Protocol Title:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults.
Protocol Number:	IAVI A003/ CHOP HVDDT 001
Phase:	Phase 1
EudraCT Number:	2013-002268-14
Sponsor:	International AIDS Vaccine Initiative (IAVI) 125 Broad Street, 9th Floor New York, New York 10004 USA
Sponsor Status:	Not for-Profit Organization
Sponsor's Legal Representative:	Jill Gilmour, Ph.D. International AIDS Vaccine Initiative (IAVI) Chelsea and Westminster Hospital Second Floor, Lift Bank D 369 Fulham Road London SW10 9NH UK
Investigational Medicinal Product Developer:	The Children's Hospital of Philadelphia 3615 Civic Center Boulevard Philadelphia, PA 19104 USA
Product Development Collaborator:	National Institutes of Allergy and Infectious Diseases U.S. Department of Health and Human Services National Institutes of Health USA
Date of Protocol:	Version 7.0 06 Apr 2017

SYNOPSIS

TITLE	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults.
PROTOCOL NUMBER	IAVI A003/ CHOP HVDDT 001
EUDRACT NUMBER	2013-002268-14
PHASE	Phase 1
SPONSOR	International AIDS Vaccine Initiative (IAVI) 125 Broad Street, 9th Floor New York, New York 10004, USA
SPONSOR STATUS	Not for Profit Organization
INVESTIGATIONAL MEDICINAL PRODUCTS	rAAV1-PG9DP virus vector: a recombinant adeno-associated virus type-1 (rAAV1) virus vector encoding the whole PG9 antibody suspended in a isotonic buffered solution Placebo: the excipient solution used to suspend the active product
SPONSOR'S LEGAL REPRESENTATIVE	Jill Gilmour, Ph.D. International AIDS Vaccine Initiative (IAVI) Chelsea and Westminster Hospital Second Floor, Lift Bank D 369 Fulham Road London SW10 9NH UK
INVESTIGATIONAL MEDICINAL PRODUCT DEVELOPER	The Children's Hospital of Philadelphia 3615 Civic Center Boulevard Philadelphia, PA 19104 USA
PRODUCT DEVELOPMENT COLLABORATOR	National Institutes of Allergy and Infectious Diseases U.S. Department of Health and Human Services National Institutes of Health USA

OBJECTIVES	***Primary:*** 1. To evaluate the safety of rAAV1-PG9DP when administered intramuscularly at $4x10^{12}$ vg, $4x10^{13}$ vg, $8x10^{13}$ vg and $1.2x10^{14}$ vg in healthy male adults ***Secondary:*** 1. To evaluate the level and duration of HIV neutralization activity in serum 2. To evaluate the level and duration of PG9 antibody in serum 3. To evaluate the development of anti-PG9 antibodies 4. To evaluate the development of anti-AAV1 antibodies 5. To evaluate the distribution and persistence of AAV1 vector in the blood 6. To evaluate the development of AAV1-specific T cell responses
ENDPOINTS	***Primary****:* *Safety and Tolerability:* **1.** Proportion of volunteers with moderate or greater reactogenicity (i.e., solicited adverse events) during a 7 day follow-up period after the injection 2. Proportion of volunteers with moderate or greater adverse events (i.e., unsolicited adverse events) including safety laboratory (biochemical, haematological) parameters, from the day of the injection up to 168 days post injection 3. Proportion of volunteers with serious adverse events (SAEs) related to the IMP throughout the study period 4. Proportion of volunteers in each group with potential Immune Mediated Diseases (pIMDs), defined as adverse events potentially caused by antigen-antibody complexes or immune responses directed to cells producing the transgene ***Secondary****:* *Pharmacokinetics and Immunogenicity:* 1. Potency and breadth of HIV neutralization activity on a standard HIV panel 2. Level of PG9 serum antibody 3. Level of anti-PG9 serum antibodies 4. Level of anti-AAV1 antibodies 5. Proportion of volunteers with detectable AAV1 in blood (by RT-PCR) in each group 6. AAV1-specific T cell responses

STUDY DESIGN TABLE

Group	Number Active/ Placebo	Dose (vg)	Route	Month 0
A	3/1	$4x10^{12}$ or Placebo	IM	X
SRB review				
B	3/1	$4x10^{13}$ or Placebo	IM	X
SRB review				
C	3/1	$8x10^{13}$ or Placebo	IM	X
SRB review to determine progression to *either* C1, *or* D and D1				
C1	9/3	$8x10^{13}$ or Placebo	IM	X
Total Number of Volunteers: 24 (18/6)				
OR				
D	3/1	$1.2x10^{14}$ or Placebo	IM	X
SRB review				
D1	4/1	$1.2x10^{14}$ or Placebo	IM	X
Total Number of Volunteers: 21 (16/5)				

IM=Intramuscular

METHODS

See Schedule of Procedures, Appendix A

STUDY POPULATION

Healthy male adults, 18 to 45 years of age, who are willing to undergo HIV testing, and who, in the opinion of the principal investigator or designee, understand the study and who provide written informed consent.

Principal exclusion criteria include confirmed HIV-1 or HIV-2 infection; significant acute or chronic disease; certain HIV high-risk criteria; clinically significant laboratory abnormalities; recent vaccination or receipt of a blood product; previous receipt of an HIV vaccine, AAV vector, monoclonal antibody product or polyclonal immunoglobulin; previous severe local or systemic reactions to vaccination or infusions; history of severe allergic reactions; serum antibodies to AAV1, AAV2, and PG9 at baseline.

NUMBER OF VOLUNTEERS

Up to 24 volunteers (18 active/6 placebo recipients) will be included in the study.

DOSAGE ESCALATION	The dose of rAAV1-PG9DP escalates as indicated in the study design table. In Groups A-D, each of the first three participants will receive the active Investigational Medicinal Product (IMP) or placebo at least 7 days apart, to allow for observation of IMP-related adverse events in the first two recipients. Because there is one placebo in each group, the first two recipients will be treated as sentinel recipients (at least one will receive the active IMP). Once all volunteers in the first dosage group (Group A) have received their injection, assignment of new allocation numbers will pause. Safety data through the Day 42 post-injection visit for all volunteers in the dosage group will be reviewed by the SRB prior to allowing volunteers into the second dosage group (Group B). Specific safety criteria to allow dose escalation are detailed in Section 17.2.2. If SRB review indicates that criteria for dose escalation are met in Group A, Group B enrolment will proceed. Once safety data through Day 42 post-injection visit are available for all volunteers in Group B, all available safety data (Groups A and B) will be reviewed by the SRB prior to allowing volunteers into Group C. If SRB review indicates that criteria for dose escalation are met in Groups A and B, Group C enrolment will proceed. Once safety data through Day 56 post-injection visit are available for all volunteers in Group C, all available safety data (Groups A, B and C) will be reviewed by the SRB. In addition, PG9 antibody expression will be evaluated. - If safety criteria to allow dose escalation are met in groups A, B and C and PG9 antibody expression is sufficient in Group C (~150ug/ml), the study will proceed to enrol an expanded number of volunteers at the Group C dose (Group C1) and enrolment into Group D will not occur. - Otherwise, if PG9 antibody expression is not sufficient, but safety criteria for dose escalation are met, the study will proceed to enrol the highest dose level (Group D). Safety and tolerability will be evaluated by the SRB over at least 56 days for volunteers in Group D. Once safety data through Day 56 post-injection visit are available for all volunteers in Group D, all available safety data (Groups A, B, C and D) will be reviewed by the SRB. If criteria for dose escalation are met in Groups A, B, C and D, the study will proceed to enrol an expanded number of volunteers at the Group D dose (Group D1).

FORMULATIONS, VOLUMES AND ROUTES OF INJECTIONS

Active/ Placebo	Group	Dose Con-centration	Total Volume in Vial (mL)	Directions & Total Volume to Inject (mL) Intramuscularly	Total Number of Vials
rAAV1-PG9DP	A	$4x10^{12}$ vg	0.6 mL	1mL (after 1:10 dilution of IMP with excipient)	1 vial IMP +1 vial excipient
	B	$4x10^{13}$ vg		1mL (after drawing 0.5mL from each of 2 vials of IMP)	2 vials IMP
	C &C1	$8x10^{13}$ vg		2mL (1mL in each deltoid after drawing 0.5mL from each of 4 vials of IMP)	4 vials IMP
	D	$1.2x10^{14}$ vg		3mL (1.5mL in each deltoid after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
	D1	$1.2x10^{14}$ vg		3mL (3 injections of 0.5mL each in each quadriceps, after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
Placebo	A & B	N/A	1.1 mL	1mL	1 vial excipient
	C &C1			2ml (1mL in each deltoid after drawing 1.0mL from each of 2 vials of excipient)	2 vials excipient
	D			3mL (1.5mL in each deltoid after drawing 1.0mL from each of 3 vials of excipient)	3 vials excipient
	D1			3mL (3 injections of 0.5mL each in each quadriceps, after drawing 0.5mL 6 times from a total of 3 vials of excipient)	3 vials excipient

DURATION OF STUDY PARTICIPATION

Volunteers will be screened up to 42 days before injection and will be followed for 12 months after the single administration. It is anticipated that it will take approximately 13 months to enrol the study.

Upon completion of this study, volunteers will be asked to participate in a follow up study to assess safety and PG9 expression for at least 4 additional years.

RANDOMIZATION AND BLINDING	Volunteers will be randomly assigned IMP or placebo within each of the dose groups described in the study design table above depending on which group is enrolling. Study staff and volunteers will be blinded only with respect to the allocation of placebo or IMP. Blinding will not apply to the assignment of dosage levels.
EVALUATION FOR INTERCURRENT HIV INFECTION	Volunteers will be tested for HIV according to the Schedule of Procedures. Test results will be interpreted according to a pre-determined diagnostic algorithm. Should one or more serological HIV test(s) post vaccination be positive, a nucleic-acid-based HIV test will be performed to distinguish a natural HIV infection acquired through exposure in the community from serologic reactivity due to the PG9 antibody. HIV testing at additional time points may be performed upon the request of the volunteer and Principal Investigator or designee as medical or social circumstances warrant. To maintain blinding of study staff who have direct contact with the volunteers, HIV test results will be reported to the clinical team as “HIV-uninfected” or as “HIV-infected” (ONLY once intercurrent HIV infection is confirmed). Volunteers who acquire HIV infection during the study, despite counselling, will have an Early Termination visit conducted and be immediately referred for treatment and care.
SAFETY MONITORING AND STATISTICAL CONSIDERATIONS	All clinical trial data collected, identified only by a study identification number, will be entered into a database. Safety will be continually monitored by the Investigators, the Sponsor’s Medical Monitor and a Protocol Safety Review Team (PSRT); detailed stopping criteria are pre-defined. All safety data will be reviewed by an independent Safety Review Board (SRB). *Ad-hoc* safety review may be specifically requested by the Sponsor, the Principal Investigators, Ethics Committees, Regulatory Authorities, or by the SRB. At the end of the study, a full analysis will be prepared according to a pre-specified statistical analysis plan. All clinical and routine laboratory data will be included in the safety analysis. Antibody and immunogenicity analyses will be performed according to a predefined analysis plan for all volunteers who received IMP or placebo.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Term
AAT	Alpha-1 Antitrypsin
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine-Aminotransferase
AST	Aspartate-Aminotransferase
CFC	Cytokine Flow Cytometry
CRF	Case Report Form
CTL	Cytotoxic T Lymphocyte
DCC	Data Coordinating Centre
DCF	Data Collection Form
DNA	Deoxyribonucleic Acid
DP	Dual Promoter
ELISA	Enzyme Linked Immunosorbent Assay
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSV	Herpes Simplex Virus
IAVI	International AIDS Vaccine Initiative
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
ITR	Inverted Terminal Repeats
kg	Kilogram
mg	Milligram
mL	Millilitre
pIMD	Potential Immune Mediated Disease, AE of Special Interest
PCR	Polymerase Chain Reaction
PBMC	Peripheral Blood Mononuclear Cells
PSRT	Protocol Safety Review Team
rAAV1	Recombinant Adeno-Associated Virus Type 1
REC	Research Ethics Committee
RPR	Rapid Plasma Reagin
RSV	Respiratory Syncytial Virus
SAE	Serious Adverse Event
SIV	Simian Immunodeficiency Virus
SOP	Standard Operating Procedure
SOM	Study Operations Manual
SRB	Safety Review Board
STI	Sexually Transmitted Infection
TPHA	Treponema Pallidum Hemagglutination
vg	viral genomes
wtAAV	wild type AAV

CONTACT INFORMATION

Detailed contact information provided in the Study Operation Manual (SOM)

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1.0 SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the appendices and provide the necessary assurances that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirement(s).

Principal Investigator:

Signed: _____ Date: _____

Name (please print): _____

Name of institution (please print): _____

2.0 INTRODUCTION AND BACKGROUND INFORMATION

According to the Joint United Nations Programme on HIV/AIDS and the World Health Organization, as of the end of 2011, 34 million people [31.4-35.9], were estimated to be living with HIV/AIDS, with 69% residing in sub-Saharan Africa. It is estimated that in 2011 alone, 2.5 million [2.2-2.8] were newly infected with HIV and 1.7 million people died of AIDS [1].

Despite the benefits of existing prevention methods such as behavioural interventions, condom use, sexually transmitted disease treatment, harm reduction, male circumcision and new prevention strategies such as pre-exposure prophylaxis, treatment as prevention, and microbicides, the development of a vaccine against HIV remains the best hope for controlling the HIV/AIDS pandemic [2].

Data from a community-based efficacy trial in Thailand (RV144) demonstrated a modest 31.2% protection against HIV acquisition. The vaccination regimen consisted of priming with the canarypox vector ALVAC-HIV, expressing HIV *gag, pro*, and *env* genes, and boosting with recombinant HIV Env protein. The regimen did not decrease HIV viral load in vaccine recipients who acquired HIV. Limited cellular immune responses were detected, while binding antibodies were detected in a majority of vaccine recipients, with very limited detectable neutralizing antibody responses to HIV. Studies of the immune correlates of protection are ongoing [3]. These results suggest that more potent vaccine regimens will be required to generate HIV-1 immune responses providing more significant protection.

The effort to develop an effective preventive vaccine against HIV-1 infection is challenged by the wide genetic diversity of HIV among different isolates [4]. Neutralizing antibodies against circulating isolates are induced principally by the envelope glycoprotein of HIV (Env) and could confer sterilizing immunity against HIV as suggested by non-human primate SHIV challenge studies [4,5,6]. However, attempts to design appropriate immunogens have failed. This has been a major drawback for Env-based HIV vaccines, since current immunogens afford only very narrow neutralization against HIV strains that are closely related to the vaccine antigen [7,8]. The search for an immunogen able to induce broad cross-protective neutralizing antibodies remains difficult and critical [9,10].

Passive immunization (immunoprophylaxis) schemes using neutralizing antibodies have been used to prevent other infections, for example, respiratory syncytial virus (RSV) and rabies. These therapies have involved short-term protection using infusions of monoclonal antibodies or polyclonal antiserum. Such a prophylactic approach for HIV prevention could potentially be developed for certain populations but it is unlikely to be practical or cost-effective. To be effective, an immunoprophylactic product for HIV prevention must provide potent and long-lasting protection.

Since 2009, IAVI has identified a number of new HIV-specific broadly neutralizing antibodies (bnAbs) with exceptional neutralization potency and breadth that target highly conserved epitopes preferentially expressed on trimeric HIV Env[11]. One of these bnAbs, PG9, was selected for further development since its exceptional potency *in vitro* suggests it might provide protection from HIV at relatively modest serum antibody concentrations. The immunoprophylaxis concept for HIV may be tested using gene transfer techniques for direct intramuscular injection of a viral vector containing the PG9 gene. Unlike traditional vaccination, gene transfer employs recombinant vectors to deliver transgenes of interest into cells, resulting in long-term expression of the transgene. An adeno-associated virus type-1 (rAAV1) vector encoding PG9 antibody gene is proposed as a prophylactic agent that will

result in production of HIV neutralizing antibody prior to HIV exposure by virtue of long-term expression of the transgene after injection into muscle. AAV-vectored gene transfer products are under development as therapies for a variety of diseases, and an AAV-vectored product was recently shown to be effective for treatment of factor IX deficiency/haemophilia B [12].

In this trial, we will test in humans the hypothesis that rAAV1 vector-mediated PG9 antibody gene delivery will be safe, tolerable and result in sustained and adequate quantities of PG9 monoclonal antibodies, potentially capable of preventing HIV infection by blocking HIV binding on target cells.

2.1 Study Rationale

We propose to conduct a Phase 1 randomized, placebo-controlled, double-blind, dose-escalation clinical trial in HIV-uninfected, healthy male volunteers to evaluate the safety and expression of a single intramuscular administration of rAAV1-PG9DP at $4x10^{12}$, $4x10^{13}$, $8x10^{13}$ and $1.2x10^{14}$ viral genomes (vg).

The hypotheses to be tested are:

- The proposed dosages of rAAV1-PG9DP will be safe and tolerable
- A single rAAV1-PG9DP intramuscular administration will result in expression of PG9 antibody for at least 12 months
- Serum from rAAV1-PG9DP recipients will demonstrate similar potency and breadth of HIV neutralization *in vitro* as the PG9 monoclonal antibody
- Development of anti-PG9 antibody will not impact HIV neutralization capacity

Proof-of-concept was achieved in a non-human primate (NHP) SIV efficacy study using an SIV-specific antibody-like molecule that was derived from macaque Ig and neutralized SIV, delivered by AAV1 (AAV1-immunoadhesin)[13]. After a single rAAV serotype 1-immunoadhesin injection, neutralizing activity in serum lasted at least 12 months and completely protected a proportion of animals against intravenous challenge with virulent SIV. A surrogate model was required as there is no good animal model for HIV prevention. Passive antibody infusion immediately before challenge has been shown to prevent infection of macaques with SHIV (SIV with an HIV envelope)[14,15,16,17]. This SHIV model would not be useful to test long-term protection, as expression of a human antibody in a macaque will result in rapid development of an immune response to the human antibody. Thus the SIV challenge model was used to demonstrate that the expression of SIV antibody–like molecules was capable of preventing infection with SIV. Please see the Investigator Brochure for details of the proof-of-concept study.

IAVI has identified HIV infected individuals who have HIV antibodies capable of neutralizing a broad range of HIV virus strains. By selecting and cloning B-cells from these individuals, monoclonal antibodies have been identified which have potent and broadly neutralizing activity against a wide range of HIV virus strains. One of these antibodies, termed PG9, was selected as the candidate for passive immunization based on its ability to neutralize a variety of HIV isolates. The PG9 monoclonal antibody was isolated from a HIV clade A-infected donor[11]. PG9 is a highly potent broadly neutralizing antibody with a median 90% Inhibitory Concentration (IC90) of 0.36µg/mL against a broad range of diverse circulating HIV isolates. *In vitro* studies have shown that the PG9 antibody is able to effectively neutralize a large number of different HIV virus strains. PG9 antibody is more potent than antibodies previously studied; the median

concentration required to achieve 90% inhibition of a diverse set of pseudotyped HIV viruses was 10 to 60 times lower than for b12, 2G12, 2F5 and 4E10.

Taken together, the efficacy data from the NHP SIV challenge study and the *in vitro* characterization of PG9 indicate that rAAV gene transfer using a PG9 transgene appears to be a viable strategy for generating long lasting, broadly neutralizing antibodies (bNAbs) and provide rationale to initiate clinical testing. As a result of preclinical experiments comparing the expression levels of various constructs (dual promoter vs single promoter, immunoadhesin vs full Ig molecule) a whole antibody approach expressing the transgene PG9 under the control of two promoters, PG9DP, was selected as the clinical candidate.

2.2 Experience with recombinant AAV vectors

The rAAV vectors are derived from the wild type AAV (wtAAV), a naturally occurring non-pathogenic, non-integrating, replication-defective virus that requires a helper virus, such as adenovirus for replication[18]. The wtAAV infect humans and other primates, but do not replicate independently and are not known to cause any human diseases[18]. AAV has been widely used in gene transfer research, since it is minimally immunogenic in humans, allowing more efficient vector transduction and potentially minimizing undesirable immune responses to the vector. Recent data indicate that *in vivo* wtAAV exist predominantly as large unintegrated episomal concatamers[19,20] which may reduce the risk of insertional mutagenesis. The rAAV vectors do not code for viral genes and incorporate only the inverted terminal repeats (ITR), which contains less than 300 nucleotides of the non-coding DNA from wtAAV. Thus, the vectors cannot replicate even in the presence of a helper virus. Perhaps for these reasons, AAV has not been associated with oncogenesis in large animals or humans. A single study in mice injected as neonates with an rAAV2 vector reported an increase in hepatocellular carcinoma (HCC) in mice at advanced age (older than 13 months[21].) The tumors were shown to contain integrated rAAV2 vector DNA, but at very low copy numbers[22]. Subsequent studies following large numbers of mice for longer time periods did not show an increase in HCC in mice injected with AAV vectors[23,24,25].

Multiple serotypes of wtAAV have been described[18]. wtAAV2 and AAV3 are most frequently isolated from humans. AAV2 was the first to be cloned and developed for gene delivery. As other serotypes were cloned and investigated, differences in cell tropism and efficiency of DNA delivery to the nucleus were observed. Recently, rAAV1 has shown significantly higher transgene expression than other serotypes in both murine and canine skeletal muscle[26]. The AAV vector based on serotype 1 was selected because it is non-integrating, non-pathogenic, and can transduce muscle cells at high efficiency[18,26]. For skeletal muscle, AAV serotype 8 has similar transduction efficiency as AAV1, however, there is clinical experience with rAAV1[27] and not AAV8 vectors for intramuscular delivery. Thus, AAV1 serotype was the preferred choice for the first clinical study.

2.2.1 Experience with rAAV1

Clinical experience with rAAV1 vectors, although limited, has not revealed any significant safety issues. Studies using rAAV1 vectors in gene therapy clinical trials of alpha-1 antitrypsin deficiency, lipoprotein lipase deficiency and cardiac

disease have been conducted with no indications of rAAV1 safety issues[28, 29, 30]. In the CUPID study, a rAAV1 vector encoding the cardiac calcium cycling protein, SERCA2a, was administered directly into the coronary artery of 39 subjects in a randomized, double-blind, placebo controlled, parallel-group, dose-ranging phase 2 clinical trial of therapy for congestive heart failure[31]. The 39 subjects were randomized to doses ranging from $6x10^{11}$ to $3x10^{12}$ to $1x10^{13}$ vg. No significant safety issues were reported in any dose group, with a trend for clinical improvement at the highest dose. A phase 1 clinical trial examining the use of AAV1 to deliver alpha-1 antitrypsin (AAT) to AAT deficient subjects showed that $6x10^{13}$ vg given intramuscularly is safe and well tolerated with only mild reactions at the injection site. In this study, patients received $6.9x10^{12}$, $2.2x10^{13}$ or $6x10^{13}$ vg[32]. There was a dose response in terms of AAT protein expression. Three phase 1 trials evaluated an AAV1 vector encoding a gene for lipoprotein lipase (LPL) given intramuscularly to 27 adult subjects with lipoprotein lipase deficiency at doses of $1x10^{11}$ genome copies (gc)/kg to $1x10^{12}$ gc/kg (n=8). The product was administered via multiple (over 20) intramuscular injections in both quadriceps muscles and was well tolerated with no significant safety issues over 2 years of follow-up.[29, 33] The product, alipogene tiparovec (Glybera), was approved for treatment of LPL deficiency in Europe in 2013 at a dose of $1x10^{12}$ gc/kg ($6x10^{13}$ gc in a 60 kg person).

Pre-existing immunity against AAV is common in humans, but neutralizing antibody titres vary between serotypes[34]. The prevalence of anti-AAV neutralizing titres was determined using a cell based immunofluorescent assay in a total of 281 serum samples from Europe. Approximately 28% of volunteers had neutralizing titres to AAV1 at 1:20, and approximately 17% at 1:80[35]. In unpublished data from IAVI, the prevalence of anti-AAV1 neutralizing titres was determined using a cell based immunofluorescent assay in a total of 239 serum samples from vaccine preparedness studies, including 44 from India and 195 from African countries. Results are shown in Table 1. Anti-AAV1 neutralizing titres were present in ~90% of Indian sera, but less common in African sera, ranging from 43% of Kenyan sera to 80% of South African sera. In both India and Africa, anti-AAV1 neutralizing titres were lower than anti-AAV2 neutralizing titres, with geometric means ranging from 1:7-1:23. It is not known whether pre-existing antibodies may alter the ability of rAAV1-PG9DP to transduce cells and direct the production of the whole antibody. In animal models, neutralizing antibodies to AAV have not impeded AAV transduction in muscle[36]. Similarly, in human trials of AAV1 vectors, transgene expression has been seen in volunteers with AAV1 neutralizing antibodies at baseline[37,27]. In other trials, volunteers with pre-existing AAV1 immunity have been excluded[38,39] . An IAVI trial of an AAV2-vectored HIV vaccine candidate in Africa found no difference in immune response between 23 volunteers with pre-existing anti- AAV2 neutralizing titres ≤ 1:8 and 68 with neutralizing titres >1:8[40]. Volunteers with pre-existing neutralizing antibodies to AAV1 will be excluded from this initial study of the rAAV1-PG9DP product. Because group sizes are small, excluding these volunteers may improve the chances for successful transgene expression.

Table 2.2.1-1: Anti-AAV1 Antibodies in India and Africa

	India N=44	*Kenya N=14*	*Rwanda N=42*	*South Africa N=50*	*Uganda N=89*
Geometric mean titer	**1:23**	**1:7**	**1:10**	**1:19**	**1:9**
Low titer (≤1/8), n (%)	**16 (36)**	**11 (79)**	**28 (67)**	**21 (42)**	**56 (63)**
Detectable titer (≥1/4), n (%)	**41 (93)**	**6 (43)**	**27 (64)**	**40 (80)**	**64 (72)**

2.2.2 Experience with other AAV vectors for gene therapy

Most clinical experience with AAV vectors for gene therapy is with AAV2 vectors. Studies of AAV2 vector gene transfer for cystic fibrosis, alpha 1-antitrypsin, haemophilia and limb girdle muscular dystrophy have not identified safety concerns, but have shown minimal efficacy [28, 38,39,40,41,42,43,44]. A recent trial of AAV8 gene delivery for factor IX deficiency haemophilia showed long-term expression of the factor IX transgene at therapeutic levels in all participants [12]. Participants received a single dose by peripheral vein infusions of $2x10^{11}$ vg/kg, $6x10^{11}$ vg/kg or $2x10^{12}$ vg/kg. AAV-mediated expression of factor IX was observed in all participants through 6 to 16 months of follow-up. No significant toxicity was observed. Neutralizing antibody and T-cell mediated immune responses to factor IX were not detectable. Both participants in the high dose group had asymptomatic elevation of serum aminotransferase levels to 5-fold and 2-fold the upper limit of normal. In one participant this was hypothesized to be due to immune-mediated destruction of AAV transduced hepatocytes, as elevated levels of AAV1-capsid-specific T cells were detected. The aetiology for the second participant was not clear. Both were treated with a short-course of oral steroids and the transaminase levels returned to normal.

2.3 Experience with immunoprophylaxis

There are no similar genetically delivered immunoglobulin products from which to draw comparable safety data. However, polyclonal serum-derived immunoglobulins have been widely used for prophylaxis and treatment of a variety of conditions. In addition, several monoclonal antibodies are now licensed. Most of these antibodies are directed against human proteins (i.e. anti-self) for the purpose of suppressing immune responses or inflammation, and their primary risk is increased susceptibility to infection. In one example of a monoclonal antibody directed against a pathogen, passive transfer of humanized monoclonal antibody to respiratory syncytial virus (RSV), palivizumab (Synagis), has been used to protect high-risk infants against serious RSV disease. In two double-blind, placebo-controlled trials, intramuscular palivizumab 15 mg/kg every 30 days for 5 months significantly reduced RSV-related hospitalizations by 55% in 1502 infants with prematurity and/or bronchopulmonary dysplasia/chronic lung disease and by 45% in 1287 infants with hemodynamically significant congenital heart disease. Palivizumab was generally well tolerated, with ≤1.9% of recipients discontinuing treatment for tolerability reasons. In placebo controlled trials, the most common potentially drug-related adverse events were fever, nervousness, injection-site reactions, and diarrhoea. Drug-related events occurred in 7.2–11% of palivizumab recipients in controlled trials (vs. 6.9–10% with placebo) and

0–7.9% in open label trials. Very few serious potentially drug related adverse events occurred in clinical trials; four occurred in 2 of 285 patients in one open label trial. No significant anti-palivizumab antibodies developed during palivizumab use, even after a second season of use. Where low-level titres occurred, antibodies were generally nonspecific and transient[47].

Passive transfer of humanized monoclonal antibodies against HIV has been evaluated as a therapy in HIV-infected patients with no evidence of benefit or harm. These trials did not demonstrate development of anti-monoclonal antibodies, clinical disease or exacerbation of existing HIV disease[45][46,47,48,49,50] These examples demonstrate no particular safety concern associated with the development of antibodies directed against monoclonal antibodies or of clinical immunologic disease. However, passive transfer of humanized monoclonal antibodies differ from our approach of delivering a transgene intramuscularly to induce production of a human monoclonal directed to HIV envelope.

2.4 Experience with rAAV1-PG9DP

There is no human experience with this product.

Expressed PG9, derived from infection of HeLa cells with the PG9DP expression cassette, was assayed for HIV neutralization activity by Monogram Biosciences using a pseudovirus assay. The results of the neutralization analyses against a panel of viruses show the neutralization activity of PG9 produced from the PG9DP vector has a median IC50 (0.14 µg/mL) almost identical to the recombinant PG9 protein control (0.16 µg/mL). To examine the activity of rAAV1-PG9DP *in vivo*, immunocompromised mice were injected intramuscularly. PG9 expression was maintained throughout the 8 week study. These data show that, in mice, PG9 is expressed *in vivo* from a single intramuscular injection with a rAAV1 vector. Furthermore, the PG9 expression levels achieved are conservatively estimated to be approximately 50 times the median 90% inhibitory concentration (IC90) of 0.36 µg/mL.

Toxicity and biodistribution of rAAV1-PG9DP was assessed in immunocompetent mice. High levels of anti-PG9 antibodies were detected in the serum of treated animals. Local inflammation at the muscle injection site was seen in all animals, as well as in the nearby skeletal muscle tissue of animals given the highest dose. The inflammation was dose related; it ranged from subacute to necrotizing, with myofiber destruction and replacement by adipocytes. These inflammatory responses were likely normal immune responses in immunocompetent mice to very large amounts of foreign antibody production. In addition 3 of 40 animals receiving the highest dose, $5x10^{12}$ vg, 100-fold higher than the planned highest dose in humans on a vg/kg basis, had cardiac inflammation/fibrosis characterized as minimal which was not considered clinically significant. Cardiac inflammation/fibrosis can occur incidentally in mice, however, in the absence of findings in the control animals in this study, a rAAV1-PG9-related effect cannot be ruled out. Therefore, cardiotoxicity will be monitored in this trial using sensitive cardiac troponin assays and electrocardiograms.

2.5 Rationale for the vector doses proposed

Data from animal models may be used to derive a working estimate of the concentration of PG9 that may be required to protect against HIV infection. *In vivo* protection data for PG9 are not currently available. The assumptions made are that PG9 has the same half-life and access *in vivo* to epitopes as the related PGT121 MAb. PGT121 is another broadly neutralizing antibody which is approximately 10-fold more potent than PG9 in vitro[46]. In a high dose SHIV/macaque vaginal challenge, PGT121 protected all animals (5/5) at a 1 mg/kg dose which resulted in serum concentration of 15 ug/mL and mucosal concentration of 0.2 ug/mL[47]. At the 0.2 mg/kg dose 3/5 animals were protected (2 ug/mL serum concentration and below the level of detection in mucosal fluids). Since a 15 ug/mL serum concentration of PGT121 completely protected against a high-dose challenge of a pathogenic SHIV, we can estimate that a 10-fold increase in concentration, ~150 ug/mL would be necessary for PG9 to protect. However, data from other animal models suggests that even lower concentrations may still be protective *in vivo*. In the repeat, low-dose SHIV vaginal challenge model, which may more closely approximate human heterosexual transmission, significant benefit has been seen from MAbs at serum and mucosal concentrations lower than those required to protect against the stringent high dose model[7].

The highest AAV1 dose tested previously in humans is $6x10^{13}$ vg/patient using rAAV1-AAT given intramuscularly[32]. Patients received $6.9x10^{12}$, $2.2x10^{13}$ or $6x10^{13}$ vg. There was a dose response in terms of AAT protein expression: the lowest dose gave no detectable expression, the mid-dose gave 0.5 ug/mL in serum, while the highest dose, $6x10^{13}$ vg, gave 1.5-3 ug/mL. This dose is equivalent to $1x10^{12}$ vg/kg. Extrapolating from the AAT study, this is the minimum dose for which reasonable transgene expression can be expected in human. It is unlikely that 1.5 ug/ml of PG9 would be sufficient to protect against HIV, however, lower levels of expression may be acceptable for the first-in-human study since the final product will contain multiple, more potent bNAbs.

Preclinical animal studies show that a dose of $4x10^{12}$ vg/kg of rAAV1-immunoadhesin is required in mice for transgene expression, and in non-human primates for efficacy[13]. This is equivalent to $3x10^{14}$ vg in a 70 kg human. Therefore, the target human dose should be close to $3x10^{14}$ vg. Manufacture of concentrations higher than $3x10^{13}$ vg/mL are likely to cause virus aggregation. Therefore, the highest doses in this study, $8x10^{13}$ vg and $1.2x10^{14}$ vg will be administered by two 1.0 mL injections of $4x10^{13}$ vg/mL (in different deltoids), and two 1.5 ml or six 0.5mL injections of $4x10^{13}$ vg/mL (divided evenly over both deltoids (Group D) or quadriceps muscles in the thighs (Group D1) respectively (at the same time point).

2.5.1 Rationale for quadriceps administration and muscle biopsy

Quadriceps administration

It is unknown if administering the IMP intramuscularly in a single injection or multiple injections affects the level of gene expression. It is also unknown if the size of the muscle in which the IMP is administered affects the level of gene expression. Therefore in the highest dose groups, where all subjects will receive the same total dose, those in Group D will receive IMP or placebo in 2 injections, one in each deltoid, and those in Group D1 will receive the same amount of IMP but divided over 6 injections, 3 in each quadriceps, a larger muscle than the deltoid. Taken together, this would result in a greater number

of muscle cells, in larger muscles, being exposed to AAV1-PG9DP. The total dose in groups D and D1 will remain the same, i.e., $1.2x10^{14}$ vg. The supportive preclinical toxicology study administered IMP into the leg muscles (gastrocnemius) of mice. Most AAV gene therapy studies with intramuscular administration have administered AAV vectors with multiple injections in the vastus lateralis muscles[33,41,45].

Muscle biopsy
All volunteers in group D1 will undergo a muscle biopsy 3 months and 12 months after administration of IMP at one of the six sites where the IMP was injected into the quadriceps. Muscle biopsy will be analysed to determine if the vector genome and/or PG9 is present in the tissue at the site of IMP delivery.

2.6 Rationale for excluding women and for contraception requirements in men

As a precaution, women will be excluded from the first in human study and only male volunteers willing to use male condoms with both male and female partners until 3 months after injection will be enrolled to avoid the possibility of horizontal (to sexual partners) or vertical transfer (to a fetus) of the vector and/or PG9. AAV is considered a non-integrating vector. Although the transmission via germ line to offspring would be of significant concern the evidence does not support integration into the germ line. In a study of AAV2 vector, 6 of 7 subjects who received the vector via intrahepatic artery infusion were observed to have vector in semen (but not in sperm). The vector was cleared in all subjects by 3 months[51]. In 8 subjects who received vector via the intramuscular route, semen samples from 7 participants were tested at one or more time points and vector was not detected[32]. In the biodistribution study conducted in mice with rAAV1-PG9DP, vector was detected in the blood and all the major organs at Day 4 but declined to undetectable in the ovaries, testis, prostate and uterus by Day 64. The risk of inadvertent germ line transmission of vector sequences would appear to be very low for AAV delivered to skeletal muscle.

2.7 Rationale for frequency and type of safety assessments

Safety will be monitored closely and evaluated throughout the trial. In particular, the potential for an immune response to the expressed foreign protein and resulting clinical disease will be evaluated closely. Since the full-length PG9 antibody will be expressed, it is less likely to present neoantigens than an immunoadhesin construct. However, there are no validated predictive analyses to determine if an expressed antibody will be immunogenic and empirical evaluation in humans in small studies is required. Each volunteer will be monitored closely in the clinic for 4 hours after injection for symptoms of immediate hypersensitivity (anaphylaxis). Injections for the first three volunteers in each dose group will be separated by at least 7 days to evaluate for the development of adverse events in at least one volunteer receiving active product. Volunteers will be monitored clinically, including clinical safety laboratories, at Day 3 and Day 7 after injection, then weekly through 6 weeks and then monthly to 6 months and quarterly to 12 months. Dose escalation will proceed only after Safety Review Board (SRB) review of safety data for at least 6 weeks of follow-up for each dosage group. Specific criteria for dose escalation are detailed in Section 17.2.2. This interval was chosen to evaluate reactogenicity and short-term events after peak protein expression, which is estimated from macaque studies to be as early as 4 weeks.

Routine clinical safety laboratory tests – complete blood count, electrolytes, and renal and liver function – will be assessed at every scheduled visit. If an immune response is generated to the expressed foreign protein, potential clinical disease could consist of an inflammatory response either locally in the muscle, or systemically as immune-mediated disease: for example immune hepatitis, nephritis, vasculitis or arthritis. Volunteers will be monitored carefully at each scheduled visit for evidence of immune-mediated diseases with history and physical examination as well as renal and liver function tests, C-reactive protein as a general screen for inflammation, creatine kinase as a general screen for muscle inflammation, and urinalysis to look for evidence of immune-mediated nephritis. The Medical Advisory Panel (see Section 7.4) will evaluate all potential immune-mediated diseases and advise on drug treatment if necessary. A short course of oral corticosteroid will be the initial treatment but additional or alternative medications will be considered depending on the type of disease and volunteer characteristics. See Appendix C for detailed treatment plan.

Because one preclinical study found cases of mild cardiac fibrosis in mice, cardiac troponin I will be assessed at baseline and each scheduled visit to look for evidence of cardiac inflammation and electrocardiogram will be evaluated baseline and at each scheduled visit for evidence of clinically significant cardiac disease.

Anti-PG9 levels will also be evaluated. An elevated anti-PG9 level or a drop in neutralization titre may indicate an immune response to the expressed protein. Alone these findings will not represent adverse events but may be needed to interpret causality of clinical events.

Because this delivery system is designed to produce long-term protein expression, evaluation of long-term safety is an important component of product development. After the trial-specified safety monitoring for 48 weeks after a single administration, volunteers will be asked to enrol in a long-term follow-up protocol to monitor safety for a total of at least 5 years after IMP administration.

See the rAAV1-PG9DP Investigator Brochure for a full description of the preclinical and clinical immunogenicity and safety profile of this candidate product, including potential on-target and off-target effects.

3.0 STUDY OBJECTIVES

3.1 Primary Objective

1. To evaluate the safety of rAAV1-PG9DP when administered intramuscularly at $4x10^{12}$ vg, $4x10^{13}$ vg, $8x10^{13}$ vg and $1.2x10^{14}$ vg in healthy male adults

3.2 Secondary Objectives

1. To evaluate the level and duration of HIV neutralization activity in serum
2. To evaluate the level and duration of PG9 antibody in serum
3. To evaluate the development of anti-PG9 antibodies
4. To evaluate the development of anti-AAV1 antibodies
5. To evaluate the distribution and persistence of AAV1 vector in the blood
6. To evaluate the development of AAV1-specific T cell responses

4.0 STUDY ENDPOINTS

4.1 Study Endpoints

4.1.1 Primary Endpoints

Safety and tolerability:

1. Proportion of volunteers with moderate or greater reactogenicity (i.e., solicited adverse events) during a 7 day follow-up period after each injection
2. Proportion of volunteers with moderate or greater adverse events (i.e., unsolicited adverse events), including safety laboratory (biochemical, haematological) parameters, from the day of injection up to 168 days post injection
3. Proportion of volunteers with serious adverse events (SAEs) related to the IMP throughout the study period
4. Proportion of volunteers in each group with potential Immune Mediated Diseases (pIMDs), defined as adverse events potentially caused by antigen-antibody complexes or immune responses directed to cells producing the transgene

4.1.2 Secondary Endpoints

Pharmacokinetics and Immunogenicity:

1. Potency and breadth of HIV neutralization activity on a standard HIV panel
2. Level of PG9 serum antibody
3. Level of anti-PG9 serum antibodies
4. Level of anti-AAV1 antibodies
5. Proportion of volunteers with detectable AAV1 in blood (by RT-PCR) in each group
6. AAV1-specific T cell responses

5.0 STUDY DESIGN

The study is a Phase 1 randomized, placebo-controlled, double-blind, dose-escalation clinical trial in healthy male adult volunteers to evaluate the safety and expression of a single intramuscular administration of rAAV1-PG9DP at $4x10^{12}$vg, $4x10^{13}$vg, $8x10^{13}$vg and $1.2x10^{14}$vg.

Sentinel recipients

The rAAV1-PG9DP administrations escalate by dosage as shown below in Table 1, Study Design. In Groups A-D, the first 3 volunteer injections will be separated by 7 days, to allow for observation of IMP-related adverse events in the first two participants. Because there is one placebo in each group, the first two recipients will be treated as sentinel recipients (at least one will receive the IMP). If no reactogenicity and adverse events that are considered to be related to IMP (possibly, probably or definitely related) and are graded as severe or worse (Grade 3 or 4 on the DAIDS Toxicity Table) occur within 7 days after injection, the second volunteer may be injected. The same process will be followed after the second injection. If no events meeting the criteria described above occur within 7 days after the second volunteer is injected, then the remainder of volunteers in that group will be injected. If events meeting the criteria described

above do occur for the first two volunteers, they will be reviewed by the Safety Review Board (SRB) to determine whether further injections may proceed.

Dose escalation

Once all volunteers in the first dosage group (Group A) have received their injection, enrolment into the next higher dosage group / assignment of new allocation numbers will pause. Safety data through the Day 42 post-injection visit for all volunteers in the dosage group will be reviewed by the SRB prior to allowing volunteers into the second dosage group (Group B). Specific safety criteria to allow dose escalation are detailed in Section 17.2.2. If criteria for dose escalation are met, Group B enrolment will proceed. Once safety data through Day 42 post-injection visit is available for all volunteers in Group B, all available safety data (Groups A and B) will be reviewed by the SRB prior to allowing volunteers into Group C. If criteria for dose escalation are met, Group C enrolment will proceed. Once safety data through Day 56 post-injection visit is available for all volunteers in Group C, all available safety data (Groups A, B and C) will be reviewed by the SRB. In addition, PG9 antibody expression will be evaluated.

- If safety criteria to allow dose escalation are met and PG9 antibody expression is sufficient (~150ug/ml), the study will proceed to enrol an expanded number of volunteers at the Group C dose (Group C1) and enrolment into Group D will not occur.

- Otherwise, if PG9 antibody expression is not sufficient but safety criteria for dose escalation are met, the study will proceed to enrol the highest dose level (Group D). Safety and tolerability will be evaluated by the SRB over at least 56 days for volunteers in Group D. Once safety data through Day 56 post-injection visit is available for all volunteers in Group D, all available safety data (Groups A, B, C and D) will be reviewed by the SRB. If criteria for dose escalation are met, the study will proceed to enrol an expanded number of volunteers at the Group D dose (Group D1).

In the study all groups will be randomized in a 3:1 ratio of active product to placebo with the exception of Group D1, where 5 volunteers will be randomized in a 4:1 ratio of active product to placebo.

Table 5.0-1
Study Design

Group	Number Active/ Placebo	Dose (vg)	Route	Month 0
A	3/1	$4x10^{12}$ or Placebo	IM	X
SRB review				
B	3/1	$4x10^{13}$ or Placebo	IM	X
SRB review				
C	3/1	$8x10^{13}$ or Placebo	IM	X
SRB review to determine progression to *either* C1, *or* D and D1				
C1	9/3	$8x10^{13}$ or Placebo	IM	X
Total Number of Volunteers: 24 (18/6)				
OR				
D	3/1	$1.2x10^{14}$ or Placebo	IM	X
SRB review				
D1	4/1	$1.2x10^{14}$ or Placebo	IM	X
Total Number of Volunteers: 21 (16/5)				

IM=Intramuscular

5.1 Duration of the Study

Volunteers will be screened up to 42 days before injection and will be followed for 12 months after the single administration.

It is anticipated that it will take approximately 13 months to enrol the study. The anticipated study duration for each volunteer is approximately 13 months from screening through last study visit.

Upon completion of this study, volunteers will be asked to participate in a follow up study to assess safety and PG9 expression for at least 4 additional years.

5.2 Study Population

The study population consists of healthy male adults aged 18 to 45 years who are willing to undergo HIV testing, and who, in the opinion of the investigator or designee, understand the study and provide written informed consent.

Up to 24 volunteers (18 active/6 placebo recipients) who meet all eligibility criteria will be included in the study.

5.3 Inclusion Criteria

1. Healthy male, as assessed by a medical history, physical exam, and laboratory tests

2. At least 18 years of age on the day of screening and has not reached his 46th birthday on the day of injection

3. Willing to comply with the requirements of the protocol and available for follow-up for the planned duration of the study

4. In the opinion of the Principal Investigator or designee and based on Assessment of Informed Consent Understanding results, has understood the information provided and potential impact and/or risks linked to injection and participation in the trial; written informed consent will be obtained from the volunteer before any study-related procedures are performed

5. Willing to undergo HIV testing, risk reduction counselling and receive HIV test results

6. All sexually active males must be willing to use male condoms with all sexual partners (female or male) from the day of first injection until 3 months after the injection

7. Willing to forgo donations of blood, or any other tissues during the study and, for those who test HIV-positive due to IMP-induced antibodies, until the anti-HIV antibody titres become undetectable

8. Group D1 only: Willing to undergo muscle biopsy 3 months and 12 months after receiving rAAV1-PG9DP or placebo.

5.4 Exclusion Criteria

1. Confirmed HIV-1 or HIV-2 infection

2. Any clinically relevant abnormality on history or examination including history of immunodeficiency or autoimmune disease; use of systemic corticosteroids, immunosuppressive, anticancer, or other medications considered significant by the investigator within the previous 6 months

The following exceptions are permitted and will not exclude study participation: use of corticosteroid nasal spray for rhinitis, topical corticosteroids for an acute uncomplicated dermatitis; or a short course (duration of 10 days or less, or a single injection) of corticosteroid for a non-chronic condition (based on investigator clinical judgment) at least 2 weeks prior to enrolment in this study.

3. Any clinically significant acute or chronic medical condition that is considered progressive or in the opinion of the investigator makes the volunteer unsuitable for participation in the study.

4. Any of the following risk behaviour for HIV infection *within 6 months prior to injection:*
   - Unprotected sexual intercourse with a known HIV infected person or a partner known to be at high risk for HIV infection or a casual partner (i.e., no continuing established relationship)
   - Unprotected anal intercourse with another man (either insertive or receptive) Three or more sexual partners
   - Engaged in sex work
   - Frequent excessive daily alcohol use or frequent binge drinking or chronic marijuana abuse or any other use of illicit drugs
   - History of newly-acquired syphilis, gonorrhoea, non-gonococcal urethritis, HSV-2, chlamydia, epididymitis, proctitis, lymphogranuloma venereum, chancroid, or hepatitis B

5. Bleeding disorder that was diagnosed by a physician (e.g., factor deficiency, coagulopathy or platelet disorder that requires special precautions). Note: A volunteer who states that he or she has easy bruising or bleeding, but does not have a formal diagnosis and has IM injections and blood draws without any adverse experience, is eligible.

6. Any of the following abnormal laboratory parameters listed below:
   Hematology
   - Hemoglobin <11 g/dL or <6.79 mmol/L in males
   - Absolute Neutrophil Count (ANC): ≤1300/mm^3 or ≤ 1.3 x 10^9/L
   - Absolute Lymphocyte Count (ALC): ≤ 650/mm^3 or ≤ 0.65 x 10^9/L
   - Platelets: < 125,000/mm^3 or > 550,000/mm^3 or ≤ 125 x 10^9/L ≥ 550 x 10^9/L

   Coagulation
   - aPTT: >1.25 x ULN
   - INR: ≥1.1 x ULN

   Chemistry
   - Sodium ≤135 mEq/L or ≥ 146 mEq/L **or** ≤135 mmol/L or ≥146 mmol/L
   - Potassium ≤3.4 mEq/L or ≥ 5.6 mEq/L or ≤3.4 mmol/L or ≥5.6 mmol/L
   - Creatinine ≥1.1 x ULN
   - AST ≥1.25 x ULN
   - ALT ≥1.25 x ULN
   - Total bilirubin ≥1.25 x ULN
   - Alkaline phosphatase ≥1.25 x ULN
   - Albumin ≤ 3.0 g/dL or ≤ 30 g/L

- Creatine kinase ≥3.0 x ULN
- C-reactive protein >10 mg/L
- Cardiac troponin I > 0.04 ng/mL
- C3 complement ≤ 0.9g/L
- C4 complement ≤ 0.1g/L
- Gamma glutamyl transferase (GGT) >73 U/L

Urinalysis
Clinically significant abnormal dipstick confirmed by microscopy:
- Protein = 1+ or more
- Blood = 1+ or more

Infectious disease
Positive for hepatitis B surface antigen: (HbsAg); hepatitis C (HCV antibodies); active syphilis (RPR confirmed by TPHA)

7. Anti-AAV1 and anti-AAV2 antibody level above the cut-off

8. PG9 level above the cut-off

9. Receipt of live attenuated vaccine within the previous 60 days or planned receipt within 60 days after injection with IMP; or receipt of other vaccine within the previous 14 days or planned receipt within 14 days after injection with IMP (exception is live attenuated influenza vaccine within 14 days)

10. Receipt of blood transfusion or blood-derived products within the previous 3 months

11. Participation in another clinical trial of an IMP currently, within the previous 3 months or expected participation during this study

12. Prior receipt of another AAV vector, investigational HIV vaccine candidate, monoclonal antibody or polyclonal immunoglobulin (note: receipt of placebo in a previous HIV vaccine or monoclonal antibody trial will not exclude a volunteer from participation if documentation is available and the Medical Monitor gives approval)

13. History of severe local or systemic reactogenicity to vaccines or infusions (e.g., anaphylaxis, respiratory difficulties, angioedema)

14. Psychiatric condition that compromises safety of the volunteer and precludes compliance with the protocol. Specifically excluded are persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years.

15. In the opinion of the Principal Investigator, it is not in the best interest of the volunteer to participate in the trial

16. Seizure disorder: a participant who has had a seizure in the last 3 years

17. ECG with clinically significant findings or features including but not limited to:

- Conduction disturbance (atrio-ventricular or intra-ventricular condition, incomplete or complete left or right bundle branch block, QT prolongation (QTc interval of ≥450 for men) or AV block of any degree (PR interval >200ms)
- Repolarization (ST segment or T wave) abnormality
- Significant atrial or ventricular arrhythmia
- Frequent atrial or ventricular arrhythmia
- Frequent atrial or ventricular ectopy (e.g., frequent premature atrial contractions, two premature ventricular contractions in a row)
- ST elevation consistent with ischemia
- Evidence of past or evolving myocardial infarction (heart attack)

18. History of, or known active cardiovascular disease, including but not limited to:
    - Previous myocardial infarction
    - Angina pectoris
    - Congestive heart failure
    - Valvular heart disease, including mitral valve prolapse
    - Cardiomyopathy
    - Pericarditis
    - Stroke or transient ischemic attack
    - Chest pain or shortness of breath with activity (such as walking up stairs)
    - Other heart conditions under the care of a doctor.

19. Have 3 or more of the following risk factors:
    - Hypertension diagnosed by a doctor
    - Hypercholesterolemia diagnosed by a doctor
    - Diabetes mellitus
    - Hyperglycemia diagnosed by a doctor
    - First degree relative (i.e., mother, father, brother, sister) who had a heart condition before the age of 50
    - Currently smoke cigarettes

20. Body mass index ≥30.0

5.5 Recruitment of Volunteers

Healthy adult male volunteers may be recruited through information presented in community organizations, hospitals, colleges, online, other institutions and/or advertisements to the general public or from existing cohorts. The information distributed will contain contact details of the trial site.

6.0 STUDY VISITS

6.1 Screening Period

During Screening, study staff will perform the following procedures:

- Provide and/or review the Informed Consent Document and answer any questions about the study prior to obtaining written informed consent.

- Complete Assessment of Informed Consent Understanding (AOU). Please refer to the Study Operations Manual (SOM).

If the volunteer agrees to participate, passes the AOU and provides written informed consent, study staff will:

- Conduct screening assessment
- Conduct HIV testing, HIV test counselling and HIV risk reduction counselling
- Perform a comprehensive medical history
- Collect concomitant medication information
- Perform a general physical examination (refer to Section 7.2)
- Collect specimens as indicated in Sections 9.1.10 and 9.1.11
- Perform ECG

The screening laboratory tests will be reviewed by the trial physician. Screening laboratory test(s) may be repeated once at the discretion of the Principal Investigator or designee to investigate any isolated abnormalities.

If a screening visit occurs more than 42 days prior to the date of injection, all screening procedures must be repeated except that the comprehensive medical history may be replaced by an interim medical history and the Volunteer Information Sheet of the Informed Consent Document should be reviewed.

If a volunteer has signed the Consent Form but does not meet the eligibility criteria, the records must be kept at the site.

6.2 Injection Visit

Prior to the injection, study staff will:

- Answer any questions the volunteer may have about the study
- Review the Informed Consent Document with the volunteer
- Review screening safety laboratory data
- Conduct HIV risk assessment
- Conduct HIV testing, HIV test counselling and HIV risk reduction counselling
- Review interim medical history
- Collect concomitant medication information
- Perform a symptom-directed physical examination (refer to Section 7.2)
- Assess cervical and axillary lymph nodes
- Prior to injection, assess local and systemic signs and symptoms (this includes an examination of injection site)
- Collect specimens as indicated in Section 9.1.10
- Perform ECG
- Assign an allocation number to the volunteer according to the instructions specified in the Study Operations Manual (SOM)

Administer the IMP as specified in Section 8.4, Administration of IMP and according to the instructions specified in the SOM.

Observe volunteer closely for at least 30 minutes and then for a total of 4 hours after injection for any acute reactogenicity and symptoms of anaphylaxis.

At the end of each observation period, or if any clinical signs of adverse reactions are observed, study staff will:

- Record vital signs (pulse, respiratory rate, blood pressure and temperature)
- Assess any local and systemic reactogenicity
- Assess any other adverse events

6.3 Post-Injection Visits

The volunteer will be asked to return to the clinic on Day 3 (+/- 1 day), Day 7 (+/- 3 days), Day 14 (+/- 3 days), Day 21 (+/- 3 days), Day 28 (+/- 3 days), Day 35 (+/- 3 days), and Day 42 (+/- 3 days) after injection for an assessment by clinic staff. The volunteer will be asked to maintain a Memory Aid from the day of the injection and for the next 7 days. Study staff will review the Memory Aid with the volunteer and determine the severity of the reactions through discussion with the volunteer.

The following procedures will be conducted at these visits:

- Review interim medical history
- Collect concomitant medication information
- Perform a symptom-directed physical examination if any signs or symptoms are present
- Assess cervical and axillary lymph nodes
- Assess local and systemic reactogenicity (Days 3 and 7 only)
- Assess any adverse events
- Collect specimens as indicated in the Schedule of Procedures (Appendix A)
- Perform ECG

6.4 Additional Follow-up Visits

Assessments and procedures will be performed according to the Schedule of Procedures (Appendix A).

6.5 Unscheduled Visits

Unscheduled Visits/Contacts are visits/contacts that are not described in the Schedule of Procedures (Appendix A). Unscheduled visits may occur any time during the study:

- For administrative reasons, e.g., the volunteer may have questions for study staff or may need to re-schedule a follow-up visit
- To obtain laboratory test results from a previous visit
- For other reasons as requested by the volunteer or site investigator

All unscheduled visits will be documented in the volunteers' study records on applicable source documents and entered into the Case Report Form (CRF).

6.6 Final Study Visit or Early Termination Visit

Assessments and procedures will be performed according to the Schedule of Procedures (Appendix A).

7.0 STUDY PROCEDURES

7.1 Informed Consent Process

A Master Informed Consent Document consisting of a Volunteer Information Sheet and a Consent Form is provided by the Sponsor to the trial site. This document is made site-specific and translated (if necessary), submitted and approved by the Research Ethics Committee (REC).

Volunteer Information Sheet
A qualified member of the study staff will conduct the informed consent process by reviewing the Volunteer Information Sheet with the potential volunteer and document it in the clinic notes.

Consent Form
The volunteer's consent to participate must be obtained by him signing and dating the Consent Form. The person obtaining consent will also sign.

The signed and dated Informed Consent Document must remain at the study site. A copy of the signed and dated Informed Consent Document will be offered to the volunteer to take home. Those volunteers who do not wish to take a copy will be required to document that they declined to do so.

7.2 Medical History and Physical Examination

Medical History
At screening, a comprehensive medical history will be collected including previous vaccinations and reaction to vaccinations, history of STI and contraceptive practices. The Registered General Practitioner of each volunteer will be contacted to obtain medical history relevant to his participation in this trial. At subsequent visits, an interim medical history will be obtained.

Physical Examination
General Physical Examination
A general physical examination includes examination of skin, respiratory, cardiovascular, abdominal, neurological and musculoskeletal systems at the time points indicated in the Schedule of Procedures (Appendix A).

Symptom-Directed Physical Examination
A symptom-directed physical examination includes the examination of skin, respiratory, cardiovascular, abdominal, neurologic and genital systems that are indicated by history or observation. This examination is done at the time points indicated in the Schedule of Procedures (Appendix A).

Measuring Height and Weight
Height and weight are measured at the time points indicated in the Schedule of Procedures (Appendix A).

Assessment of Lymph Nodes
An assessment of cervical and axillary lymph nodes is performed at the time points indicated in the Schedule of Procedures (Appendix A).

Vital Signs
Vital signs including pulse, respiratory rate, blood pressure and temperature are measured and recorded at the time points indicated in the Schedule of Procedures (Appendix A)

7.3 HIV Testing and HIV Test Counselling

Study staff will perform pre-HIV test counselling prior to collecting blood for an HIV test and post-HIV test counselling when HIV test results are available. This counselling is referred to as HIV test counselling and done according to the national guidelines. For more information on HIV testing and HIV test counselling, see Section 11.0.

7.4 HIV Risk Reduction Counselling

Study staff will provide HIV risk reduction counselling, based on reported individual risk and provide free condoms and water soluble lubricant, as appropriate, at every visit.

7.4 Contraception Counselling

Volunteers are required to use condoms with any female or male partners until 3 months after receiving the study injection. This is to avoid the possibility of horizontal (to sexual partners) or vertical transfer (to a fetus) of the vector and/or PG9.

7.5 Specimens

Approximately 40mL of blood will be collected at the Screening Visit and up to approximately 90mL of blood will be collected at later visits, usually from the antecubital fossa.

Muscle tissue biopsy will be collected from all volunteers in group D1 at 3 months and 12 months after administration of IMP at one of the six sites where the IMP was injected into the quadriceps.

All specimens will be handled according to the procedures specified in instructions provided by the Sponsor.

In the event of an abnormal laboratory value, volunteers may be asked to have a repeat/ additional sample collected at the discretion of the Principal Investigator or designee.

7.6 Reimbursement

Volunteers will be reimbursed for their time, effort and for costs to cover their travel expenses to the study site and any inconvenience caused due to study participation. Reimbursement amounts will be documented in the Volunteer Information Sheet and approved by the Research Ethics Committee (REC).

7.7 Randomization and Blinding

Volunteers will be identified by a unique study identification number.

Volunteers will be randomized according to the randomization schedule prepared by the statisticians at the Data Coordinating Centre (DCC) prior to the start of the study. Volunteers will be automatically assigned a specific IMP allocation number as they are enrolled into the data entry system.

This study is a double-blind clinical trial. Volunteers and study staff (investigator and clinical personnel monitoring the safety and laboratory assay results), with the exception of the pharmacist (or other suitably trained and delegated investigator) will be blinded with respect to the allocation of IMP (active or placebo).

Blinding will not apply to group assignment or dose levels.

A volunteer will be considered enrolled once he/she has been assigned an allocation number. If an enrolled volunteer is discontinued from the study before receiving IMP, another volunteer may be enrolled with an assignment to match the assignment that was previously assigned to that discontinued volunteer.

Volunteers will be informed about their assignments (active/placebo) at/after study completion, once the database is locked.

7.8 Unblinding Procedure for Individual Volunteers

Unblinding of an individual volunteer may be indicated in the event of a medical emergency if the clinical management of the volunteer would be altered by knowledge of the treatment assignment. In the event of an emergency, the investigator is able to unblind treatment for a study volunteer without first contacting IAVI.
The unblinded information should be restricted to a small group of individuals involved in clinical management/medical treatment of the volunteer (e.g. treating physician) and the blind must be maintained for those responsible for the study assessments.

The reasons for unblinding should be documented and the IAVI Chief Medical Officer, the Medical Monitor and the DCC should be notified as soon as possible. The procedures and contact numbers for un-blinding are outlined in the SOM.

7.9 Referral to Long Term Follow-Up Study

To assess the long-term safety of the IMP, study volunteers will be offered participation in a follow-up study for approximately 5 years following their study injection. This study will include a health assessment questionnaire and HIV testing. Additional blood samples may be collected to assess the persistence of the study endpoints in rAAV1-PG9DP recipients and persistence of product-induced HIV seroreactivity. Separate informed consent will be obtained for this long-term follow-up study.

8.0 INVESTIGATIONAL MEDICINAL PRODUCT

8.1 Description

Both the rAAV1-PG9DP vector and excipient (placebo/diluent) were manufactured by the Clinical Vector Core (CVC) division of the Centre for Cellular and Molecular Therapeutics (CCMT) at the Children's Hospital of Philadelphia (CHOP) in the US.

8.1.1 rAAV1-PG9DP Vector

The rAAV1-PG9DP is a replication deficient recombinant AAV-1 vector containing genes of human IgG1-PG9 variable heavy (VH) and variable light (VL) domains. The vector is filled into single-use vials for intramuscular (IM) injection. The vector is formulated at a concentration of 4×10^{13} vg/mL in an isotonic buffered solution composed of 180mM sodium chloride, 10mM sodium phosphate and 0.001% Poloxamer 188, at pH 7.3.

The vector is supplied as a frozen sterile solution in a 1.5mL polypropylene vial with polypropylene closure and silicone gasket. Each vial contains 0.6 mL of vector. The volume of administration is dependent upon the group assignment, but will be either a single administration of 1 mL (Groups A and B) or two separate 1 mL administrations for a total of 2 mL administered to the volunteer (Groups C and C1), or two separate 1.5 ml (Group D), or six separate 0.5 ml (Group D1), administrations for a total of 3 mL (Groups D and D1). The dose of the vector is expressed as total vector genomes (vg), as measured by quantitative PCR. The vector for Group A will be diluted 1:10 prior to administration, whereas doses for B, C, C1, D and D1 groups do not require dilution. The vector for Groups B, C, C1, D and D1 will require multiple vials to be thawed and prepared.

8.1.2 Excipient (Placebo and Diluent)

The excipient, which will be used as both a placebo and a diluent, is an isotonic buffered saline solution consisting of 180mM sodium chloride, 10mM sodium phosphate and 0.001% Poloxamer 188, at pH 7.3. The excipient is supplied as a frozen sterile solution in a 1.5mL polypropylene vial with polypropylene closure and silicone gasket. The excipient is filled into single-use vials for intramuscular injection. Each vial contains 1.1 mL of excipient. The volume of administration is dependent upon the group number, but will be either 1 mL, 2 mL or 3 mL per administration. A summary of the Investigational Medicinal Product is shown in Table 8.1.2-1.

Table 8.1.2-1 Formulation of Investigational Medicinal Product

Active/ Placebo	Group	Dose Con-centration	Total Volume in Vial (mL)	Directions & Total Volume to Inject (mL) Intramuscularly	Total Number of Vials
rAAV1-PG9DP	A	$4x10^{12}$ vg	0.6 mL	1mL (after 1:10 dilution of IMP with excipient)	1 vial IMP +1 vial excipient
	B	$4x10^{13}$ vg		1mL (after drawing 0.5mL from each of 2 vials of IMP)	2 vials IMP
	C &C1	$8x10^{13}$ vg		2mL (1mL in each deltoid after drawing 0.5mL from each of 4 vials of IMP)	4 vials IMP
	D	$1.2x10^{14}$ vg		3mL (1.5mL in each deltoid after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
	D1	$1.2x10^{14}$ vg		3mL (1.5mL in each quadriceps, 6 injections of 0.5mL each, after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
Placebo	A & B	N/A	1.1 mL	1mL	1 vial excipient
	C &C1			2ml (1mL in each deltoid after drawing 1.0mL from each of 2 vials of excipient)	2 vials excipient
	D			3mL (1.5mL in each deltoid after drawing 1.0mL from each of 3 vials of excipient)	3 vials excipient
	D1			3mL (1.5mL in each quadriceps, 6 injections of 0.5mL each, after drawing 6 times 0.5mL from 3 vials of excipient)	3 vials excipient

8.2 Shipment and Storage

Authorization to ship the IMP to the site will be provided in writing by the Sponsor, upon confirmation that all regulatory approvals are in place and all required critical documents for shipment authorization are completed. The IMP will be shipped maintaining the required storage conditions and stored in a secure location in the clinical site's freezer room.

The rAAV1-PG9DP vector and Excipient are stored as frozen vials inside cryoboxes at less than -60°C, in a secure freezer in the freezer room. All of the vials will have an Annexe 13 compliant affixed printed label that will include the following: product name, clinical study number, product lot number, dose volume and route of administration, vial number, storage temperature, cautionary statement, and name of the sponsor. Box labels will contain this same information, plus the sponsor contact information and the Principal Investigator's contact information.

8.3 Preparation of Investigational Medicinal Product

Vector or excipient vials will be removed from the freezer and allowed to equilibrate to room temperature. Depending on the group assignment, the vector is diluted to the appropriate concentration Group A, or multiple vials are combined prior to administration (Groups B, C, C1, D, D1). Detailed instructions for preparing the IMP will be provided to the pharmacist (or other suitably trained and delegated investigator) in the SOM.

The pharmacist (or other suitably trained and delegated investigator) will not be blinded, but the study physician/designee administering the vector will be blinded. A verifier will be required to double check and document each step of IMP preparation. The time for preparation of excipient and active vector should be similar to avoid any accidental unblinding. The injection should be given within 4 hours after preparation. Instructions for storing used vials until the end of the study and subsequent disposal will be provided in the SOM. Syringes or other components in direct contact with IMP will be disposed of in a biohazard container and disposed of according to the clinical research centre-specific GM Risk Assessment.

8.4 Administration of Investigational Medicinal Product

IMP will be administered according to the Schedule of Procedures (Appendix A).

The preferred site for administration is the deltoid muscle of the non-dominant upper arm (for example, injection in the left arm if the volunteer uses mainly the right arm) for groups A, B, C and D, unless contraindicated for another reason. For group D1, the site for administration is the vastus lateralis muscles in the quadriceps muscles of the upper legs. In groups with 2mL volume, one injection of 1.0mL each will be given in each deltoid. In groups with 3mL volume, one injection of 1.5mL each will be given in each deltoid for Group D, or three injections of 0.5mL each, will be given in each vastus lateralis for Group D1. In group D1, the administration will be conducted under ultrasound guidance.

Further information on the administration of the IMP is supplied in the SOM.

8.5 Accountability and Disposal of Investigational Medicinal Product

All used vials will be returned to the pharmacy at the end of each vaccination visit. The date, vial allocation number and location of storage of the returned vials will be recorded.

During the study, the IMP accountability forms including dispensing and returning of vials will be kept and monitored.

At the end of the study, the used and unused vials will be handled according to instructions of Sponsor.

Further information on Accountability and Disposal is supplied in the SOM.

8.6 Containment and Transmission of rAAV1-PG9DP Vector

The rAAV1-PG9DP is a replication deficient recombinant AAV-1 vector containing genes of human IgG1-PG9 variable heavy (VH) and variable light (VL) domains and hence does not have the ability to replicate or be transmitted. As part of our testing strategy, the vector product will be tested for replication competent AAV particles. AAV vectors can be handled in Containment Level 1 conditions. AAV is not listed as a human pathogen and although the genetic modification introduces the IgG1-PG9 gene sequences into the AAV vector this does not alter the classification of Containment Class 1.

9.0 ASSESSMENTS

9.1 Safety Assessments

Data on local and systemic reactogenicity will be collected by structured interview and medical examination (i.e., solicited AEs). Data on other (unsolicited) adverse events will be collected with open-ended questions. All data will be recorded on the appropriate source documents and entered into the study database. Volunteers will be given a Memory Aid, which is a tool to assist with collecting reactogenicity data.

Local and systemic reactogenicity events will be assessed by study staff prior to injection and at least 30 minutes and for a total of at least 4 hours post-injection as specified in the Schedule of Procedures (Appendix A). Volunteers will be closely monitored on-site at the clinical facility for symptoms of anaphylaxis.

9.1.1 Local reactogenicity

The presence of local reactogenicity will be assessed at the time points specified in the Schedule of Procedures (Appendix A).

Pain, tenderness, erythema/skin discoloration, swelling/hardening or thickening will be assessed and graded using Appendix B, Adverse Event Severity Assessment Table, as a guideline.

9.1.2 Systemic reactogenicity

The presence of systemic reactogenicity will be assessed at the time points specified in the Schedule of Procedures (Appendix A).

Fever, chills, headache, nausea, vomiting, malaise and myalgia will be assessed and graded using the Appendix B, Adverse Event Severity Assessment Table as a guideline.

9.1.3 Assessment of Lymph Nodes

At injection visit, an assessment of cervical and axillary lymph nodes is performed by study staff prior to injection, and at the first post-injection visit (Day 3). If lymphadenopathy is increased relative to baseline, it will be followed until resolution. For the other study visits, lymph nodes will be assessed at the time points specified in the Schedule of Procedures (Appendix A).

9.1.4 Vital Signs

At injection visit, vital signs (pulse, respiratory rate, blood pressure and temperature) will be measured by study staff prior to injection and at least 30 minutes and 4 hours post-injection. For the other study visits vital signs will be assessed at the time points specified in the Schedule of Procedures (Appendix A).

9.1.5 Other Adverse Events

Other adverse events (AEs) will be collected through Day 168. Serious Adverse Events (SAEs) will be collected throughout the entire study period. Open ended

questions will be asked at time points according to the Schedule of Procedures (Appendix A). All adverse events will be graded using Appendix B, Adverse Event Severity Assessment Table, as a guideline and will be assessed for causality to the IMP. For more information regarding adverse events refer to Section 10.0, Adverse Events.

9.1.6 Potential Immune Mediated Diseases (pIMDs)

Potential Immune Mediated Diseases (pIMDs), as defined in Section 10.5, will be collected throughout the study period, using the SAE reporting process.

9.1.7 12-lead ECG

A 12-lead ECG will be performed as described in the Schedule of Procedures to assess for evidence of cardiac adverse events. ECGs will be interpreted formally by a trained cardiologist.

9.1.8 Muscle biopsy

In the event of an extensive local inflammatory reaction, or evidence of systemic inflammatory or immune disease that requires further evaluation to understand the potential relationship to IMP, biopsy of the muscle in which the IMP was administered may be performed by an experienced operator. Volunteers will be informed about this potential procedure in the study informed consent document. If a muscle biopsy is indicated as a safety assessment, volunteers would provide informed consent for the procedure itself at that time.

9.1.9 Concomitant Medications

Concomitant receipt of other IMPs is prohibited during the study.

All medication ongoing at study entry will be documented. (See DCF instructions on DCC website)

During the study, information regarding concomitant medications and reasons for their use will be solicited from the study volunteers until six months after the injection. Ongoing concomitant medications will be recorded until end of study.

9.1.10 Routine laboratory parameters (Safety Labs)

Table 9.1.10-1 shows the laboratory parameters that will be measured routinely. The samples for these tests will be collected at Screening and all subsequent visits, as indicated in the Schedule of Procedures (Appendix A). Note that the Day 3 (+/- 1 day) visit does not include total bilirubin, alkaline phosphatase, albumin, creatine kinase and all tests listed in Table 9.1.10-1 as “Other”. Also note that the Day 49 and Day 70 safety labs include Cardiac Troponin I only.

Table 9.1.10-1: Laboratory Parameters

Laboratory Parameter	Test
Haematology	Haemoglobin, haematocrit, leukocytes, platelets, absolute neutrophil count, absolute lymphocyte count
Clinical Chemistry	Sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase, albumin, creatine kinase,
Urinalysis	Dipstick test for protein, blood glucose, ketones, esterase (leukocytes) and nitrite. If clinically significant abnormalities (e.g. blood, protein, leukocytes) are found on dipstick test, then further test(s) will be performed (e.g. microscopy, culture).
Other	C-reactive protein, cardiac troponin I, aPTT, INR, complement C3 and C4

9.1.11 Specific screening tests:

In addition to the tests listed in the table above, Screening Labs will include:

- Anti-AAV1 and anti-AAV2 antibodies
- PG9 antibodies
- Gamma glutamyl transferase (GGT)

In addition, volunteers will be screened to exclude the following diseases:

- Hepatitis B: positive for hepatitis B surface antigen (HBsAg)
- Hepatitis C: positive for hepatitis C antibodies (HCV antibodies)
- Active syphilis: confirmed diagnosis (i.e., positive RPR confirmed by TPHA)

9.2 Immunogenicity Assessments

9.2.1 PG9 antibody responses

- PG9 antibody activity in the volunteer serum and or plasma will be measured according to time points as indicated in the Schedule of Procedures (Appendix A) through the following: Binding antibodies to a gp120 protein known to bind to PG9 (rates and antibody titres)
- Neutralizing antibodies to a panel of viruses (rates, neutralization titres and breadth)

Anti-PG9 antibody activity will be measured in the volunteer serum and or plasma according to time points as indicated in the Schedule of Procedures

9.2.2 Laboratory assessments specific to AAV

Anti-AAV1 antibody Titres: titres to AAV1 capsid will be measured at screening/baseline and after rAAV-PG9DP administration, at time points specified in the Schedule of Procedures.

AAV1 Systemic Distribution of rAAV1-PG9DP: A DNA PCR assay using primers specific to the PG9DP insert will be performed on whole blood to determine if the vector genome is present in the systemic circulation after IM delivery at time points specified in the Schedule of Procedures.

9.2.3 Cellular Responses

IFN-g ELISPOT and/or Intracellular cytokine staining (ICS) will be performed to monitor the number of AAV-1 specific circulating T cells that can be stimulated to produce cytokines and other effector molecules at time points indicated in the Schedule of Procedures using AAV1 capsid peptide pools or other antigen preparations of AAV1.

Selected T cell responses may be further characterized using additional markers on the responding cells, such as markers for activation or homing to mucosal tissues (ICS).

At each time point indicated in the Schedule of Procedures using the procedure provided by the IAVI Human Immunology Laboratory, at least four vials of frozen peripheral blood mononuclear cells (PBMC) each containing approximately 10^7 PBMC will be collected for immunogenicity analysis (ELISPOT,ICS) and/or quality control assays at the IAVI Human Immunology Laboratory. These samples will be shipped promptly, according to an agreed schedule.

9.2.4 PBMC, Serum and Plasma Storage

Samples of cryopreserved PBMC, plasma and serum will be stored as indicated in the SOP and may be used for the purposes of standardization, quality control and for future assays related to HIV vaccine research and development. These samples will be archived and the testing laboratories will be blinded to the volunteer's identity.

For the immunogenicity assessments, the laboratory personnel will be trained as necessary by the sponsor and provided with a written procedure manual. The sponsor will also provide specific instructions on reagents.

The samples described in Sections 9.2.2 and 9.2.3 will be sent from the CRC to the IAVI Human Immunology Laboratory and will be identified only by the volunteer's study ID number. The immunological testing will be performed at the IAVI Human Immunology Laboratory in accordance with IAVI standard operating procedures and standard reagents.

9.2.5 Muscle Biopsies

All volunteers in group D1 will undergo a muscle biopsy 3 months and 12 months after administration of IMP at one of the sites where the IMP was injected.

Microscopy and immunohistochemical staining will be performed on biopsy tissue to assess in situ parameters at the site of IMP administration, such as PG9 expression, inflammation, fibrosis.

AAV1 in situ presence of rAAV1-PG9DP: A DNA PCR assay using primers specific to the PG9DP insert will be performed on biopsy tissue to determine if the vector genome is present in the tissue at the site of IMP delivery.

9.3 Other Assessments

9.3.1 HLA Typing

Samples for HLA typing will be collected as specified at the time point specified in the Schedule of Procedures and may be analyzed as warranted.

9.3.2 HIV test

Samples will be tested at the time points indicated in the Schedule of Procedures (Appendix A). Further information is specified in Section 11.1 HIV Testing.

9.3.3 Screening Assessment

The screening assessment is a conducted by going through the Inclusion and Exclusion Criteria.

9.3.4 HIV Risk Assessment

Study staff will assess volunteers at time points indicated in Schedule of Procedures (Appendix A) for their past and current risk of acquiring HIV.

9.3.5 Social Impact Assessment

Each volunteer will have a social impact assessment administered at the time points specified in the Schedule of Procedures (Appendix A). This assessment is intended to assess the impact of trial participation on the volunteer's personal and professional life.

10.0 ADVERSE EVENTS

10.1 Definition

An adverse event (AE) is any untoward medical occurrence in a volunteer administered IMP and which does not necessarily have a causal relationship with the IMP. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease, temporally associated with the use of the IMP whether or not related to the IMP.

Assessment of severity of all AEs, including expectedness and seriousness of AEs, is ultimately the responsibility of the Principal Investigator.

10.2 Assessment of Severity of Adverse Events

The following general criteria should be used in assessing adverse events as mild, moderate, severe or very severe at the time of evaluation:

Grade 1 (Mild): Symptoms causing no or minimal interference with usual social and functional activities

Grade 2 (Moderate): Symptoms causing greater than minimal interference with usual social and functional activities

Grade 3 (Severe): Symptoms causing inability to perform usual social and functional activities

Grade 4 (Very Severe): Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

Guidelines for assessing the severity of specific adverse events and laboratory abnormalities are listed in Appendix B, Adverse Event Severity Assessment Table.

10.3 Relationship to Investigational Medicinal Product

Assessment of relationship of an AE or SAE to IMP is the responsibility of the Principal Investigator or designee. All medically indicated and available diagnostic methods (e.g., laboratory, blood smear, culture, X-ray, etc.) should be used to assess the nature and cause of the AE/SAE. Best clinical and scientific judgment should be used to assess relationship of AE/SAEs to the IP and/or other cause.

The following should be considered:

- Presence/absence of a clear temporal (time) sequence between administration of the IMP and the onset of AE/SAE
- Presence/absence of another cause that could more likely explain the AE/SAE (concurrent disease, concomitant medication, environmental or toxic factors)
- Whether or not the AE/SAE follows a known response pattern associated with the IMP

The relationship assessment should be reported as one of the following:

Not Related: clearly explained by another cause (concurrent disease, concomitant medication, environmental or toxic factors, etc.).

Unlikely: more likely explained by another cause (concurrent disease, concomitant medication, environmental or toxic factors, etc.).

Possibly: equally likely explained by another cause but the possibility of the IMP relationship cannot be ruled out (e.g., reasonably well temporally related and/or follows a known IMP response pattern but equally well explained by another cause).

Probably: more likely explained by the IMP (e.g., reasonably well temporally related and/or follows a known Investigational Medicinal Product response pattern and less likely explained by another cause).

Definitely: clearly related and most likely explained by the IMP.

For the purpose of expedited safety reporting, all possibly, probably or definitely related SAEs are considered IMP-related SAEs.

10.4 Serious Adverse Events

An adverse event is reported as a "Serious Adverse Event" if it meets any of the following criteria (as per International Conference on Harmonisation [ICH] Good Clinical Practice [GCP] Guidelines):

- Results in death
- Is life threatening
- Results in persistent or significant disability/incapacity
- Requires in-patient hospitalization or prolongs existing hospitalization
- Is a congenital anomaly/birth defect or spontaneous abortion
- Any other important medical condition that requires medical or surgical intervention to prevent permanent impairment of a body function or structure

Elective surgery for pre-existing condition that did not increase in severity or frequency is not considered an SAE.

Serious Adverse Events (SAEs) should be reported to IAVI within 24 hours of the site becoming aware of the event, and sent to the Sponsor as described in the SOM.

To discuss IMP-related SAEs or any urgent medical questions related to the SAE, the site investigator should contact one of the IAVI Medical Monitors directly (see Contact List in the SOM).

The IAVI SAE Report Form should be completed with all the available information at the time of reporting and sent to the Sponsor as described in the SOM. The minimum data required in reporting an SAE are the study identification number, date of birth, gender, event description (in as much detail as is known at the time), onset date of event (if available), reason event is classified as serious, reporting source (name of Principal Investigator or designee), and relationship to the IMP as assessed by the investigator.

The Principal Investigator or designee is required to prepare a detailed written report with follow up until resolution or until it is judged by the Principal Investigator or designee to have stabilized.

The Principal Investigator or designee must notify the responsible Ethics Committee of all SAEs as appropriate. In case of IMP-related SAEs, the Sponsor will notify responsible regulatory authorities, Safety Review Board (SRB), and other study sites where the same IMP is being tested.

More details on SAE definitions and reporting requirements are provided in the SOM.

<u>Serious Event Prior to Investigational Medicinal Product Administration</u>

If a serious event occurs in the period between the volunteer signing the Informed Consent Form and receiving the study injection, the event will be reported using the SAE form and following the same procedures for SAE reporting, as indicated in Section 10.4. The timing of the event will be indicated by using the relevant checkbox on the SAE form.

10.5 Reporting Potential Immune-Mediated Diseases

Potential immune-mediated diseases (pIMDs) are a subset of AEs that include both clearly autoimmune diseases and also other inflammatory and/or neurologic disorders which may or may not have an autoimmune aetiology. These events are of special interest since they could potentially be caused by immune responses to the transgene. The investigator should report such adverse events within the same time limits (following confirmation of an AE as a pIMD; see last paragraph of this section below), and using the same CRF pages, as utilized for SAEs. The investigator will evaluate the occurrence of pIMDs at every visit/contact during the study. IAVI will also expect investigators to provide additional information about pIMD events. AEs to be reported and documented as pIMDs include:

Neuroinflammatory disorders: optic neuritis, cranial nerve disorders (including Bell's palsy), multiple sclerosis, demyelinating disease, transverse myelitis, Guillain-Barré syndrome, myasthenia gravis, encephalitis, neuritis.

Musculoskeletal disorders: systemic lupus erythematosus, cutaneous lupus, Sjögren's syndrome, scleroderma, dermatomyositis, polymyositis, myopathy, rheumatoid arthritis and juvenile rheumatoid arthritis, polymyalgia rheumatica or temporal arteritis, reactive arthritis, psoriatic arthropathy, ankylosing spondylitis, undifferentiated spondyloarthropathy.

Gastrointestinal disorders: Crohn's disease, ulcerative colitis or proctitis, celiac disease.

Metabolic diseases: autoimmune thyroiditis, Grave's or Basedow's disease, Hashimoto thyroiditis, insulin-dependent diabetes mellitus (IDDM), Addison's disease.

Skin disorders: psoriasis, vitiligo, Raynaud's phenomenon, erythema nodosum, autoimmune bullous skin diseases.

Others: autoimmune hemolytic anemia, thrombocytopenia, antiphospholipid syndrome, *vasculitis, pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune glomerulonephritis, autoimmune uveitis, autoimmune myocarditis/cardiomyopathy, sarcoidosis, Stevens-Johnson syndrome, Behçet's syndrome.

Injection site reactions: Grade 3 or 4 injection site reactions lasting more than 2 days.

*Vasculitis: Vasculitis, Diffuse vasculitis, leucocytoclastic vasculitis, polyarteritis nodosa, microscopic polyangiitis,Wegener's granulomatosis, anti-neutrophil cytoplasmic antibody positive vasculitis, Henoch-schönlein purpura, allergic granulomatous angiitis (Churg-Strauss disease), Kawasaki disease, Takayasu's arteritis, temporal arteritis (giant cell arteritis), renal vasculitis.

Medical judgement should be exercised in deciding whether other disorders/diseases have an autoimmune origin and should also be reported as described above, and this judgement is the investigator's prerogative. Whenever sufficient data exist to substantiate any of the diagnoses in the above list, the event must be reported as a pIMD. While the intent of pIMD reporting is to be inclusive, isolated nonspecific

symptoms, which might (or might not) represent the above diagnoses, should be captured as AEs but not reported as pIMDs until the diagnosis can be defended.

10.6 Clinical Management of Adverse Events

Adverse events (AEs) will be managed by the clinical study team who will assess, provide first line of care as appropriate and refer to health care and treatment facilities as warranted. If any treatment/medical care is required as a result of the harm caused by the IMP or study procedures, this care will be provided free of charge.

If a volunteer has an AE and/or abnormal laboratory value that is known at the time of study injection, the specifications of Section 12.0 will be followed.

Volunteers will be followed until the AE resolves or stabilizes or up to the end of the study, whichever comes last. If at the end of the study, an AE (including clinically significant laboratory abnormality) that is considered possibly, probably or definitely related to the IMP is unresolved, follow-up will continue until resolution if possible and/or the volunteer will be referred.

10.7 Intercurrent HIV Infection

HIV infection cannot be directly caused by the IMP. If a volunteer acquires HIV through exposure in the community, the volunteer should have an Early Termination (ET) visit and offered referral to appropriate care and treatment facilities. The volunteer will be invited to enrol into a long term follow-up protocol for HIV infected volunteers, as described in Section 11.3.2.

Intercurrent HIV infection in study volunteers, although not considered an SAE, must be reported promptly to IAVI using the designated forms. However, medical conditions associated with the HIV infection that meet criteria for serious specified in the Section 10.4 of this Protocol (e.g., sepsis, *Pneumocystis jiroveci [carinii] pneumonia*, etc.) should be reported as SAEs using the SAE Report Form.

11.0 MANAGEMENT OF HIV ISSUES DURING AND FOLLOWING STUDY

11.1 HIV Testing

All volunteers will be tested for HIV as indicated in the Schedule of Procedures (Appendix A) or as needed, if medical or social circumstances arise. All volunteers will receive HIV risk reduction counselling and pre- and post-HIV-test counselling, as specified in Section 11.3.1 Counselling.

Volunteers who have a positive HIV serology test as a result of receipt of rAAV1-PG9DP and injection-induced HIV antibodies will have their test results reported as “Not infected with HIV-1 or HIV-2” (to prevent unblinding of volunteer and staff). A product recipient who still tests HIV positive due to injection-inducted antibodies at the end of the study will be informed of his/her positive test result and offered continuing follow-up until the test becomes negative.

If a volunteer is found to be HIV-infected through exposure to HIV in the community during the course of the study, the volunteer will be followed up as specified in Section 11.3.

Should a volunteer require an HIV test outside the study for personal reasons, it is recommended that the volunteer contact the study staff first. The HIV test should be done at the study site and then processed at an independent laboratory as above. Written evidence of HIV status (HIV-infected or HIV-uninfected) will be provided upon request.

11.2 Social Discrimination as a Result of a Seroreactive Diagnostic Test for HIV

To minimize the possibility of social discrimination in volunteers (if any) who test positive on a diagnostic HIV antibody test due to PG9 antibodies, appropriate diagnostic HIV testing and documentation/information will be provided both during and after the study as needed.

11.3 HIV infection

Volunteers who are found HIV infected at screening and volunteers who acquire HIV infection after screening, during the study (intercurrent HIV-infection) will be provided the following:

11.3.1 Counselling

The volunteer will be counselled by appropriately trained study investigational staff. The counselling process will assist the volunteer with the following issues:

- Psychological and social implications of HIV infection
- Whom to inform and what to say
- Implications for sexual partners
- Avoidance of transmission to others in future

11.3.2 Referral for Support/Care and Long Term Follow-Up Protocol for HIV Infection

Volunteers will be referred to a patient support centre or institution of his/her choice for a full discussion of the clinical aspects of HIV infection. Referral will be made to a designated physician or centre for discussion of options of treatment of HIV-infection.

For those individuals who become HIV infected after enrolment in the study (i.e., from injection through final study visit), antiretroviral therapy will be provided when clinically indicated according to accepted treatment guidelines.

12.0 DEFERRAL OF INJECTION AND/OR WITHDRAWAL FROM STUDY

12.1 Deferral of Injection

Injection may be temporarily deferred if the volunteer is clinically ill at the time of the injection visit and/or presents with fever at the time of the injection visit. A volunteer must be clinically well and afebrile for a minimum of a 24-hour consecutive period prior to injection.

12.2 Withdrawal from the Study (Early Termination)

Volunteers may be withdrawn from the study permanently for the following reasons:

1. Volunteers may withdraw from the study at any time if they wish, for any reason
2. The Principal Investigator or designee has reason to believe that the volunteer is not complying with the protocol
3. If the Sponsor decides to terminate or suspend the study
4. If a volunteer becomes infected with HIV (through exposure in the community)

If a volunteer withdraws or is withdrawn from the study, all termination visit procedures will be performed according to the Schedule of Procedures (Appendix A) when possible. Every effort will be made to determine and document the reason for withdrawal.

13.0 DATA HANDLING

13.1 Data Collection and Record Keeping at the Study Site

Data Collection: All study data will be collected by the clinical study staff using designated source documents and entered onto the appropriate case report forms (CRFs). CRFs will be provided by IAVI and should be handled in accordance with the instructions from IAVI. All study data must be verifiable to the source documentation. A file will be held for each volunteer at the clinic(s) containing all the source documents. Source documentation will be available for review to ensure that the collected data are consistent with the CRFs.

All CRFs and laboratory reports will be reviewed by the clinical team, who will ensure that they are accurate and complete.

Source documents and other supporting documents will be kept in a secure location. Standard GCP practices will be followed to ensure accurate, reliable and consistent data collection.

Source documents include but are not limited to:

- Signed Informed Consent Documents
- Progress notes
- Data collection forms
- Documentation of any existing conditions or past conditions relevant to eligibility

- Printed laboratory results
- Print out of the IDES generated enrolment confirmation
- All Adverse Events
- Concomitant medications
- Local and systemic reactogenicity events

13.2 Data Collection and Transfer at the Human Immunology Laboratory

Data generated at the IAVI Human Immunology Laboratory will be transferred directly to the Data Coordinating Centre.

13.3 Data Entry at the Study Site

The data collected at the site will be recorded onto the CRFs by the study staff and entered into a database. To provide for real time assessment of safety, data should be entered as soon as reasonably feasible after a visit occurs.

13.4 Data Analysis

The data analysis plan will be developed and agreed upon by the Sponsor, PI and IMP Developer prior to unblinding of the study. The statistician at the Data Coordinating Centre (DCC) in collaboration with the Principal Investigator, Sponsor and Developer of AAV-PG9DP will create tables according to this data analysis plan.

The DCC will conduct the data analysis and will provide interim and final study reports for the Sponsor, Principal Investigator, the SRB and the regulatory authorities, as appropriate.

14.0 STATISTICAL CONSIDERATIONS

14.1 Sample Size

The total number enrolled will depend on the course of dose escalation. Up to 24 total volunteers (18 active, 6 placebo) may be enrolled.

14.2 Statistical Power and Analysis

Safety and Tolerability:

The frequency of local and systemic reactogenicity events will be determined and compared between IMP regimens.

The frequency of SAEs judged possibly, probably or definitely related to the IMP will be determined. AEs that may be temporarily incapacitating (for example, loss or cancellation of work or social activities), which could make an IMP impractical for large scale use if they occur in more than a small proportion of cases, will also be described.

All AEs will be analysed and, grouped by seriousness, severity and relationship to the IMP (as judged by the investigator).

For specific adverse events related to the IMP: if none of the volunteers receiving the IMP experience such reactions, then the 95% upper confidence bound for the rate of

these adverse events in the population is 26.5%, 21.8% and 18.5%, depending on whether the total number of enrolled volunteers is 12 (Groups A, B, C, D), 15 (Groups A, B, C, D, D1) or 18 (Groups A, B, C, C1), respectively. If we consider a single dose group with only 3 volunteers receiving the IMP, then the 95% upper confidence bound for the rate of these adverse events in the population is 70.8%. All calculations are based on exact (Clopper-Pearson) confidence limits.

There is limited power for comparing the combined event rates between placebo and treatment arms. If all volunteers receiving the IMP are combined into a single group and compared with all placebo recipients, then the table below shows the difference (%) in event rates, depending on the final sample size, that will be detectable with 80% power at alpha levels of 0.05 and 0.10 using Fisher's exact 1-tailed test (assuming that event rates are lower in the placebo group). At the 5% level of significance the minimum detectable difference is 70% and at the 10% level of significance it is 47%. All calculations were made using PASS 2008 (Version 08.0.6).

Detectable difference (%) in event rates that will be detectable with 80% power at alpha levels of 0.05 and 0.10 using Fisher's exact 1-tailed test

Alpha	Sample size Vaccine/Placebo	True event rate in the placebo group					
		0.01	0.05	0.10	0.15	0.2	0.25
0.05	15/5	67%	68%	70%	70%	69%	67%
	18/6	57%	59%	60%	62%	62%	61%
0.10	15/5	54%	56%	58%	59%	60%	60%
	18/6	47%	51%	55%	56%	57%	56%

Considering the low power available for group comparison and the associated risk of false safety signal in the context of multiple exploratory analyses, the analysis of safety events will be based on within-group descriptive summaries for the incidence rate of each event. The associated 95% confidence intervals for the incidence rate within each group will also be provided.

Immunogenicity:
Cellular immune response rates (including ELISPOT and ICS), PG9 titres and neutralizing antibody response rates will be reported and analyzed using binomial methods. Descriptive statistics of the magnitude of response (ELISPOT and ICS counts, PG9 titres and neutralizing antibody titres) will also be provided, including mean, standard deviation and median, as well as the geometric mean titre (GMT) and corresponding 95% confidence interval. The breadth of neutralizing antibody responses to the various viruses will also be summarised (frequencies and proportions). Due to the small sample sizes per group, these analyses will be mainly descriptive. Assays will be performed using the IAVI HIL and trials site SOPs and standard reagents for all volunteers.

Based on the previous experience with IAVI Phase 1/2 IMP studies, it is expected that the amount of missing, unused or spurious data will be insignificant. Unused and

spurious data will be listed separately and excluded from the statistical analysis. Missing data will be excluded from the statistical analysis.

15.0 QUALITY CONTROL AND QUALITY ASSURANCE

To ensure the quality and reliability of the data collected and generated and the ethical conduct of this study, a Study Operations Manual (SOM) will be developed. All deviations will be reported and investigated. The SOM describes reporting and deviation documentation requirements and procedures.

Regular study monitoring will be performed according to ICH-GCP as indicated in Section 17.5.

An independent audit of the study and study site may be performed by the Sponsor or designee to establish the status of applicable quality systems. Inspection by regulatory authorities may also occur.

By signing the protocol, the Principal Investigator agrees to facilitate study related monitoring, audits, IRB/IEC review and regulatory inspection(s) and direct access to source documents. Such information will be treated as strictly confidential and under no circumstances be made publicly available.

16.0 DATA AND BIOLOGICAL MATERIAL

All data and biological material collected through the study shall be managed in accordance with the Clinical Trial Agreement (CTA). Distribution and use of these data will be conducted by agreement of all parties.

The computerized raw data generated will be held by the DCC on behalf of the Sponsor. The study site will also hold the final data files and tables generated for the purpose of analysis. Principal investigators or designees will have access to the clinical study database with appropriate blinding.

17.0 ADMINISTRATIVE STRUCTURE

The Principal Investigator will be responsible for all aspects of the study at the study site.

17.1 Protocol Safety Review Team (PSRT)

A PSRT will be formed to monitor the clinical safety data. Until 6 weeks after the last volunteer is injected, the PSRT will review the clinical safety data on a weekly basis via electronic distribution of reports. An ad hoc PSRT review meeting will occur if any of the members of the PSRT requests a special review to discuss a specific safety issue or as specified in the Study Operations Manual. After the injection phase the PSRT will review the clinical safety data at least monthly.

The PSRT will consist of the IAVI Medical Monitor(s) and PI or designee from the clinical team. The study chair or an IAVI Medical Monitor may be the PSRT chair. *Ex officio*

members will include the IAVI Chief Medical Officer, IAVI Medical Monitor and representatives from the IMP Developer.

Additional PSRT participants may include the following, as needed:

- Co-investigators and trial site senior clinical research nursing staff
- Laboratory directors
- Data management, study statistician and regulatory staff

The PSRT membership and procedures are detailed in the PSRT charter.

17.2 Safety Review Board (SRB)

The SRB will oversee the progress of the study and determine whether the criteria to dose-escalate have been met at each stage. The SRB will consist of independent clinicians/scientists/statisticians who are not involved in the study. Investigators responsible for the clinical care of volunteers or representative of the Sponsor may not be a member of the SRB. Details of membership, chair and co-chair and responsibilities are outlined in the SRB charter. Principal Investigator(s) or designee and/or a Sponsor representative may be asked to join an open session of the SRB meeting to provide information on study conduct, present data or to respond to questions. Safety data will be reviewed by the SRB at pre-specified time points and at an ad-hoc basis.

17.2.1 Timing and content of Interim Safety Reviews

Interim safety reviews will be conducted by the SRB at the following times:

- Once all volunteers in Group A have been followed for at least 42 days after injection
- Once all volunteers in Group B have been followed for at least 42 days after injection
- Once all volunteers in Group C have been followed for at least 56 days after injection
- Once all volunteers in Group D have been followed for at least 56 days after injection

The SRB will be asked to review the following unblinded data at the planned interim reviews. Data will include all accumulated safety data available at the time:

- Summary of reactogenicity (i.e., solicited adverse events)
- All clinical adverse events judged by the Principal Investigator or designee to be possibly, probably or definitely related to the IMP and all pIMD
- All laboratory adverse events confirmed on retest and judged by the Principal Investigator or designee to be possibly, probably, or definitely related to IMP and/or clinically significant
- Summary of ECG findings. The SRB will be provided copies of specific ECGs if they request.
- Summary of cardiac troponin I results. The SRB will be provided individual cardiac troponin I results if they request.
- All SAEs
- PG9 antibody level (for Group C review only, to determine if higher dose is indicated or if enrolment should be expanded to C1)

17.2.2 Criteria for Dose Escalation

The following criteria must be met to proceed to the next dose level:

- No new ECG findings suggestive of cardiac disease
- No confirmed and clinically significant elevated cardiac troponin I levels
- No case of a disease that might be immune mediated and is judged to be possibly, probably or definitely related to IMP, such as acute arthritis, severe generalized rash, or glomerulonephritis
- No pattern of reactogenicity or adverse events suggestive of autoimmune disease or immune mediated disease
- No serious adverse event which is assessed as probably or definitely related to the IMP
- No volunteer death which is assessed as possibly, probably or definitely related to the IMP

17.3 Medical Advisory Panel

In addition to the SRB, a Medical Advisory Panel with physicians with expertise in the use of immunosuppressive drugs and the management of immune-mediated disease such as immune nephritis and hepatitis will be formed at the clinical site institution. The Medical Advisory Panel will be knowledgeable about the protocol and available to provide rapid evaluation and management of potential immune mediated diseases, if they occur in study volunteers.

17.4 Criteria for Pausing the Study

Enrolments and injections will be paused, a formal study halt will be submitted to the MHRA as a substantial amendment, and a safety review conducted by the SRB for any of the following criteria:

1. One volunteer develops a Grade 3 or 4 adverse event that is judged to be possibly, probably or definitely related to IMP;
2. Grade 2 adverse events that persists for more than 7 days that is possibly, probably or definitely related to the IMP;
3. One volunteer experiences a serious adverse event that is probably or definitely related to the IMP, or two or more subjects experience similar serious adverse events which are possibly related to the IMP;
4. There is a volunteer death assessed as possibly, probably or definitely related to the IMP;
5. One volunteer shows evidence of disease that might be due to immune complexes and is judged to be possibly, probably or definitely related to IMP, such as acute arthritis, severe generalized rash, or glomerulonephritis;
6. A 5-fold increase in ALT within 10 weeks after IMP administration which is not responsive to oral corticosteroids and is judged to be possibly, probably or definitely related to IMP.

The Sponsor will request a review by the SRB, (or the SRB chair if other SRB members cannot be convened), to be held within 2 business days of the Sponsor learning of the

event. The individual volunteer(s)/or study may be unblinded at the discretion of the SRB.

Following this review, the SRB will make a recommendation regarding the continuation or suspension of the vaccinations or the trial and communicate this decision immediately to the Sponsor. If the SRB decision is to restart enrolments and injections, then a further substantial amendment will be submitted to the MHRA including all appropriate safety data and justification to restart. The Sponsor then will inform the Principal Investigator of the MHRA decision.

Additional *ad hoc* review may be specifically requested by the Sponsor, the Principal Investigator(s) or by the SRB.

17.5 Study Supervision

The SRB, the IAVI Chief Medical Officer (CMO) and the IAVI Medical Monitor(s) have access to progress report(s) of this study. Close cooperation will be necessary to track study progress, respond to queries about proper study implementation and management, address issues in a timely manner, and assure consistent documentation, and share information effectively. Rates of accrual, retention, and other parameters relevant to the site's performance will be regularly and closely monitored by the study team.

17.6 Study Monitoring

On-site monitoring will ensure that the study is conducted in compliance with human subjects' protection and other research regulations and guidelines, recorded and reported in accordance with the protocol, is consistent with SOPs, GCP, applicable regulatory requirements and locally accepted practices. The monitor will confirm the quality and accuracy of data at the site by validation of CRFs against the source documents, such as clinical records. The investigators, as well as volunteers through consenting to the study, agree that the monitor may inspect study facilities and source records (e.g., informed consent forms, clinic and laboratory records, other source documents), as well as observe the performance of study procedures. Such information will be treated as strictly confidential and will under no circumstances be made publicly available.

The monitoring will adhere to GCP guidelines. The Principal Investigator will permit inspection of the facilities and all study-related documentation by authorized representatives of IAVI, and Government and Regulatory Authorities responsible for this study.

17.7 Investigator's Records

Study records include administrative documentation—e.g., reports and correspondence relating to the study—as well as documentation related to each volunteer screened and/or enrolled in the study—including informed consent forms, case report forms, and all other source documents. The investigator will maintain and store, in a secure manner, complete, accurate, and current study records for a minimum of 2 years after marketing application approval or the study is discontinued and applicable national and

local health authorities are notified. IAVI will notify the Principal Investigator of these events.

18.0 INDEMNITY

The Sponsor and Institution are responsible to have appropriate liability insurance. For research-related injuries and/or medical problems determined to result from receiving the IMP, treatment including necessary emergency treatment and proper follow-up care will be made available to the volunteer free of charge at the expense of the Sponsor.

19.0 PUBLICATION

A primary manuscript describing safety and immune responses in this trial will be prepared promptly after the data analysis is available.

Authors will be representatives of the trial site, the data management and statistical analysis centre, the laboratories, the product developers, and IAVI, subject to the generally accepted criteria of contributions to the design and conduct of the study, the analysis of data and writing of the manuscript. Precedence will be given to authors from the site enrolling the greatest number of volunteers. Manuscripts will be reviewed by representatives of each participating group as specified in the CTA**.**

20.0 ETHICAL CONSIDERATIONS

The Principal Investigator will ensure that the study is conducted in compliance with the protocol and SOPs in accordance with guidelines formulated by the ICH for GCP in clinical studies, the ethical principles that have their origins in the Declaration of Helsinki and applicable local standards and regulatory requirements. All required approvals will be obtained before recruitment of volunteers.

APPENDIX A: SCHEDULE OF PROCEDURES

Study Month		0							1				2		3		4	5	6	9	12[1]	
Study Day	Screen	0	0	0	3	7	14	21	28	35	42	49	56	70	84	98[5]	112	140	168	252	336[1]	350[5]
Visit Windows (Days)	-42	0	0	0	± 1	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	±3	± 7	± 7	± 7	± 7	± 7	±3
VISIT	01	02	02A (30min post)	02B (4hrs post)	02C	02D	03	04	05	06	07	07M	08	08M	09	09M	10	11	12	13	14	14M
INVESTIGATIONAL MEDICINAL PRODUCT																						
Investigational Medicinal Product/Placebo		X																				
CONSENT/ASSESSMENTS/ COUNSELLING																						
Informed Consent	X																					
Screening Assessment	X																					
HIV Risk Assessment		X																			X	
HIV Risk Reduction Counselling	X	X																	X		X	
HIV Test Counselling	X	X																	X		X	
Contraception Counselling	X	X							X				X									
Social Impact Assessment																					X	
CLINICAL SAFETY ASSESSMENTS																						
Comprehensive Medical History	X																					
Interim Medical History		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
General Physical Exam	X																				X	
Symptom Directed Physical Exam		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Weight	X																				X	
Height	X																					
Vital Signs	X	X	X	X					X				X		X						X	
Cervical & Axillary Lymph Nodes	X	X			X	X	X	X	X	X	X	X	X									

Study Month		0							1				2		3		4	5	6	9	12[1]	
Study Day	Screen	0	0	0	3	7	14	21	28	35	42	49	56	70	84	98[5]	112	140	168	252	336[1]	350[5]
Visit Windows (Days)	-42	0	0	0	± 1	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	±3	± 7	± 7	± 7	± 7	± 7	±3
VISIT	01	02	02A (30min post)	02B (4hrs post)	02C	02D	03	04	05	06	07	07M	08	08M	09	09M	10	11	12	13	14	14M
Local & Systemic Reactogenicity		X	X	X	X	X																
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Serious Adverse Events and pIMD	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Muscle biopsy (group D1 only)															X						X	
CLINICAL LABORATORY TESTS																						
Screening Labs	X																					
Safety Labs	X	X			X[2]	X	X	X	X	X	X	X[4]	X	X[4]	X		X	X	X	X	X	
12- lead ECG	X	X			X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	
HIV Diagnostics	X	X																	X[3]		X	
HLA sample		X																				
IMMUNOGENICITY ASSAYS																						
PG9 antibody	X	X			X	X	X	X	X	X	X		X		X		X	X	X	X	X	
Anti-PG9 antibody		X							X		X				X		X	X	X	X	X	
Anti-AAV1 and Anti-AAV2 antibodies	X								X		X				X		X	X	X	X	X	
HIV neutralization		X							X		X				X		X	X	X	X	X	
AAV1 distribution (blood)		X			X	X	X	X	X						X							
AAV1-specific T cell responses		X									X				X						X	
PBMC collection		X									X				X						X	

[1] Early Termination (ET): Procedures to be performed at ET are the same as last visit procedures
[2] See Section 9.1.10 for tests not included at Day 3
[3] If HIV infected, conduct ET visit, see SOM for details
[4] Cardiac troponin I only
[5] Muscle biopsy safety assessment follow-up visit for Group D1 volunteers only. This visit may be conducted by telephone or as a clinic visit. If visit is conducted by phone then symptom-directed physical exam is not required. Study clinician may request the volunteer to return to the clinic for assessment if clinically indicated.

APPENDIX B: ADVERSE EVENT SEVERITY ASSESSMENT TABLE

Adapted from: DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS Version 1.0, December, 2004; Clarification AUGUST 2009

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ("DAIDS AE Grading Table") is a descriptive terminology that can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

This clarification of the DAIDS Table for Grading the Severity of Adult and Pediatric AE's provides additional explanation of the DAIDS AE Grading Table and clarifies some of the parameters.

I. Instructions and Clarifications

Grading Adult and Pediatric AEs

The DAIDS AE Grading Table includes parameters for grading both Adult and Pediatric AEs. When a single set of parameters is not appropriate for grading specific types of AEs for both Adult and Pediatric populations, separate sets of parameters for Adult and/or Pediatric populations (with specified respective age ranges) are given in the Table. If there is no distinction in the Table between Adult and Pediatric values for a type of AE, then the single set of parameters listed is to be used for grading the severity of both Adult and Pediatric events of that type.

Note: In the classification of adverse events, the term "severe" is not the same as "serious." Severity is an indication of the intensity of a specific event (as in mild, moderate, or severe chest pain). The term "serious" relates to a participant/event outcome or action criteria, usually associated with events that pose a threat to a participant's life or functioning.

Estimating Severity Grade for Parameters Not Identified in the Table

In order to grade a clinical AE that is not identified in the DAIDS AE grading table, use the category "Estimating Severity Grade" located on Page 3.

Determining Severity Grade for Parameters "Between Grades"

If the severity of a clinical AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE. If a laboratory value that is graded as a multiple of the ULN or LLN falls between two grades, select the higher of the two grades for the AE. For example, Grade 1 is 2.5 x ULN and Grade 2 is 2.6 x ULN for a parameter. If the lab value is 2.53 x ULN (which is between the two grades), the severity of this AE would be Grade 2, the higher of the two grades.

Values Below Grade 1

Any laboratory value that is between either the LLN or ULN and Grade 1 should not be graded.

Determining Severity Grade when Local Laboratory Normal Values Overlap with Grade 1 Ranges

In these situations, the severity grading is based on the ranges in the DAIDS AE Grading Table, even when there is a reference to the local lab LLN.

For example: Phosphate, Serum, Low, Adult and Pediatric > 14 years (Page 70) Grade 1 range is 2.50 mg/dL - < LLN. A particular laboratory's normal range for Phosphate is 2.1 – 3.8 mg/dL. A participant's actual lab value is 2.5. In this case, the value of 2.5 exceeds the LLN for the local lab, but will be graded as Grade 1 per DAIDS AE Grading Table.

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II. Definitions of terms used in the Table:

Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding. Young Children Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).
LLN	Lower limit of normal
Medical Intervention	Use of pharmacologic or biologic agent(s) for treatment of an AE.
NA	Not Applicable
Operative Intervention	Surgical OR other invasive mechanical procedures.
ULN	Upper limit of normal
Usual Social & Functional Activities	Adult Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc. Young Children Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
ESTIMATING SEVERITY GRADE				
Clinical adverse event NOT identified elsewhere in this DAIDS AE Grading Table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYSTEMIC				
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions
Fever (nonaxillary)	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Pain (indicate body site) DO NOT use for pain due to injection (See Injection Site Reactions: Injection site pain) See also Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than emergency room visit) indicated
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
INFECTION				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (e.g., septic shock)
INJECTION SITE REACTIONS				
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Injection site reaction (localized)				
Adult > 15 years	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm^2 – 81cm^2)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
Pediatric ≤ 15 years	Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
Pruritis associated with injection See also Skin: Pruritis (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA
SKIN – DERMATOLOGICAL				
Alopecia	Thinning detectable by study participant (or by caregiver for young children and disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	NA

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	NA	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
CARDIOVASCULAR				
Cardiac arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic, non-life-threatening AND Non-urgent medical intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Cardiac-ischemia/infarction	NA	NA	Symptomatic ischemia (stable angina) OR Testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Hemorrhage (significant acute blood loss)	NA	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≤ 2 units packed RBCs (for children ≤ 10 cc/kg) indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children > 10 cc/kg) indicated
Hypertension				
Adult > 17 years (with repeat testing at same visit)	140 – 159 mmHg systolic OR 90 – 99 mmHg diastolic	160 – 179 mmHg systolic OR 100 – 109 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Correction: in Grade 2 to 160 - 179 from > 160-179 (systolic) and to ≥ 100 -109 from > 100-109 (diastolic) and in Grade 3 to ≥ 180 from > 180 (systolic) and to ≥ 110 from > 110 (diastolic).				
Pediatric ≤ 17 years (with repeat testing at same visit)	NA	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Pericardial effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no intervention	Effusion with non-life threatening physiologic consequences OR Effusion with non-urgent intervention indicated	Life-threatening consequences (e.g., tamponade) OR Urgent intervention indicated
Prolonged PR interval				
Adult > 16 years	PR interval 0.21 – 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec	Complete AV block
Pediatric ≤ 16 years	1st degree AV block (PR > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block	Complete AV block
Prolonged QTc				
Adult > 16 years	Asymptomatic, QTc interval 0.45 – 0.47 sec OR Increase interval < 0.03 sec above baseline	Asymptomatic, QTc interval 0.48 – 0.49 sec OR Increase in interval 0.03 – 0.05 sec above baseline	Asymptomatic, QTc interval ≥ 0.50 sec OR Increase in interval ≥ 0.06 sec above baseline	Life-threatening consequences, e.g. Torsade de pointes or other associated serious ventricular dysrhythmia
Pediatric ≤ 16 years	Asymptomatic, QTc interval 0.450 – 0.464 sec	Asymptomatic, QTc interval 0.465 – 0.479 sec	Asymptomatic, QTc interval ≥ 0.480 sec	Life-threatening consequences, e.g. Torsade de pointes or other associated serious ventricular dysrhythmia
Thrombosis/embolism	NA	Deep vein thrombosis AND No intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Deep vein thrombosis AND Intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Embolic event (e.g., pulmonary embolism, life-threatening thrombus)
Vasovagal episode (associated with a procedure of any kind)	Present without loss of consciousness	Present with transient loss of consciousness	NA	NA
Ventricular dysfunction (congestive heart failure)	NA	Asymptomatic diagnostic finding AND intervention indicated	New onset with symptoms OR Worsening symptomatic congestive heart failure	Life-threatening congestive heart failure
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
Comment: Please note that, while the grading scale provided for Unintentional Weight Loss may be used as a guideline when grading anorexia, this is not a requirement and should not be used as a substitute for clinical judgment.				

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., diuretics or therapeutic paracentesis)	Symptomatic despite intervention	Life-threatening consequences
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis or perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea				
Adult and Pediatric ≥ 1 year	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Pediatric < 1 year	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Liquid stools resulting in severe dehydration with aggressive rehydration indicated OR Hypotensive shock
Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated	Life-threatening reduction in oral intake
Mucositis/stomatitis (clinical exam) Indicate site (e.g., larynx, oral) See Genitourinary for Vulvovaginitis See also Dysphagia-Odynophagia and Proctitis	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences (e.g., aspiration, choking)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than emergency room visit)	Symptomatic AND Hospitalization indicated (other than emergency room visit)	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Proctitis (functional-symptomatic) Also see Mucositis/stomatitis for clinical exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
NEUROLOGIC				
Alteration in personality-behavior or in mood (e.g., agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with usual social & functional activities	Alteration causing inability to perform usual social & functional activities	Behavior potentially harmful to self or others (e.g., suicidal and homicidal ideation or attempt, acute psychosis) OR Causing inability to perform basic self-care functions
Altered Mental Status For Dementia, see Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions
Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
CNS ischemia (acute)	NA	NA	Transient ischemic attack	Cerebral vascular accident (CVA, stroke) with neurological deficit

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Developmental delay – **Pediatric ≤ 16 years**	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Difficulty sleeping causing inability to perform usual social & functional activities	Disabling insomnia causing inability to perform basic self-care functions
Neuromuscular weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal interference with usual social & functional activities	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizure: (new onset) – **Adult ≥ 18 years** See also Seizure: (known pre-existing seizure disorder)	NA	1 seizure	2 – 4 seizures	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure: (known pre-existing seizure disorder) **– Adult ≥ 18 years** For worsening of existing epilepsy the grades should be based on an increase from previous level of control to any of these levels.	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR Infrequent break-through seizures while on stable medication in a previously controlled seizure disorder	Change in seizure character from baseline either in duration or quality (e.g., severity or focality)	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Seizure **– Pediatric < 18 years**	Seizure, generalized onset with or without secondary generalization, lasting < 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5 – 20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Syncope (not associated with a procedure)	NA	Present	NA	NA
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
RESPIRATORY				
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 – 80%	FEV1 or peak flow 50 – 69%	FEV1 or peak flow 25 – 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress				
Adult ≥ 14 years	Dyspnea on exertion with no or minimal interference with usual social & functional activities	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities	Dyspnea at rest causing inability to perform usual social & functional activities	Respiratory failure with ventilatory support indicated
Pediatric < 14 years	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 – 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilatory support indicated
MUSCULOSKELETAL				
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss				
Adult ≥ 21 years	BMD t-score -2.5 to -1.0	BMD t-score < -2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Pediatric < 21 years	BMD z-score -2.5 to -1.0	BMD z-score < -2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	Asymptomatic with radiographic findings AND No operative intervention indicated	Symptomatic bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
GENITOURINARY				
Cervicitis (symptoms) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions
Cervicitis (clinical exam) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Minimal cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption < 25% of total surface	Moderate cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption of 25 – 49% total surface	Severe cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption 50 – 75% total surface	Epithelial disruption > 75% total surface
Inter-menstrual bleeding (IMB)	Spotting observed by participant OR Minimal blood observed during clinical or colposcopic examination	Inter-menstrual bleeding not greater in duration or amount than usual menstrual cycle	Inter-menstrual bleeding greater in duration or amount than usual menstrual cycle	Hemorrhage with life-threatening hypotension OR Operative intervention indicated
Urinary tract obstruction (e.g., stone)	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences
Vulvovaginitis (symptoms) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Vulvovaginitis (clinical exam) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Minimal vaginal abnormalities on examination OR Epithelial disruption < 25% of total surface	Moderate vaginal abnormalities on examination OR Epithelial disruption of 25 - 49% total surface	Severe vaginal abnormalities on examination OR Epithelial disruption 50 - 75% total surface	Vaginal perforation OR Epithelial disruption > 75% total surface
OCULAR/VISUAL				
Uveitis	Asymptomatic but detectable on exam	Symptomatic anterior uveitis OR Medical intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Visual changes (from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
ENDOCRINE/METABOLIC				
Abnormal fat accumulation (e.g., back of neck, breasts, abdomen)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	NA
Diabetes mellitus	NA	New onset without need to initiate medication OR Modification of current medications to regain glucose control	New onset with initiation of medication indicated OR Diabetes uncontrolled despite treatment modification	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma)
Gynecomastia	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 VERY SEVERE
Lipoatrophy (e.g., fat loss from the face, extremities, buttocks)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 VERY SEVERE**
HEMATOLOGY	***Standard International Units are listed in italics***			
Absolute CD4+ count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	300 – 400/mm^3 *300 – 400/µL*	200 – 299/mm^3 *200 – 299/µL*	100 – 199/mm^3 *100 – 199/µL*	< 100/mm^3 *< 100/µL*
Absolute lymphocyte count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	600 – 650/mm^3 *0.600 x 10^9 – 0.650 x 10^9/L*	500 – 599/mm^3 *0.500 x 10^9 – 0.599 x 10^9/L*	350 – 499/mm^3 *0.350 x 10^9 – 0.499 x 10^9/L*	< 350/mm^3 *< 0.350 x 10^9/L*
Comment: Values in children ≤ 13 years are not given for the two parameters above because the absolute counts are variable.				
Absolute neutrophil count (ANC)				
Adult and Pediatric, > 7 days	1,000 – 1,300/mm^3 *1.000 x 10^9 – 1.300 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	500 – 749/mm^3 *0.500 x 10^9 – 0.749 x 10^9/L*	< 500/mm^3 *< 0.500 x 10^9/L*
Infant*†, 2 – ≤ 7 days	1,250 – 1,500/mm^3 *1.250 x 10^9 – 1.500 x 10^9/L*	1,000 – 1,249/mm^3 *1.000 x 10^9 – 1.249 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	< 750/mm^3 *< 0.750 x 10^9/L*
Infant*†, ≤1 day	4,000 – 5,000/mm^3 *4.000 x 10^9 – 5.000 x 10^9/L*	3,000 – 3,999/mm^3 *3.000 x 10^9 – 3.999 x10^9/L*	1,500 – 2,999/mm^3 *1.500 x 10^9 – 2.999 x 10^9/L*	< 1,500/mm^3 *< 1.500 x 10^9/L*
Comment: Parameter changed from "Infant, < 1 day" to "Infant, ≤1 day"				
Fibrinogen, decreased	100 – 200 mg/dL *1.00 – 2.00 g/L* OR 0.75 – 0.99 x LLN	75 – 99 mg/dL *0.75 – 0.99 g/L* OR 0.50 – 0.74 x LLN	50 – 74 mg/dL *0.50 – 0.74 g/L* OR 0.25 – 0.49 x LLN	< 50 mg/dL *< 0.50 g/L* OR < 0.25 x LLN OR Associated with gross bleeding

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 VERY SEVERE**
Hemoglobin (Hgb)				
Comment: The Hgb values in mmol/L have changed because the conversion factor used to convert g/dL to mmol/L has been changed from 0.155 to 0.6206 (the most commonly used conversion factor). For grading Hgb results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for that lab.				
Adult and Pediatric ≥ 57 days (HIV POSITIVE ONLY)	8.5 – 10.0 g/dL *5.24 – 6.23 mmol/L*	7.5 – 8.4 g/dL *4.62–5.23 mmol/L*	6.50 – 7.4 g/dL *4.03–4.61 mmol/L*	< 6.5 g/dL *< 4.03 mmol/L*
Adult and Pediatric ≥ 57 days (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL *6.18 – 6.79 mmol/L* OR Any decrease 2.5 – 3.4 g/dL *1.58 – 2.13 mmol/L*	9.0 – 9.9 g/dL *5.55 - 6.17 mmol/L* OR Any decrease 3.5 – 4.4 g/dL *2.14 – 2.78 mmol/L*	7.0 – 8.9 g/dL *4.34 - 5.54 mmol/L* OR Any decrease ≥ 4.5 g/dL *> 2.79 mmol/L*	< 7.0 g/dL *< 4.34 mmol/L*
Comment: The decrease is a decrease from baseline				
Infant*†, 36 – 56 days (HIV POSITIVE OR NEGATIVE)	8.5 – 9.4 g/dL *5.24 – 5.86 mmol/L*	7.0 – 8.4 g/dL *4.31 – 5.23 mmol/L*	6.0 – 6.9 g/dL *3.72 – 4.30 mmol/L*	< 6.00 g/dL *< 3.72 mmol/L*
Infant*†, 22 – 35 days (HIV POSITIVE OR NEGATIVE)	9.5 – 10.5 g/dL *5.87 - 6.54 mmol/L*	8.0 – 9.4 g/dL *4.93 – 5.86 mmol/L*	7.0 – 7.9 g/dL *4.34 – 4.92 mmol/L*	< 7.00 g/dL *< 4.34 mmol/L*
Infant*†, ≤ 21 days (HIV POSITIVE OR NEGATIVE)	12.0 – 13.0 g/dL *7.42 – 8.09 mmol/L*	10.0 – 11.9 g/dL *6.18 – 7.41 mmol/L*	9.0 – 9.9 g/dL *5.59- 6.17 mmol/L*	< 9.0 g/dL *< 5.59 mmol/L*
Correction: Parameter changed from "Infant < 21 days" to "Infant ≤ 21 days"				
International Normalized Ratio of prothrombin time (INR)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Methemoglobin	5.0 – 10.0%	10.1 – 15.0%	15.1 – 20.0%	> 20.0%
Prothrombin Time (PT)	1.1 – 1.25 x ULN	1.26 – 1.50 x ULN	1.51 – 3.00 x ULN	> 3.00 x ULN
Partial Thromboplastin Time (PTT)	1.1 – 1.66 x ULN	1.67 – 2.33 x ULN	2.34 – 3.00 x ULN	> 3.00 x ULN
Platelets, decreased	100,000 – 124,999/mm^3 *100.000 x 10^9 – 124.999 x 10^9/L*	50,000 – 99,999/mm^3 *50.000 x 10^9 – 99.999 x 10^9/L*	25,000 – 49,999/mm^3 *25.000 x 10^9 – 49.999 x 10^9/L*	< 25,000/mm^3 *< 25.000 x 10^9/L*
WBC, decreased	2,000 – 2,500/mm^3 *2.000 x 10^9 – 2.500 x 10^9/L*	1,500 – 1,999/mm^3 *1.500 x 10^9 – 1.999 x 10^9/L*	1,000 – 1,499/mm^3 *1.000 x 10^9 – 1.499 x 10^9/L*	< 1,000/mm^3 *< 1.000 x 10^9/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 VERY SEVERE**
CHEMISTRIES	***Standard International Units are listed in italics***			
Acidosis	NA	pH < normal, but ≥ 7.3	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, serum, low	3.0 g/dL – < LLN *30 g/L – < LLN*	2.0 – 2.9 g/dL *20 – 29 g/L*	< 2.0 g/dL *< 20 g/L*	NA
Alkaline Phosphatase	1.25 – 2.5 x ULN†	2.6 – 5.0 x ULN†	5.1 – 10.0 x ULN†	> 10.0 x ULN†
Alkalosis	NA	pH > normal, but ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bicarbonate, serum, low	16.0 mEq/L – < LLN *16.0 mmol/L – < LLN*	11.0 – 15.9 mEq/L *11.0 – 15.9 mmol/L*	8.0 – 10.9 mEq/L *8.0 – 10.9 mmol/L*	< 8.0 mEq/L *< 8.0 mmol/L*
Comment: Some laboratories will report this value as Bicarbonate (HCO_3) and others as Total Carbon Dioxide (CO_2). These are the same tests; values should be graded according to the ranges for Bicarbonate as listed above.				
Bilirubin (Total)				
Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Infant*†, ≤ 14 days (non-hemolytic)	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	25.1 – 30.0 mg/dL *429 – 513 µmol/L*	> 30.0 mg/dL *> 513.0 µmol/L*
Infant*†, ≤ 14 days (hemolytic)	NA	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	> 25.0 mg/dL *> 428 µmol/L*
Calcium, serum, high				
Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL *2.65 – 2.88 mmol/L*	11.6 – 12.5 mg/dL *2.89 – 3.13 mmol/L*	12.6 – 13.5 mg/dL *3.14 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Infant*†, < 7 days	11.5 – 12.4 mg/dL *2.88 – 3.10 mmol/L*	12.5 – 12.9 mg/dL *3.11 – 3.23 mmol/L*	13.0 – 13.5 mg/dL *3.245 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Calcium, serum, low				
Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL *1.95 – 2.10 mmol/L*	7.0 – 7.7 mg/dL *1.75 – 1.94 mmol/L*	6.1 – 6.9 mg/dL *1.53 – 1.74 mmol/L*	< 6.1 mg/dL *< 1.53 mmol/L*
Infant*†, < 7 days	6.5 – 7.5 mg/dL *1.63 – 1.88 mmol/L*	6.0 – 6.4 mg/dL *1.50 – 1.62 mmol/L*	5.50 – 5.90 mg/dL *1.38 – 1.51 mmol/L*	< 5.50 mg/dL *< 1.38 mmol/L*
Comment: Do not adjust Calcium, serum, low or Calcium, serum, high for albumin				

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 VERY SEVERE**
Cardiac troponin I (cTnI)	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cardiac troponin T (cTnT)	NA	NA	NA	≥ 0.20 ng/mL OR Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cholesterol (fasting)				
Adult ≥ 18 years	200 – 239 mg/dL *5.18 – 6.19 mmol/L*	240 – 300 mg/dL *6.20 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Pediatric < 18 years	170 – 199 mg/dL *4.40 – 5.15 mmol/L*	200 – 300 mg/dL *5.16 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Creatine Kinase	3.0 – 5.9 x ULN†	6.0 – 9.9 x ULN†	10.0 – 19.9 x ULN†	≥ 20.0 x ULN†
Creatinine	1.1 – 1.3 x ULN†	1.4 – 1.8 x ULN†	1.9 – 3.4 x ULN†	≥ 3.5 x ULN†

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 VERY SEVERE**
Glucose, serum, high				
Nonfasting	116 – 160 mg/dL *6.44 – 8.88 mmol/L*	161 – 250 mg/dL *8.89 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Fasting	110 – 125 mg/dL *6.11 – 6.94 mmol/L*	126 – 250 mg/dL *6.95 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Glucose, serum, low				
Adult and Pediatric ≥ 1 month	55 – 64 mg/dL *3.05 – 3.55 mmol/L*	40 – 54 mg/dL *2.22 – 3.06 mmol/L*	30 – 39 mg/dL *1.67 – 2.23 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Infant*†, < 1 month	50 – 54 mg/dL *2.78 – 3.00 mmol/L*	40 – 49 mg/dL *2.22 – 2.77 mmol/L*	30 – 39 mg/dL *1.67 – 2.21 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Lactate	ULN - < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences
Comment: Added ULN to Grade 1 parameter				
LDL cholesterol (fasting)				
Adult ≥ 18 years	130 – 159 mg/dL *3.37 – 4.12 mmol/L*	160 – 190 mg/dL *4.13 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Pediatric > 2 - < 18 years	110 – 129 mg/dL *2.85 – 3.34 mmol/L*	130 – 189 mg/dL *3.35 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Lipase	1.1 – 1.5 x ULN	1.6 – 3.0 x ULN	3.1 – 5.0 x ULN	> 5.0 x ULN
Magnesium, serum, low	1.2 – 1.4 mEq/L *0.60 – 0.70 mmol/L*	0.9 – 1.1 mEq/L *0.45 – 0.59 mmol/L*	0.6 – 0.8 mEq/L *0.30 – 0.44 mmol/L*	< 0.60 mEq/L *< 0.30 mmol/L*
Pancreatic amylase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Phosphate, serum, low				
Adult and Pediatric > 14 years	2.5 mg/dL – < LLN *0.81 mmol/L – < LLN*	2.0 – 2.4 mg/dL *0.65 – 0.80 mmol/L*	1.0 – 1.9 mg/dL *0.32 – 0.64 mmol/L*	< 1.00 mg/dL *< 0.32 mmol/L*
Pediatric 1 year – 14 years	3.0 – 3.5 mg/dL *0.97 – 1.13 mmol/L*	2.5 – 2.9 mg/dL *0.81 – 0.96 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Pediatric < 1 year	3.5 – 4.5 mg/dL *1.13 – 1.45 mmol/L*	2.5 – 3.4 mg/dL *0.81 – 1.12 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Potassium, serum, high	5.6 – 6.0 mEq/L *5.6 – 6.0 mmol/L*	6.1 – 6.5 mEq/L *6.1 – 6.5 mmol/L*	6.6 – 7.0 mEq/L *6.6 – 7.0 mmol/L*	> 7.0 mEq/L *> 7.0 mmol/L*
Potassium, serum, low	3.0 – 3.4 mEq/L *3.0 – 3.4 mmol/L*	2.5 – 2.9 mEq/L *2.5 – 2.9 mmol/L*	2.0 – 2.4 mEq/L *2.0 – 2.4 mmol/L*	< 2.0 mEq/L *< 2.0 mmol/L*
Sodium, serum, high	146 – 150 mEq/L *146 – 150 mmol/L*	151 – 154 mEq/L *151 – 154 mmol/L*	155 – 159 mEq/L *155 – 159 mmol/L*	≥ 160 mEq/L *≥ 160 mmol/L*
Sodium, serum, low	130 – 135 mEq/L *130 – 135 mmol/L*	125 – 129 mEq/L *125 – 129 mmol/L*	121 – 124 mEq/L *121 – 124 mmol/L*	≤ 120 mEq/L *≤ 120 mmol/L*
Triglycerides (fasting)	NA	500 – 750 mg/dL *5.65 – 8.48 mmol/L*	751 – 1,200 mg/dL *8.49 – 13.56 mmol/L*	> 1,200 mg/dL *> 13.56 mmol/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 VERY SEVERE**
Uric acid	7.5 – 10.0 mg/dL *0.45 – 0.59 mmol/L*	10.1 – 12.0 mg/dL *0.60 – 0.71 mmol/L*	12.1 – 15.0 mg/dL *0.72 – 0.89 mmol/L*	> 15.0 mg/dL *> 0.89 mmol/L*
URINALYSIS	***Standard International Units are listed in italics***			
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Proteinuria, 24 hour collection				
Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h *1.000 – 1.999 g/d*	2,000 – 3,500 mg/24 h *2.000 – 3.500 g/d*	> 3,500 mg/24 h *> 3.500 g/d*
Pediatric > 3 mo - < 10 years	201 – 499 mg/m^2/24 h *0.201 – 0.499 g/d*	500 – 799 mg/m^2/24 h *0.500 – 0.799 g/d*	800 – 1,000 mg/m^2/24 h *0.800 – 1.000 g/d*	> 1,000 mg/ m^2/24 h *> 1.000 g/d*

APPENDIX C: TREATMENT OF POTENTIAL IMP-RELATED DISEASES

Potential diseases, including immune-mediated diseases, caused by reaction to the expressed antibody will be rapidly assessed by the Medical Advisory Panel. Individual treatment plans will be recommended by the Panel based on type of disease (ie nephritis, arthritis, vasculitis) and characteristics of the volunteer.

In general, a course of corticosteroids – oral prednisolone - will be the first line treatment. Corticosteroids are highly effective as first-line agents in severe autoimmune diseases such as glomerulonephritis, immune hepatitis, and vasculitis.

Regimen:
Week 1 60mg daily
Week 2 40mg daily
Week 3 30mg daily
Week 4 20mg daily
Maintenance 20mg daily, until symptoms or laboratory indicator (eg renal function, liver function) returns to base line
Then reduce by 5mg/week

Additional or alternative medications and treatments will be considered as necessary such as: azathioprine, cyclosporine, mycophenolate mofetil, IV immunoglobulin, rituximab and plasmapheresis.

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SUMMARY OF CHANGES TO PROTOCOL	
Protocol Title:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy adult males at risk of HIV infection.
Protocol Number:	IAVI A003/ CHOP HVDDT 001
Regulatory Investigational Product Number:	MHRA Reference: 33372/0003/001-0001 Eudract Number: 2013-002268-14
Sponsor:	International AIDS Vaccine Initiative (IAVI) 125 Broad Street, 9th Floor New York, New York 10004 USA
Date of Protocol Version:	3 July 2013 1.0
Date of Amendment Version:	5 November 2013 2.0

The following are the primary reasons for the changes to the protocol:

- Change study population from MSM at risk of HIV infection to males at low risk of HIV infection
- Clarification on when a substantial amendment is to be submitted to MHRA
- Correct mistakes in Schedule of Procedures
- Minor corrections and clarifications

Section Number & Title:	Protocol Title; 2.1 Study Rationale, 5.0 Study Design
Previous Text:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults ~~at risk of HIV infection~~
Revised Text:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults
Rationale:	Change study population per request of Oxford A REC

Section Number & Title:	Synopsis (Primary Objective); 3.1 Primary Objective
Previous Text:	To evaluate the safety of rAAV1-PG9DP when administered intramuscularly at $4x10^{12}$ vg, $4x10^{13}$ vg, $8x10^{13}$ vg and $1.2x10^{14}$ vg in healthy male adults ~~at risk of HIV infection~~
Revised Text:	To evaluate the safety of rAAV1-PG9DP when administered intramuscularly at $4x10^{12}$ vg, $4x10^{13}$ vg, $8x10^{13}$ vg and $1.2x10^{14}$ vg in healthy male adults
Rationale:	Change study population per request of Oxford A REC

Section Number & Title:	Synopsis (Study Population); 5.2 Study Population; 5.5 Recruitment of Volunteers
Previous Text:	Healthy male adults ~~at risk of HIV infection~~, 18 to 45 years of age, who are willing to undergo HIV testing, and who, in the opinion of the principal investigator or designee, understand the study and who provide written informed consent.
Revised Text:	Healthy male adults, 18 to 45 years of age, who are willing to undergo HIV testing, and who, in the opinion of the principal investigator or designee, understand the study and who provide written informed consent.
Rationale:	Change study population per request of Oxford A REC

Section Number & Title:	Synopsis (Duration of Study Participation); 5.1 Duration of Study
Previous Text:	Upon completion of this study, volunteers will be asked to participate in a follow up study to assess safety and PG9 expression for at least 4 additional years.
Revised Text:	Upon completion of this study, volunteers will be asked to participate in a follow up study to assess safety and PG9 expression for at least 5 additional years.
Rationale:	Correct typographical error

Section Number & Title:	2.6 Rationale for testing rAAV1-PG9DP in healthy volunteers at risk of HIV infection
Previous Text:	HIV-infected volunteers could have been considered for initial safety evaluation of this clinical candidate. However, for several reasons, this protocol initiates clinical development in healthy HIV at-risk volunteers, the population for whom the eventual product, if approved, would be indicated. First, the HIV neutralizing activity naturally present in the serum of HIV-infected persons could make difficult or impossible the measurement of the primary measure of activity: *in vitro* HIV neutralization by expressed PG9. Second, passive immunization with humanized monoclonal antibodies against HIV has been evaluated and shown to have limited clinical benefit in HIV positive individuals; at best, decreases in virus load were transient, and antibody-resistant viruses were selected[48,49,50,51,52]. Third, evaluation of any adverse events caused by antibodies directed against the product may be limited by immunosuppression in HIV-infected individuals or obscured by HIV-related symptoms. Fourth, the concentration of the expressed protein could be altered by interaction with actively produced HIV particles, and detection of the expressed protein would be difficult due to previously produced HIV antibodies.
Revised Text:	(Text deleted)
Rationale:	Delete section due to change in study population

Section Number & Title:	5.3 Inclusion Criteria
Previous Text:	At least 18 years of age on the day of screening and has not reached his ~~45~~th birthday on the day of injection
Revised Text:	At least 18 years of age on the day of screening and has not reached his 46th birthday on the day of injection
Rationale:	Correct typographical error

Section Number & Title:	5.3 Inclusion Criteria
Previous Text:	3. Reported at-risk behaviour for HIV infection, defined as having anal sex with another male in the past 6 months 4. Has undergone standard screening exams for sexually transmitted infections for men who have sex with men in the past 6 months
Revised Text:	(Text deleted)
Rationale:	Delete Inclusion Criteria that allow MSM only into study

Section Number & Title:	Section 5.4 Exclusion Criteria
Previous Text:	Any of the following ~~specific~~ risk behaviour for HIV infection *within 6 months prior to injection:* • Unprotected sexual intercourse with a known HIV infected person or ~~≥4 casual partners (i.e. no continuing established relationship)~~ • Engaged in sex work • Frequent excessive daily alcohol use or frequent binge drinking or chronic marijuana abuse or any other use of illicit drugs • History of newly-acquired syphilis, gonorrhoea, non-gonococcal urethritis, HSV-2, chlamydia, epididymitis, proctitis, lymphogranuloma venereum, chancroid, or hepatitis B
Revised Text:	Any of the following risk behaviour for HIV infection *within 6 months prior to injection:* • Unprotected sexual intercourse with a known HIV infected person or a partner known to be at high risk for HIV infection or a casual partner (i.e., no continuing established relationship) • Unprotected anal intercourse with another man (either insertive or receptive) • Three or more sexual partners • Engaged in sex work • Frequent excessive daily alcohol use or frequent binge drinking or chronic marijuana abuse or any other use of illicit drugs • History of newly-acquired syphilis, gonorrhoea, non-gonococcal urethritis, HSV-2, chlamydia, epididymitis, proctitis, lymphogranuloma venereum, chancroid, or hepatitis B
Rationale:	Recruiting men at low risk of HIV infection instead of MSM at risk of HIV infection

Section Number & Title:	7.4 Contraception Counselling
Previous Text:	(Text added)
Revised Text:	Volunteers are required to use condoms with any female or male partners until 3 months after receiving the study injection. This is to avoid the possibility of horizontal (to sexual partners) or vertical transfer (to a fetus) of the vector and/or PG9.
Rationale:	Add section regarding contraception counseling that will be given to volunteers

Section Number & Title:	7.8 Unblinding Procedure for Individual Volunteers
Previous Text:	Unblinding of an individual volunteer may be indicated in the event of a medical emergency if the clinical management of the volunteer would be altered by knowledge of the treatment assignment.
Revised Text:	Unblinding of an individual volunteer may be indicated in the event of a medical emergency if the clinical management of the volunteer would be altered by knowledge of the treatment assignment. In the event of an emergency, the investigator is able to unblind treatment for a study volunteer without first contacting IAVI.
Rationale:	Clarification of unblinding procedures

Section Number & Title:	8.2 Shipment and Storage
Previous Text:	The rAAV1-PG9DP vector and Excipient are stored as frozen vials inside cryoboxes at -60°C ~~or below~~, in a secure freezer in the freezer room
Revised Text:	The rAAV1-PG9DP vector and Excipient are stored as frozen vials inside cryoboxes at less than -60°C, in a secure freezer in the freezer room
Rationale:	Correction of storage temperature

Section Number & Title:	Table 9.1.10-1: Laboratory Parameters
Previous Text:	Haemoglobin, haematocrit, leukocytes, ~~lymphocytes, neutrophils~~, platelets
Revised Text:	Haemoglobin, haematocrit, leukocytes, platelets, absolute neutrophil count, absolute lymphocyte count
Rationale:	Clarify hematology assays to be performed

Section Number & Title:	12.1 Deferral of Injection
Previous Text:	~~Injection should be deferred temporarily if the volunteer has known abnormal laboratories that do not meet inclusion criteria and have not resolved, at the time of injection.~~ Injection may be temporarily deferred if the volunteer is clinically ill at the time of the injection visit and/or presents with fever at the time of the injection visit. A volunteer must be clinically well and afebrile for a minimum of a 24-hour consecutive period prior to injection.
Revised Text:	Injection may be temporarily deferred if the volunteer is clinically ill at the time of the injection visit and/or presents with fever at the time of the injection visit. A volunteer must be clinically well and afebrile for a minimum of a 24-hour consecutive period prior to injection.
Rationale:	If volunteer has laboratory abnormalities that do not meet inclusion criteria, volunteer would not be eligible.

Section Number & Title:	17.4 Criteria for Pausing the Study
Previous Text:	Enrolments and injections will be paused, and a safety review conducted by the SRB for any of the following criteria:
Revised Text:	Enrolments and injections will be paused, a formal study halt will be submitted to the MHRA as a substantial amendment, and a safety review conducted by the SRB for any of the following criteria:
Rationale:	Clarification requested by MHRA

Section Number & Title:	17.4 Criteria for Pausing the Study
Previous Text:	Following this review, the SRB will make a recommendation regarding the continuation or suspension of the vaccinations or the trial and communicate this decision immediately to the Sponsor. ~~The Sponsor then will inform the Principal Investigators without delay.~~
Revised Text:	Following this review, the SRB will make a recommendation regarding the continuation or suspension of the vaccinations or the trial and communicate this decision immediately to the Sponsor. If the SRB decision is to restart enrolments and injections, then a further substantial amendment will be submitted to the MHRA including all appropriate safety data and justification to restart. The Sponsor then will inform the Principal Investigator of the MHRA decision.
Rationale:	Clarification requested by MHRA

Section Number & Title:	Appendix A: Schedule of Procedures
Previous Text:	
Revised Text:	• Add Month 4 and Month 5 visits which were accidentally left off table • Remove Risk Reduction Counselling Visits at Month 3 and Month 9 since these should only take place at time points when HIV Test Counselling is also given. • Add Contraception Counselling at Screening, Month 0, Month 1 and Month 2
Rationale:	Correct mistakes in previous version of Schedule of Procedures

SUMMARY OF CHANGES TO PROTOCOL	
Protocol Title:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults
Protocol Number:	IAVI A003/ CHOP HVDDT 001
Regulatory Investigational Product Number:	MHRA Reference: 33372/0003/001-0001 Eudract Number: 2013-002268-14
Sponsor:	International AIDS Vaccine Initiative (IAVI) 125 Broad Street, 9th Floor New York, New York 10004 USA
Date of Protocol Version:	5 November 2013 2.0 22 July 2014 3.0 (Note that this version was submitted as an Urgent Safety Measure to MHRA)
Date of Amendment Version:	7 October 2014 4.0

The following are the primary reasons for the changes to the protocol:

- Increase the frequency of clinical follow-up to weekly through Day 56, then every two weeks through Day 84
- Add two new exclusion criteria and modify a third exclusion criteria
- Remove anogenital systems examination at screening
- Minor corrections and clarifications

Section Number & Title:	Sponsor Contact Information
Previous Text:	*Claudia Schmidt, MD, MPH, DTMH* *Medical Monitor* *International AIDS Vaccine Initiative* *125 Broad Street, 9th Floor* *New York, New York 10004* *USA*
Revised Text:	(Text deleted)
Rationale:	Change in sponsor staffing.

Section Number & Title:	Synopsis (Dosage Escalation); Section 5.0 Study Design (Dose Escalation)
Previous Text:	Safety and tolerability will be evaluated by the SRB over at least ~~42~~ days for volunteers in Group D.
Revised Text:	Safety and tolerability will be evaluated by the SRB over at least 56 days for volunteers in Group D.
Rationale:	Increase the frequency of clinical follow-up to weekly through Day 56 with corresponding change in cut-off date for safety data review by SRB.

Section Number & Title:	Section 5.4 Exclusion Criteria
Previous Text:	(Text added)
Revised Text:	PG9 level above the cut-off
Rationale:	Exclude potential volunteers who have a PG9 level above the assay cut-off at baseline. This would most likely be due to false-positive assay result.

Section Number & Title:	Section 5.4 Exclusion Criteria
Previous Text:	ECG with clinically significant findings or features including but not limited to: Conduction disturbance (atrio-ventricular or intra-ventricular condition, left or right bundle branch block, QT prolongation or AV block of any degree, ~~except first degree type 1 AV block which is allowed~~
Revised Text:	17. ECG with clinically significant findings or features including but not limited to: Conduction disturbance (atrio-ventricular or intra-ventricular condition, incomplete or complete left or right bundle branch block, QT prolongation (QTc interval of ≥450 for men) or AV block of any degree (PR interval >200ms)
Rationale:	Exclude potential volunteers with pre-existing cardiac conduction delays.

Section Number & Title:	Section 5.4 Exclusion Criteria
Previous Text:	(Text added)
Revised Text:	Body mass index ≥30.0
Rationale:	Exclude potential volunteers with obesity which may make ECG interpretation difficult.

Section Number & Title:	6.1 Screening Period
Previous Text:	If ~~the~~ screening visit occurs more than 42 days prior to the date of injection, all screening procedures must be repeated except that the comprehensive medical history may be replaced by an interim medical history and the Volunteer Information Sheet of the Informed Consent Document should be reviewed.
Revised Text:	If a screening visit occurs more than 42 days prior to the date of injection, all screening procedures must be repeated except that the comprehensive medical history may be replaced by an interim medical history and the Volunteer Information Sheet of the Informed Consent Document should be reviewed.
Rationale:	Wording revised as screening procedures may occur over more than one visit.

Section Number & Title:	Section 5.4 Exclusion Criteria
Previous Text:	General Physical Examination A general physical examination includes examination of skin, respiratory, cardiovascular, abdominal, neurological and musculoskeletal systems ~~and external ano-genital systems~~ at the time points indicated in the Schedule of Procedures (Appendix A).
Revised Text:	General Physical Examination A general physical examination includes examination of skin, respiratory, cardiovascular, abdominal, neurological and musculoskeletal systems at the time points indicated in the Schedule of Procedures (Appendix A).
Rationale:	Requirement for external genital examination was removed, since men at low risk of HIV and STIs are being recruited, instead of MSM at risk of HIV infection

Section Number & Title:	9.1.7 12-lead EKG
Previous Text:	A 12-lead ECG will be performed as described in the Schedule of Procedures to assess for evidence of cardiac adverse events. ECGs will be interpreted formally by a trained cardiologist ~~at the study centre~~.
Revised Text:	A 12-lead ECG will be performed as described in the Schedule of Procedures to assess for evidence of cardiac adverse events. ECGs will be interpreted formally by a trained cardiologist.
Rationale:	Formal ECG interpretation will be performed by a trained cardiologist not at the study centre but under contract by the Sponsor.

Section Number & Title:	9.1.10 Routine Laboratory Parameters
Previous Text:	(Text added)
Revised Text:	Also note that the Day 49 and Day 70 safety labs include Cardiac Troponin I only.
Rationale:	Clarification of safety lab schedule.

Section Number & Title:	Appendix A: Schedule of Procedures
Previous Text:	(Text added)
Revised Text:	• Add Day 49 (V07M) and Day 70 (V08M) visits • Remove symptom directed physical exam at final study visit • Add vital sign collection at Day 28, 56 and 84
Rationale:	Increase the frequency of clinical follow-up to weekly through Day 56 and every two weeks through Day 84; Correct mistakes in previous version of Schedule of Procedures

SUMMARY OF CHANGES TO PROTOCOL	
Protocol Title:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults
Protocol Number:	IAVI A003/ CHOP HVDDT 001
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Sponsor:	International AIDS Vaccine Initiative (IAVI) 125 Broad Street, 9th Floor New York, New York 10004 USA
Date of Protocol Version:	5 November 2013 2.0 22 July 2014 3.0 (Note that this version was submitted as an Urgent Safety Measure to MHRA) 7 October 2014 4.0
Date of Amendment Version:	24 February 2015 5.0

Section Number & Title:	Section 5.4 Exclusion Criteria
Previous Text:	Any of the following risk behaviour for HIV infection *within 6 months prior to injection:* • Unprotected sexual intercourse with a known HIV infected person or a partner known to be at high risk for HIV infection ~~or a casual partner (i.e., no continuing established relationship)~~ • ~~Unprotected anal intercourse with another man (either insertive or receptive)~~ • ~~Three or more sexual partners~~ • Engaged in sex work • Frequent excessive daily alcohol use or frequent binge drinking or chronic marijuana abuse or any other use of illicit drugs • History of newly-acquired syphilis, gonorrhoea, non-gonococcal urethritis, HSV-2, chlamydia, epididymitis, proctitis, lymphogranuloma venereum, chancroid, or hepatitis B

Revised Text:	4. Any of the following risk behaviour for HIV infection *within 6 months prior to injection:* • Unprotected sexual intercourse with a known HIV infected person or a partner known to be at high risk for HIV infection • Unprotected sex with three or more sexual partners • Engaged in sex work • Frequent excessive daily alcohol use or frequent binge drinking or chronic marijuana abuse or any other use of illicit drugs • History of newly-acquired syphilis, gonorrhoea, non-gonococcal urethritis, HSV-2, chlamydia, epididymitis, proctitis, lymphogranuloma venereum, chancroid, or hepatitis B
Rationale:	For a healthy male study population at low risk for HIV, the sexual behavior exclusion criteria were overly restrictive.

SUMMARY OF CHANGES TO PROTOCOL	
Protocol Title:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults
Protocol Number:	IAVI A003/ CHOP HVDDT 001
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Date of Protocol Version:	5 November 2013 2.0 22 July 2014 3.0 (Note that this version was submitted as an Urgent Safety Measure to MHRA) 7 October 2014 4.0
Date of Amendment Version:	6.0 12 Apr 2016

Note: This summary reflects changes between approved protocol IAVI A003/ CHOP HVDDT 001 v4.0 07 Oct 2014 and amendment version 6.0 12 Apr 2016. Formatting, minor grammar and word choice changes or clarifications are not indicated. Changes are noted in track changes.

<table>
<tr><td>1.</td><td>Section Number & Title:</td><td colspan="2">Synopsis: Study Design Table, and Table 5.0-1: Study Design</td></tr>
<tr><td></td><td>Previous Text:</td><td colspan="2">
<table>
<tr><th rowspan="2">Group</th><th>Number</th><th rowspan="2">Dose (vg)</th><th rowspan="2">Route</th><th rowspan="2">Month 0</th></tr>
<tr><th>Active/ Placebo</th></tr>
<tr><td>A</td><td>3/1</td><td>4x10¹² or Placebo</td><td>IM</td><td>X</td></tr>
<tr><td colspan="5">SRB review</td></tr>
<tr><td>B</td><td>3/1</td><td>4x10¹³ or Placebo</td><td>IM</td><td>X</td></tr>
<tr><td colspan="5">SRB review</td></tr>
</table>
</td></tr>
</table>

			C	3/1	$8x10^{13}$ or Placebo	IM	X
			SRB review to determine progression to *either* C1, *or* D and D1				
			C1	9/3	$8x10^{13}$ or Placebo	IM	X
			Total Number of Volunteers: 24 (18/6)				
			OR				
			D	3/1	$1.2x10^{14}$ or Placebo	IM	X
			SRB review				
			D1	3/1	$1.2x10^{14}$ or Placebo	IM	X
			Total Number of Volunteers: 20 (15/5)				
	Revised Text:		**Group**	**Number Active/ Placebo**	**Dose (vg)**	**Route**	**Month 0**
			A	3/1	$4x10^{12}$ or Placebo	IM	X
			SRB review				
			B	3/1	$4x10^{13}$ or Placebo	IM	X
			SRB review				
			C	3/1	$8x10^{13}$ or Placebo	IM	X
			SRB review to determine progression to *either* C1, *or* D and D1				
			C1	9/3	$8x10^{13}$ or Placebo	IM	X
			Total Number of Volunteers: 24 (18/6)				
			OR				
			D	3/1	$1.2x10^{14}$ or Placebo	IM	X
			SRB review				
			D1	4/1	$1.2x10^{14}$ or Placebo	IM	X
			Total Number of Volunteers: 21 (16/5)				

	Rationale:	The number of volunteers to be enrolled in Group D1 has changed from 4 to 5. 4 volunteers will be randomized to receive active IMP and 1 volunteer will be randomized to receive placebo. Adding one volunteer would allow us to collect the maximum amount of data in this small phase I study, since there are enough vials of active IMP remaining for a fifth volunteer to be enrolled in the high-dose group.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

2.	***Section Number & Title:***	**Synopsis: Study Population**
	Previous Text:	Principal exclusion criteria include confirmed HIV-1 or HIV-2 infection; significant acute or chronic disease; certain HIV high-risk criteria; clinically significant laboratory abnormalities; recent vaccination or receipt of a blood product; previous receipt of an HIV vaccine, AAV vector, monoclonal antibody product or polyclonal immunoglobulin; previous severe local or systemic reactions to vaccination or infusions; history of severe allergic reactions; serum antibodies to AAV1 at baseline.
	Revised Text:	Principal exclusion criteria include confirmed HIV-1 or HIV-2 infection; significant acute or chronic disease; certain HIV high-risk criteria; clinically significant laboratory abnormalities; recent vaccination or receipt of a blood product; previous receipt of an HIV vaccine, AAV vector, monoclonal antibody product or polyclonal immunoglobulin; previous severe local or systemic reactions to vaccination or infusions; history of severe allergic reactions; serum antibodies to AAV1, AAV2, and PG9 at baseline.
	Rationale:	The principal exclusion criteria include the presence of serum antibodies to AAV2 and PG9. These were inadvertently omitted from the study population description in the previous protocol version.
	Impact on other study documents:	The study operations manual will be updated. This addition is already included in the lab analytical plans for the study.

3.	***Section Number & Title:***	**Synopsis: Formulations, Volumes, and Routes of Injections and Table 8.1.2-1 Formulation of Investigational Medicinal Product**

Previous Text:

Active/ Placebo	Group	Dose Con-centration	Total Volume in Vial (mL)	Directions & Total Volume to Inject (mL) Intramuscularly	Total Number of Vials
rAAV1-PG9DP	A	$4x10^{12}$ vg	0.6 mL	1mL (after 1:10 dilution of IMP with excipient)	1 vial IMP +1 vial excipient
	B	$4x10^{13}$ vg		1mL (after drawing 0.5mL from each of 2 vials of IMP)	2 vials IMP
	C &C1	$8x10^{13}$ vg		2mL (1mL in each deltoid after drawing 0.5mL from each of 4 vials of IMP)	4 vials IMP
	D &D1	$1.2x10^{14}$ vg		3mL (1.5mL in each deltoid after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
Placebo	A & B	N/A	1.1 mL	1mL	1 vial excipient
	C &C1			2ml (1mL in each deltoid after drawing 1.0mL from each of 2 vials of excipient)	2 vials excipient
	D &D1			3mL (1.5mL in each deltoid after drawing 1.0mL from each of 3 vials of excipient)	3 vials excipient

Revised Text:

Active/ Placebo	Group	Dose Con-centration	Total Volume in Vial (mL)	Directions & Total Volume to Inject (mL) Intramuscularly	Total Number of Vials
rAAV1-PG9DP	A	$4x10^{12}$ vg	0.6 mL	1mL (after 1:10 dilution of IMP with excipient)	1 vial IMP +1 vial excipient
	B	$4x10^{13}$ vg		1mL (after drawing 0.5mL from each of 2 vials of IMP)	2 vials IMP
	C &C1	$8x10^{13}$ vg		2mL (1mL in each deltoid after drawing 0.5mL from each of 4 vials of IMP)	4 vials IMP
	D	$1.2x10^{14}$ vg		3mL (1.5mL in each deltoid after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
	D1	$1.2x10^{14}$ vg		3mL (3 injections of 0.5mL each in each quadriceps, after drawing 0.5mL from each of 6 vials of IMP)	6 vials IMP
Placebo	A & B	N/A	1.1 mL	1mL	1 vial excipient
	C &C1			2ml (1mL in each deltoid after drawing 1.0mL from each of 2 vials of excipient)	2 vials excipient
	D			3mL (1.5mL in each deltoid after drawing 1.0mL from each of 3 vials of excipient)	3 vials excipient
	D1			3mL (3 injections of 0.5mL each in each quadriceps, after drawing 0.5mL 6 times from a total of 3 vials of excipient)	3 vials excipient

Rationale: While the total dose (vector genomes) of IMP is the same in Groups D and D1, we have increased the number of injections from two to six and changed the location of injections from the

		deltoid to the quadriceps for Group D1 volunteers in order to assess if increasing the number of injections and the amount of muscle tissue exposed to the IMP will affect the level of PG9 expression. Each volunteer will receive six 0.5mL injections of $4x10^{13}$ vg/mL, divided evenly over both quadriceps muscles in the thighs.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

4.	***Section Number & Title:***	**Contact Information: IMP Developer**	
	Previous Text:	Philip R. Johnson, MD Chief Scientific Officer & Executive Vice President The Children's Hospital of Philadelphia Abramson Research Center, Room 1216B 3615 Civic Center Boulevard Philadelphia, PA 19104-4318 USA	Phone: + 1 267 426 0351 Fax : + 1 267 426 0363 E-mail : johnsonphi@email.chop.edu
	Revised Text:	Philip R. Johnson, MD Senior Advisor, R&D Executive Office International AIDS Vaccine Initiative 125 Broad Street, 9th Floor New York, New York 10004 USA	Phone : +1-212-847-1061 Fax : E-mail : PJohnson@iavi.org
	Rationale:	Phil Johnson has joined IAVI as the Senior Advisor to the R&D Executive Office and his contact information has changed.	
	Impact on other study documents:	No impact on other study documents.	

5.	***Section Number & Title:***	**Contacts: Clinical Research Centres**	
	Previous Text:	N/A	
	Revised Text:	Site 47 NIHR Southampton Wellcome Trust Clinical	Phone: +44 23 8120 6455 Email: R.C.Read@soton.ac.uk

		Research Facility Professor Rob Read Principal Investigator Southampton Centre for Biomedical Research Mailpoint 218 University Hospital Southampton NHS Foundation Trust Southampton General Hospital Tremona Road Southampton, SO16 6Y	
	Rationale:	The NIHR Southampton Wellcome Trust Clinical Research Facility has been added as a second clinical research centre in the study. The addition of the second site was approved by the MHRA on 05 May 2015 (Amendment 5) and by the NRES ethics committee (South Central – Oxford A) on 08 June 2015.	
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.	

6.	***Section Number & Title:***	**Section 2.5: Rationale for the vector doses proposed**
	Previous Text:	Preclinical animal studies show that a dose of $4x10^{12}$ vg/kg of rAAV1-immunoadhesin is required in mice for transgene expression, and in non-human primates for efficacy[13]. This is equivalent to $3x10^{14}$ vg in a 70 kg human. Therefore, the target human dose should be close to $3x10^{14}$ vg. Manufacture of concentrations higher than $3x10^{13}$ vg/mL are likely to cause virus aggregation. Therefore, the highest doses in this study, $8x10^{13}$ vg/mL and $1.2x10^{14}$ vg/mL will be administered by two 1.0 mL injections of $4x10^{13}$ vg/mL (in different deltoids), and two 1.5 ml injections of $6x10^{13}$ vg/mL (in different deltoids) respectively(at the same time point).
	Revised Text:	Preclinical animal studies show that a dose of $4x10^{12}$ vg/kg of rAAV1-immunoadhesin is required in mice for transgene expression, and in non-human primates for efficacy[13]. This is equivalent to $3x10^{14}$ vg in a 70 kg human. Therefore, the target human dose should be close to $3x10^{14}$ vg. Manufacture of concentrations higher than $3x10^{13}$ vg/mL are likely to cause virus aggregation. Therefore, the highest doses in this study, $8x10^{13}$ vg and $1.2x10^{14}$ vg will be administered by two 1.0 mL injections of $4x10^{13}$ vg/mL (in different deltoids), and two 1.5 ml or six 0.5mL injections of $4x10^{13}$ vg/mL (divided evenly over both deltoids (Group D) or quadriceps muscles in the thighs (Group D1)

		respectively (at the same time point).
	Rationale:	See previous explanation above in item #3.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

7.	***Section Number & Title:***	**Section 2.5.1: Rationale for quadriceps administration and muscle biopsy**
	Previous Text:	N/A
	Revised Text:	Rationale for quadriceps administration and muscle biopsy *Quadriceps administration* IIt is unknown if administering the IMP intramuscularly in a single injection or multiple injections affects the level of gene expression., It is also unknown if the size of the muscle in which the IMP is administered affects the level of gene expression. Therefore in the highest dose groups, where all subjects will receive the same total dose, those in Group D will receive IMP or placebo in 2 injections, one in each deltoid, and those in Group D1 will receive the same amount of IMP but divided over 6 injections, 3 in each quadriceps, a larger muscle than the deltoid. Taken together, this would result in a greater number of muscle cells, in larger muscles, being exposed to AAV1-PG9DP. The total dose in groups D and D1 will remain the same, i.e., 1.2x10E14 vg. The supportive preclinical toxicology study administered IMP into the leg muscles (gastrocnemius) of mice. Most AAV gene therapy studies with intramuscular administration have administered AAV vectors with multiple injections in the vastus lateralis muscles33,41,45. *Muscle biopsy* All volunteers in group D1 will undergo a muscle biopsy 3 months and 12 months after administration of IMP at one of the six sites where the IMP was injected into the quadriceps. Muscle biopsy will be analysed to determine if the vector genome and/or PG9 is present in the tissue at the site of IMP delivery.
	Rationale:	See previous explanation above in item #3. Additionally: Volunteers in Group D1 will provide a muscle biopsy sample in order to measure presence of AAV and PG9 expression in situ.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

8.	***Section Number & Title:***	**5.0: Study Design – Dose Escalation**

	Previous Text:	In the study, up to 24 volunteers will be randomized in a 3:1 ratio of active product to placebo.
	Revised Text:	In the study, all groups will be randomized in a 3:1 ratio of active product to placebo with the exception of Group D1, where 5 volunteers will be randomized in a 4:1 ratio of active product to placebo.
	Rationale:	See previous explanation above in item #1.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

9.	***Section Number & Title:***	**Section 5.3: Inclusion Criteria**
	Previous Text:	N/A
	Revised Text:	Group D1 only: Willing to undergo muscle biopsy 3 months and 12 months after receiving rAAV1-PG9DP or placebo.
	Rationale:	All Group D1 volunteers will provide a muscle biopsy sample at months 3 and 12 post-injection in order to assess presence of AAV and PG9 expression in the muscle.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

10.	***Section Number & Title:***	**Section 5.4: Exclusion Criteria**
	Previous Text:	- Cardiac troponin I > 0.04 ug/mL
	Revised Text:	- Cardiac troponin I > 0.04 ng/mL
	Rationale:	The units indicated for cardiac troponin were incorrect in the previous protocol version. The correct units for cardiac troponin in the exclusion criteria are ng/mL.
	Impact on other study documents:	The study operations manual will be updated.

11.	***Section Number & Title:***	**Section 5.4: Exclusion Criteria**
	Previous Text:	7. Anti-AAV1 antibody level above the cut-off
	Revised Text:	7. Anti-AAV1 and anti-AAV2 antibody level above the cut-off
	Rationale:	See previous explanation above in item #2.
	Impact on	The study operations manual will be updated. This addition is

	other study documents:	already included in the lab analytical plans for the study.

12.	***Section Number & Title:***	**Section 7.5: Specimens**
	Previous Text:	N/A
	Revised Text:	Muscle tissue biopsy will be collected from all volunteers in group D1 at 3 months and 12 months after administration of IMP at one of the six sites where the IMP was injected into the quadriceps.
	Rationale:	See previous explanation above in item #7.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

13.	***Section Number & Title:***	**Section 8.1.1: rAAV1-PG9DP Vector**
	Previous Text:	The volume of administration is dependent upon the group assignment, but will be either a single administration of 1 mL (Groups A and B) or two separate 1 mL administrations for a total of 2 mL administered to the volunteer (Groups C and C1), or two separate 1.5 ml administrations for a total of 3 mL (Groups D and D1).
	Revised Text:	The volume of administration is dependent upon the group assignment, but will be either a single administration of 1 mL (Groups A and B) or two separate 1 mL administrations for a total of 2 mL administered to the volunteer (Groups C and C1), or two separate 1.5 ml (Group D), or six separate 0.5 ml (Group D1), administrations for a total of 3 mL (Groups D and D1).
	Rationale:	See previous explanation above in item #3.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

14.	***Section Number & Title:***	**Section 8.3: Preparation of Investigational Medicinal Product**
	Previous Text:	Syringes or other components in direct contact with IMP will be disposed of in a biohazard container and disposed of according to the Surrey CRC GM Risk Assessment.
	Revised Text:	Syringes or other components in direct contact with IMP will be disposed of in a biohazard container and disposed of according

		to the clinical research centre-specific GM Risk Assessment.
	Rationale:	See previous explanation above in item #5.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

15.	***Section Number & Title:***	**Section 8.4: Administration of Investigational Medicinal Product**
	Previous Text:	The preferred site for administration is the deltoid muscle of the non-dominant upper arm (for example, injection in the left arm if the volunteer uses mainly the right arm), unless contraindicated for another reason. In groups with 2mL volume, two injections of 1.0mL each will be given in each deltoid. In groups with 3mL volume, two injections of 1.5mL each will be given in each deltoid.
	Revised Text:	The preferred site for administration is the deltoid muscle of the non-dominant upper arm (for example, injection in the left arm if the volunteer uses mainly the right arm) for groups A, B, C and D, unless contraindicated for another reason. For group D1 the site for administration is the vastus lateralis muscles in the quadriceps muscles of the upper legs. In groups with 2mL volume, one injection of 1.0mL each will be given in each deltoid. In groups with 3mL volume, one injection of 1.5mL each will be given in each deltoid for Group D, or three injections of 0.5mL each, will be given in each vastus lateralis for Group D1. In group D1, the administration will be conducted under ultrasound guidance.
	Rationale:	See previous explanation above in item #3.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

16.	***Section Number & Title:***	**9.1.8 Muscle Biopsy**
	Previous Text:	In the event of an extensive local inflammatory reaction, or evidence of systemic inflammatory or immune disease that requires further evaluation to understand the potential relationship to IMP, muscle biopsy of the deltoid muscle in which the IMP was administered may be performed by an experienced operator. Volunteers will be informed about this potential procedure in the study informed consent document. If a muscle biopsy is indicated, volunteers would provide informed consent for the procedure itself at that time.

	Revised Text:	In the event of an extensive local inflammatory reaction, or evidence of systemic inflammatory or immune disease that requires further evaluation to understand the potential relationship to IMP, biopsy of the muscle in which the IMP was administered may be performed by an experienced operator. Volunteers will be informed about this potential procedure in the study informed consent document. If a muscle biopsy is indicated as a safety assessment, volunteers would provide informed consent for the procedure itself at that time.
	Rationale:	The language in this section has been generalized as a biopsy may be required from the deltoid or quadriceps muscle in the event that a safety assessment is needed.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual will be updated as well.

17.	***Section Number & Title:***	**9.1.11: Specific screening tests:**
	Previous Text:	In addition to the tests listed in the table above, Screening Labs will include: • Anti-AAV1 antibodies • Gamma glutamyl transferase (GGT)
	Revised Text:	In addition to the tests listed in the table above, Screening Labs will include: • Anti-AAV1 and anti-AAV2 antibodies • PG9 antibodies • Gamma glutamyl transferase (GGT)
	Rationale:	See previous explanation above in item #2.
	Impact on other study documents:	The study operations manual will be updated. This addition is already included in the lab analytical plans for the study.

18.	***Section Number & Title:***	**9.2.5 Muscle Biopsies**
	Previous Text:	N/A
	Revised Text:	**Muscle Biopsies** All volunteers in group D1 will undergo a muscle biopsy 3 months and 12 months after administration of IMP at one of the sites where the IMP was injected. Microscopy and immunohistochemical staining will be performed on biopsy tissue to assess in situ parameters at the site of IMP

		administration, such as PG9 expression, inflammation, fibrosis. AAV1 in situ presence of rAAV1-PG9DP: A DNA PCR assay using primers specific to the PG9DP insert will be performed on biopsy tissue to determine if the vector genome is present in the tissue at the site of IMP delivery.
	Rationale:	Volunteers in Group D1 will provide a muscle biopsy sample in order to measure PG9 expression in situ.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

19.	***Section Number & Title:***	**Appendix A: Schedule of Procedures**
	Previous Text:	N/A
	Revised Text:	The following changes have been made to the Schedule of Procedures: 1. A muscle biopsy has been added for Group D1 volunteers at 3 and 12 months post-injection. 2. Additional visits have been added after each visit where a biopsy is collected. The new visits are at study day 98 (visit 9m) and study day 350 (visit 14m.) Procedures at visits 9m and 14m are indicated with "X" marks throughout the table. These visits may be conducted by telephone or the volunteers may come into the clinic if they prefer or if there is a medical reason for an in-clinic assessment. 3. PG9 antibody and anti-AAV2 antibody assessments have been added to the screening visit. These assessments were inadvertently omitted in the previous protocol version but they are required at screening.
	Rationale:	Volunteers in Group D1 will provide a muscle biopsy sample in order to measure PG9 expression in situ. Additional visits have been added after each biopsy visit in order to assess the volunteers' health after the biopsy procedure. Corrections were made to add assays that were inadvertently omitted in the previous protocol version.
	Impact on other study documents:	Updates are required in the site-specific informed consent documents (ICDs.) The revised ICDs will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plans will be updated as well.

SUMMARY OF CHANGES TO PROTOCOL	
Protocol Title:	A Phase 1, randomized, blinded, dose-escalation study of rAAV1-PG9DP recombinant AAV vector coding for PG9 antibody in healthy male adults
Protocol Number:	IAVI A003/ CHOP HVDDT 001
Regulatory Investigational Product Number:	MHRA Reference: 33372/0003/001-0001 Eudract Number: 2013-002268-14
Sponsor:	International AIDS Vaccine Initiative (IAVI) 125 Broad Street, 9th Floor New York, New York 10004 USA
Date of Protocol Version:	2.0, 5 November 2013 3.0, 22 July 2014 (Note that this version was submitted as an Urgent Safety Measure to MHRA) 4.0, 7 October 2014 5.0, 05 March 2015 (not approved by ethics committee and therefore not implemented) 6.0, 11 Apr 2016
Date of Amendment Version:	7.0, 06 April 2017

Note: This summary reflects changes between approved protocol IAVI A003/ CHOP HVDDT 001 version 6.0 11 Apr 2016 and amendment version 7.0 06 Apr 2017. Formatting, minor grammar and word choice changes or clarifications are not indicated.

1.	***Section Number & Title:***	**Contact Information: Clinical Research Centre Contacts**	
	Previous Text:	Professor David JM Lewis, MD Principal Investigator Clinical Research Centre University of Surrey, Guildford, GU2 7XP UK	Phone: +44 1483689797 Email: d.j.lewis@surrey.ac.uk
	Revised Text:	Dr Daryl Bendel, MBChB MBA Dip Pharm Med MFPM Principal Investigator Consultant in Pharmaceutical and Translational Medicine School of Biosciences and	Phone: +44 7900682275 Email: daryl@xideasolutions.com

		Medicine Faculty of Health and Medical Sciences Surrey Clinical Research Centre University of Surrey, Guildford, GU2 7XP UK	
	Rationale:	The Principal Investigator for A003 has changed from Prof. David Lewis to Dr. Daryl Bendel. The clinical research centre contact information has been updated accordingly.	
	Impact on other study documents:	Updates are required to the site-specific informed consent document (ICD.) The revised ICD will be submitted to ethics and regulatory committees per local guidelines. The study operations manual and lab analytical plan will be updated as well. The GP letter that is provided to the volunteer's GP (General Practitioner) also needs to be updated and will also be submitted to the ethics committee for approval.	

2.	***Section Number & Title:***	**Contact Information: Clinical Research Centre Contacts**	
	Previous Text:	Site 47 NIHR Southampton Wellcome Trust Clinical Research Facility	
		Professor Rob Read Principal Investigator Southampton Centre for Biomedical Research Mailpoint 218 University Hospital Southampton NHS Foundation Trust Southampton General Hospital Tremona Road Southampton, SO16 6Y	Phone: +44 23 8120 6455 Email: R.C.Read@soton.ac.uk
	Revised Text:	Removed contact information for the Southampton CRF.	
	Rationale:	The A003 trial completed enrollment before the Southampton CRF could enroll volunteers and therefore the site will not be participating in the trial. Accordingly the contact information for the Southampton CRF has been removed from the protocol.	
	Impact on other study documents:	The study operations manual will need to be updated to remove the Southampton CRF.	