CONFIDENTIAL

Ad4HIV

A Phase I Single-Blind Trial Investigating Immunisation Strategies Using Ad4-EnvCN54, MVA-CN54 and CN54gp140/MPLA Combinations in order to Maximise Antibody Responses to Human Immunodeficiency Virus

Version: 7.3

Date: 01 Nov 2019

EUDRACT: 2016-000052-94

REC REF: 17/LO/0302

IRAS ID: 186866

Authorised by:	
Name:	**Dr Katrina Pollock**
Role:	**Principal Investigator**
Signature:	
Date:	**04 Nov 2019**
Name:	
Role:	**Sponsor – Imperial College London**
Signature:	
Date	

1 GENERAL INFORMATION

This protocol describes the Ad4HIV trial and provides information about procedures for entering participants into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial.

COMPLIANCE

The trial will be conducted at the clinical site in compliance with the approved protocol, the Principles of Helsinki (2008), the principles of Good Clinical Practice (GCP), Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act (DPA number: Z5886415), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

SPONSOR

Imperial College London
Room 221
Level 2, Medical School Building
Norfolk Place
London
W2 1PG

COLLABORATING ORGANISATION

Surrey Clinical Research Centre, University of Surrey, Egerton Road, Guildford GU2 7XP, UK

VACCINES WILL BE SUPPLIED BY IMPERIAL COLLEGE LONDON AND MANUFACTURED AS BELOW:

MVA-CN54: Bavarian Nordic, Hejreskovvej 10 A, Kvistgård, 3490 Denmark

CN54gp140 / MPLA: Polymun Scientific Immunbiologische Forschung GmbH, Honaustrasse 99, 3400 Klosterneuburg, Austria

Ad4-EnvCN54 encoding CN54: PaxVax, Redwood City, California, USA

GMP STORAGE, LABELLING, PACKAGING, QP RELEASE AND DISTRIBUTION OF VACCINE TO SITES

PCI, Biotech House, Central Park

Western Avenue, Bridgend Industrial Estate

Bridgend CF31 3RT

UK

FUNDING

UK MRC

AUTHORISATIONS AND APPROVALS

This protocol will not be implemented until approvals have been received from all the necessary regulatory and ethical bodies.

TRIAL REGISTRATION

This trial has been registered in the EudraCT database where it is identified as 2016-000052-94.

SAE REPORTING AND IMPORTANT AE NOTIFICATION
Within 24 hours of becoming aware of an SAE or important AE, please complete the SAE form, and email the coordinating centre (Clinical Trials Manager) to notify on:
Ad4HIV_trial@imperial.ac.uk

Additionally, please email a completed paper SAE form to the Imperial Joint Research Compliance Office (JRCO) on:
Email jrco.ctimp.team@imperial.ac.uk
SAE REPORTING AND IMPORTANT AE NOTIFICATION

POSSIBLE SERIOUS BREACH NOTIFICATION
Within 24 hours of becoming aware of a possible serious breach, please complete the Protocol Deviation Form, and email the coordinating centre (Clinical Trials Manager) to notify on:
Ad4HIV_trial@imperial.ac.uk

Additionally, please email a completed paper form to the Imperial Joint Research Compliance Office (JRCO) on:
Email jrco.ctimp.team@imperial.ac.uk

TRIAL ADMINISTRATION

Please direct all queries to the Clinical Trial Manager at the Coordinating Center in the first instance; clinical queries will be passed to the Prinicpal Investigator.

COORDINATING CENTER – IMPERIAL COLLEGE LONDON (ICL)

Imperial College London **Group of Mucosal Infection & Immunity**
Department of Medicine St Mary's Campus Imperial College London Wright Fleming Wing Norfolk Place London W2 1PG

IMPERIAL COLLEGE LONDON - STAFF

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Project Manager	Jennifer O'Connor	Tel: +44 (0)207 594 2510	jennifer.oconnor@imperial.ac.uk
Chief Scientific Investigator:	Prof Robin Shattock	Tel: +44 (0)207 594 5206	r.shattock@imperial.ac.uk

Clinical Trial Site

NIHR Imperial CRF Imperial Centre for Translational and Experimental Medicine (L-Block) Hammersmith Hospital Imperial College Healthcare NHS Trust Du Cane Road London W12 0HS Fax: 0203 313 1763

Chief/Principal Investigator (PI)

Name	Telephone Number	Email Address
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Trial Monitor

Ms Sia Gravani	Tel: +44 (0)203 313 6199	a.gravani@nhs.net

Pharmacy at Trial Site

Mrs Regina Storch	Tel: +44 (0)203 313 4333	regina.storch@nhs.net

Laboratory investigators – Shattock Laboratory at Imperial College London

Dr Hannah Cheeseman	Tel: +44 (0)20 7594 2574	hannah.cheeseman@imperial.ac.uk

Data Management Center & statistical analysis

Name	Telephone Number	Email Address
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Surrey Clinical Research Centre University of Surrey Guilford Surrey GU2 7XH		

2 SUMMARY OF TRIAL

Summary Information Type	Summary Details
ACRONYM (or short Title of Trial)	Ad4HIV
Long Title of Trial	**Ad4HIV:** A Phase I Single-Blind randomised trial investigating immunisation strategies using Ad4-EnvCN54, MVA-CN54 and CN54gp140/MPLA combinations in order to maximise antibody responses to Human Immunodeficiency Virus
Version	6.0
Date	20 Dec 2018
EudraCT	2016-000052-94
REC Reference	17/LO/0302
IRAS ID	186866
Rationale and Methodology	This is a randomised two part Phase I study which will explore the impact of different boosting options (MVA-C and protein) for oral Adenovirus serotype 4 vector prime expressing HIV-1 CN54 envelope (Ad4-EnvCN54) designed to optimize systemic and mucosal antibody response. The participants will be blind to the dose administered and the laboratory staff will be blind to the regimen throughout. **Part 1** is exploratory and designed to select conditions capable of promoting enhanced systemic and mucosal B cell responses in a limited number of participants. **Part 2** is dependent upon Part 1 and is designed to study groups selected on performance in part 1 in an expanded number of subjects. Data from both stages will be combined for safety and immunological analyses.
Investigational Products	1) Ad4-EnvCN54 encoding CN54 envelope 2) MVA-C encoding CN54 gp120 3) CN54gp140: recombinant HIV-1 subtype C trimeric envelope protein adjuvanted in monophosphopyl lipid A (MPLA) and lyposomes 4) Placebo 1: Capsule administered orally to match Ad4-EnvCN54 5) Placebo 2: Buffered saline administered by I.M needle injection

<table>
<tr><th>Summary Information Type</th><th>Summary Details</th></tr>
<tr><td>Type of Participants to be Studied</td><td>Healthy male or female adults, 18 to 50 years of age, at low risk for HIV infection, who are available for the duration of the trial. They must be willing to undergo HIV testing, use an effective method of contraception. They must, 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, prexisting Ad4 serum neutralising antibodies, pregnancy and lactation, significant acute or chronic disease, clinically significant laboratory abnormalities, recent vaccination or receipt of a blood product, previous receipt of an HIV vaccine, previous severe local or systemic reactions to vaccination or history of severe allergic reactions.</td></tr>
<tr><td>Study Design</td><td>Part 1
The study will enrol 48 participants to Part 1. Recruitment will select potential participants who are willing to undergo serial blood draws and mucosal secretion sampling. Additional participants may be enrolled to replace any early withdrawals.

Vaccine & Immunisation Schedule for Part 1 & Part 2
Table 1
<table>
<tr><th>Group</th><th>N</th><th>Month 0</th><th>Month 3</th><th>Month 6</th></tr>
<tr><td>A</td><td>6</td><td>Oral placebo</td><td>CN54gp140
+Oral placebo
+I.M placebo</td><td>CN54gp140
+Oral placebo
+I.M placebo</td></tr>
<tr><td>B</td><td>6</td><td>Ad4-EnvCN54</td><td>CN54gp140
+Oral placebo
+I.M placebo</td><td>CN54gp140
+Oral placebo
+I.M placebo</td></tr>
<tr><td>C</td><td>6</td><td>Ad4-EnvCN54</td><td>CN54gp140
+ Ad4-EnvCN54
+ I.M placebo</td><td>CN54gp140
+Oral placebo
+ I.M placebo</td></tr>
<tr><td>D</td><td>6</td><td>Ad4-EnvCN54</td><td>CN54gp140
+ Ad4-EnvCN54
+I.M placebo</td><td>CN54gp140
+ Ad4-EnvCN54
+I.M placebo</td></tr>
<tr><td>E</td><td>6</td><td>Oral placebo</td><td>CN54gp140
+MVA-CN54
+Oral placebo</td><td>CN54gp140
+MVA-CN54
+Oral placebo</td></tr>
<tr><td>F</td><td>6</td><td>Ad4-EnvCN54</td><td>CN54gp140
+MVA-CN54
+Oral placebo</td><td>CN54gp140
+MVA-CN54
+Oral placebo</td></tr>
<tr><td>G</td><td>6</td><td>Ad4-EnvCN54</td><td>CN54gp140
+MVA-CN54</td><td>CN54gp140
+MVA-CN54</td></tr>
</table></td></tr>
</table>

			+ Ad4-EnvCN54	+Oral placebo
H	**6**	Ad4-EnvCN54	CN54gp140 +MVA-CN54 + Ad4-EnvCN54	CN54gp140 +MVA-CN54 + Ad4-EnvCN54
	48			

Participants will be randomised for recruitment into groups A-H in Part 1. The number of Ad4-EnvCN54 administrations combined with either gp140 protein boosts (A-D) or co-administration of gp140 with MVA-CN54 (E-H) will vary. Groups A (CN54gp140 alone) and E (MVA-CN54/CN54gp140 alone) will serve as control groups to assess the added benefit of the Ad4 vector. Following completion of Part 1, each vaccine group (A-H) will be assessed for number of mucosal responders (mucosal antibody (rectal/genital/oral) as measured by ELISA), and titre (mucosal and systemic) of antigen specific binding antibody. The primary immunogenicity endpoint will be Week 26; Visit 9. The vaccine groups and regimes (one from B–D and one from F–H) that have the best positive response at this timepoint will be expanded in Part 2 of the study.

Based on data from the MUCOVAC2 study, in which participants received 3 intramuscular doses of CN54gp140 with GLA-AF adjuvant, we anticipate that there will be no mucosal responders in group A (although there will be detectable systemic IgG responses). Enrollment through to completion of the primary immunological assessment for each group will be 7 months.

Vaccine Schedule for Part 2

The study will enrol an additional 12 participants to Part 2. The best regimes from A-D (Ad4-EnvCN54 / CN54gp140) and F-H (Ad4-EnvCN54 / MVA-CN54 / CN54gp140) will be expanded to n=12. Immunogenicity data will be analysed in 32 participants at week 26 (Primary Immunogenicity Endpoint). The study design for Part 2 will be the same as that used for Part 1 (Table 1: Vaccine and Immunisation Schedule) for the best responding groups only.

Selection will be based on total number of responders. In the event that more than one group has the same number of responders, mucosal antibody titre will be used to rank groups to determine which to take forward to Part 2. Should mucosal titers be indistinguishable between groups, then serum antibody levels will be taken into consideration in the selection of groups to progress to Part 2. The criterion for a positive mucosal response, and methods for ranking groups on the basis of mucosal and serum antibody titers, will be specified in the Laboratory Analysis Plan (LAP). If any group is associated with unacceptable product related adverse events, it will be discontinued from further study.

The decision to progress any group to Part 2 will be taken by the Trial Management Group.

Summary	**Summary Details**

<table>
<tr><th>Information Type</th><th></th></tr>
<tr><td>Formulations</td><td>

Ad4-EnvCN54 at the dosage of 10^{10} VP will be administered orally
MVA-CN54 at the dosage of 1.0 x 10^8 $TCID_{50}$ will be administered by IM needle injection
CN54gp140 at the dosage of 100μg will be administered with 5μg of MPLA in a liposomal formuation and administered by IM needle injection
Placebo 1: Capsule administered orally
Placebo 2: Buffered saline administered by I.M needle injection

<table>
<tr><th>Vaccine</th><th>Dosage Level(s)</th><th>Total Administered Volume (per site)</th><th>Route of Administration</th></tr>
<tr><td>Ad4-EnvCN54</td><td>10^{10}VP</td><td>Lyophilized powder in capsule</td><td>Oral</td></tr>
<tr><td>MVA-CN54</td><td>1.0 x 10^8 $TCID_{50}$</td><td>0.5ml</td><td>I.M</td></tr>
<tr><td>CN54gp140 / MPLA + Liposome</td><td>100μg / 5μg</td><td>0.4 mL</td><td>I.M</td></tr>
<tr><td>Placebo[1]</td><td>-</td><td>Powder in capsule</td><td>Oral</td></tr>
<tr><td>Placebo[2]</td><td>-</td><td>0.5ml</td><td>I.M</td></tr>
</table>
</td></tr>
<tr><td>Site:</td><td>Clinical investigations will be carried out at the NIHR Imperial CRF, Hammersmith Hospital, Imperial College Healthcare NHS Trust.

Immunogenicity laboratory investigations will be performed in the Department of Infection and Immunity at Imperial College London, and safety laboratory investigations will be performed at Imperial College Healthcare NHS Trust.</td></tr>
<tr><td>Aim:</td><td>To explore the impact of different boosting options (MVA-C and protein) for oral Adenovirus serotype 4 vector prime expressing HIV-1 CN54 envelope (Ad4-EnvCN54) designed to optimize systemic and mucosal antibody response.</td></tr>
<tr><td>Primary Objectives</td><td>Part 1 & Part 2:
To evaluate the immunogenicity, safety and tolerability of oral Ad4 vector expressing HIV-1 CN54 envelope (Ad4-EnvCN54) when combined with different boosting options (MVA-CN54 and CN54gp140/MPLA) designed to optimise systemic and mucosal responses.</td></tr>
</table>

Summary Information Type	Summary Details
Secondary Objectives	***Immunogenicity*** **Part 1 & Part 2:** To select conditions capable of promoting enhanced B cell responses based on

	the following HIV-specific immune responses: • Kinetics and magnitude of induced HIV-specific serum binding antibodies • Kinetics and magnitude of induced HIV-specific mucosal binding antibodies (cervico-vaginal for women, semen for men and rectal and nasal for all)
Exploratory Objectives	***Immunogenicity*** **Part 1 & Part 2** To assess and characterize the following HIV-specific immune responses: • HIV-specific neutralizing antibodies in the systemic compartment. • Frequency and titer of serum binding antibodies to other HIV Env antigens (alternative clades) by ELISA or other assays. • Characterisation of non-neutralising antibody function including Antibody Dependent Cellular Cytotoxicity (ADCC) and Antibody Dependent Cellular Viral Inhibition (ADCVI), Antibody dependent phagocytosis (ADCP), viral capture and aggregation assays. • Anti-vector mediated immune responses i.e. anti-MVA-CN54 and anti_Ad4 antibody responses and functionality if present • HIV-specific T-cell-mediated responses by ICS or Elispot analysis. • Epitope mapping of B- and T-cell responses. • Ex vivo analysis of colorectal biopsies (Part 2 participants only) • PBMC ex-vivo HIV suceptibility assay

Summary Information Type	**Summary Details**
Primary Endpoints Measure(s)	**Part 1 & Part 2:** ***Immunogenicity*** Antigen specific mucosal (cervico-vaginal for women, semen for men, and rectal

	and nasal for all) antibody (µg/ml) responses two weeks after the final immunisation. ***Safety and Tolerability:*** • Proportion of participants with severe or greater (Grades 3-4) adverse reactions during the study • Proportion of participants with vaccine-related serious adverse events (SAEs) throughout the study period
Secondary Endpoints Measure(s)	***Immunogenicity:*** **Part 1 & Part 2** To assess the kinetics of immune responses elicited by each of the vaccine regimens: • Frequency of serum and mucosal binding antibodies to HIV CN54gp140 antigen measured by binding ELISA.

Summary Information Type	**Summary Details**
Exploratory Endpoints Measure(s)	***Immunogenicity:*** **Part 1 & Part 2** • Frequency and magnitude of HIV-gp140 specific B-cell-mediated responses in the systemic compartment measured by B-cell ELISPOT. • Frequency, titer and avidity of serum binding antibodies to other HIV Env antigens (alternative clades) by ELISA or other assays. • Frequency and titer of serum neutralising antibodies to homologous virus, and, if warranted a wider a panel of viruses representing different clades.

	• Longevity of serum and mucosal responses at 12 months following final immunization • Frequency and magnitude of HIV-specific T-cell mediated responses measured by T-cell and ICS (Intracellular Cytokine Staining). • PBMC ex-vivo HIV suceptibility assay • Measurement of CN54gp140 serum IgA responses over time • Frequency and magnitude of T-cell chemokine and cytokine release following ex-vivo antigen stimulation quantified by Luminex. • Epitope mapping of B- and T-cell responses • Serum antibody responses to Ad4-EnvCN54 and MVA-CN54 as a marker of anti-vector immunity and vaccine "take" • Ex vivo analysis of colorectal biopsies for activation markers by ICS (Part 2 only) • Susceptibility of rectal biopsies to HIV-infection – infection assays • Durability of responses will also be determined by measurement of response at weeks 26 (visit 9) and 48 (visit 11) of the trial schedule
Group selection	**Part 1:** Participants will be recruited and randomised into groups A-H with an oral and I.M placebo control (dependent on treatment allocaiton). This will be a participant blinded study where the particpants are blinded to the group and dosing regime to which they are assigned. The laboratory undertaking immunological analysis will also be blinded to group and dosing regimen to prevent bias in analysis. **Part 2:** Participants will be randomised to the groups chosen for expansion. If only one group is expanded, there will be no randomisation. Participants will be blind to the vaccine regime group to which they have been assigned. The laboratory undertaking immunological analysis will also be blinded to group and dosing regimen to prevent bias in analysis.

Summary Information Type	**Summary Details**
Number of Participants to be Studied	The study will enrol 48 participants to Part 1 and 12 participants for Part 2. In Part 1, n=6 participants per group will be randomised to groups A-H. In Part 2, n=6 participants will be vaccinated to the best responding regimes. Recruitment will select potential participants who are willing to undergo serial

	blood draws. Additional participants may be enrolled to replace any early withdrawals.
Duration of Study Participation	Participants will be screened up to 90 days before the first vaccination and will be followed up for 24 weeks after the last vaccine administration. Excluding screening, participants will attend for 11 visits in 48 weeks.
Evaluation for Inter-Current HIV Infections	Participants will be tested for HIV according to the Trial Assesment Schedule (Table 2). 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 (if clinically indicated) to distinguish a true HIV infection acquired through exposure in the community from HIV seropositivity due to the vaccine-induced antibody response. HIV testing at additional time points may be performed upon request of the volunteer and Principal Investigator or designee as medical or social circumstances warrant.
Organisation	The Coordinating Center will be the Group of Mucosal Infection & Immunity at ICL. Drafting of the SAP will be lead by the Surrey Clinical Research Centre.
Safety Monitoring And Statistical Considerations	Only a volunteer Trial ID, (and the Date of Birth) will identify collected CRF data. Safety will be continually monitored by the Investigators; pause criteria are pre-defined to facilitate consultation with the Trial Management Group prior to deciding on a permanent stop to protocol. *Ad hoc* safety review may be specifically requested by the Sponsor and the Principal Investigator, based on on-going review of the data. At the end of the study, a Clinical Study Report (CSR) will be drafted. Safety and tolerability will be addressed by examining the overall rates of solicited and unsolicited adverse events and serious adverse events that might be associated with vaccination and the number of participants who experience these events. All clinical and routine laboratory data will be included in the safety analysis.

Summary Information Type	**Summary Details**
Sponsor	Imperial College London (ICL)
Sponsor Status	Non-Profit Organization
Funder	MRC UK
Chief/Principal Investigator	Dr Katrina Pollock
Chief Scientific	Professor Robin Shattock

Investigator	
Clinical Trial Manager (Coordinating Center)	Dr Aleisha Miller
Clinical Trial Manager (ICRF – Clinical Site)	Dr Tom Cole
Lead Trial Nurse (ICRF – Clinical Site)	Mr Allan Listanco

3 TRIAL SCHEMA

Table 1: VACCINATION SCHEDULE

The doses, methods and schedule of immunisation are described below.

PART I							PART II	
	Group	**N**	**Month 0**	**Month 3**	**Month 6**		**N**	**Vaccinations 1,2,&3**
ARM 1	**A**	**6**	Oral placebo	CN54gp140 +Oral placebo +I.M placebo	CN54gp140 +Oral placebo +I.M placebo		**12**	Selected dosage from Part I
	B	**6**	Ad4-EnvCN54	CN54gp140 +Oral placebo +I.M placebo	CN54gp140 +Oral placebo +I.M placebo			
	C	**6**	Ad4-EnvCN54	CN54gp140 Ad4-EnvCN54 +I.M placebo	CN54gp140 +Oral placebo +I.M placebo			
	D	**6**	Ad4-EnvCN54	CN54gp140 Ad4-EnvCN54 +I.M placebo	CN54gp140 Ad4-EnvCN54 +I.M placebo			
ARM 2	**E**	**6**	Oral placebo	CN54gp140 +MVA-CN54 +Oral placebo	CN54gp140 +MVA-CN54 +Oral placebo			
	F	**6**	Ad4-EnvCN54	CN54gp140 +MVA-CN54 +Oral placebo	CN54gp140 +MVA-CN54 +Oral placebo			
	G	**6**	Ad4-EnvCN54	CN54gp140 +MVA-CN54 +Ad4-EnvCN54	CN54gp140 +MVA-CN54 +Oral placebo			
	H	**6**	Ad4-EnvCN54	CN54gp140 +MVA-CN54 +Ad4-EnvCN54	CN54gp140 +MVA-CN54 + Ad4-EnvCN54			
		48					**max 12**	

4 TRIAL ASSESSMENT SCHEDULE

TABLE 2 - SCHEDULE OF VISITS, IMMUNISATIONS AND ASSESSMENTS - PART 1 & PART 2

Visit		1	2	3	4	5	6	7	8	9	10	11
Study Month		M0			M3			M6				M12
Study Week		W0	W1	W4	W12	W13	W16	W24	W25	W26	W28	W48
Study Day		D0	D7	D28	D84	D91	D112	D168	D175	D182	D196	D336
Visit Windows (Days)	-90		± 1	± 2	(1) -7/+14	± 1	± 2	(1) -7/+14	± 1	± 2	± 2	± 14
Immunizations		X			X			X				
Informed consent	X											
Medical history/ demographics	X											
General physical examination	X											
Entry screen for Ad4 neutralising antibodies	X											
Symptom directed physical exam		X	X	X	X	X	X	X	X	X	X	
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	
Vital signs	X	X[6]	X		X[6]	X		X[6]	X	X	X	
Inspection of administration sites (I.M)	X				X[6]	X		X[6]	X			
Solicited Adverse Events		X[6]	X		X[6]	X		X[6]	X			
Review Solicited AE on diary card			X			X			X			
Unsolicited Adverse events		X[6]	X	X	X[6]	X	X	X[6]	X	X	X	
Serious adverse events		X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test (women) [2]	X	X			X			X				X
Routine safety bloods[3]	X	X	X		X	X		X	X	X	X	
Antinuclear antibody and immunoglobulin[7] tests	X											
HIV test[4]	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X
HBV, HCV and syphilis tests	X											
STI screen[5]	X											
Blood for serum – *Immunogenicity*		X		X	X		X	X	X	X	X	X
Blood for PBMC – *Immunogenicity*		X		X	X		X	X	X	X	X	X
Blood for PBMC – *Infectivity*		X		X			X		X	X	X	X
Mucosal antibody responses (rectal / genital / nasal)		X		X	X		X	X		X		X
Rectal swab for Ad4-EnvCN54 PCR		X	X	X		X	X		X	X	X	X
Rectal biopsies (optional; Part 2 only)		X[8]								X[9]		X[10]

[1] The second and third vaccinations (visits 4 and 7) can be shifted by -7 or up to +14 days. Subsequent visits would be shifted by the same number of days to ensure the interval to subsequent visits is maintained

[2] Confirmed negative prior to vaccination

[3] The parameters are detailed in section 12.3.3

[4] HIV testing at additional time points may be performed upon request of the volunteer and principal investigator or designee as medical or social circumstances warrant

[5] Genital swabs or urine for Neisseria gonorrhoea and chlamydia trachomatis will be collected

[6] Pre-dose, and at 60 (+/–10) min post-dose

[7] Immunoglobulins IgA, IgG and IgM

[8] Biopsy may be performed up to 2 weeks before vaccination

[9] Window for biopsy is –2 to +7 days

[10] Window for biopsy –56 to +56 days

5 CONTENTS

6 ABBREVIATIONS

Ab	Antibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
ARD	Acute respiratory disease
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Adverse reaction
AST	Aspartate aminotransferase
BMI	Body Mass Index
CF	Consent form
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CSR	Clinicial Study Report
CTM	Clinical Trial Manager
EDC	Electronic Data Capture
ELISA	Enzyme Linked Immunosorbent Assay
ELISpot	Enzyme Linked Immunosorbent Spot Assay
ERC	Endpoint Review Committee
EUDRACT	European Union Drug Regulatory Agency Clinical Trial
GCP	Good Clinical Practice
HHC	Household Contact
HIV	Human immunodeficiency virus
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICL	Imperial College London
ICS	Intracellular cytokine staining
ID	Intradermal
IDES	Internet Data Entry System
IDMC	Independent Data Management Committee
IM	Intramuscular
IMP	Investigational Medicinal Product

IRB	Institutional Review Board
ISF	Investigator Site File
ITT	Intention-to-treat
JRCO	Joint Research Compliance Office
LC	Langerhans Cell
mBC	Memory B cell
MHRA	Medicines and Healthcare Products Regulatory Agency
NHS	National Health Service
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PIS	Participant Information Sheet
PI	Principal Investigator
POC	Proof of Concept
PP	Per-Protocol
QA	Quality Assurance
QC	Quality Control
R&D	Research and Development
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TOPS	The Overvolunteering Prevention System
UAR	Unexpected Adverse Reaction
UKHVC	UK HIV Vaccine Consortium

7 INTRODUCTION AND BACKGROUND

At the end of 2012, an estimated 35.3 million people were living with HIV worldwide, up 17% from 2001. This reflects the continued large number of new HIV infections and a significant expansion of access to antiretroviral therapy (ART), which has helped reduce AIDS-related deaths, especially in more recent years (UNAIDS Report on the global AIDS epidemic 2013. Geneva 2013). The majority of new HIV infections continue to occur in sub-Saharan Africa. There is an urgent need to strengthen and scale-up existing and new prevention methods such as HIV testing and counselling, behavioural interventions, condom use, treatment of sexually transmitted diseases, harm reduction, male circumcision (Wamai et al. 2011) and antiretroviral drugs for prevention (Granich et al. 2011). New prevention strategies to control the epidemic and prevent new infections, including pre-exposure prophylaxis (Kim et al. 2010), antiviral treatment for prevention (Granich et al. 2011), topical microbicides (Krakower et al. 2011), and HIV preventive vaccines must be explored and their access ensured. Although combination ART has transformed life expectancy, and global initiatives such as PEPFAR and the Global Fund to fight AIDS, TB and Malaria have made these drugs widely available, treatment has had limited impact on the number of new HIV infections. In the UK, even against a backdrop of comprehensive treatment and care services, sexual transmission of HIV continues unabated with an estimated incidence of 1-3 per 100 person years amongst young homosexual men. Whilst we hope that the strategic use of ART to prevent infection will increase, the need for an efficient and realistic vaccine regimen against HIV-1 remains a priority for global public health (Kim et al 2010; Rerks-Ngarm et al 2009; Koff 2010).

7.1 THE STATUS OF THE FIELD

Important findings from the Thai RV144 trial indicate that a vaccine regimen based on a non-replicating vector prime and a recombinant monomeric HIV envelope subunit protein (rgp120) boost could reduce the incidence of HIV-1 acquisition (Kim et al 2015).

The RV144 phase IIb clinical efficacy trial provided an important proof of concept that HIV acquisition can be reduced with a vaccine regimen based on a non-replicating pox vector prime, and an Envelope subunit protein boost. However, the observed level of efficacy (31%) is likely insufficient to provide a cost effective vaccine. Interestingly the reported efficacy of the RV144 vaccine at 12 months was 60%, but rapidly waned in conjunction with systemic antibody levels. Mucosal antibody levels, thought to be critical in preventing HIV acquisition and known to wane more rapidly than those in the systemic compartment, were not measured in the RV144 trial. Nevertheless it is widely accepted that a vaccine capable of delivering a sustained efficacy of ≥60% would be clinically useful in both developing (Andersson et al 2011) and developed world settings (Long et al 2011). However this is dependent on the level of coverage and the required frequency of boosting to maintain protective immunity. This trial has now become the benchmark for future studies. The products to be evaluated in this study (Ad4HIV phase I) offer significant potential to improve on this initial and important proof of concept. Specifically, we anticipate that the novel use of a mucosal replicating vector will maximize and maintain mucosal responses providing a durable efficacy of ≥60%, significantly lengthening the potential interval between required re-vaccination, a critical consideration for cost effectiveness.

The modest protective efficacy of the RV144 regime over 36 months (31%) highlights the need for both increased efficacy and durability of immune protection. In this respect live-

attenuated viral vaccines have been far more effective in preventing viral infections in humans and animal than any other approach, providing a compelling case for delivery of an HIV vaccine with a live vector (Parks et al. 2013). This is mirrored by results in the rhesus macaque model in which live-attenuated viruses like the nef deleted SIV-mac239 have proven to be the most effective experimental vaccine for preventing AIDS (Koff et al. 2006). However a live attenuated HIV vaccine is impractical based on significant inherent safety risks. Given that sexual transmission of HIV-1 is the predominant mode of HIV acquisition in adults, a key element for a successful preventative vaccine may be the ability to generate potent immune responses not only within the systemic circulation but also at the mucosal portals of entry (genital tract and rectum). Therefore a mucosal replication competent (live) virus vector may have additional advantages compared to non-replicating vaccines for priming mucosal and systemic responses.

7.2 CURRENT VACCINE STRATEGIES

Currently only six replicating viral vectors have progressed to testing in phase I clinical trials: Adenovirus serotype 4 vaccine for CN54 (current protocol), Adenovirus serotype 4 vaccine for H5N1 Influenza (Gurwith et al. 2013), Tiantan Vaccinia virus (National Center for AIDS/STD Control and Prevention, China CDC), Measles virus (Institut Pasteur), Sendai virus (International AIDS Vaccine Initiative) and Vesicular stomatitis virus (NIAID USA). There are a range of other replicating vectors in preclinical development but the transfer to clinical programs is unclear and depends on appropriate toxicology and manufacturing processes.

We believe Ad4 vectors have a significant advantage over other vector-based strategies for priming mucosal and systemic responses due to their oral application. Here they are non-pathogenic but replicate within the GI tract. Furthermore, the “parent wild-type” Ad4 vaccine has a substantial safety record having been administered orally to more than 10 million US military recruits without safety concern (Radin et al. 2014). In contrast, the non-replicative Ad5 vector was associated with a trend toward increased HIV-1 acquisition in Ad5 seropositive subject in the Merck phase IIb STEP trial and the HVTN503 Phambili trials (Gray et al. 2010). However, the Ad4 vector is substantially biologically different from Ad5 vectors in terms of tropism, cellular receptor usage, innate immune profile, and adaptive immune phenotypes. Adenovirus 4 tropism for the mucosal surface of the GI tract initiates replication in submucosal tissues, thus maximises effective expression of native HIV envelope trimers on the surface of infected cells (Alexander et al. 2013). This localised mucosal expression of native envelope has the strongest potential to induce potent immunity to strengthen defences of the mucosal barriers at the front lines of HIV acquisition.

There is currently no clinical data on expression of HIV immunogens using the Ad4 vector, however recent clinical studies using the Ad4 vector to induce responses to the haemagglutinin of avian influenza A H5N1 virus show the platform to be safe and tolerable (Gurwith et al. 2013). Although Ad4 proved efficient for priming systemic and mucosal responses to the haemagglutinin, optimal antibody responses were only observed on protein boosting (Gurwith et al 2013). We intend to build on these promising initial findings by boosting responses through the use of recombinant protein alone or in combination with a non-replicative MVA vector.

This builds on previous studies indicating that priming with non-replicative adenovirus vectors, can be significantly boosted by MVA vectors and/or recombinant protein (Draper et al. 2013) and our own preclinical modelling indicating that co-administration of MVA-CN54 and GLA-AF adjuvanted HIV CN54gp140 protein in primed animals promotes enhanced responses over protein alone (McKay et al 2014). This approach is currently under evaluation in a phase I clinical trial (UKHVC SPOKE003; EudraCT: 2012-003277-26) funded by the MRC DCS. While these studies will inform our own vaccine development program, indication that a replicating Adenovirus 4 vector prime can elevate the magnitude and duration of pox-protein strategies would also have significant impact for the wider field.

7.3 RESEARCH LEADING TO THE PROPOSAL OF CLINICAL STUDY

7.3.1 ADENOVIRUS TYPE 4

Adenovirus serotype 4 belongs to the family of Adenoviridae, is frequently the causative agent for conjunctivitis or pharyngoconjunctival fever and is responsible for acute respiratory disease (ARD) among susceptible populations worldwide. Adenovirus serotype 4 is a non-enveloped, icosahedral virion, 70-90nm in diameter containing a double-stranded linear DNA genome. Adenovirus 4 transmission occurs through direct human to human contact, faecal-oral and occasionally water transmission.

The incubation period after transmission is between 1-10 days, and Ad4 infections vary in clinical manifestation and severity. Symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough, or conjunctivitis. Usually a syndrome of fever, pharyngitis, and conjunctivitis (pharyngoconjunctival fever) is associated with an Ad4 infection. In addition, it has been documented that Ad4, the only known serotype of Adenovirus species E, is the leading causative agent of febrile respiratory infections (acute respiratory disease [ARD]) in military trainees. In rare instances, Ad4 has caused severe pneumonia in military recruits or immunocompromised individuals.

The Adenovirus serotype 4 parental vaccine protects against respiratory disease and is attenuated by the oral route of delivery. Previous studies of the oral replication competent Ad4 vector encoding hemagglutinin (HA) of H5N1 influenza have detected rectal, but not respiratory, shedding for up to 28 days after administration. Most importantly no shedding or evidence of seroconversion to the encoded transgene HA was observed in household contacts, but shedding was likely to be detected in vaccinees receiving the high dose of 10^{11} VP after oral immunisation.

7.3.2 THE VACCINES

7.3.2.A Recombinant Adenovirus type 4 vector encoding CN54-Env (Ad4-EnvCN54)

The vaccine vector virus designated as Ad4-EnvCN54, is a recombinant, replication-competent Ad4 encoding the full length of a HIV-1 membrane expressed trimeric envelope protein (CN54-Env). The recombinant Ad4 virus was derived from the US Military Ad4 vaccine virus which had been originally isolated from a military recruit with acute respiratory disease before being passaged in WI-38 cells (Lyons et al. 2008). Ad4 will encode the HIV-1 clade C envelope protein from the clade C Chinese viral isolate 97CN54. This subunit has been shown to be immunogenic, raising high titre antibodies when given systemically in B6D2F1 mice, and intra-vaginally in New Zealand white rabbits. Ad4-EnvCN54 (or matching placebo) will be administered orally as a lyophilized powder in a capsule at each immunisation visit. The vaccine has been manufactured by PaxVax (USA).

7.3.2.B MVA-C encoding CN54gp120

The MVA-CN54 has been developed by M. Esteban at the Centro Nacional de Biotecnologia of CSIC and expresses the HIV-1 protein gp120 and the fusion protein gag-pol-nef from HIV-1 97CN54. Ad4HIV trial participants will receive 1.0 x 10^8 $TCID_{50}$ (or matching placebo) in a volume of 0.5mls. MVA-CN54 will be injected into the **left deltoid muscle** at the 2nd and 3rd immunisation visits. The vaccine has been manufactured by Bavarian Nordic (Denmark).

7.3.2.C Recombinant CN54gp140 + MPLA

The CN54gp140 + monophosphoryl lipid A (MPLA) is a recombinant gp140 protein, derived from the HIV-1 97CN54 coding sequence and a trimeric recombinant clade C envelope protein, mixed with MPLA liposomes. The protein comprises a sequence of 670 amino acids, and has been shown to be immunogenic in humans. Trial participants will receive 100μg of CN54gp140 and 5μg of MPLA in a volume of 0.4mls. CN54gp140 + MPLA will be injected into the **right deltoid muscle** at the 2nd and 3rd immunisation visits. The vaccine has been manufactured by Polymun Scientific (Austria).

7.3.3 PRE-CLINICAL IMMUNOGENICITY STUDIES

Studies have shown in mice, non-human primate and human studies that vaginal vaccination in the absence of strong adjuvants is inefficient in induction of genital tract antibody responses to HIV envelope (Buffa et al. 2012; Cranage et al. 2011; Lewis et al. 2011). This has also proven to be the case with direct rectal immunisation in macaques. Furthermore, concerns over the impact of local immune activation on HIV acquisition preclude the use of immune potentiators at the mucosal portals of HIV entry. Indeed the possibility that vaccination with human Ad5 vector increased mucosal T-cell activation at the portals of HIV acquisition remains a central hypothesis to explain the potential enhancement of HIV acquisition within the STEP and Phambili trials (Gray et al. 2014). In contrast oral immunisation has previously been shown to induce rectal and genital tract antibody responses to both replicating vaccines (poliovirus; Ogra and Ogra, J Immunol. 1973) and adjuvanted subunit immunogens (Rudin et al. 1998). Furthermore the oral route of immunization offers significant advantages in relation to ease of application and acceptability, and is not complicated by the influence of reproductive hormones on local immune responsiveness to vaccination. While there is considerable evidence to support the oral administration of the Ad4-EnvCN54 vector, currently there is no available data on the safety or efficacy of vaginal or rectal administration of Ad4-EnvCN54.

A prototype HIV-1 candidate vaccine based on Ad4 recombinant vectors expressing an HIV-1 clade C full-length Env (Alexander et al. 2013), designed to express and present on the cell membrane the correct conformation of Env appropriate for immunogenicity, and has already been evaluated in preclinical studies. This vaccine was compared with other Ad4Env vectors expressing gp140 or gp120, secreted from recombinant vector infected cells. The immune sera from Ad4 immunised animals efficiently neutralized tier 1 clade C pseudovirus and the homologous and heterologous tier 2 pseudoviruses to a lesser extent, reflective of the modest antibody titres achieved by repeat vaccination with a homologous vector. Nevertheless these promising preclinical data likely underestimate the real potential of the Ad4 vector platform given its exclusive restriction for replication in human cells.

Although the mechanistic correlates of protection in the RV144 trial remain obscure, reduced risk of HIV acquisition correlated with a range of functional antibody characteristics. This modest and short-lived immune protection, based on a non-replicating canarypox vector prime and recombinant envelope protein boost regime, suggests the need for much more potent vaccine-induced protective antibody responses at the mucosal portals of viral entry. A mucosal replicating vector system generating local Env antigen persistence could potentially lead to higher levels of protective mucosal and systemic neutralizing antibodies.

7.3.4 CLINICAL STUDIES IN HUMANS

In **MucoVac 01** (EudraCT number 2007-000781-20), was the first human clinical trial to have used the trimeric CN54gp140 to healthy participants, although the vaccine was not given systemically and was administered in the absence of an adjuvant. The trial was conducted in 17 healthy women that were given nine intravaginal (IVAG) immunisations and exposed to a total of 900µg CN54gp140 in carbopol gel over the course of one menstrual cycle. The vaccine formulation did not cause any serious adverse events. Most of the adverse events were genital, and mild in nature, except for 4 which involved genital bleeding. Three of the 4 were moderate, and in one case a woman on hormonal contraception met the criteria for severe, which was defined as bleeding heavier than menses for more than 4 days. There were no detectable antibodies seen either locally or systemically, and this might have been explained by the proximity of the immunizations which were administered 3 times a week for 3 consecutive weeks in one menstrual cycle (Lewis et al. 2011).

In **Mucovac 02** (EudraCT: 2010-019103-27) built directly on Mucovac 1, and continued to focus on mucosal immune responses. The trial was conducted in 36 healthy women in two centres in the UK who were randomised to one of four groups to receive CN54gp140 with or without GLA-AF/Chitosan and by a variety of methods. Mucovac 02 was the only completed trial to have generated safety data on the systemic administration of the CN54gp140 in GLA-AF in healthy participants. The results showed that 1/11 and 0/5 women in the IM IVAG or IN groups made systemic IgG, and after a single I.M boost of the intranasal (I.N) group increased to 3/3, suggestive of amnestic responses and some effect of priming via the IN route.

Analysis of the (presumed) peak antibody responses measured after the last immunisation demonstrated induction of robust antibody responses, with moderate neutralization activity against easy to neutralize tier 1 viral isolates. There were no vaccine related serious adverse events although participants experienced at least one mild or moderate solicited adverse event. There were no safety concerns of note attributable to the study products and all participants completed all their allocated immunisations.

In **UKHVC SPOKE003** (EudraCT: 2012-003277-26) built directly on Mucovac 2, continued to focus on inducing durable systemic binding antibodies to CN54gp140 at levels exceeding those seen in the RV144 trial. In addition, an accelerated regimen, with 5 rather than 7 sets of immunisations and shortened by 8 weeks, was implemented to investigate whether augmenting humoral responses compromised participant safety. This approach is currently under evaluation.

7.3.5 STUDIES DIRECTLY SUPPORTING THE DESIGN OF AD4HIV

7.3.5.1 Scientific Hypothesis

The RV144 efficacy trial demonstrated that a vaccine based on a non-replicating pox vector prime, and Envelope subunit protein boost can provide protection up to 60% protection against HIV-1 infection for 12 months, although rapidly waning to 31% by 36 months. Our hypothesis is that priming with an orally delivered mucosal replicating vector (Ad4) will significantly enhance the magnitude and durability of mucosal and systemic antibody responses to HIV-1 gp140 on boosting with recombinant protein, with or without co-administration of a non-replicating pox vector (MVA-C).

We believe there is a compelling case to test this hypothesis as: Ad4 has the longest safety record of currently available replicating vectors; is the only available viral vector adapted for oral administration; naturally infects mucosal surfaces initiating replication in sub-mucosal tissues; and transduces expression of native HIV envelope on the surface of infected cells. Hence we believe the proposed strategy has enhanced potential to strengthen antibody-mediated defenses of the mucosal barriers at the front lines of HIV acquisition.

To test this hypothesis we will deliver a two arm phase I clinical trial conducted in the NIHR Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital, London.

Arm 1 (Groups A-D): Depending on the allocation of groups, participants will receive Ad4-EnvCN54 or placebo orally, CN54gp140/MPLA, and placebo I.M injections at month 0 (Week 0), 3 (Week 12) & 6 (Week 24).

Arm 2 (Groups E-H): Depending on the allocation of groups, participants will receive Ad4-EnvCN54 or placebo orally, CN54gp140/MPLA and MVA-CN54 I.M injections at month 0 (Week 0), 3 (Week 12) & 6 (Week 24).

The proposed assessment of combined MVA-CN54/protein boost in the second of two arms in this study is intended to determine maximal responses. The choice of the MVA-CN54 /protein combination is based on our own preclinical and clinical modelling of a DNA /MVA / CN54 -protein combination trialed in a now closed to recruitment phase I clinical trial study (UKHVC SPOKE 003) funded by the MRC-DCS.

Data from animal studies suggests that co-administration of MVA-CN54 and GLA-AF adjuvanted HIV CN54gp140 protein promotes enhanced responses over DNA followed by protein alone (McKay PF, et al. 2014). Care has been taken in the design of the proposed study to facilitate direct comparison between the two experimental arms, and data obtained from the on-going clinical assessment of the more complex (x3)-DNA (2x)- MVA-C -protein-GLA strategy in the UKHVC SPOKE003.

7.4 STUDY RATIONALE

We propose to evaluate the use of new HIV-1 vaccine products in a phase I clinical trial by exploring the impact of different boosting options for MVA-C and CN54gp140 for oral Ad4 serotype 4 vector prime expressing HIV-1 CN54 (Ad4-EnvCN54). This regime will identify the most immunogenic strategy for the induction of durable mucosal and systemic protective antibody responses. This will facilitate up-selection of the most promising regime for accelerated development towards a phase II efficacy trial.

The overall aim of this phase I clinical study is to evaluate the impact of priming with an orally delivered mucosal replicating competent Ad4 vectored prime expressing HIV-1 CN54Env (Ad4-EnvCN54), by evaluating potential enhancement of the magnitude and durability of mucosal and systemic antibody responses to HIV-1 CN54Env on boosting with recombinant trimeric envelope protein boosts, with or without co-administration of a non-replicating pox vector (MVA-C) to identify the most immunogenic strategy for the induction of durable mucosal and systemic protective antibody responses. The proposed study will provide critical data needed to support and inform the further exploration of immunisation regimen based on Ad4 vectors for HIV vaccines in a phase II/III trial.

7.5 INVESTIGATIONAL PRODUCTS / INTERVENTIONS

7.5.1 Justification of selected dose for the Investigational product: Ad4-EnvCN54

Ad4-EnvCN54 encoding rgp150 HIV-1 membrane expressed trimeric envelope protein subtype C CN54 will be administered orally at the dose of 10^{10}VP. This dose was well tolerated in a recent study of the Ad4 vaccine for H5N1 influenza performed in the US (Gurwith et al. 2013), and demonstrated superior vaccine uptake (Ad4 seroconversion) and observed immunogenicity (cellular and humoral response to influenza) than lower doses.

7.5.2 Justification of selected dose for MVA-C encoding CN54gp120

The MVA-CN54 has been developed by M. Esteban at the Centro Nacional de Biotecnologia of CSIC and expresses the HIV-1 protein gp120 and the fusion protein gag-pol-nef from HIV-1 97CN54. Participants will receive 1.0 x 10^8 $TCID_{50}$ in a volume of 0.5mls intramuscular injections (I.M) into the **left deltoid muscle**.

The dose 1.4 x 10^8 $TCID_{50}$ was used in the EuroVacc trials and the doses of MVA-CN54 used in HIVIS/TaMoVac trials (10^8 $TCID_{50}$) in which there have been no concerns about safety. This dose has been shown to be well tolerated and immunogenic and has now been administered to many individuals in these and many other trials of other vaccines. The vaccine has been manufactured by Bavarian Nordic (Denmark).

7.5.3 Justification of selected dose for CN54gp140 / MPLA: recombinant HIV-1 subtype C trimeric envelope protein adjuvanted in monophosphoryl lipid A adjuvant (MPLA)

Recombinant CN54gp140 / MPLA will be administered I.M at the dose of 100µg adjuvanted with 5µg MPLA in a liposomal formulation into the **right deltoid muscle**. The dose to be used in this protocol is identical to the dose of CN54gp140 (100µg) and MPLA (5µg), in the same administered volume of 0.4ml, used in three previous UK clinical trials: MUCOVAC2 (EudraCT 2010-019103-27); Spoke 003 (EudraCT 2012-003277-26); and X001 (EudraCT 2013-001032-22). In these trials the MPLA was called GLA (glucopyranosyl lipid A). The combination was safe, and participants made good immune responses to the CN54gp140.

For the proposed clinical study, the CN54gp140 / MPLA will be manufactured by Polymun Scientific. CN54gp140 is a trimeric recombinant HIV-1 clade C envelope glycoprotein derived from a Chinese viral isolate 97CN54 (Rodenburg et al. ; Su et al. 2000).

The HIV-derived amino acid sequence of CN54gp140, as predicted from the primary DNA sequence of the clone, comprises 634 residues. The molecular mass predicted by the

polypeptide sequence alone is approximately 70 kD. However, the protein is heavily glycosylated and has a mass of approximately 140 kD as determined by SDS-PAGE and size-exclusion chromatography. Furthermore, the CN54gp140 secreted by CHO cells is oligomeric, and following purification is essentially trimeric, with a projected mass of 420 kD. CN54gp140 / MPLA vaccine is provided at a concentration of 250 µg/ml of protein and 12.5 µg/ml of MPLA, in a volume of 0.6ml, as a clear, colourless, sterile liquid, presented in translucent polypropylene vials.

7.6 CLINICAL STUDY DESIGN

To identify an effective Ad4 based oral vaccine that promotes systemic and mucosal responses to the HIV-1 envelope glycoprotein, our group will adopt a two part adaptive clinical trial. The study will assist in rapidly screening the impact of different routes, combination regimes and boosting options to Ad4-EnvCN54, MVA-CN54 and CN54gp140 / MPLA, to augment systemic and mucosal antibody responses following initial priming with Ad4.

The study will enrol 48 participants to Part 1 and 12 participants in Part 2. Recruitment will select potential participants who are willing to undergo serial blood draws. Additional participants may be enrolled to replace any early withdrawals.

In Part 1, n=6 participants per group will be randomised to groups A-H (Table 1). This design varies the number of Ad4 administrations, from 1 to 3, combined with either CN54gp140 protein boosts (A-D) or co-administration of gp140 with MVA (E-H). Groups A (gp140 alone) and E (MVA-CN54/CN54gp140 alone) will serve as control groups to assess the added benefit of the Ad4 vector. Each vaccine group (A-H) will be assessed for number of mucosal responders (mucosal antibody (rectal/genital/nasal) as measured by ELISA), and titre (mucosal and systemic) of antigen specific binding antibody. Based on data from the MUCOVAC2 study, in which participants received 3 intramuscular doses of CN54gp140 with GLA-AF adjuvant, we anticipate that there will be no mucosal responders in group A (although there will be detectable systemic responses). Enrolment through to completion of the primary immunological assessment for each group will be 7 months.

In Part 2, the best regimes from A-D (Ad4-EnvCN54/CN54gp140) and E-H (Ad4-EnvCN54/MVA-CN54/CN54gp140) will be expanded to n=12 (additional n=6) in Part 2. Immune responses at the Primary Immunogenicity Endpoint (Week 26) will be analysed. Selection will be based on total number of responders. In the event that more than one group has the same number of responders, mucosal antibody titre will be used to rank groups to determine which to take forward to Part 2. Should mucosal titers be indistinguishable between groups, then serum antibody levels will be taken into consideration in the selection of groups to progress to Part 2.

The criteria for a positive mucosal response, and methods for ranking groups on the basis of mucosal and serum antibody titers, will be specified in the LAP. If any groups are associated with unacceptable product related adverse events, they will be discontinued from further study. The decision to progress any group to Part 2 will be taken by the Trial Management Group.

7.7 ANTICIPATED NEXT STEPS

The selected HIV Envelope insert is based on subtype C, the most common infecting subtype globally, and particularly in southern Africa. Should the proposed trial identify a particularly promising vaccine strategy, the aim will be to transfer the clinical studies to collaborators in southern Africa. Although protection is likely to be clade specific, proof of concept in this study would allow the rapid development of additional Ad4/MVA/protein HIV Envelope vaccines to generate a multi-clade vaccine approach. The European & Developing Countries Clinical Trials Partnership (EDCTP) will be the ideal organization through which to take the best results from this study into a phase II study. Funding will be sought from the Wellcome Trust, MRC, DFID, EDCTP and International Trials program for phase II support if this trial meets its endpoints.

7.8 RISKS AND BENEFITS

This is a phase I trial in healthy male and female participants.

There is currently no clinical data for Ad4-EnvCN54, and limited data available on the systemic use of CN54gp140 and MVA-C but our group is directly involved in the only trial using CN54gp140 delivered I.M in DNA-MVA-protein strategy (UKHVC SPOKE 003; EudraCT number 2012-003277-26) initiated in July 2013 and now completed, and for which our group have direct access to safety data as it becomes available.

There are extensive safety data on the use of similar recombinant HIV protein vaccines including the large Phase III Vaxgen and RV144 trials - albeit formulated with different adjuvants (Andersson et al. 2011; Su et al. 2000; UNIADS Report 2013). In the RV144 trial, the ALUM adjuvanted gp120 protein (AIDSVAX B/E) was injected along with ALVAC (canarypox expressing gag pro and env genes) after two priming immunisations with ALVAC alone. There were no safety concerns in that trial of over 16,000 healthy participants in Thailand.

Adenovirus 4 and MVA-C based vaccines are widely used alone and in combination regimens, and previous experience with similar vaccine products is reassuring with respect to safety. Adverse reactions similar to those seen with previous vaccines and licensed vaccines are expected. There is no direct benefit to the participants. They will be reimbursed for their time and travel. As there are placebo groups, there is a risk that adverse events will be over-reported, but this should not influence severity grading and is not a concern at this stage of evaluation.

Immunisation will be carried out by qualified health care professionals who have had appropriate training, according to written procedures for each clinical centre. The dispensing records will be checked at each monitoring visit including used vials.

The vaccination schedules are complex and the study may be difficult to recruit to, and there is an increased risk of loss to follow-up as a result. We have previous experience of similarly complex regimens. Participants will be issued a diary card in addition to a 7 day follow-up visit.

The Immunogenicity endpoints will be analysed in laboratories at Imperial College London, and serum samples may also be shipped to a specialist immunology laboratories in the USA for wider measurement of antibodies of interest. Procedures will be put in place to ensure the secure chain of custody of samples when transporting from clinic to laboratory and between laboratories; therefore we consider that the risk of loss or compromise of samples is low.

8 CLINICAL TRIAL SITE

8.1 SITE/INVESTIGATOR INCLUSION CRITERIA

The Principal Investigator, Dr Katrina Pollock (Imperial Clinical Research Facility, Hammersmith Hospital) is experienced in Phase I clinical trials. Participants will be seen by clinical staff from the Imperial Clinical Research Facility, Imperial College Healthcare NHS Trust, who have experience of running Phase 1 vaccine trials.

8.2 CI'S QUALIFICATIONS & AGREEMENTS

1. The investigator should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial at their site and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the REC and/or the regulatory authorities.

2. The investigator should be thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, in the current Investigator Brochures, in the product information and in other information sources provided by the Coordinating Centre.

3. The investigator should be aware of, and should comply with, the principles of ICH GCP and the applicable regulatory requirements. A record of GCP training should be accessible for all investigators.

4. The investigator/site should permit monitoring and auditing by the Sponsor, and inspection by the appropriate regulatory authority(ies)

5. The investigator should maintain a delegation log of appropriately-qualified persons to whom the investigator has delegated significant trial-related duties.

6. The investigator should sign an investigator statement, which verifies that the site is willing and able to comply with the requirements of the trial.

8.3 ADEQUATE RESOURCES

1. The investigator should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

2. The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

3. The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

4. The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related

duties and functions.

5. The site should have sufficient data management resources to allow prompt data entry into the CRF. Sites that have previously participated in trials coordinated by the same centre should have a proven track record of timely data entry.

8.4 SITE ASSESSMENT

In addition and in compliance with the principles of ICH GCP, all site staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to the Coordinating Centre. An up-to-date copy of this log must be stored in the trial master file at the Coordinating Centre and the Investigator Site File (ISF) at the Site.

9 SELECTION OF PARTICIPANTS

The eligibility criteria for this trial have been carefully considered. There will be **no exceptions** to eligibility requirements at the time of randomisation. The eligibility criteria are the standards used to ensure that only medically appropriate participants are considered for this study. Participants not meeting the criteria will not be permitted to join the study. For the safety of the participants, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other individuals as well as scientific conclusions, it is important that no exceptions be made to these admission criteria.

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

9.1 PARTICIPANT INCLUSION CRITERIA

1. Men and women aged between 18 and 50 years on the day of screening
2. BMI between 18-30
3. Seronegative for Adenovirus 4 serum neutralising antibodies
4. Available for follow-up for the duration of the study
5. Willing and able to give written informed consent
6. At low risk of HIV infection and willing to remain so for the duration of the study defined as:
    - no history of injecting drug use in the previous ten years
    - no gonorrhoea or syphilis in the last six months
    - no high risk partner (e.g. injecting drug use, HIV positive partner) either currently or within the past six months
    - no unprotected anal or vaginal intercourse in the last six months, outside a relationship with a regular partner known to be HIV negative
7. Willing to undergo HIV testing
8. Willing to undergo a STI screen for chlamydia, gonorrhoea and syphilis
9. Must agree to require male sexual partner to use condoms, from at least 14 days before the first vaccination until at least 4 months after the last
10. If heterosexually active female capable of becoming pregnant, must (in addition to requiring male partner to use condoms) agree to use hormonal contraception, or to complete abstinence, from at least 30 days before the first vaccination until at least 4 months after the last. [Note: Acceptable hormonal contraception is combined (estrogen and progestogen containing) or progestogen-only hormonal contraception associated with inhibition of ovulation. Complete abstinence can be used, when in line with the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, lactational amenorrhoea method, and IUD/IUS are not acceptable methods of contraception.]
11. If sexually active male, must agree to use condoms from the day of first vaccination until at least 4 months after the last. [Note: Additional use of an effective method of contraception is recommended for any non-pregnant female partner over the same period.]
12. Agree to abstain from donating blood, eggs or sperm from the day of first vaccination until at least 3 months after the end of their participation in the trial

13. Registered with a GP for at least the past month
14. Entered and clearance obtained from The Overvolunteering Prevention System (TOPS) database[1]

[F1] *Participants will be advised that they cannot enrol in any other trials of medicinal products during the period from screening to the final visit in Ad4HIV. All participants will be entered onto the TOPS database as a measure to prevent over volunteering. If staff discover that a participant is enrolled on another study during this period, they must immediately contact the Principal Investigator for advice. Decisions will be reviewed on a case by case basis and participant safety will be the primary concern*

9.2 PARTICIPANT EXCLUSION CRITERIA

1. Are pregnant or breast feeding, or living with anyone under the age of 5 years old or over 75 years old
2. Have close contact with an immunocompromised individual thought to be at clinical risk from Adenovirus infection
3. Clinically relevant abnormality on history or examination including:
    a. Liver disease with inadequate hepatic function
    b. Any skin condition which may interfere with the trial assessment of the injection sites
    c. Haematological, metabolic, gastrointestinal or cardio-pulmonary disorders
    d. Uncontrolled infection; autoimmune disease, immunodeficiency
4. Known hypersensitivity to any component of the vaccine formulations used in this trial, or have severe or multiple allergies to drugs or pharmaceutical agents
5. History of severe local or general reaction to vaccination defined as
    - Local: extensive, indurated redness and swelling involving most of the antero-lateral thigh or the arm, not resolving within 72 hours
    - General: fever ≥39.5^{o}C within 48 hours; anaphylaxis; bronchospasm; laryngeal oedema; collapse; convulsions or encephalopathy within 72 hours
6. Receipt of live attenuated vaccine within 60 days or other vaccine within 30 days of enrolment
7. Receipt of an experimental vaccines containing HIV antigens, Ad4 and MVA-C products at any time in the past
8. Receipt of blood products or immunoglobin within 4 months of screening, or drugs that suppress the immune system, such as steroids (including inhaled steroids, excluding topical steroids unless applied to the upper arm), in the preceding 3 months
9. Participating in another trial of a medicinal product, completed less than 30 days prior to enrolment
10. HIV 1 or 2 positive or indeterminate on screening
11. Positive for antibodies to hepatitis B surface antigen, hepatitis C antibody or serology indicating active syphilis requiring treatment
12. Clinically significant positive reaction in antinuclear antibody screen or clinically significant immunoglobulin (IgA, IgG or IgM) values
13. Grade 1 or above clinically significant routine laboratory parameters
14. Unable to read and/or speak English to a fluency level adequate for the full comprehension of procedures required in participation and consent

15. Women with a history of toxic shock syndrome
16. Women using an intrauterine device for contraception (as incompatible with softcup sampling)
17. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data
18. Unlikely to comply with protocol

9.3 NUMBER & SOURCE OF PARTICIPANTS

Forty eight healthy participants for Part 1, plus an additional 12 healthy participants for Part 2 will mainly be recruited through advertising, and through the investigator site's healthy volunteer database, and given contact details. They will be provided with further information about the study and asked to complete a short interview (by telephone or in person) to assess their suitability. They will be given or sent an information sheet.

9.4 SCREENING PROCEDURES & PRE-ENROLMENT INVESTIGATIONS

Written informed consent must be obtained from participants after explanation of the aims, methods, benefits and potential hazards of the trial and **BEFORE** any trial-specific procedures are performed or any blood is taken for the trial. Participants should be given a minimum of 24 hours after receiving the information sheet to sign a consent form. It must be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without affecting their treatment.

At the screening visit the volunteer will be allocated a Trial ID. The trial will be discussed in detail, and any questions about the study will be answered. If participants are willing and interested they will be asked to sign the informed consent form.

Signed consent forms must be kept by the investigator and documented in the medical notes and case report form (CRF) and a copy given to the participant.

After written informed consent has been collected, assessments and investigations will be undertaken according to the schedule in Table 2. These include demographic, sexual and medical histories, general examination, and collection of urine and blood samples for routine laboratory investigations. Samples for tests for sexually transmitted infections including HIV will be collected. As soon as the required test results are available, data will be entered into the CRF and medical notes and the results of the screening investigations will be reviewed and eligibility signed off by a physician.

10 REGISTRATION & DISTRIBUTION INTO TREATMENT GROUPS

10.1 RANDOMISATION PRACTICALITIES

10.1.1 PART 1

The enrolment visit will take place within 90 days after the screening visit and will be the same day as the first vaccination. Assessments and procedures will be undertaken according to the schedules in Table 2, and data entered on the CRF.

The study will use sentinel dosing as a safety precaution. The first 6 participants successfully enrolled into the trial will be sequentially pre-allocated to treatment groups B, C, D, F, G and H. The first participant will be enrolled to group B. The safety data for this participant will be reviewed at day 7 before enrolling the next participant to group C. This process will be repeated for the other groups. This will ensure an escalation of the number of Ad4-EnvCN54 administrations combined with either CN54gp140 protein boosts (B-D) or co-administered CN54gp140 and MVA-CN54 (F-H).

After the accumulated safety data (up to day 7) for the 6th participant has been reviewed, on their first day of dosing the remaining participants will be block randomised into groups as follows:

- Block 1 (n=10): A, A, B, C, D, E, E, F, G, H
- Blocks 2-6 (n=8/block): A, B, C, D, E, F, G, H

10.1.2 PART 2

Six additional healthy participants per group will be recruited and randomised to the best two regimes from A-D (Ad4-EnvCN54/CN54gp140) and E-H (Ad4-EnvCN54/MVA-CN54/CN54gp140). The visits schedule for participants in Part 2 will be the same as that for participants in Part 1. Assessments and procedures will be undertaken according to the schedules in Table 2, and data entered on the CRF.

10.1.3 PASS CRITERION FOR PART 2

The group selection for Part 2 is based on results generated from the primary immunogenicity endpoint (Week 26, Visit 9). In the event that more than one group has the same number of responders, mucosal antibody titre will be used to rank groups to determine which to take forward to Part 2. Should mucosal titers be indistinguishable between groups, then serum antibody levels will be taken into consideration in the selection of groups to progress to Part 2. The criterion for a positive mucosal response, and methods for ranking groups on the basis of mucosal and serum antibody titers, will be specified in the LAP. If any groups are associated with unacceptable product related adverse events, they will be discontinued from further study.

10.1.4 GP NOTIFICATION

With the participant's consent, shortly after randomisation a letter should be sent to the general practitioner informing him/her of the trial and the participant's involvement in the trial.

10.2 RANDOMISATION CODES AND & UN-BLINDING

This is a single-blind, randomised trial with placebo controls. Participants will be block-randomised centrally to the study groups shown in Table 1, using a computer-generated algorithm with a back-up manual procedure. Randomisation will be stratified on the basis of gender. All participants in Part 1 and Part 2 will be blinded to the vaccine regime group to which they have been assigned. The laboratory teams undertaking immunological analysis will also be blinded to group and dosing regimen to prevent bias in analysis. The clinical team will remain unblinded to the vaccine regime of trial participants throughout.

10.3 CO-ENROLMENT GUIDELINES

Participants will be advised that they cannot enrol in any other trials during the period from screening to the final visit in Ad4HIV. All participants will be entered onto the TOPS (The Over volunteering Prevention System) database as a measure to prevent over volunteering.

If staff discover that a participant is enrolled on another study during this period, they must immediately contact the Principal Investigator for advice. Decisions will be reviewed on a case by case basis and participant safety will be the primary concern.

10.4 PARTICIPANT'S COMPLIANCE & ADHERENCE

In consenting to the trial, participants are consenting to trial treatment, trial follow-up and data collection. However, an individual participant may stop treatment early or be stopped early for any of the following reasons:

- Unacceptable adverse event
- Inadequate compliance with the protocol in the judgement of the treating physician
- Withdrawal of consent by the participant

As the volunteer's participation in the trial is voluntary, they may choose to discontinue the trial treatment at any time without loss of benefits to which they are otherwise entitled. Although the participant is not required to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason while fully respecting his/her rights.

Participants should remain in the trial for the purpose of follow-up and data analysis (unless the volunteer withdraws their consent from all stages of the trial). Data will be kept and included for volunteer who stop follow-up early.

11 TREATMENT OF PARTICIPANTS

11.1 VACCINE PRODUCTS & ADMINISTRATION

CN54gp140 / MPLA:

CN54gp140 / MPLA will be provided by UK HIV Vaccine Consortium (UKHVC) in 2 mL Schott type 1 glass vials. One dose of CN54gp140/MPLA will be 100µg of protein and 5µg of MPLA, in a volume of 0.4mL.

MVA-CN54:

MVA-CN54 will be provided by UK HIV Vaccine Consortium in 2 mL Schott type 1 glass vials. One dose of MVA-CN54 delivers 1.0 x 10^8/$TCID_{50}$ in a volume of 0.5 mL.

Ad4-EnvCN54:

Ad4-EnvCN54 will be provided by PaxVax as a lyophilised powder. One dose of Ad4-EnvCN54 delivers 10^{10}VP per capsule.

Oral Placebo

Oral placebo capsules were developed by Patheon UK Ltd, to match the active Ad4-EnvCN54 oral capsule. Hypromellose (HPMC) capsule shells were filled with sucrose supplied as a white crystalline powder, and then coated with an enteric solution.

Intramuscular Placebo

Intramuscular placebo for injection vials were developed by Polymun Scientific Ltd; Austria, to match the active MVA-C investigational product. The placebo comprises a liquid formulation containing 20 mM Tris / 0.15 M NaCl (pH 7.5). Tris is used as buffer substance, sodium chloride for appropriate osmolality and hydrochloric acid for buffer pH adjustment.

Investigational medicinal product vials and bottles will be labelled and packaged according to regulatory requirements. The PI will ensure that staff administering the vaccines have been appropriately trained in the written procedures for delivery of the vaccines.

Investigators and trained members of the clinical team will be encouraged to administer the vaccine as soon as possible and within 2 hours of being removed from cold storage. The date and time of administration will be recorded in the Case Report Form (CRF) and medical notes.

Participants will receive I.M injections, and Ad4 or placebo administered orally, as described in the schedule of doses (Table 1). In Part I, 36 participants will receive Ad4CN54-Env in combination with CN54gp140, or MVA-CN54 and CN54gp140, whilst 12 participants will receive an oral placebo in combination MVA-CN54 and/or CN54gp140. Dosing will be at weeks 0 (Month 0), 12 (Month 3) and 24 (Month 6). The two best vaccine regimes from B-D (Ad4/gp140) and F-H (Ad4/MVA-C/gp140), provided they meet the pass criterion, will be expanded and 12 participants will be screened and enrolled for Part 2 of the study.

11.2 ACCOUNTABILITY FOR USED AND UNUSED SUPPLIES

The PI will ensure that the IMPs and placebos are stored and dispensed in accordance with the protocol and local pharmacy procedures, and that records are maintained of receipt, dispensing and return/destruction of all supplies. The PI must ensure that all IMP and placebo supplies are kept in a secure area accessible only to authorised individuals, and maintained in storage at the following temperatures:

- Ad4-EnvCN54 at –20 ±5 °C
- Matched oral placebo at –20 ±5 °C
- MVA-CN54 at ≤–70 °C
- CN54gp140/MPLA at 2–8 °C
- Matched I.M placebo at 2–8 °C

Upon receipt of supplies, a designated member of staff will conduct an inventory and acknowledge receipt to the supplier. A record must be kept of all Ad4-EnvCN54, MVA-CN54 and CN54gp140 / MPLA used during the trial. This will include the description (lot numbers and expiry dates) and quantity of IMP received at the trial site and date of receipt, as well as a record of when (date of administration) and to whom (volunteer trial ID) it was dispensed.

At the end of the trial, IMP accountability will be checked by the designated member of staff responsible for the inventory and trial monitors. The Sponsor and the PI will retain copies of the complete IMP accountability records. All unused supplies will be retained at the trial site until the Sponsor gives instructions for their return/destruction.

11.3 VACCINATION SCHEDULE – PART 1 & PART 2

The doses, methods and schedule of immunisation are described below.

Table 1: Schedule of Doses and Method of Vaccinations

PART I							PART II	
	Group	**N**	**Month 0**	**Month 3**	**Month 6**		**N**	**Vaccinations 1,2,&3**
ARM 1	**A**	**6**	Oral placebo	CN54gp140 + Oral placebo +I.M placebo	CN54gp140 + Oral placebo +I.M placebo		**12**	Selected dosage from Part I
	B	**6**	Ad4-EnvCN54	CN54gp140 + Oral placebo +I.M placebo	CN54gp140 + Oral placebo +I.M placebo			
	C	**6**	Ad4-EnvCN54	CN54gp140 + Ad4-EnvCN54 +I.M placebo	CN54gp140 + Oral placebo +I.M placebo			
	D	**6**	Ad4-EnvCN54	CN54gp140 + Ad4-EnvCN54 +I.M placebo	CN54gp140 + Ad4-EnvCN54 +I.M placebo			
ARM 2	**E**	**6**	Oral placebo	CN54gp140 + MVA-CN54 + Oral placebo	CN54gp140 + MVA-CN54 + Oral placebo			
	F	**6**	Ad4-EnvCN54	CN54gp140 + MVA-CN54 + Oral placebo	CN54gp140 + MVA-CN54 + Oral placebo			
	G	**6**	Ad4-EnvCN54	CN54gp140 + MVA-CN54 + Ad4-EnvCN54	CN54gp140 + MVA-CN54 + Oral placebo			
	H	**6**	Ad4-EnvCN54	CN54gp140 + MVA-CN54 + Ad4-EnvCN54	CN54gp140 + MVA-CN54 + Ad4-EnvCN54			
		48					**max 12**	

11.4 COMPLIANCE & ADHERENCE

All vaccinations will be administered by clinically trained site staff to ensure compliance.

11.5 DOSE MODIFICATIONS, INTERRUPTIONS & DISCONTINUATIONS

There are no planned modifications to dose, other than discontinuation.

The schedule may be modified if a participant has symptoms or signs on the day of scheduled immunisation, and the investigator considers it best to defer the immunisation. The participant will be asked to return for review within the window period of a scheduled immunisation.

The Principal Investigator may decide to permanently discontinue dosing in a participant who has received one or more vaccinations, if it is deemed that continuing might compromise the participant's wellbeing or interfere with the achievement of the trial's objectives. Participants will be encouraged to continue to attend trial visits for sampling and safety monitoring.

Discontinuation of dosing is essential following a grade 3 or 4 clinical or laboratory event (confirmed on examination or repeat testing respectively) which is considered possibly, probably or definitely related to the vaccine and which did not resolve within 72 hours. All participants will be followed up fo the duration of the study and samples will be collected at the allotted times.

Dosing must be discontinued in participants who become pregnant or HIV infected.

Participants may decide to discontinue dosing. They will be encouraged to provide a reason, and to remain in follow-up. If the participant explicitly states their wish not to contribute further data to the study, the PI should be informed in writing.

Both the PI and the coordinator centre should be informed as soon as possible if a participant chooses to stop receiving vaccines or to withdraw from the trial, and within 1 working day of a decision being taken to discontinue a participant when that decision is informed by an adverse event (**Section 13.1**).

11.6 CLINICAL MANAGEMENT OF ADVERSE EVENTS

Adverse events will be managed by the clinical trial team who will assess and treat the event as appropriate, including referral to an independent physician and/or the participant's General Practitioner if required. Clinically significant abnormalities detected following clinical examination or routine laboratory tests will be managed as determined by local PI or delegate.

11.7 NON-TRIAL TREATMENT

As stated in the exclusion criteria, participants should not have received other immunisations, blood products, immunoglobulin, immunosuppressive medicines or other trial medication within specified periods prior to enrolment. This applies during the trial through

to the final safety visit 28 weeks after enrolment, 4 weeks after the last scheduled immunisation, unless the treatment is required for an emergency.

Should a participant require immunisation for the purposes of travel, occupation or other clinical need during the trial, the request will be reviewed by the Principal Investigator who will advise on timing and whether or not the trial immunisation schedule needs to be amended.

Participants will be allowed to continue with hormonal contraception if this forms part of their regular appropriate contraception plan. Details will be recorded on the screening and concomitant medication CRF.

All concomitant medication will be recorded on the CRF, including any dispensed by the investigators in the management of adverse events or reactions.

11.8 ISSUES RELATED TO HIV

A 4th generation HIV antibody/antigen assay, the standard laboratory method for diagnosing HIV infection, will be used to screen participants. An HIV test will be performed at two time-points in the study: the screening visit, and the final visit 48 weeks from enrolment. During the study clinic staff will determine whether the risk status for HIV has changed and repeat tests if necessary.

After completing the study, regardless of whether or not they are reactive in HIV tests, participants will be provided with appropriate certification of their HIV antibody status, and (if clinically indicated) invited to return to the clinical centre for re-testing until such time as the test becomes negative.

11.9 VERIFICATION OF HIV STATUS OF PARTICIPANTS

In the event of an equivocal or positive result which the PI is not convinced to be vaccine-induced, a specimen will be processed through a range of assays according to the local laboratory operating procedures to establish the HIV status of the individuals. A confirmatory specimen will be collected at a later date, if the first result suggests that the participant is HIV infected.

11.10 HIV INFECTION

In the unexpected circumstances that a participant in the trial acquires HIV infection, they will be referred for clinical care and counselling. Participants will be referred initially to a specialist physician for a full discussion of the clinical management of HIV infection. Further investigations will be undertaken as necessary. Should the participant prefer to be managed

at a hospital closer to their home, this will be arranged. Referral for counselling will be arranged by the specialist physician, to a counsellor at their clinical centre.

12 ASSESSMENTS & FOLLOW-UP

12.1 ASSESSMENTS AT SCREENING

12.1.1 INFORMED CONSENT PROCESS

A site-specific Consent Form will be submitted and approved by the Independent Ethics Committee (IEC)/ Ethics Review Board (ERB).

Participant Information Sheet

A qualified member of the study staff will conduct the informed consent process by reviewing the Participant Information Sheet with the volunteer, and answering any questions. This process will be documented in the clinic notes.

Consent Form

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

The signed/marked and dated Informed Consent Document must remain at the study site. A copy of the signed/marked and dated Informed Consent Document, and PIS will be offered to the volunteer to take home.

12.1.2 DEMOGRAPHICS, MEDICAL HISTORY AND PHYSICAL EXAMINATION

Demographic information such as age and ethnic origin will be collected at screening and entered onto the CRF.

A past and current medical history will be collected at screening during a face to face structured interview using a source document, such that relevant aspects of eligibility can be adequately assessed.

The general physical examination will include weight (kg), height (cm), calculation of BMI, temperature, blood pressure and heart rate (vital signs), inspection of the skin and respiratory, cardio-vascular, abdominal, and neurological systems. An assessment of cervical, axillary and inguinal lymph nodes will also be undertaken.

12.1.3 HIV RISK ASSESSMENT & SEROLOGY TESTING

An HIV risk assessment will be performed according to the relevant eligibility criterion.

The following will be collected from all participants:

- Serology for HIV and syphilis
- Serology for markers of hepatitis B and hepatitis C infection
- Urine for Chlamydia and gonorrhoea at screening
- Serology for Ad4 neutralising antibodies
- Serology for antinuclear antibodies and immunoglobulins IgA, IgG and IgM

12.1.4 ROUTINE LABORATORY PARAMETERS

Peripheral blood will be collected by trained staff into the appropriate containers and transported to the local laboratory.

All laboratory assessments, except urine pregnancy tests which will be done by the trained individuals on site, will be done by the local laboratory.
The following parameters will be collected from all participants:

- Hb, total WBC, neutrophils, lymphocytes and platelets
- creatinine, total bilirubin, alkaline phosphatase (ALP) and alanine aminotransferase (ALT)
- Urine test (female participants) to exclude pregnancy

12.2 VACCINATION AND FOLLOW-UP VISITS

- The assessments that will be performed at each visit are described in Table 2.
- Participants will be required to make 11 scheduled outpatient visits over the course of 48 weeks. Time 0 will start on the day of enrolment, which is also the day of randomisation and the first immunisation, and must be within 90 days after screening.
- Vaccinations will take place at Weeks 0 (Month 0), 12 (Month 3) and 24 (Month 6).
- Serum and peripheral blood mononuclear cells (PBMC) will be collected for immunogenicity at Weeks 0 (enrolment), 4, 12, 16, 24, 25, 26, 28 and 48.
- Adverse events will be assessed during the enrolment visit before and after immunisations and at every visit thereafter until the final safety visit at Week 28; thereafter only serious adverse events will be recorded.
- Injection sites will be inspected vaccination days at Weeks 12 and 24 (pre-dose and 60 (+/–10) min post-dose), and at Weeks 13 and 25.
- Symptom-directed physical examinations will be done at Week 0 and at every visit thereafter until the final safety visit at Week 28.
- Vital signs will be done on vaccination days (pre-dose and 60 (+/–10) min post-dose) and at Weeks 1, 13, 25, 26 and 28.
- Concomitant medications will be collected at every visit up to the final safety safety visit.
- Blood for routine laboratory safety parameters will be collected at Weeks 0 (enrolment), 1, 12, 13, 24, 25, 26 and 28 and at any other visit if clinically indicated.
- Mucosal samples for antibody responses (genital / rectal / nasal) will be collected at Weeks 0, 4, 12, 16, 24, 26 and 48.
- Urine pregnancy test will be assessed at Weeks 0, 12, 24 and 28.
- Rectal swabs for Ad4-EnvCN54 PCR will be assessed at Weeks 0, 1, 4, 13, 16, 25, 26, 28 and 48.

- Rectal biopsies will be assessed at Weeks 0, 26 and 48 for participants in **Part 2** of the clinical trial only. Participants may opt out of this procedure.

12.2.1 ADDITIONAL VISITS

Additional visits and assessments may be required to evaluate an adverse event, and/or identify a diagnosis. These are compatible with the protocol.

The safety assessments are intense. Participants will be asked to remain in clinic for about 1 hour following each immunisation, and to complete a diary card until the day 7 safety visit. They will be advised to call the clinic staff if they are concerned, and 24 hour cover will be available.

Referral to an independent specialist with the appropriate expertise will be arranged if there is uncertainty about the relationship to vaccine.

12.2.2 VISIT WINDOWS

The second and third vaccination visits scheduled for weeks 12 and 24 will be compliant with the protocol if they take place -7 / +14 days either side of the target date determined by the date of the previous vaccination. If there is a delay >4 weeks a decision will be made by the TMG (see **section 20.1**) as to whether immunisation will continue.

The post-immunisation safety visits scheduled for weeks 1, 13 and 25 will be compliant with the protocol if they take place +/- 1 day of the target date determined by the date of the previous vaccination.

The primary endpoint visit (Visit 9; Week 26) and final safety visit scheduled for week 28 will be compliant with the protocol if they take place $\pm$ 2 days either side of the target date determined by the date of the third vaccination. The follow-up visit at Week 48 will be compliant with the protocol if it takes place ±14 days either side of the target date determined by the date of the third vaccination.

12.3 PROCEDURES FOR ASSESSING SAFETY AND TOLERABILITY

12.3.1 ADVERSE EVENT ASSESSMENT

Information on adverse events will be collected through questions at every visit from and including the enrolment visit up to week 28, as indicated in **Table 2**.

The investigator or delegate will record the diagnosis or the symptoms if a diagnosis is not apparent, the date of onset and the date of resolution if appropriate.

The relationship (to vaccine) and seriousness of the event will be determined by the investigator or delegate according to the definitions provided in **section 13.3**. All of this information will be recorded in the adverse event CRF.

12.3.2 PHYSICAL EXAMINATION AND VITAL SIGNS

The general and symptom-directed examinations, and assessment of vital signs will be performed at time points specified in **Table 2**. Additional physical examinations and vital signs assessments may be performed if indicated by the results of scheduled safety assessments.

12.3.3 ROUTINE SAFETY BLOODS AND URINE PREGNANCY

A pregnancy test will be performed by analysis of a urine sample for Human Chorionic Gonadotrophin (HCG) collected from female participants as detailed in Table 2. The analysis will be conducted by a member of the study team.

Peripheral blood will be collected and analysed for the following parameters at the time points specified in Table 2, and at additional time points if clinically indicated to further evaluate or follow up adverse events.

Blood

- Creatinine, ALT, alkaline phosphatase, total bilirubin
- Hb, total WBC, neutrophils, lymphocytes, platelets

12.3.4 SOLICITED LOCAL AND SYSTEMIC ADVERSE EVENTS

Various local and systemic adverse events are known to be associated with licensed vaccines, and are referred to as 'solicited adverse events'.

This information will be collected from all study participants on the day of immunisation and at the day 7 safety visit following each immunisation, through direct questions asked during a structured face to face interview and through examination according to the schedule.

Participants will also be asked to complete a diary card recording solicited adverse events that start from each immunisation until the day 7 safety visit.

TABLE 3: SOLICITED ADVERSE EVENTS

Type	Event
Local AEs (Immunisation site)	Pain Tenderness Erythema / Discolouration Warmth Itching Swelling
Systemic Clinical AEs	Abnormally raised temperature Chills / rigors Myalgia / flu-like general muscle aches Malaise Excess fatigue Headache Nausea / Vomiting Abdominal Pain Diarrhoea Sore throat

The events will be graded according to **Appendix 1.**

Relationship to the product, the assumption is that any of these solicited events starting within 7 days of an immunisation are at least possibly related. If the onset is beyond 7 days, the event will be recorded on the adverse event CRF and a relationship determined by the investigator or delegate.

12.3.5 NON-SOLICITED ADVERSE EVENTS

Non-solicited AEs starting from the first vaccination until the final safety visit (Week 28) will be captured and reported on the adverse event CRF. When making a non-solicited AE enquiry, staff should ask a non-leading question such as 'how have you been feeling?'

12.3.6 FOLLOW-UP OF ADVERSE EVENTS AND PREGNANCY

The Investigator will make every effort to monitor all adverse events, regardless of severity, until resolution or stabilisation in order to report this on the CRF (see section 15.1.1) during the trial.

After the database is locked and the trial is closed, any additional information about adverse events or pregnancy that comes to the attention of an investigator should be reported by email to the Principal Investigator.

12.3.7 SERIOUS ADVERSE EVENTS

A serious adverse event is defined in section 13.1. These will be collected throughout the entire study period.

All serious adverse events should be reviewed by the PI and discussed at the next TMG call. The assessment should include consideration of whether or not to discontinue dosing or to withdraw a participant from the trial.

For all serious adverse events, the investigator will complete the serious adverse event form as soon as becoming aware of the event and, within 24 hours, will:

- Email it to the Coordinating Centre (Clinical Trials Manager) as well as notify by phone the form has been sent
- Email it to the Imperial Joint Research Compliance Office

12.4 PROCEDURES FOR ASSESSING IMMUNOGENICITY

Samples (indicated below) will be collected at the timepoints specified in Table 2 and transferred to the Shattock Laboratory for analysis.

Cervico-vaginal secretion samples will be collected from female participants using a commercially available, self-inserted menstrual cup, at the time points indicated in Table 2, except at visits when female participants are menstruating in which case cervico-vaginal secretions will not be collected. In the case of light spotting, a sample **will** be collected.

Rectal secretion samples will be collected from male and female participants. Absorbent swabs will be held against the rectal wall for 2 minutes. Should a participant not wish to have a proctoscopy the swab may be inserted by the nurse or doctor or self-inserted by the participant, 3 cm into the anal canal.

Semen samples will be collected from male participants whilst within the clinical trials unit or brought into the clinic following donation at the time points indicated in Table 2. Semen samples should be donated following 24 hours of sexual abstinence, and produced ≤2 hours before being handed to clinic staff.

Nasal secretion samples will be collected from male and female participants using nasal synthetic absorptive matrix at the time points indicated in Table 2.

Rectal biopsies (Part 2 only)

Participants may opt out of this procedure. Rectal biopsies will be collected from the rectal walls of male and female participants with a Sarratt biopsy forcep (or similar sterile, single-use disposable device). Biopsies may be taken on the left and/or right rectal wall at each scheduled time-point, from sites selected by the doctor performing the procedure such that high quality samples can be safely taken. The time-points are sufficiently spaced to allow the mucosa to heal completely between biopsies.

Blood and Serology Specimens

For measurement of antibodies in serum, 6ml peripheral venous blood will be collected into an appropriate blood collection tube, and then processed to obtain serum.

Blood for Immunogenicity assays requiring PBMC

For assessment of T-cell responses, 40-50ml ml of peripheral venous blood will be collected into tubes containing sodium heparin as an anti-coagulant, mixed by inverting gently several times and then processed to obtain PBMC which will be frozen and stored for future batch immunogenicity analysis.

12.5 PROCEDURES FOR ASSESSING AD4 SHEDDING

Rectal swabs will be used to collect stool samples from male and female participants and the samples assessed for viral shedding by a polymerase chain reaction (PCR) assay.

12.6 CRITERIA FOR STOPPING TREATMENT GROUPS OR WHOLE TRIAL

All trial immunisations will be put on hold in the event of a SAR or two severe ARs, and in the event of every subsequent SAR or severe AR.

In the event of a SAR, the PI should notify the sponsor within 24 hours of becoming aware, and the PI will arrange for any necessary expert reviews to take place.

Further immunisations will be put on hold until the review is completed.

A component of any expert review will be to consider whether or not further immunisations should be discontinued in the individual, and/or the trial.

If immunisations are put on hold, the TMG will determine whether or not to call an unscheduled meeting of the Independent Data Monitoring Committee (IDMC) to review the safety data, and whether or not to hold further immunisations until this has taken place. If an unscheduled IDMC is warranted, the Sponsor will be informed and the IDMC asked to make a recommendation to the Principal Investigator and the Sponsor about continuing further immunisations.

At any time if the study is put on hold (for example following the occurrence of a SAR) the Regulatory authority has to be informed of the temporary halt and a substantial amendment with relevant data has to be submitted to the MHRA for approval in case a decision to resume dosing is taken. The Sponsor reserves the right to stop the whole trial at any time.

12.7 EARLY STOPPING & FOLLOW-UP

If a participant chooses to discontinue their trial treatment, they should always be followed up providing they are willing, that is, they should be encouraged to not leave the trial. If they do not wish to remain on trial follow-up, however, their decision must be respected and the participant will be withdrawn from the trial completely. The trial team should be informed of this in writing using the appropriate documentation.

If the medical data collected during the participant's participation in the trial are kept for research and analysis purposes, they must remain anonymised. Consent for future use of stored samples already collected can be refused when leaving the trial early (but this should be discouraged and should follow a discussion).

12.8 WITHDRAWAL OF PARTICIPANTS

Withdrawal means stopping all further visits.

The reason(s) for withdrawal should be recorded in the CRF.

All participants are free to withdraw from the trial at any time, for any reason, without affecting their future medical care. The PI may decide to withdraw a participant if the investigator deems that continuing might compromise participant wellbeing or interfere with the achievement of the trial's objectives.

Participants who are withdrawn due to an adverse event (AE) will be followed-up until the event has stabilised.

Withdrawn participants who have received any immunisations will be asked to undergo the procedures scheduled for the primary endpoint visit (Visit 9; Week 26), with the addition of an HIV test and (women only) a pregnancy test.

12.8.1 Policy for Replacing Withdrawals

As the number of participants per group is small and hence each participant is essential to the study we will replace those that withdraw from the study before completion of the week 26 visit. Based on previous experience we would expect potential withdrawal of 10-20% of participants, predominantly as a result of changes in their personal circumstance that prevents visit attendance. Should we observe a >20% drop out in any group based on perceived safety or tolerability (i.e. more than 1 participant per group), further replacement with be determined by the TMG.

12.9 END OF TRIAL DEFINITION

The trial will be closed when all participants have made their final visit (Week 48; Visit 11), the data entered into the database and all queries resolved and the database locked. There will be a final monitoring/closeout visit to the clinical site between the last visit and the database lock.

13 SAFETY REPORTING

The principles of ICH GCP require that both investigators and sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials. These procedures are described in this section of the protocol. **Section 13.1** lists definitions, **section 13.3** describes details of the responsibilities of the institutions/investigators.

13.1 DEFINITIONS

The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial protocol. These definitions are given in Table 4.

TABLE 4 TYPES OF ADVERSE EVENTS AND DEFINITIONS

Type of Event	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product. The Principal Investigator will use the following criteria when deciding whether to report a laboratory parameter that falls outside the normal range according to the local laboratory guidelines as an adverse event: - If it is clinically significant[1] and Grade 1 (or above) according to the grading system in Appendix 1 the abnormal result will be recorded as an AE -
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered. 'Related' means possibly, probably or definitely as defined below. All solicited adverse events which start within 7 days of a vaccination will be automatically classified as an adverse reaction.

1 An abnormal result will be deemed to be clinically significant if any of the following apply:
- The test result is associated with relevant accompanying symptoms
- Additional diagnostic tests or medication are indicated

- As a consequence of the test result, an immunisation is delayed or further immunisations are discontinued
- The investigator considers the result to constitute an adverse event for any other reason

TABLE 4 TYPES OF ADVERSE EVENTS AND DEFINITIONS CONTINUED

Type of Event	Definition
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: • **Results in death** • **Is life-threatening*** • **Requires hospitalisation or prolongation of existing hospitalisation**** • **Results in persistent or significant disability or incapacity** • **Consists of a congenital anomaly or birth defect** • **Is another important medical condition***** **Notes:** '**A threat to life**' refers to an event or reaction in which the patient was at risk of death at the time of the event; it does not refer to an event or reaction which hypothetically might have caused death had it been more severe. **If an AE is serious and unexpected and considered possibly, probably or definitely related to study product according to the classification below then it meets the criteria for a SUSAR and should be reported accordingly.**

*The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, that has not worsened or for an elective procedure do not constitute an SAE.

*** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation or development of drug dependency.

13.2 REPORTING ADVERSE EVENTS

Adverse Events should be recorded on the appropriate CRF.

All **SAEs** should be reported to the sponsor within 24 hours of the Clinical Investigator becoming aware of the event fulfilling the criteria. The SAE form should be completed and emailed to the Clinical Trials Manager and Joint Research Compliance Office. The minimum criteria required in reporting a SAE are the participant identifiers (Trial ID/Date of Birth), reporting source (name of Investigator), and why the adverse event is identifiable as serious.

Other important adverse events that should be reported to the sponsor within 24 hours of the Clinical Investigator becoming aware of the event, include

- allergic bronchospasm requiring intensive emergency treatment
- a seizure
- any adverse event that results in **discontinuation of the immunisation schedule**
- any adverse event that requires intervention to prevent a threat to life or death

SAE REPORTING AND IMPORTANT AE NOTIFICATION
Within 24 hours of becoming aware of an SAE or important AE, please complete the SAE form, and email the coordinating centre (Clinical Trials Manager) to notify on:
Ad4HIV_trial@imperial.ac.uk

Additionally, please email a completed paper SAE form to the Imperial Joint Research Compliance Office (JRCO) on:
Email jrco.ctimp.team@imperial.ac.uk
SAE REPORTING AND IMPORTANT AE NOTIFICATION

Investigators should notify the Coordinating Centre of all SAEs within 24 hours of the investigator becoming aware of the event, from the time of registration until the end of trial. SARs and SUSARs must be notified to the Coordinating Centre until trial closure.

Upon receipt of a SAE form at the Coordinating Centre, the PI (or a medically-qualified delegate) will review all SAE reports received. The causality assessment given by the local investigator at the hospital cannot be overruled; in the case of disagreement, both opinions will be provided in any subsequent reports.

The PI will assess whether the event qualifies in seriousness and relationship as a **Suspected Unexpected Serious Adverse Reaction (SUSAR**). Fatal and life-threatening SUSARs must be reported to the MHRA within 7 days of day 0 which is defined as the day the sponsor became aware of the event. Relevant follow-up information should be sought and a further report completed as soon as possible and submitted within 8 additional days. SUSARs which do not result in death or a threat to life should be reported within 15 days of day 0. Ultimate responsibility for classification resides with the study Sponsor.

The team at the Coordinating Centre is primarily responsible for the reporting of SUSARs and other SARs to the sponsors and regulatory authorities (MHRA and competent authorities of other European member states and any other countries in which the trial is taking place) and the research ethics committees, as appropriate. The study team at the Imperial CRF will act as back-up to the Coordinating Centre in this respect.

13.2.1 PREGNANCY

Pregnancy is not an adverse event. However, it is a reportable event in a Phase I trial, and should be reported to the PI within 24 hours of the Clinical Investigator becoming aware of the pregnancy by email, fax or phone.

All pregnancies will be followed up to collect information about the outcome which will be recorded in the clinical study report.

13.2.2 REPORTING SERIOUS BREACHES

In the event of a serious breach, the site will inform the Coordinating Centre as soon as they are aware of a possible serious breach of compliance, so that the Coordinating Centre can report this breach if necessary within 7 days as per the UK regulatory requirements. The study team at the Imperial CRF will provide back-up in this respect. For the purposes of this regulation, a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects in the trial, or
- The scientific value of the trial

13.3 INVESTIGATOR RESPONSIBILITIES AND ASSESSMENTS

All non-serious AEs and ARs, whether expected or not, should be recorded in the source documents and CRF. SAEs and SARs should be notified to the Coordinating Centre and the JRCO within 24 hours of the investigator becoming aware of the event.

13.3.1 SERIOUSNESS

When an AE or AR occurs, the investigator must first assess whether or not the event is serious using the definition given **Table 4**. If the event is serious then an SAE Form must be completed and the Coordinating Centre notified within 24 hours.

13.3.2 SEVERITY OR GRADING OF ADVERSE EVENTS

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded using the toxicity gradings in **Appendix 1**.

13.3.3 CAUSALITY

The investigator must assess the causality of all non-solicited adverse events or reactions in relation to each of the trial IMPs using the definitions in **Table 5**. There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an AE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR.

TABLE 5 - ASSIGNING TYPE OF AE THROUGH CAUSALITY
Relationship to study product classified as:

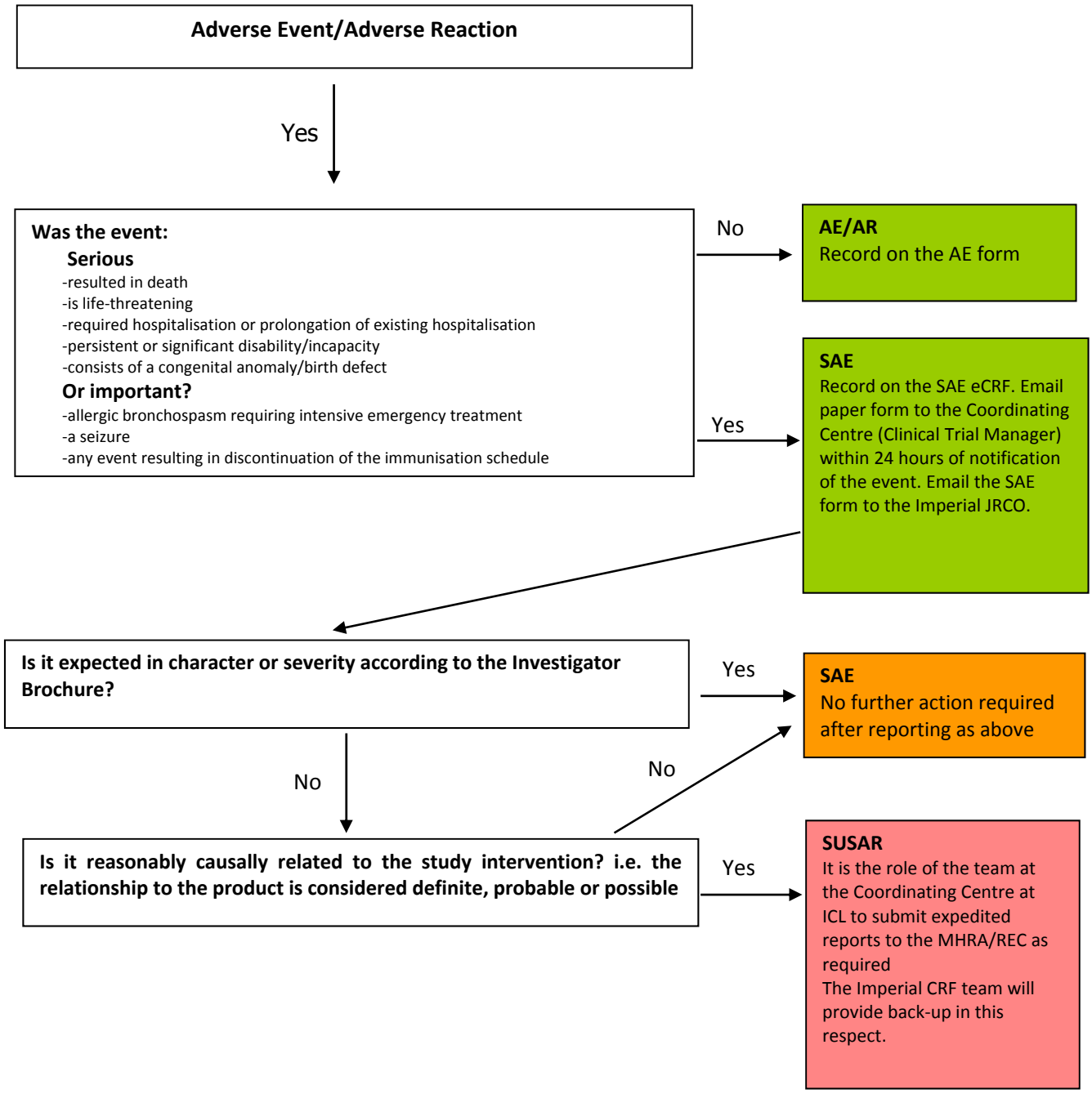
Relationship	Description	AE Type
Unrelated	There is no evidence of any causal relationship	**Unrelated AE**
Unlikely	There is little evidence to suggest that there is a causal relationship (for example, the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (for example, the patient's clinical condition, other concomitant treatment).	**Unrelated AE**
Possible	There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (for example, the patient's clinical condition, other concomitant treatments).	**AR**
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	**AR**
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	**AR**

13.3.4 Reference Safety Information and Expectedness of SARs

If there is at least a possible causal relationship of a SAR with an IMP, the investigator must assess the expectedness of the event. An unexpected adverse reaction is one not previously listed in the reference safety information (RSI) in the current Investigator Brochure (IB) or one that is listed but is more frequent or more severe than stated in the RSI.

13.4 SAFETY REPORTING FLOWCHART

Figure 1: Safety Reporting Flowchart



AE:	Adverse Event
AR:	Adverse Reaction
CRF:	Case report form
SAE:	Serious adverse event
SUSAR:	Suspected unexpected serious adverse reaction

14 STATISTICAL CONSIDERATIONS

14.1 METHOD OF RANDOMISATION

The first 6 participants successfully enrolled into the trial will be sequentially pre-allocated to treatment groups B, C, D, F, G and H.

Participants thereafter will be block-randomised centrally using a computer-generated algorithm with a back-up manual procedure. Randomisation will be stratified on the basis of gender.

14.2 OUTCOME MEASURES

14.2.1 PRIMARY ENDPOINTS

Immunogenicity

Antigen specific mucosal (cervico-vaginal for women, semen for men, and rectal and nasal for all) antibody (µg/ml) responses two weeks after the final immunisation.

Safety and Tolerability:

- Proportion of participants with severe or greater (Grades 3-4) adverse reactions during the study
- Proportion of participants with vaccine-related serious adverse events (SAEs) throughout the study period

14.2.2 SECONDARY ENDPOINTS

Immunogenicity:

To assess the kinetics of immune responses elicited by each of the vaccine regimens:

- Frequency of serum and mucosal binding antibodies to HIV CN54gp140 antigen measured by binding ELISA.

14.2.3 EXPLORATORY ENDPOINTS

Immunogenicity:

- Frequency and magnitude of HIV-gp140 specific B-cell-mediated responses in the systemic compartment measured by B-cell ELISPOT.
- Frequency, titer and avidity of serum binding antibodies to other HIV Env antigens (alternative clades) by ELISA or other assays.
- Frequency and titer of serum neutralising antibodies to homologous virus, and, if warranted a wider a panel of viruses representing different clades.

- Longevity of serum and mucosal responses at 12 months following final immunization
- Frequency and magnitude of HIV-specific T-cell mediated responses measured by T-cell and ICS (Intracellular Cytokine Staining).
- PBMC ex-vivo HIV suceptibility assay
- Measurement of CN54gp140 serum IgA responses over time
- Frequency and magnitude of T-cell chemokine and cytokine release following ex-vivo antigen stimulation quantified by Luminex.
- Epitope mapping of B- and T-cell responses
- Serum antibody responses to Ad4-EnvCN54 and MVA-CN54 as a marker of anti-vector immunity and vaccine "take"
- Ex vivo analysis of colorectal biopsies for activation markers by ICS (Part 2 only)
- Susceptibility of rectal biopsies to HIV-infection – infection assays
- Durability of responses will also be determined by measurement of response at weeks 26 (visit 9) and 48 (visit 11) of the trial schedule

14.3 SAMPLE SIZE

It is not the remit of this Phase I trial to recruit a sufficient number of participants to be statistically confident about the differences between groups. By the end of this study 6 participants will have been exposed to each schedule in Part 1 (groups A – H) and this provides confidence around the response/event proportions of 0–100% in Table 7.

Table 7: Sample Size

Number of "responders"	Proportion if n=6	95% confidence interval[1]
0	0%	0 – 39%
1	17%	0 – 64%
2	33%	4 – 78%
3	50%	12 – 88%
4	67%	22 – 96%
5	83%	36 – 100%
6	100%	54 – 100%

[1] Clopper-Pearson method **(suitable for small sample sizes)**

It is difficult to give an estimate of the power of group comparisons using quantitative antibody titre outcomes at this stage as this is dependent on the number of responders.

14.4 INTERIM MONITORING AND ANALYSIS

Part 1

The accumulating safety and tolerability data will be reviewed by the IDMC (see **section 20.2**) after **twenty participants have reached the primary endpoint of the immunisation schedule (Week 26; Visit 9)**. The IDMC will make a recommendation to the statistical team should any modification to the analysis plan or statistical tables be required. An unscheduled meeting of the IDMC may be required at the request of the Principal

Investigator **(see section 20.2)**, in which case the IDMC will make a recommendation about whether or not to continue further immunisations in the trial.

14.5 DATA ANALYSES AND PRESENTATIONS

Main analysis population will be Intention-to-treat population. Per-protocol population and Modified-Intention-to-treat populations will be considered if important differences between populations arise.

14.5.1 PARTICIPANT POPULATIONS

- **Intention-to-treat (ITT) population:** all participants randomised and given at least one vaccination in the trial

- **Per-protocol (PP) population:** all participants randomised and immunised with all scheduled vaccinations, and who completed the trial with no major protocol deviations

- **Modified-Intention-to-treat (MITT):** all participants randomised and given at least one immunisation in the trial (including those participants whose sample collection occurred outside the allowed windows)

14.5.2 PRIMARY AND SECONDARY IMMUNOGENICITY OUTCOMES

All mucosal and serum samples will be screened for antigen specific antibodies IgG (and IgA). The absolute levels of antibody in samples that are found to be positive will be determined using a standardised and quantitative ELISA developed in Robin Shattock's laboratory at Imperial College London. In this sandwich capture ELISA, the Ab of interest is captured by the relevant target antigen and then detected using a labelled isotype specific secondary Ab. An estimate of the concentration of Ab in the sample is calculated by interpolation relative to a standard curve based on titration of purified human standards IgG or IgA captured by anti-human kappa/lambda-specific antibodies.

The number of 'responders' in each assay will be presented by time-point and group as a proportion with 95% confidence interval. A 'responder' will be defined as a participant in whom a response was detected in two weeks after the final vaccination immunogenicity sample. A positive result will be defined relative to a pre-defined cut-off threshold value and assays will be validated using predefined thresholds based on the responses to positive and negative control stimuli. More information on the assay and definition of positive results will be supplied in the LAP. Titres of antigen specific antibodies will be described by time-point and group, and compared using rank tests where appropriate.

14.5.3 EXPLORATORY IMMUNOLOGICAL ASSESSMENT OUTCOMES

Exploratory immunological assessment outcomes will include evaluation of the proportion of individuals with systemic HIV-specific neutralising antibody responses (breadth and magnitude), the characterisation of non-neutralising antibody function including Antibody Dependent Cellular Viral Inhibition (ADCVI), Antibody dependent viral capture and aggregation assays, and assessment of anti-vector mediated immune responses i.e. anti-

MVA-CN54 and anti-Ad4-EnvCN54 antibody responses. CN54 gp140 specific antibody isotope responses (IgG1-4 and IgA) will be analysed and compared between vaccine groups.

14.5.4 SAFETY OUTCOMES

The AEs will be coded using MedDRA. If a participant reports the same event more than once then the worst severity and worst relationship to trial vaccine will be taken. Discrepancies between diary card and CRF reports will be queried by the monitor if unclear. It is assumed that the grade assigned by the clinician is more accurate, and this will be the grade reported in the tables. If the diary card grade is worse, this will be foot noted.

Safety outcomes will be reported overall with proportion and 95% confidence interval, and by group and time-point, and by relationship to study product, and method.

For the primary analysis of safety and tolerability endpoints (as defined in section 14.2), results will be expressed as a proportion with confidence interval.

15 DATA MANAGEMENT

Data management, analysis and reporting of all trial data will be prepared by the Surrey Clinical Research Centre and ICL.

15.1 DATA MANAGEMENT AND RECORD KEEPING AT THE CLINICAL TRIAL SITE

The dates of visits including immunisation dates, dates and results of pregnancy tests, and details of clinical management (description of adverse events and concomitant medication) will be documented in the clinical / medical notes and other source documents.

The CRFs will not bear the participant's name. The participant's date of birth and Trial ID (which will have been given at screening) will be used for identification.

15.1.1 STAFF AT THE CLINICAL CENTRE WILL BE RESPONSIBLE FOR:

- Creating medical notes for each participant which includes paper copies of the consent documents and the blood results
- Entering information relevant to eligibility and emergent adverse events and documenting the result of any pregnancy tests in the medical notes and other source documents
- The accurate completion of CRFs (CRFs)
- Collection and review of the diary cards from participants
- Notification of SAEs within 24 hours of becoming aware of the event to the PI, Clinical Trial Manager and the JRCO
- Notification of pregnancy within 24 hours of the PI becoming aware of the pregnancy
- Recording data directly into the source documents and then entered onto the CRF ideally within 24 hours. Additional detail will be expected should there be a clinical abnormality relevant to eligibility. **Note: the paper CRFs used to capture solicited and unsolicited adverse events, and concomitant medications, are source documents.**
- Notifying the coordinationg site of SUSARs within the timelines defined in **section 13.2**
- Ensuring all written source data is entered legibly in black ink with a ball-point pen (If an error is made, the error will be crossed through with a single line in such a way that the original entry can still be read. The correct entry will then be clearly inserted and the alterations will be initialed and dated by the person making the alteration. Overwriting or use of correction fluid will not be permitted).
- Preserving the participant confidentiality (CRF will not bear the participant's name). The participant's date of birth and Trial ID (given at screening) will be used for identification.
- Ensuring source documentation are available for review to ensure that the collected data are consistent with the CRFs.
- Ensuring all CRFs and laboratory reports are reviewed by the PI or delegate, who will ensure that they are accurate and complete.
- Ensuring that all source documents and other supporting documents are kept in a secure location. Standard GCP practices will be followed to ensure accurate, reliable

and consistent data collection.
- Maintaining the Investigator Site File
- Communication with the coordinating Center by telephone, mail and email

Please note the list above is not an exhaustive list

A member of the clinical trial team must sign all laboratory reports. In the event of an abnormality, an indication should be given whether or not the abnormality is clinically significant (and therefore gradable per Appendix 1), the date of review and the signature of the clinician reviewing the result.

The database and source documents should be kept in a secure location for 2 years after the last approval of a marketing application or until 2 years have elapsed since formal discontinuation of product development, and at least 25 years after the clinical trial has ended.

15.1.2 DATA MANAGEMENT IN THE IMMUNOLOGY LABORATORIES

Standard operating procedures will be followed in all laboratories to ensure the quality of the data. Data will be stored electronically and transferred in an agreed format for analysis in accordance to GCP.

15.2 ELECTRONIC DATA MANAGEMENT BY PROMASYS

The computerized raw data, final data files and tables will be held by the Surrey Clinical Research Facility and/or the Coordinating Center on behalf of the Sponsor. Principal investigators or designees will have access to the clinical study database.

15.2.1 Staff at Surrey Clinical Research Centre for Promasys will be responsible for:

- Creating a database for data entry (Internet data entry system (IDES))
- Training staff at the clinical centre in data collection and overseeing data entry which may include data entry at the clinical centre directly into the database
- Assisting with the drafting of the SAP and conducting the analyses

The data analysis plan will be developed and agreed upon by Surrey Clinical Research Centre, PI and/or the Coordinating Center on behalf of the Sponsor. The Surrey Clinical Research Centre, in collaboration with the PI, and/or the Coordinating Centre on behalf of the Sponsor will create tables according to this data analysis plan.

The Surrey Clinical Research Centre will conduct the data analysis and will provide interim and final data tables for the Coordinating Centre, Sponsor, PI, and the regulatory authorities, as appropriate.

15.3 DATA MANAGEMENT AT THE COORDINATING CENTER

The Coordinating Center and the Sponsor must be informed of any SAE that occurs during this trial regardless of relationship to the investigational product.

The Sponsor will ensure, by delegating this responsibility to the Principal Investigator, that an annual safety report is provided to REC and the MHRA, and that this includes a description of all suspected unexpected serious adverse reaction (SUSAR) reports.

Staff at the Coordinating Centre will be responsible for:

- Arranging clinical site monitoring
- Maintaining the Trial Master File on behalf of the sponsor

The Coordinating Center will promptly inform the Sponsor if they suspect a serious breach of GCP or the trial protocol has occurred according to the criteria stated in the MHRA's guidance. The Sponsor, using the same criteria, will make the decision and notify the MHRA and other bodies such as main REC and NHS R&D office, within seven days of becoming aware of a serious breach.

The Coordinating Center will inform the Sponsor of all breaches of GCP and deviations that impact on safety or validity. The Coordinating Centre will report to the main REC and to NHS R&D office any breaches or deviations that are, in their opinion, of major significance.

Within 90 days after the end of the trial, the main REC and the MHRA will be notified that the trial has finished. If the trial is terminated prematurely, those reports will be issued within 15 days after the termination date which is defined as the final participant visit.

All study results will be fed back to the participants in an open end of study meeting and by internet webex presentation once all data is available and has been analysed. Individual participant data will not be available for that participant.

The study team at the Imperial CRF will provide back-up to the Coordinating Centre where required due to staff absence etc.

16 TRIAL MONITORING

16.1 RISK ASSESSMENT

The Clinical Trial Manger at the Coordinating Center will perform a risk assessment to assess the risks and benefits of trial participation to individual participant safety, as well as the risks that underlie the validity of the trial results with respect to safety and immunogenicity outcome measurements.

The risk assessment will be discussed by the PI, the Clinical Trial Manger and the Sponsor's representative.

This assessment will be used to guide the development of procedures with respect to informed consent, confidentiality, trial monitoring and audit.

16.2 STUDY MONITORING BY THE COORDINATING CENTER

Monitoring will be performed according to ICH-GCP. A monitoring plan will be written based on the risk assessment. An independent audit of the study may be performed by the Sponsor or designee to establish the status of applicable quality systems. Inspection by regulatory authorities may also occur.

On-site monitoring will be delegated to a trained monitor by the Sponsor's Institution to 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 investigators, as well as participants 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 Principal Investigator will permit inspection of the facilities and all study-related documentation by authorized representatives of the Sponsor, and Government and Regulatory Authorities responsible for this study. Monitoring visits will be performed before, during and after the trial has finished as required by the procedures set out in the monitoring plan to ensure patient safety, accurate data collection and reporting. Monitoring visits will also be dependent on rates of and numbers of participants recruited.

Prior to the first volunteer being screened, an initiation visit will be completed at the clinical site , and will consist of review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), review of recruitment strategy, review of site facilities and equipment, essential document receipt, collection and filing, and archiving and inspection. Copies of the trial specific procedure manuals and related documents will be given to the investigators. The approved version of the protocol should be followed at all times, and protocol deviations will be documented in a Protocol Deviation Form and submitted to the Trial Coordinating Centre as soon as possible. The investigators will allow the monitors to:

- Inspect the site, the facilities, IMP management and materials used for the trial

- Meet all members of the team involved in the trial, and ensure all staff working on the trial are experienced and appropriately trained, and have access to review all of the documents relevant to the trial
- Have access to the CRF and source data
- Discuss with the Principal Investigator and site staff trial progress and any issues on a regular basis
- Train investigators and site staff at the beginning of the trial and then as appropriate.

16.3 CLINICAL SITE MONITORING

The trial site will be monitored to ensure that:

- All participant records will be inspected for confirmation of existence, eligibility and informed consent
- There is adherence to the protocol, including consistency with inclusion/exclusion criteria
- There is compliance with the principles of GCP and regulatory requirements
- Trial Documentation is complete and up to date (e.g. correct versions of documents being used, source data captured) and relevant documents are collected for the trial master file
- The CRFs have been completed correctly and accurately, and all entries correspond to data captured in source documents
- The IMP accountability records are in order (receipt, dispensing and destruction), storage is under appropriate conditions and secure, expiry dates are being checked and adhered to, and dispensing is according to the protocol and trial procedures.

All information dealt with during such visits will be treated as strictly confidential. At the end of the trial, a close out visit will be performed by the monitor after the final participant visit has been completed and prior to database lock. During this visit the monitor will verify that all trial close out activities are completed – all queries resolved, missing data completed, monitoring completed, archiving arrangements in place, IMP accountability complete and all used and unused IMP destroyed, ISF (investigator site file) completed and trial master file documents collected, and end of trial notified.

Each investigator will also be notified that an audit or inspection may be carried out - by the sponsor; sponsor's representatives or the regulatory authorities - at any time, before, during or after the end of the trial. The investigator must allow the representatives of the audit or inspection team:

- To inspect the site, facilities and material used for the trial
- To meet all members of his/her team involved in the trial
- To have direct access to trial data and source documents, to consult all of the documents relevant to the trial

16.4 MONITORING BY THE TRIAL MANAGEMENT GROUP (TMG)

The TMG (see **section 20.1**) will monitor the following:

- Screening and enrolment / Trial ID
- Immunisations completed and any missed or outside the window

- Adverse events of note
- Missed visits and loss to follow-up
- Logistical difficulties at the clinical centre
- Data management issues (timeliness of CRFs, completeness)
- Immunology core lab issues (completeness of specimen collection, next batch transfer or analysis)
- GCP issues (minor or other breaches)

16.5 CONFIDENTIALITY

The principles for the UK DPA will be followed. Identifiable personal details of the participants and the results of the trial will be kept strictly confidential. The Sponsor, as represented by the Imperial JRCO, will not keep any material on file containing the participants' full names; this information will be kept by trial team in the clinical trial facilities in a secure location. The confidentiality of participants will be respected and maintained at all times. Each participant's GP will be informed of the nature and timing of the trial.

17 ETHICAL CONSIDERATIONS AND APPROVAL

17.1 ETHICAL ISSUES

There are three aspects of this trial that could raise potential ethical issues.

First, safety: Wild type Ad4 and MVA-CN54 and protein based vaccines have been widely used in man at similar doses to those employed in this trial to vaccinate against a wide range of infectious diseases such as HIV as well as non-infectious diseases such as cancer. To date, there have been no safety concerns. The nature of the product may lead participants to erroneously conclude they are protected against HIV and to engage in riskier behaviour as a consequence. They will be warned against this with the PIS.

Second, confidentiality: because the product under investigation is a **candidate HIV vaccine**, and HIV is transmitted sexually, it is necessary to collect sensitive personal information and the participants will need to undergo STI screening. It is possible that following immunisations, participants may have equivocal results in the standard laboratory tests for HIV. However, it will be possible for any accredited laboratory to distinguish between a response to the vaccine and the occurrence of a natural infection using routine assays.

Third, financial: **the reimbursement** to compensate for the intense follow-up schedule, which is a feature of healthy volunteer trials, could be sufficient incentive for individuals to take part against their better judgement.

17.2 ETHICAL CONSIDERATIONS

The trial will be conducted in compliance with the approved protocol, UK Clinical Trial Regulations and any amendments, which include compliance with the principles of GCP and will abide by the principles of the Declaration of Helsinki, the UK Data Protection Act (DPA number: Z5886415), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

The trial proposal including the trial specific information to be provided to participants will be reviewed by a recognised Research Ethics Committee (REC), The trial will not proceed unless the sponsor obtains a favorable opinion from the REC.

All participants must give written consent to participate in this trial, before any screening evaluation. Before giving consent, participants will be asked to read the information sheet about the trial and raise questions. They must also read the consent form. They will have the opportunity to discuss the trial with the Principal Investigator or delegate, and be asked to explain what the trial involves in their own words, to ensure the volunteer understands the intensity of the schedule and the issues associated with taking part in a trial of a candidate HIV vaccine.

The safety assessments are intense. Participants will be asked to remain in clinic for about 1 hour following each immunisation, to complete a diary card until the day 7 safety visit. They will be advised to call the clinic staff if they are concerned, and 24 hour cover will be available.

18 INDEMNITY

The Sponsor for the trial is Imperial College London (ICL).

The Sponsor undertakes to compensate any participants for injuries which are considered, on the balance of probabilities to have arisen as a result of their participation in the trial regardless of whether the injuries were caused by negligence or not.

ICL holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that IC has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant in the clinical trial. ICL does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of the hospital employees. This applies whether the hospital is an NHS Trust or not. This does not affect the participant's right to seek compensation via the non-negligence route.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of ICL or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Principal Investigator, who will pass the claim to the Sponsor's Insurers, via the Joint Research Compliance Office.

19 FINANCE

The clinical trial activities and acquisition of product, data management and analysis are funded by the Medical Research Council.

Participants 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. Participants will receive £150 per vaccination visit, £100 for screening / safety and follow-up visits up to a total of £1350 over the course of the study. Only enrolled participants will be paid for the screening visit.

Participants who undergo rectal biopsies in Part 2 will be paid an extra £50 per biopsy if it is done on the same day as other procedures/assessments scheduled at the same time-point, or an extra £100 if they have to make an additional visit to the site for the biopsy.

There are no bonuses or per participant incentives paid to staff.

20 OVERSIGHT & TRIAL COMMITTEES

There are a two committees involved with the oversight of the trial. These committees are detailed below.

20.1 TRIAL MANAGEMENT GROUP

A TMG will be formed comprising the Principal Investigator, other lead investigators (clinical and non-clinical) and members of the Coordinating Centre. The TMG will be responsible for the day-to-day running and management of the trial and will be accountable to the Sponsor.

The TMG will monitor the clinical safety data. During the vaccination phase of the trial, the PI will review clinical safety data on an on-going basis. The TMG will also be responsible for the composition of the expert panel to review any emerging SUSAR data.

20.2 INDEPENDENT DATA MONITORING COMMITTEE (IDMC) INTERIM MONITORING AND ANALYSES

Analysis of the safety data will be carried out at Surrey Clinical Research Centre where the main Promasys database will be held. The immunological data will not form part of the main database and will not be analysed by the Surrey Clinical Research Centre.

An IDMC will be invited to oversee this trial, and none of its members have direct involvement with the trial. The IDMC will report to the Sponsor for scheduled meetings, and directly to the Sponsor should an unscheduled meeting be required either to review a SUSAR, other significant adverse event or at the request of the Principal Investigator or TMG.

A charter will be developed to describe the functioning of the IDMC. There will be one scheduled interim analysis after **twenty participants have completed their immunisations and before Part 2 of the trial commences. Data for all participants will be analysed and annotated in a report to be reviewed by the IDMC.**

Further details of IDMC functioning, and the procedures for interim analysis and monitoring are provided in the IDMC Charter.

21 PUBLICATION

The preparation of a manuscript for publication in a peer-reviewed professional journal or an abstract for presentation, oral or written, to a learned society or symposium will be discussed on the TMG calls.

The Sponsor will be notified of this intention through the PI and the TMG notes. Every effort will be made to allow the Sponsor and other relevant parties involved in the clinical trial and named in the clinical trial agreement prepared by the Sponsor, 30 days to comment before any results are submitted. This timeline will be strictly observed for peer-review journals, but may be more difficult to adhere to for conference presentations. Approval from the Principal Investigator, the clinical centre Principal Investigator and Project Lead must be obtained as a minimum before submission to a conference.

Authorship should reflect work done by the investigators and personnel of the sponsor, in accordance with generally recognised principles of scientific collaboration.

Any publications based on the results of the Clinical Trial and originating from ICL or the Investigators will be submitted for review to UKHVC and will require their acceptance for further publications, according to the Ad4HIV agreement.

22 PROTOCOL AMENDMENTS

After the protocol has been approved by the main REC and the MHRA, no changes may be made without the written agreement of the PI and the sponsor.

The MHRA and main REC do not need to approve any substantial change to the protocol that needs to be implemented urgently to avoid an immediate hazard to trial participants. The sponsor will ensure that the MHRA and main REC are informed of urgent amendments in accordance with UK clinical trials regulatory guidance.

The REC and/or MHRA must approve substantial amendments before they are implemented.

23 REFERENCES

Andersson, K. M., Palteil, A. D., and Owens, D. K., (2011) "The Potential impact of an HIV vaccine with rapidly waning protection on the epidemic in Southern Africa: examining the RV144 trial results" Vaccine 18; 29(36):6107-6112.

Alexander, J., Mendy, J., Vang, L., Avanzini, J. B., Garduno, F., Manayani, D. J., Ishioka, G., Farness P., Ping, L. H., Swanstrom, R., Parks, R., Liao, H. X., Haynes, B. F., Montefiori, D. C., LaBranche, C., Smith, J., Gurwith, M., and Mayall, T., (2013) "Pre-clinical development of a recombinant, replication-competent adenovirus serotype 4 vector vaccine expressing HIV-1 envelope 1086 clade C" PLoS One 3;8(12):e82380.

Buffa, V., Klein, K., Fischetti, L., and Shattock, R. J., (2012) "Evaluation of TLR agonists as potential mucosal adjuvants for HIV gp140 and tetanus toxoid in mice" 7(12): e50529

Draper, S. J., Cottingham, M. G., and Gilbert, S. C., (2013) "Utilizing poxviral vectored vaccines for antibody induction-progress and prospects" Vaccine 6;31(39): 4223-4230

Granich, R., S. Gupta, A. B. Suthar, C. Smyth, D. Hoos, M. Vitoria, M. Simao, C. Hankins, B. Schwartlander, R. Ridzon, B. Bazin, B. Williams, Y. R. Lo, C. McClure, J. Montaner and G. Hirnschall (2011). "Antiretroviral therapy in prevention of HIV and TB: update on current research efforts." Curr HIV Res **9**(6): 446-469.

Granich, R., Y. R. Lo, A. B. Suthar, M. Vitoria, R. Baggaley, C. M. Obermeyer, C. McClure, Y. Souteyrand, J. Perriens, J. G. Kahn, R. Bennett, C. Smyth, B. Williams, J. Montaner and G. Hirnschall (2011). "Harnessing the prevention benefits of antiretroviral therapy to address HIV and tuberculosis." Curr HIV Res **9**(6): 355-366.

Gray, G., Buchbinder, S., and Duerr, A., (2010) "Overview of STEP and Phambili trial results: two phase IIb test-of-concept studies investigating the efficacy of MRK adenovirus type 5 gag/pol/nef subtype B HIV vaccine" Curr Opin HIV AIDS 5(5): 357-361

Gray, G. E., Moodie, Z., Metch, B., Gilbert, P. B., Bekker, L. G., Churchyard, G., Nchabeleng, M., Mlisana, K., Laher, F., Roux, S., Mngadi, K., Innes, C., Mathebula, M., Allen, M., McElrath, M. J., Robertson, M., Kublin, J., Corey, L., and HVTN 503/Phambili study team. (2014) "Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study" Lancet Infect Dis 14(5): 388-396

Gurwith, M., Lock, M., Taylor, E. M., Ishioka, G., Alexander, J., Mayall, T., Ervin, J. E., Greenberg R. N., Strout, C., Treanor, J. J., Webby, R. and Wright, P. F., (2013) "Safety and immunogenicity of an oral, replicating adenovirus serotype 4 vector vaccine for H5N1 influenza: a randomised, double-blind, placebo-controlled, phase 1 study" Lancet 13(3); 238-250

Kim, J. H., S. Rerks-Ngarm, J. L. Excler and N. L. Michael (2010). "HIV vaccines: lessons learned and the way forward." Curr Opin HIV AIDS **5**(5): 428-434.

Kim, S. C., S. Becker, C. Dieffenbach, B. S. Hanewall, C. Hankins, Y. R. Lo, J. W. Mellors, K. O'Reilly, L. Paxton, J. S. Roffenbender, M. Warren, P. Piot and M. R. Dybul (2010). "Planning for pre-exposure prophylaxis to prevent HIV transmission: challenges and opportunities." J Int AIDS Soc **13**: 24.

Kim, S. C., Exceler, J. L., (2015) "Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlations of protection" Annu Rev Med 66: 423-437

Koff, W. C. (2010). "Accelerating HIV vaccine development." Nature **464**(7286): 161-162.

Koff, W. C., Johnson, P. R, Watson, D. I., Burton, D. R., Lifson, J. D., Hasenkrug, K. L., McDermott, A. B., Schultz, A., Zamb, T. J., Boyle, R. and Desrosiers, R. C., (2006). HIV Vacine Design: Insights from live attunated SIV vaccines. Nat Immunol 7(1): 19-23

Krakower, D. and K. H. Mayer (2011). "Promising prevention approaches: tenofovir gel and prophylactic use of antiretroviral medications." Curr HIV/AIDS Rep **8**(4): 241-248.

Lewis, D. J., Fraser, C. A., Mahmoud, A. N., Wiggins, R. C., Woodrow, M., Cope, A., Cai, C., Giemza, R., Jeffs, S. A, Manoussaka, M., Cole, T., Cranage, M. P., Shattock, R. J., and Lacey, C. J., (2011) "Phase I randomised clinical trial of an HIV-1(CN54), clade C, trimeric envelope vaccine candidate delivered vaginally" PLosOne 6(9):e25165

Long, E. F., and Owens, D. K (2011) "The cost-effectiveness of a modestly effective HIV vaccine in the United States" Vaccine 18;29(36): 6113-6124

Lyons A, Longfield J and Kuschner R, "A double-blind, placebo-controlled study of the safety and immunogenicity of live, oral type 4 and type 7 adenovirus vaccines in adults" Vaccine (2008); 26:2890-8.

McKay, P. F., Cope, A. V., Mann, J. F., Joseph, S., Esteban, M., Tatoud, R., Carter, D., Reed, S. G., Weber, J., and Shattock, R. J., "Glucopyranosyl lipid A adjuvant significantly enhances HIV specific T and B cell responses elicited by a DNA-MVA-protein vaccine regimen" Plos One (2014) 23;9(1);e84707

Ogra, P. L., Ogra, S. S., Al-Nakeeb, S., and Coppola, P. R., (1973) "Local antibody response to experimental poliovirus infection in the central nervous system of rhesus monkeys" Infect Immun 8(6):931-937

Radin, J. M., Hawksworth, A. W., Blair, P. J., Faix, D. J,. Raman, R., Russell, K. L. and Gray, G. C., (2014) "Dramatic decline of respiratory illness among US military recruits after the renewed use of adenovirus vaccines" Clin Infect Dis 59(7): 962-968

Rerks-Ngarm, S., P. Pitisuttithum, S. Nitayaphan, J. Kaewkungwal, J. Chiu, R. Paris, N. Premsri, C. Namwat, M. de Souza, E. Adams, M. Benenson, S. Gurunathan, J. Tartaglia, J. G. McNeil, D. P. Francis, D. Stablein, D. L. Birx, S. Chunsuttiwat, C. Khamboonruang, P. Thongcharoen, M. L. Robb, N. L. Michael, P. Kunasol and J. H. Kim (2009). "Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand." N Engl J Med **361**(23): 2209-2220.

Rodenburg, C. M., Li, Y., Trask, S. A., Chen, Y., Decker, J., Robertson, D. L., Kalish, M. L., Shaw, G. M., Allen, S., Hahn, B. H., Gao, F. and UNAIDS and NIAID Networks for HIV Isolation and Characterization. (2001) "Near full-length clones and reference sequences for subtype C isolates of HIV type 1 from three different continents" AIDS Res Hum Retroviruses 20;17(2): 161-168

Rudin, C. M., and Thompson, C. B., (1998) "B-cell development and maturation" Semin Oncol 25(4):435-446

Su, L., Graf, M., Zhang, Y., von Briesen, H., Xing, H., Köstler, J., Melzl, H., Wolf, H., Shao, Y., and Wagner, R., (2000) "Characterization of a virtually full-length human immunodeficiency virus type 1 genome of a prevalent intersubtype (C/B') recombinant strain in China" J Virol 74(23):11367-11376

UNAIDS Report on the global AIDS epidemic 2013. Geneva, S. (2013). UNAIDS.

Wamai, R. G., B. J. Morris, S. A. Bailis, D. Sokal, J. D. Klausner, R. Appleton, N. Sewankambo, D. A. Cooper, J. Bongaarts, G. de Bruyn, A. D. Wodak and J. Banerjee (2011). "Male circumcision for HIV prevention: current evidence and implementation in sub-Saharan Africa." J Int AIDS Soc **14**: 49.

24 APPENDICES

APPENDIX 1 ADVERSE EVENTS GRADING

All AEs (except those listed in the tables below) should be graded according to the following criteria:

- **Symptom not experienced (Grade 0)**
- **Mild (Grade 1):** No interference with daily activity
- **Moderate (Grade 2):** Some interference with daily activity
- **Severe (Grade 3):** Prevents daily activity
- **Potentially life-threatening (Grade 4):** Emergency treatment or hospitalisation

REACTION	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Fever (oral temperature)	38.0 – 38.4	38.5 – 38.9	39.0 – 40.0	≥ 40.1
Tachycardia (bpm)*	101 – 115	116 – 130	> 130	Emergency treatment or hospitalisation
Bradycardia (bpm)**	50 – 54	45 – 49	< 45	Emergency treatment or hospitalisation
Hypertension (mm Hg) Systolic	141 – 150	151 – 155	> 155	Emergency treatment or hospitalisation
Hypertension (mm Hg) Diastolic	91 – 95	96 – 100	> 100	Emergency treatment or hospitalisation
Hypotension (mm Hg)*** Systolic	85 – 89	80 – 84	< 80	Emergency treatment or hospitalisation
Redness (cm) (greatest single diameter)	2.5 – 5	5.1 – 10	≥ 10.1	Necrosis or exfoliative dermatitis
Swelling (cm) (greatest single diameter)	2.5 – 5	5.1 – 10	≥ 10.1	Necrosis

* *At rest*

** *When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes.*

*** *Only if symptomatic (e.g. dizzy/ light-headed)*

LABORATORY PARAMETERS

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 EXTREME
HAEMATOLOGY				
Haemoglobin (g/L) Female	113 – 110	109 – 95	94 – 80	< 80
Haemoglobin (g/L) Female **(change from baseline)**	Any decrease – 15	16 – 20	21 – 50	> 50
Haemoglobin (g/L) Male	129 – 125	124 – 105	104 – 85	< 85
Haemoglobin (g/L) Male **(change from baseline)**	Any decrease – 15	16 – 20	21 – 50	> 50
White Blood Count ($x10^9$/L) Increase Decrease	11.5 – 15.0 OR 3.5 – 2.5	15.1 – 20.0 OR 2.4 – 1.5	20.1 – 29.9 OR 1.4 – 1.0	≥ 30.0 OR < 1.0
Absolute Neutrophils ($x10^9$/L)	1.9 – 1.5	1.4 – 1.0	0.9 – 0.5	< 0.5
Lymphocytes ($x10^9$/L)	1.0 – 0.8	0.7 – 0.5	0.4 – 0.2	< 0.2
Platelets ($x10^9$/L)	129 – 125	124 – 100	99 – 25	< 25
BIOCHEMISTRY				
ALT (increase by factor)	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Alkaline Phosphatase (increase by factor)	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10.0 x ULN	> 10.0 x ULN
Bilirubin (increase by factor)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Creatinine (increase by factor)	1.1 – 1.5 x ULN	1.6 – 3.0 ULN	> 3.0 ULN	Dialysis