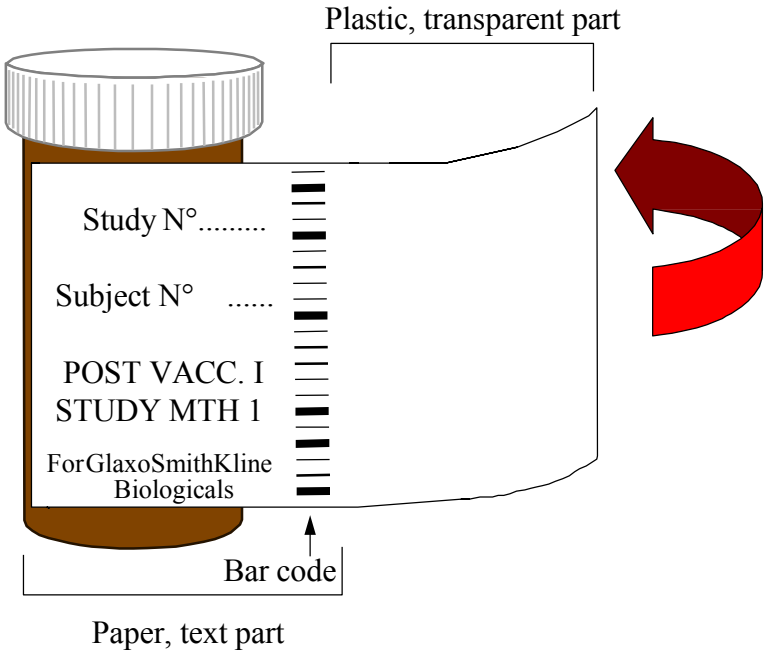
- For separation of serum using Vacutainer® tubes, the instructions provided by the manufacturer should be followed. Often the manufacturer's instruction states that the relative centrifugal acceleration known also as "G" must be "between 1000 and 1300 G" with tubes spinning for ten minutes. Error in calculation of centrifuge speed can occur when laboratory personnel confuse "G" acceleration with "RPM" (revolutions per minute). The speed of centrifugation must be calculated using the "G" rate provided in the manufacturer's instructions and the radius of the centrifuge head. After measuring the radius of the centrifuge machine, a speed/acceleration nomograph must be employed to determine the centrifuge speed in "RPM".
- Following separation, the serum should be aseptically transferred to the appropriate standard tubes using a sterile disposable pipette. The serum should be transferred as gently as possible to avoid blood cell contamination.
- The tube should not be overfilled (max. 314 of the total volume) to allow room for expansion upon freezing.
- The tube should be identified by the appropriate label provided by GSK Biologicals (see point 3).

3. Labelling

- The standard labels provided by GSK Biologicals should be used to label each serum sample.
- If necessary, any hand-written additions to the labels should be made using indelible ink.

- The label should be attached to the tube as follows (see diagram):
  - first attach the paper part of the label to the tube
  - then wrap the label around the tube so that the transparent, plastic part of the label overlaps with the label text and bar code and shields them.

This will ensure optimal label attachment.



- Labels should not be attached to caps.

4. Sorting and storage

- Tubes should be placed in the GSK Biologicals' cardboard boxes in numerical order from left to right, starting from the lower left hand corner, beginning with the pre-vaccination samples series, then with the post-vaccination sample series.
- The tubes of serum should be stored in a vertical position at approximately -20°C (alternatively at approximately -70°180°C is also acceptable) until shipment to GSK Biologicals. The storage temperature should be checked regularly and documented. Wherever possible, a backup facility for storage of serum samples should be available.
- A standard Serum Listing Form, specifying the samples being shipped for individual subjects at each timepoint, should be prepared for each shipment. A copy of this list should be retained at the study site, while the original should be sealed in a plastic envelope and shipped with the serum samples.

- Once shipment details are known, a standard Specimen Transfer Form must be completed and faxed to GSK Biologicals to the number provided below. A copy of the Specimen Transfer Form must be in the parcel.[1]

GLAXOSMITHKLINE BIOLOGICALS

Attention Biospecimen Reception
Clinical Immunology
PPD
PPD - Belgium
Telephone: PPD
PPD
E-mail: PPD

Instructions for Handling Cells for Cell-Mediated Immunity Assay

When materials are provided by GSK Biologicals, it is mandatory that all clinical samples be collected and stored using exclusively those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from analysis. The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement.

1. Collection of whole blood

Collect blood by venipuncture in Terumo tubes with heparin (or equivalent) and record time of collection. The tubes should be kept at room temperature and shipped to a designated clinical site for separation of peripheral blood mononuclear cells (PBMCs). The shipment must be timed to ensure that PBMC separation will be performed within 24 hours. Use well closed styrofoam boxes of 5 cm thickness for blood samples transport (see current version of GSK Biologicals SOP RD_HCI_001 for guidance).

2. Separation and freezing of PBMCs

PBMCs will be separated on a density gradient, aliquoted and frozen at --80°C for 24 hours and further cryopreserved in liquid nitrogen until testing (see GSK Biologicals SOP: RD_HCI_007 for guidance).

3. Labelling of cryotubes for PBMC samples

- If labels are provided by GSK, it is mandatory to use them.
- If necessary, any hand-written additions to the labels should be made using indelible ink.

4. Sorting and storage of PBMC samples

Samples should be stored in liquid nitrogen until shipment to GSK Biologicals, if needed. Wherever possible, a backup facility for storage of samples should be available.

A standard Cryotube Listing Form (see current version of GSK Biologicals SOP RD_HCI_009 for guidance) specifying the samples being shipped for individual subjects at each timepoint, should be prepared for each shipment. A copy of this list should be retained at the study site, while the original should be sealed in a plastic envelope and shipped with the PBMC samples.

Appendix E Shipment of Biological Samples

Instructions for Shipment of Serum Samples

Serum samples should be sent to GSK Biologicals at regular intervals. The frequency of shipment of samples should be decided upon by the Site Monitor, Central Study Coordinator and the investigator prior to the study start.

Serum samples should always be sent by contract courier designated by the sponsor, unless otherwise requested by the sponsor.

Serum samples must be placed with dry ice (maximum -20°C) in a container complying with International Air Transport Association (IATA) requirements. The completed standard serum listing form should always accompany the shipment.

The container must be clearly identified with the labels provided by GSK Biologicals specifying the shipment address and the storage temperature (-20°C).

Details of the shipment, including:
* number of samples
* date of transfer

should be sent by fax or by e-mail, two days before shipment, to:

GLAXOSMITHKLINE BIOLOGICALS
Attention Biospecimen Reception
Clinical Immunology
R & D Department/Building 44
Rue de l'Institut, 89
B-1330 Rixensart - Belgium
Telephone: PPD
PPD
PPD
E-mail: PPD

Instructions for Shipment of PBMC Samples

Frozen PBMCs must be transported in liquid nitrogen in an appropriate dry shipper (ideally CP400 or equivalent containing up to 4 boxes of 100 cryovials). The dry shipper must be previously filled with liquid nitrogen as described on the lid of the shipper, as follows:

1. Remove the unit from its shipping enclosure.

2. Fill the refrigerator unit completely with liquid nitrogen (do not overfill).

3. Let the unit stand undisturbed while the refrigerant is being absorbed, for 24 hours.

4. Repeat refills three times.

5. Pour out the remaining liquid standing in the central cavity of the refrigerator to prevent spillage during shipment. When completely filled, the CP400 should weigh 25 kg.

When the refrigerator is charged, transfer the material from the liquid nitrogen storage tank into the dry shipper as quickly as possible (see GSK Biologicals SOP: RD_HCI_009_E for guidance).

Details of the shipment, including number of samples and date of transfer should be sent by fax, two days before shipment, to:

GLAXOSMITHKLINE BIOLOGICALS
Attention Biospecimen Reception
Clinical Immunology
R & D Department/Building 44
Rue de l'Institut, 89
B-1330 Rixensart - Belgium
Telephone: PPD
PPD
Fax: PPD
E-mail: PPD

Appendix F Laboratory Assays

Serology: antibody responses

Blood samples for antibody determination will be collected at indicated timepoints and serum will be separated and frozen at -- 20°C.

Antibody level will be determined by evaluating antibody (IgG) responses to p17, p24, RT, Nef and F4co, using standard ELISA. The ELISA utilizes antibody and antigen interactions to test for the presence of specific antibodies in the serum sample. The antigens are chosen from the evaluated vaccine based on what is likely to be an immunogenic component.

Partially purified antigen is pre-coated onto a 96-well plate. Serum samples are added to the plate followed by a secondary anti-human antibody conjugated with an enzyme. The addition of substrate provides a means of detecting the serum antibody that is specific for the antigens. Positive control, calibrators are run on each plate in order to assess the relative titre of each test sample. Negative controls are also run on each plate to ensure specificity.

Cell-mediated immune responses

Peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood cells. 10^7 PBMC/vial will be frozen for subsequent CMI analysis. PBMC will be re-suspended in 1 mL of 90% cold fetal calf serum and 10% DMSO. Cells will then be frozen slowly to -- 70°C (± 5°C) and transferred to liquid nitrogen for storage.

Flow cytometry using Intracellular Cytokine Staining (ICS) provides information on the frequency of CD4+ and CD8+ T cells responding to the antigen. It provides simultaneously information on the frequency of CD4+ 1 CD8+ T-lymphocytes expressing molecules involved in immunity such as IFN-γ, IL-2, TNF-α and/or CD40L. Other markers may be used for exploratory analysis and further definition of the quality of the induced immune response.

PBMCs are stimulated in vitro with pools of peptides covering the sequences of p17, p24, RT or Nef antigens for 2 hours; then intracellular block is added (to inhibit cytokine secretion) for an additional overnight incubation. The cells will be stained for surface markers (e.g. CD4+ or CD8+ for T cells), than fixed and permeabilized, and subsequently stained with fluoresent antibodies to detect cytokines. The cells will then be analysed by flow cytometry.

Appendix G Vaccine supplies, packaging and accountability

1. Vaccine and/or other supplies

GSK Biologicals will supply the following study vaccines, sufficient number of vials and syringes to administer to all subjects as described in the present protocol.

- F4co (p24-RT-Nef-p17) antigen in monodose vials
- AS01B adjuvant in monodose vials
- Water for injection in single-dose syringes

At least an additional 5% of their respective amounts will be supplied for replacement in case of breakage, bad storage conditions or any other reason that would make the vaccine unusable (i.e. given by mistake to another subject).

All monodose vials must be accounted for on the form provided.

Labels for sample identification:
The investigator will receive labels from GSK Biologicals to identify samples taken from each subject at each timepoint. Each label will contain the following information: study number, identification number for the subject (e.g. **Subject number**), sampling timepoint (e.g., post vacc 3), timing (e.g., study Month 7).

Other supplies provided by GSK Biologicals:
In addition to the vaccines, the study documentation and the sample labels, the investigator will receive the following supplies:

- tubes with screw caps for serum samples,
- racks and cardboard boxes for the tubes of serum.

The investigator or pharmacist must sign a statement that he/she has received the clinical supplies for the study.

It is NOT permitted to use any of the supplies provided by GSK Biologicals for purposes other than those specified in the protocol.

2. Vaccine packaging

The vaccines will be packed in labelled boxes. The box label will contain, as a minimum, the following information: study number, treatment number, lot number (or numbers, when double-blind), instructions for vaccine administration and any other relevant regulatory requirements.

3. Vaccine shipment from GSK Biologicals Rixensart to local country medical department, dispatching center or investigational site from local country medical department to investigational site

Upon reception of the shipment,its content, quality and maintenance of the cold chain must be checked.

Supplies receipt must then be returned to:

Attention of Clinical Trials Supplies Unit
GSK Biologicals Rixensart
Fax : PPD
E-mail: PPD

In case of any temperature deviation, the official written approval for the use of vaccine must be obtained from GSK.

4. Vaccine accountability

At all times the figures on supplied, used and remaining vaccines should match. At the end of the study, it must be possible to reconcile delivery records with those of used and unused stocks. An explanation must be given of any discrepancies.

After approval from GSK Biologicals and in accordance with GSK SOP WWD-1102, used and unused vaccine vials/syringes/containers should be destroyed at the study site using locally approved biosafety procedures and documentation unless otherwise described in the protocol. If no adequate biosafety procedures are available at the study site, the used and unused vaccine vials/syringes/containers are to be returned to an appropriate GSK Biologicals site for destruction, also in accordance with current GSK SOP WWD-1102.

5. Transfers of clinical vaccines or products from country medical department or dispatch center to study sites or between sites

Storage temperatures must be maintained during transport and deviations must be reported to GSK for guidance. All transfers of clinical vaccines or products must be documented using the Clinical Supply Transfer Form.

All packaging and shipment procedures for transfer of clinical vaccines or products must follow procedures approved by the sponsor.

Clinical vaccines or products should always be sent by contract courier designated by the sponsor, unless otherwise requested by the sponsor.

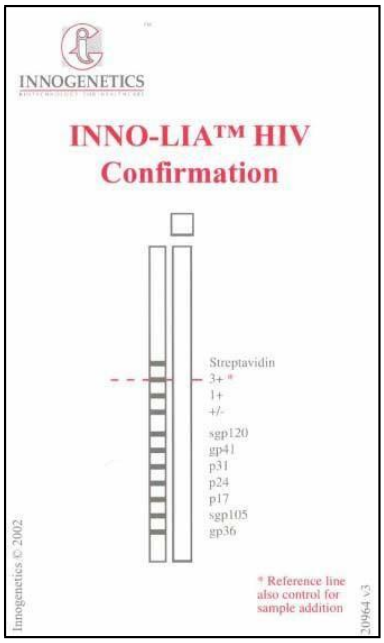
Appendix H Detection of intercurrent HIV-1 infection

At screening

The presence of HIV Ab and Ag will be tested using the Abbott AxSYM HIV Ag1Ab ELISA test. The AxSYM assay allows detection of Abs to HIV-1 group "M" gp41, HIV-2 gp36 and HIV group "O" gp41, and detection of the HIV p24 Ag.

If the result is negative, the subject can be enrolled

If the result is positive, the ELISA test will be repeated and a Western Blot performed using the Innogenetics Line Immunoblot Assay (INNO-LIA™). This assay is a blot-assay for HIV antibody detection using recombinant proteins and synthetic peptides as antigens. The following antigens are applied on the strips: sgp120, gp41, p31, p24, p17, sgp105 and gp36 and allow detection of HIV-1, HIV-2 and HIV-1 group "O" infections. .



At all the other visits

The same procedure as described for the screening will be followed.

Subjects with positive or equivalent test results will be referred to the Infectious Diseases Clinic.

Appendix I Amendments and Administrative Changes to the Protocol

GlaxoSmithKline Biologicals Clinical Research & Development **Protocol Amendment Approval**	
eTrack study number	108706
eTrack abbreviated title	PRO HIV-005
EudraCT number	2006-003796-12
Protocol Title	Dose-ranging study to evaluate the safety and immunogenicity of GSK Biologicals' HIV vaccine 732461, adjuvanted or not, when administered as a 2-dose schedule to healthy adult HIV seronegative volunteers, aged 18 to 40 years.
Amendment number:	1
Amendment date:	September 24, 2007
Co-ordinating author:	PPD (*Scientific writer, Clinical Operations*)

Rationale/background for changes:

- The third vaccination will be cancelled. A preclinical toxicology study using a prime boost combination of F4co / AS01B and a DNA vaccine (GW825780X) concluded that potentially treatment-related effects can not be excluded and further toxicology studies are required to fully understand the data. The results of the new toxicology will only become available in early 2008. As the primary study endpoint of HIV-005 has already been met and subjects will be over a year post vaccination by the time the toxicology data is available, it has been decided to cancel the third vaccine dose.
- Visit 5 FU has been cancelled as the subjects were attending for safety follow up one week post vaccination. As no third vaccination is to take place it was decided that it is preferable to subjects not to attend this visit.
- CMI and serology bloods will not be taken at visit 6 although haematology / biochemistry and virology bloods will be taken.
- The interim analysis post visit 6 will not be carried out as there is no CMI or serology analyses at visit 6.
- The study visit at Month 7 will be postponed to Month 9.
- A new Central Study Coordinator has been appointed.

Amended text has been included in *bold italics* in the following section(s):

Detailed Title: Dose-ranging study to evaluate the safety and immunogenicity of GSK Biologicals' HIV vaccine 732461, adjuvanted or not, when administered as a ~~3~~ ***2***-dose

schedule to healthy adult HIV seronegative volunteers, aged 18 to 40 years.

Title: A Phase I-II partially-blinded, randomized, dose- ranging study (10-30-90 µg) to compare the safety and immunogenicity of GSK Biologicals' candidate HIV vaccine F4co (p24-RT-Nef-p17), adjuvanted or not with AS01B, administered intramuscularly according to a vaccination schedule of 0, ~~1~~, ~~6 m~~onths to healthy adult HIV seronegative volunteers, aged 18 to 40 years.

Sponsor information:

3. Study Monitor

PPD , Central Study Coordinator
GlaxoSmithKline Biologicals
Phone: PPD
Fax: PPD

4. Study Contact for Reporting of a Serious Adverse Event

PPD ~~, Central Study Coordinator~~
~~GlaxoSmithKline Biologicals~~
~~Phone:~~ PPD
~~Fax:~~ PPD

GSK Biologicals Clinical Safety Physician
GlaxoSmithKline Biologicals
Phone: PPD
Fax: PPD ***or*** PPD
Mobile phone /or 717 day availability:
PPD
Back-up mobile phone contact:
PPD

Synopsis:

Secondary Objectives

- To evaluate the CD4+ T-cell immune response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 µg) after two ~~and three~~ vaccinations.
- To evaluate the serological response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 µg) after two ~~and three~~ vaccinations.

Study Design

- Vaccination schedule: ~~3~~ ***2*** vaccinations administered intramuscularly according to a 0, ~~1~~, ~~6~~ month schedule**.**

Primary endpoints

- Haematological and biochemical levels at months 0, 1, 2, 6, ~~7~~ **9**, 12 and at Day 44 (two weeks after the second vaccination) in all subjects.

Secondary endpoints

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing at least 2 cytokines including IL-2 equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Antibody titers to p17, p24, Nef, RT and F4co as measured by ELISA at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).

Exploratory endpoints

- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing at least 2 cytokines (IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L) equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).

Section 2.2 Secondary objectives

- To evaluate the CD4+ T-cell immune response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) after two ~~and three~~ vaccinations.
- To evaluate the serological response to the candidate vaccine F4co (p24-RT-Nef-p17) with or without AS01B at three different doses (10-30-90 μg) after two ~~and three~~ vaccinations.

Section 3. Study design overview

Figure 1 Overall study design overview

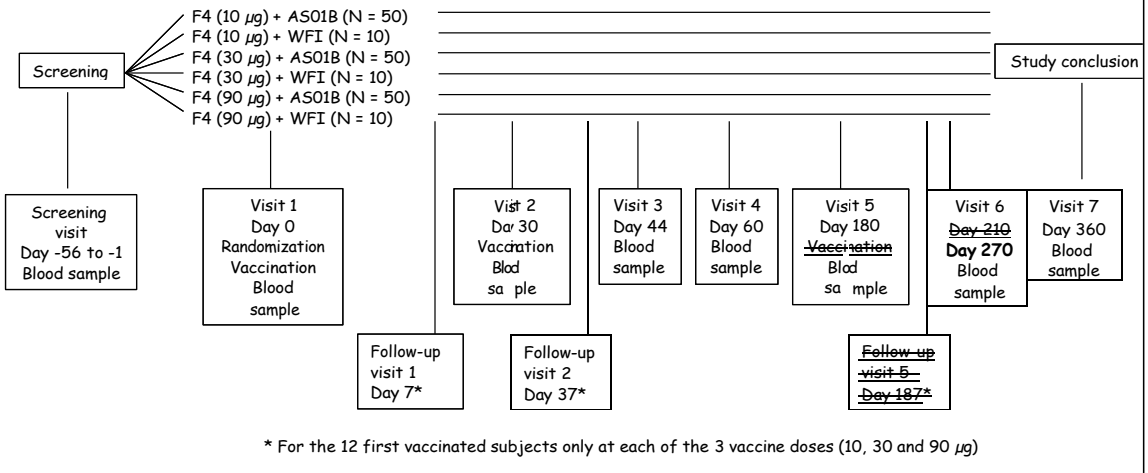
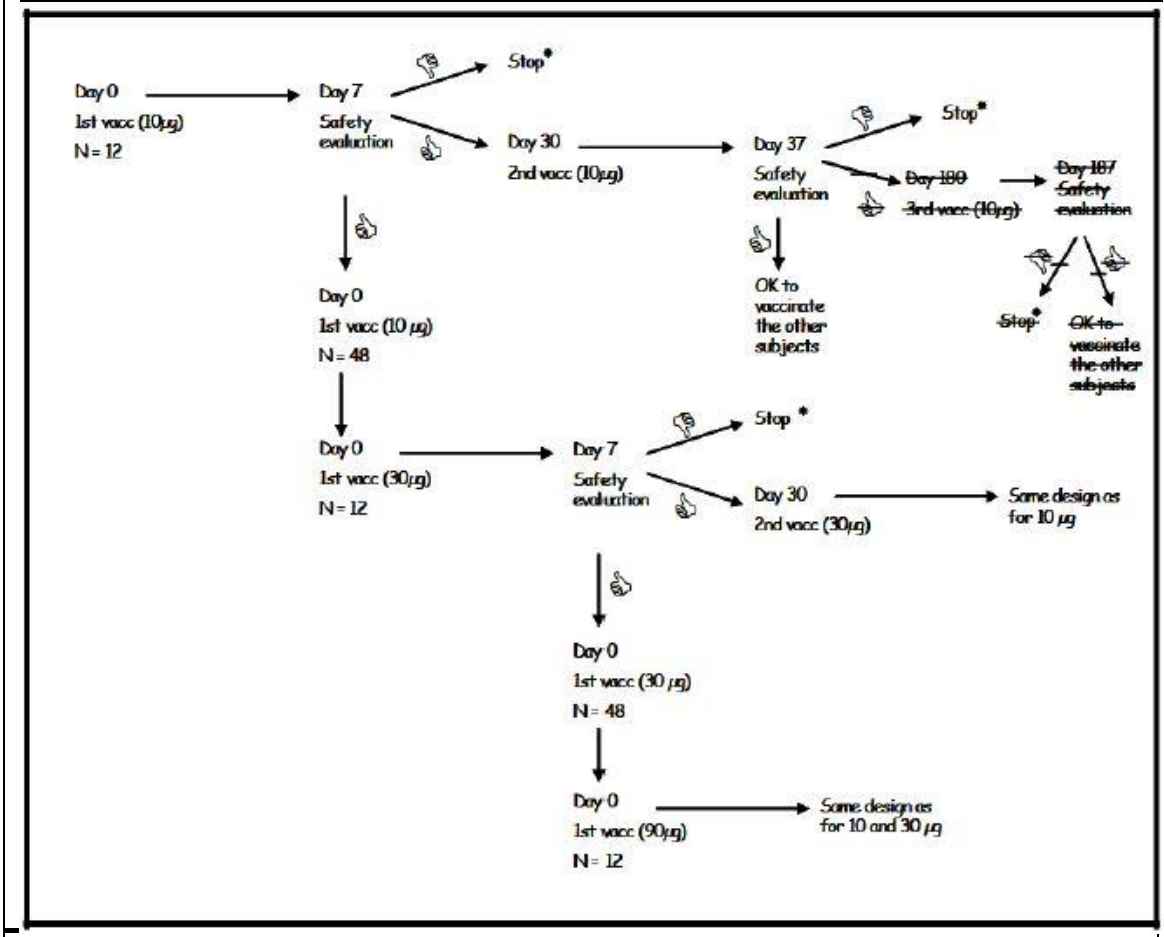


Figure 2 Staggered design overview



*The same approach will be repeated with each of the three vaccine doses (10, 30 and 90 µg) and for each of the ~~three~~ **two** vaccinations (see Section 5.6.3).*
~~Exactly the same methodological approach will be replicated for the third vaccination in each dose group~~ (see Section 5.6.3).

- ~~Three~~***Two***-vaccination course given at 0, ***and*** 1 ~~and 6~~ months. All vaccinations will be administered by the intramuscular route in the deltoid of the non-dominant arm.
- Recording of serious adverse events (SAEs) in a prospective manner throughout the study period, i.e., the period beginning with the first vaccination and ending ~~6~~ ***11*** months after the ~~third~~ ***second*** vaccination.
- ~~Three~~ ***Two*** separate analyses will be performed on cleaned data:
  ~~A second analysis will be performed when all data (immunogenicity and reactogenicity) up to and including 1 month post-vaccination III are available.~~
  A ~~third~~ ***second*** analysis ~~on the immunogenicity persistence data and SAEs data~~ will be performed at the completion of the study (Month 12).

Section 5.2.1.3 Diary Cards
Table 2 Overview of diary cards

Type of diary card	Subjects	Distribution	Return
Day 0 - Day 6	12 first subjects of each dose group 12 first subjects of each dose group ~~12 first subjects of each dose group~~	Visit 1 (Day 0) Visit 2 (Day 30) ~~Visit 5 (Day 180)~~	Follow-up Visit 1 (Day 7) Follow-up Visit 2 (Day 37) Follow-up Visit 5 (Day 187)
Day 7 - Day 29	12 first subjects of each dose group 12 first subjects of each dose group ~~12 first subjects of each dose group~~	Follow-up Visit 1 (Day 7) Follow-up Visit 2 (Day 37) ~~Follow-up Visit 5 (Day 187)~~	Visit 2 (Day 30) Visit 4 (Day 60) ~~Visit 6 (Day 210)~~
Day 0 - Day 29	48 other subjects of each dose group 48 other subjects of each dose group ~~48 other subjects of each dose group~~	Visit 1 (Day 0) Visit 2 (Day 30) ~~Visit 5 (Day 180)~~	Visit 2 (Day 30) Visit 4 (Day 60) ~~Visit 6 (Day 210)~~

Section 5.1.2.4 Laboratory parameters

Effects on the haematological and biochemical parameters will be monitored at the Screening Visit, Visit 1 (Day 0), Visit 2 (Day 30), Visit 3 (Day 44), Visit 4 (Day 60), Visit 5 (Day 180), Visit 6 (Day ~~210~~ **270**) and Visit 7 (Day 360).

Section 5.2.2 Detection of intercurrent HIV infection

- Laboratory assessment of potential intercurrent HIV-1 infection
  HIV Ag /Ab ELISA testing will be routinely performed at screening, vaccine dose 1, ~~1 month post-vaccination III~~ ***Month 9*** and ~~Month~~ 12.

Section 5.4 Outline of study procedures

Table 3 List of study procedures

Visit	Screening	1	Follow-up visit 1[1]	2	Follow-up visit 2[1]	3	4	5	~~Follow-up visit 5[1]~~	6	7
Timing (Months) Timing (Days)	Screening -56 to -1	0 0	7	1 30	37	44	2 60	6 180	~~187~~	~~7 9~~ ~~210~~ *270*	12 360
Sampling time point		PRE (M0)		P I (M1)		P II (M1.5)	P II (M2)	P II (M6)		~~P III~~ *PII* ~~(M7)~~ *(M9)*	~~P III~~ *PII* (M12)
Vaccination		I		II				~~III~~			
Examination and Procedure:											
Signed Consent Form	•										
Signed Addendum to Consent Form								•			
Randomization		•									
Physical examination and vital signs	•	•		•				•			•
Demographic data	•										
General medical history	•										
Prevention Counselling (pregnancy and HIV)	•	•		•				•			
Inclusion/exclusion criteria	•	○									

Elimination criteria Contraindications		●	●[1]	● ●	●[1]	●	●	● ~~●~~	~~●~~[1]	●	
Behavioural risk assessment [questionnaire to be filled in by the subject and high or low risk to be recorded on CRF]	●							●			●
Pre-vaccination body temperature		●		●				~~●~~			
Distribution of diary card Return of diary card Transcription of solicited symptoms recorded by the subjects (Day 0 to Day 6)		○	○[1] ○[1] ●[1]	○ ○ ●	○[1] ○[1] ●[1]		 ○ ●	~~○~~	~~○~~[1] ~~○~~[1] ~~●~~[1]	 ~~○~~ ~~●~~	
Recording of non-serious unsolicited adverse events occurring within one month post-vaccination by Investigator (Day 0 to Day 29)			●[1]	●	●[1]		●		~~●~~[1]	~~●~~	
Reporting of serious adverse events	●	●	●[1]	●	●[1]	●	●	●	~~●~~[1]	●	●
Record any concomitant medications		●	●[1]	●	●[1]	●	●	●[4]	~~●~~[1]	●[4]	●[4]
Study conclusion							●			~~●~~	●

Blood Sample (ml):	11	101		6.5		96.5	96.5	56.5		~~61~~ ***11***	69
Virology(4.5 ml)											
Hep B/C markers: anti HBc Ab, HBsAg, *Anti HCV Ab*	•										
HIV Ag/Ab[t]	•	•								•	•
Panel of HIV tests (8 ml)*											•
Biochemistry/Haematology (6.5 ml)	•	•		•		•	•	•		•	•
Serology (10 ml)											
Anti-p17,-24,-RT,-Nef, -F4co antibodies (ELISA)		•				•	•	•		~~•~~	•
Cellular-mediated immunity (40 or 80 ml)**											
p17-,24-,RT-,Nef- Specific effector T-cells (ICS)		•				•	•	•		~~•~~	•
Urine Sample:											
Pregnancy test (HCG), if applicable	•	•		•				~~•~~			

Note: The double-line border following Month 2 ~~and Month 7~~ indicates the ~~2~~ overall interim analyses that will be performed on all serology and immunogenicity data obtained up to PII (M2) ~~and PIII (M7)~~.

•: Is used to indicate a study procedure which requires documentation in the individual eCRF; O is used to indicate a study procedure that does not require documentation in the individual eCRF; •[1]: In the 12 first vaccinated subjects of each dose group only; • : Only those SAEs that are considered related to study procedures need to be recorded (see Section 8.4); • : To be recorded only if the subject is not a screening failure. . ***••[4] Recording of concomitant medications till 30 days after vaccination. Please refer to Section6.9 for description of concomitant/contraindicated medication to be recorded***

[t] Abbott AxSYM HIV Ag/Ab ELISA assay, which allows detection of Absto HIV-1 group "M" gp41, HIV-2 gp36 and HIV group "O" gp41, and detection of the HIV p24 Ag.

* At the final study visit or following a subject's withdrawal from the study.

** 80 mL of blood are needed for cellular-mediated immunity when cross-reactivity is determined pre-vaccination (Day 0), PII (M 1.5) (Day 44) and PII (M2) (Day 60). Otherwise, 40 mL of blood are enough for classical cellular-mediated immunity assessment at the other time points.

Table 4 Intervals between study visits

Interval	length of interval (Days)
1 (Screening → Visit 1)	1 to 56 (8 weeks)
2 (Visit 1 → Follow-up Visit 1)*	7- 8*
3 (Visit 1 → Visit 2)	30 - 34
4 (Visit 2 → Follow-up Visit 2)*	7 - 8*
5 (Visit 2 → Visit 3)	14 ± 2
6 (Visit 2 → Visit 4)	30 - 34
7 (Visit 1 → Visit 5)	180 ± 14
8~~(Visit 5 → Follow-up Visit 5)*~~	~~7 - 8*~~
9 (Visit ~~5~~ ***1*** → Visit 6)	~~30 - 34~~ ***270*** ± ***21***
10 (Visit 5 → Visit 7)	180 ± 14

* For the 12 first vaccinated subjects only

Section 5.5.8 Study Visit 5: ~~Third vaccination~~ (Day 180 - Month 6)
~~Prior to Vaccination~~

- ***Signed addendum to informed consent form prior to study continuation.***
- History-directed physical examination and vital signs.
- ~~Pre-vaccination assessment of body temperature~~
- Counselling on avoidance of HIV infection and pregnancy.
- Check of elimination criteria.
- ~~Check of contraindications to vaccination.~~
- Behavioural risk assessment questionnaire (hard copy) to be filled in by the subject.
- Reporting of any serious adverse events which might have occurred since vaccination. ***The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.***
- Recording of any ***contraindicated*** concomitant ~~m~~edication/treatment/vac~~ination~~ taken since the last visit (see Section 6.9).
- ~~A urine pregnancy test for females of reproductive potential will be performed.~~
- Collection of blood (56.5 mL) for biochemistry, haematology, serology and cellular-mediated immunity.
- Evaluations and blood draws must be performed prior to vaccination, but it is not necessary to have results (except for pregnancy test) prior to the administration of the vaccine.

~~**Vaccination**~~

- ~~Vaccination: intramuscular administration in the deltoid muscle of the non-dominant arm. The vaccinees will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.~~
- ~~Reporting of serious adverse events. The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.~~
- ~~Diary cards will be provided for the subjects to record temperature and any local (at the injection site) or general adverse events occurring on the day of vaccination and the 6 following days.~~
- ~~The first 12 vaccinated subjects will be instructed to return their completed Day 0 to Day 6 diary cards on Day 187 to the investigator. All the other subjects will be instructed to return their Day 0 to Day 29 diary cards on Day 210 to the investigator.~~

Section 5.5.9. ~~Follow-up Visit 5 (Day 187; in the first 12 vaccinated subjects only~~)

- ~~Check elimination criteria~~
- ~~The subjects will return their completed (Day 0 to Day 6) diary cards to the investigator, who will transcribe them into the eCRF.~~
- ~~Reporting of any unsolicited adverse events which might have occurred since vaccination.~~
- ~~Reporting of any serious adverse events which might have occurred since vaccination.~~
- ~~Recording of any concomitant medication/treatment/vaccination taken since the last visit (see Section 6.9).~~
- ~~Subjects will be distributed Day 7 to Day 29 diary cards and will be instructed to~~

~~return them completed on Day 210 to the investigator.~~

Section 5.5.10 Study Visit 6 (Day ~~210~~ *270* - Month ~~7~~ *9*)

- ~~The subjects will return their completed diary cards (Day 7 to Day 29 diary cards for the 12 first vaccinated subjects and Day 0 to Day 29 diary cards for all the other subjects) to the investigator, who will transcribe them into the eCRF.~~
- ~~The investigator will record any non-serious unsolicited adverse events which might have occurred within one month (Day 0 to Day 29) post-vaccination.~~
- Reporting of serious adverse events which might have occurred since the last visit.
- Recording of any ***contraindicated*** concomitant ~~m~~edication/treatment/vac~~cination~~ taken since the last visit (see Section 6.9).
- Collection of blood (~~61~~ ***11*** mL) for virology, biochemistry, ***and*** haematology, ~~- serology and cellular-mediated immunity~~.
- ~~Study conclusion (for interim analysis).~~

Section 5.6.1 Staggered design

~~Exactly the same methodological approach will be replicated for the third vaccination in each dose group.~~

Section 5.6.2 Review of safety data

Vaccinations are scheduled on Days 0, ~~-~~***and*** 30 ~~and 180~~ and additional safety evaluations 7 days after each vaccination. The following events must occur within the specified timeframe to ensure proceeding to the next vaccination and to the next dose as per schedule.

Section 5.6.3 Holding rules

These holding or stopping rules will be applied to each of the three vaccine doses (10, 30 and 90 μg) and to each of the ~~three~~ ***two*** vaccinations.

Section 5.7.3 Immunological read-outs

Table 6 Immunological read-outs (timings, markers, numbers of subjects and laboratory)

Blood sampling timepoint			Marker	No. subjects	laboratory
Timing	Timepoint	Visit			
Pre	Day 0	1	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA)	180	CEVAC, Belgium
			p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180	*CEVAC, Belgium
Post II	Day 44	3	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA)	180	CEVAC, Belgium
			p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180	*CEVAC, Belgium
Post II	Day 60	4	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA)	180	CEVAC, Belgium
			p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180	*CEVAC, Belgium

Post II	Day 180	5	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium
~~Post III~~	~~Day 210~~	~~6~~	~~Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA)~~ ~~p17-,p24-,RT-, Nef-Specific effector T cells (ICS)~~	~~180~~ ~~180~~	~~CEVAC, Belgium~~ ~~*CEVAC, Belgium~~
~~Post III~~ ***Post II***	Day 360	7	Anti-p17, -p24, -RT, -Nef, -F4co antibodies (ELISA) p17-,p24-,RT-, Nef-Specific effector T cells (ICS)	180 180	CEVAC, Belgium *CEVAC, Belgium

* Or GSK Biologicals

Section 8.4 Time period, frequency, and method of dectecting adverse events and serious adverse events.

The standard time period for collecting and recording SAEs will begin at the first receipt of vaccine and will end ~~6~~ ***11*** months following injection of the last dose of vaccine for each subject. See Section 8.7 for instructions for reporting and recording SAEs.

Section 8.7.2 Completion and transmission of serious adverse events reports to GSK

~~Study Contact for Reporting SAEs~~

PPD ~~, Central Study Coordinator, Clinical R & D~~
~~GlaxoSmithKline Biologicals~~
~~Tel:~~ PPD
~~Fax~~: PPD
~~E-mail address:~~ PPD

~~Back-up~~ Study Contact for Reporting SAEs

GSK Biologicals Clinical Safety Physician
Tel: PPD
Fax: PPD or PPD
Mobile phones for 717 day availability:
PPD
Back-up mobile phone contact:
PPD

24/24 hour and 7/7 day availability

Section 10.1 Primary endpoints

- Haematological and biochemical levels at months 0, 1, 2, 6, ~~7~~ **9**, 12 and at Day 44 (two weeks after the second vaccination) in all subjects.

Section 10.2 Secondary endpoints

- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Frequency of p17, p24, Nef and RT-specific CD4+ T cells expressing at least 2 cytokines including IL-2 equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Antibody titers to p17, p24, Nef, RT and F4co as measured by ELISA at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).

Section 10.3 Exploratory endpoints

- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L, as determined by ICS at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).
- Frequency of p17, p24, Nef and RT-specific CD8+ T cells expressing at least 2 cytokines (IL-2 and/or TNF-α and/or IFN-γ and/or CD40-L) equal or above the cut-off at Months 0, 2, ~~6~~, ~~7~~, 12 and at Day 44 (two weeks after the second vaccination).

Section 10.7.2 Analysis of immunogenicity

For cell-mediated immune response, the following parameters will be tabulated by vaccine groups at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination):
For CD4+ T-cells expressing IL-2 and another cytokine response, the following parameters will be tabulated by vaccine groups at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination)
For CD8+ T-cells expressing at least 2 cytokines, percentage of responders to each antigen (p17, p24, Nef, RT) will be tabulated by vaccine groups at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination).
For humoral immune response, the following parameters will be tabulated by vaccine groups for each antigen (p17, p24, Nef, RT and F4co) at months 0, 2, ~~6~~, ~~7~~, 12 and at day 44 (two weeks after the second vaccination)

Section 10.8 Planned interim analyses

~~Nine~~ ***Six*** interim analyses are planned for safety evaluation (see Section 5.6).
These analyses will be performed at the end of the 7-day follow-up period (Days 7, -***and*** 37 ~~and 187~~) after each vaccine injection in a subset of 12 subjects and for each vaccine dose (10, 30 and 90 μg).
Final analyses will be performed by groups on cleaned data and in ~~three~~ ***two*** steps.

- ~~A second analysis will be performed when all data up to and including month 7 (immogenicity and safety) will be available.~~

A ~~third~~ ***second*** analysis will be performed and a clinical report will be written at the completion of the study

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment Approval

eTrack study number	108706
eTrack abbreviated title	PRO HIV-005
EudraCT number	2006-003796-12
Protocol Title	Dose-ranging study to evaluate the safety and immunogenicity of GSK Biologicals' HIV vaccine 732461, adjuvanted or not, when administered as a 2-dose schedule to healthy adult HIV seronegative volunteers, aged 18 to 40 years.
Amendment number:	2
Amendment date:	October 08, 2007
Co-ordinating author:	PPD (*Scientific writer, Clinical Operations*)

Rationale/background for changes:

- The Addendum to the Consent Form has to been signed at Visit 6 on Day 270.

Amended text has been included in *bold italics* in the following section(s):

In Table 3 List of study procedures, row 7:

Visit	Screening	1	Follow-up visit 1[1]	2	Follow-up visit 2[1]	3	4	5	~~Follow-up visit 5[1]~~	6	7
Timing (Months) Timing (Days)	Screening -56 to -1	0 0	7	1 30	37	44	2 60	6 180	~~187~~	~~7~~ *9* ~~210~~ *270*	12 360
Sampling time point		PRE (M0)		P I (M1)		P II (M1.5)	P II (M2)	P II (M6)		~~P III~~ *P II* ~~(M7)~~ *(M9)*	~~P III~~ *P II* (M12)
Vaccination		I		II				~~III~~			
Examination and Procedure:											
Signed Consent Form	•										
***Signed Addendum to Consent Form* (Amended October 08, 2007)**								~~•~~		•	
Randomization		•									
Physical examination and vital signs	•	•		•				•			•
Demographic data	•										
General medical history	•										
Prevention Counselling (pregnancy and HIV)	•	•		•				•			

Inclusion/exclusion criteria	●	○									
Elimination criteria Contraindications		 ●	●[1]	● ●	●[1]	●	●	● ~~●~~	~~●~~[1]	●	
Behavioural risk assessment [questionnaire to be filled in by the subject and high or low risk to be recorded on CRF]	●							●			●
Pre-vaccination body temperature		●		●				~~●~~			
Distribution of diary card Return of diary card Transcription of solicited symptoms recorded by the subjects (Day 0 to Day 6)		○	○[1] ○[1] ●[1]	○ ○ ●	○[1] ○[1] ●[1]		 ○ ●	~~○~~	~~○~~[1] ~~○~~[1] ~~●~~[1]	 ~~○~~ ~~●~~	
Recording of non-serious unsolicited adverse events occurring within one month post-vaccination by Investigator (Day 0 to Day 29)			●[1]	●	●[1]		●		~~●~~[1]	~~●~~	
Reporting of serious adverse events	●	●	●[1]	●	●[1]	●	●	●	~~●~~[1]	●	●
Record any concomitant medications		●	●[1]	●	●[1]	●	●	●[4]	~~●~~[1]	●[4]	●[4]
Study conclusion							●			~~●~~	●
Blood Sample (ml):	11	101		6.5		96.5	96.5	56.5		~~61~~ ***11***	69
Virology(4.5 ml)											
Hep B/C markers: anti HBc Ab, HBsAg, *Anti HCV Ab*	●										
HIV Ag/Ab[t] **Panel of HIV tests (8 ml)***	●	●								●	● ●
Biochemistry/Haematology (6.5 ml)	●	●		●		●	●	●		●	●
Serology (10 ml)											
Anti-p17,-24,-RT,-Nef, -F4co antibodies (ELISA)		●				●	●	●		~~●~~	●
Cellular-mediated immunity (40 or 80 ml)**											
p17-,24-,RT-,Nef- Specific effector T-cells (ICS)		●				●	●	●		~~●~~	●
Urine Sample:											
Pregnancy test (HCG), if applicable	●	●		●				~~●~~			

Note: The double-line border following Month 2 ~~and Month 7~~ indicates the ~~2~~ overall interim analyses that will be performed on all serology and immunogenicity data obtained up to PII (M2) ~~and PIII (M7)~~.

•: Is used to indicate a study procedure which requires documentation in the individual eCRF; O is used to indicate a study procedure that does not require documentation in the individual eCRF; •[1]: In the 12 first vaccinated subjects of each dose group only; • : Only those SAEs that are considered related to study procedures need to be recorded (see Section 8.4); • : To be recorded only if the subject is not a screening failure. ***•[4] Recording of concomitant medications till 30 days after vaccination. Please refer to Section 6.9 for description of concomitant/contraindicated medication to be recorded.***

[t] Abbott AxSYM HIV Ag/Ab ELISA assay, which allows detection of Absto HIV-1 group "M" gp41, HIV-2 gp36 and HIV group "O" gp41, and detection of the HIV p24 Ag.

* At the final study visit or following a subject's withdrawal from the study.

** 80 mL of blood are needed for cellular-mediated immunity when cross-reactivity is determined pre-vaccination (Day 0), PII (M 1.5) (Day 44) and PII (M2) (Day 60). Otherwise, 40 mL of blood are enough for classical cellular-mediated immunity assessment at the other time points.

In section 5.5.8:

- ~~**Signed addendum to informed consent form prior to study continuation.**~~

In section 5.5.10:

- ***Signed addendum to informed consent form prior to study continuation***.

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment Approval

eTrack study number	108706
eTrack abbreviated title	PRO HIV-005
EudraCT number	2006-003796-12
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Amendment number:	Amendment 2
Amendment date:	October 08, 2007

Approved by:
Lead Clinical Development Manager

Lisa Mc Nally, MD

dd-mm-yyyy

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GlaxoSmithKline Biologicals

Clinical Research & Development

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Amendment number:	Amendment 2
Amendment date:	October 08, 2007

Agreed by:

Investigator: Prof. Dr. Geert Leroux-Roels

Investigator signature:

Date:

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