APPLICATION FOR CONSENT TO RELEASE A GMO – ORGANISMS OTHER THAN HIGHER PLANTS

Department for Environment, Food and Rural Affairs

PART A1: INFORMATION REQUIRED UNDER SCHEDULE 2 OF THE GENETICALY MODIFIED ORGANISMS (DELIBERATE RELEASE) REGULATIONS 2002

Part I: General Information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Name and address of the applicant
Chair in Mucosal Infection and Immunity
Imperial College London
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London
W2 1NY

2. The title of the project.
A Phase I Single-Blind randomised trial investigating immunisation strategies using Ad4-EnvCN54, MVA-CN54 and CN54rgp140 combinations in order to maximise antibody responses to Human Immunodeficiency Virus

Short Title: Ad4HIV

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Part II: Information relating to the Organisms

Characteristics of the donor, parental and recipient organisms

3. Scientific name and taxonomy.
Ad4-EnvCN54 is a live attenuated adenovirus serotype 4 vector based investigational vaccine product that encodes a truncated length of a HIV-1 membrane expressed trimeric envelope protein (CN54-Env gp150). Ad4-EnvCN54 will be used together in combination with other study IMP (non-replicative) for this trial in a prime-boost vaccination regimen.

Adenovirus serotype 4 is a non-enveloped, icosahedral virion, 70-90nm in diameter containing a double-stranded linear DNA genome.

Family:	Adenoviridae
Subfamily:	Mastadenoviruses
Genus:	Adenovirus
Species:	Adenovirus serotype 4

4. Usual strain, cultivar or other name.
Adenovirus serotype 4 belongs to the family of Adenoviridae

5. Phenotypic and genetic markers.
The Ad4-EnvCN54 vaccine candidate is a live, replication-competent recombinant adenovirus serotype 4 (Ad4) vaccine vector expressing an HIV-1 clade C truncated (gp150) Env. The construct was derived from the wild type, unattenuated Ad4 virus used by the US military as a vaccine and originally developed by Wyeth. The Ad4 wild type viral DNA from the Wyeth tablet was cloned into a plasmid capable of replication in *E. coli*. The entire Ad4 DNA genome was subsequently sequenced to confirm identity to the sequence on file, GenBank accession number: AY594254. The Ad4 DNA was transfected into the A549 cell substrate adherent cells with linearized plasmids and plaque-purified prior to amplification for master viral seed production.

The vector design is based on the adenovirus serotype 4 where the gene of interest is inserted downstream of the intact E3 region between the E3B poly A sequence and the L5 fiber gene. The HIV1-CN54-envelope glycoprotein-150 (GenBank AX149771.1) was synthesized by GeneArt and codon optimized which includes MPER and terminates 118 amino acids upstream of the carboxyl end of the envelope. The expression cassette consists of HIV1-CN54 envelope glycoprotein-150 transgene followed by BGH polyA allowing for robust processing of the RNA, and is driven by the endogenous E3 promoter.

To generate the virus, the shuttle plasmid containing the expression cassette is recombined with the full length viral genome plasmid in bacteria. The full length viral backbone is described in PaxVax technical report PTR-MB-GD-001. The final recombinant viral plasmid is linearized to release the viral genome from the bacterial backbone and transfected into A549 cells to generate the recombinant virus.

6. The degree of relatedness between the donor and recipient or between parental organisms.

Derivation History: The recombinant vector design is based on the adenovirus serotype 4 (Ad4) virus where the HIV-1 CN54 envelope gene of interest (GenBank AX149771.1) is inserted downstream of the intact E3 region between the E3B poly A sequence and the L5 fiber gene (Technical Report, PTR-RD-MRC-001, "Vector design, construction & characterization of Ad4 Full length-E3-HIV1-CN54 pg150 envelope clade C"). The *env* gene was synthesized by GeneArt and codon optimized which includes MPER and terminates 118 amino acids upstream of the carboxyl end on the gene. The expression cassette consists of Env glycoprotein-150 (gp150) transgene followed by BGH poly A allowing for robust processing of the RNA.

Biological characteristics of the parent viral strain: The parental wild type Ad4 virus vaccine has been orally administered to more than 10 million U.S. recruits between 1971 and 1999 and shown to be very well tolerated and safe (Gaydos et al 1995; Gurwith et al 1989; Kajon et al 2007). The vaccine was re-introduced in October 2011 (Hoke et al 2013). The Ad4 U.S. Military vaccine protects against Ad4 respiratory disease confirming the oral delivery and in vivo replication induce protective immunity against respiratory exposure to adenovirus.

Route of transmission of the parent strain: Adenovirus 4 transmission predominantly occurs via short-range droplets and aerosols, and via direct contact with contaminated fomites with self-inoculation onto mucous membranes. Transmission can also be mediated by faecal-oral and occasionally water transmission. However the parental Ad4 vaccine is attenuated by route of delivery (oral) and has been administered to more than 10 million US Military recruits without significant toxicity (Gaydos et al 1995). No significant vaccine associated illness has been reported. In a recent Phase 1 re-licensure study Ad4 vaccine virus shedding was not detected in throat swab specimens from any vaccinee; faecal shedding of the Ad4 vaccine was detected between 7 - 21 days post vaccination in 27% of vaccinees.

In a previous large phase 3 study (3031 received vaccine; (1009 received placebo) one placebo recipient developed ARD caused by type 4 vaccine virus (Lyons et al 2008). This was not unexpected because person-to-person transmission of the Ad4 vaccines was previously documented. Such transmission was rare and facilitated by close contact, such as between married couples and within families (Mueller et al 1969; Stanley et al 1969). However this was defined by serology as no molecular typing was available at the time, thus it is hard to distinguish transmission from naturally circulating strains. Intra-family spread was more common when young children received the Ad4 vaccines rather than their parents, suggesting faecal

contamination as the possible mechanism. Recruit life may be particularly conducive to transmission, with individuals living, eating, and sleeping in close quarters. In addition, training necessitates working in close proximity and physical contact (e.g., combative training). Under these conditions, it is encouraging that only one case of transmission (0.1%) was identified (upper 95% CI, 0.6%). Furthermore, the trial design likely overestimates actual future risk of transmission between recruits.

Replication competence: The parent and recombinant Ad4-EnvCN54 vector are replication competent.

Attenuation: The Ad4 respiratory virus is attenuated when delivered by an oral route (Gaydos et al 1995; Lyons et al 2008).

Tropism of the product: Tissue tropism for Ad4 is typically respiratory and ocular (Coughlan et al 2010). The best-studied Ad4 receptor is the coxsackie and adenovirus receptor (CAR) a 46-kKa protein and a member of an immunoglobulin superfamily with two immunoglobulin-like extracellular domains (Zhang et al 2005; Roelvink et al 1998). CAR is present in specialized intracellular junctions, including polarized epithelial cells. The vector can only replicate in human cells and there is no other known host capable of supporting the replication of Ad4.

7. The description of identification and detection techniques.

Detection and identification of Ad4-EnvCN54 is accomplished using the following assays:

- Viral Particle Concentration by AE-HPLC
- TCID(50) assay to measure titers of infectious virus
- Polymerase Chain Reaction (PCR) of inserted genes and recombination junctions for virus identity
- Transgene glycoprotein expression by Western Blot

8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

- Viral Particle Concentration by AE-HPLC: The test measures the total Ad4 viral particles per unit in the MVS, BDS, and final drug product. The Ad4-EnvCN54 is evaluated against an Ad4 wild type virus Reference Standard. The method is based on the retention of intact virus particles on an anion exchange resin in the presence of low ionic strength buffers. The virus is eluted with a sodium chloride gradient. The virus peak has a unique retention time and UV absorbance at 260 nm, which is discernable by analysis of the Photodiode Array (PDA) detector spectral. Routine quantitation of the virus particles is performed by monitoring the column elution profile at 260 mm (PDA) and comparing the peak area to that of known reference standard (for viral particle per millilitre between 2.70e9 and 5.38e10). The Ad4 reference standard elutes at 4.0 to 5.5 minutes.

- TCID(50) assay to measure titers of infectious virus: The test is a method for estimating adenoviral infectious titer in an end-point assay set up in a 96-well tissue culture plate. Virus (8 wells per dilution) and A549 cells are incubated for 2-3 days. The dilutions that produce fewer than 100% positive wells are used in the titer calculation per the Spearman-Karber analysis method (Finney). Spearman-Karber provides an interpolation to a midpoint, providing a dilution where 50% of the wells would have been positive.
- Polymerase Chain Reaction (PCR) of inserted genes and recombination junctions for virus identity by DNA sequencing: This assay is used to detect the absence or presence of the transgene. Primers specific for flanking upstream and downstream region of the transgene are used to generate a PCR product that spanned the envelope transgene and adjoining Ad4 E3 region. PCR amplification of extracted viral DNA generated a single band of 4.8 Kb. PCR product was sequenced to confirm the presence of the transgene and absence of inadvertent mutations. Alignment of the sequencing data with the control plasmid confirmed the presence of EnvCN54 and the absence of mutations in the transgene and flanking Ad4 sequences.
- Transgene glycoprotein expression by Western Blot: This assay uses the western blot method to detect EnvCN54 protein expression by the adenoviral vector while replicating in A549 cells. This assay may be used after production of the master viral bank, bulk drug substance and/or drug product. Test sample acceptance is based on the detection of a 150kDa band on the blot. The controls and antibodies used are as follows: positive control is HIV-1 CN54 gp120 recombinant protein, NIH AIDS Reagent Program, 7749; negative control is Ad4 wild type cell lysate from the same batch of cells used for viral amplification; primary antibody is mAb mouse V1V2[gp120/gp160(Clade B/C'/CN54)], Immune Technology, IT-001-21 1M1; and secondary antibody, Goat anti-mouse IgG (H+L)-HRP, Invitrogen, #62-6520.

9. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.

Adenovirus serotype 4 has global prevalence where 30% of adults have detectable neutralising antibodies. Epidemics of Ad4 infection may occur in healthy children or adults in closed or crowded settings (particularly military recruits). Adenovirus serotypes 4 has been associated with outbreaks of acute respiratory disease among military recruits (Gaydos et al 1995; Gurwith et al 1989). These outbreaks have resulted in hospitalization and some mortality (Gray et al 2000). Humans are the only host able to support replication of Ad4.

10. The organisms with which transfer of genetic material is known to occur under natural conditions.

None known

11. Verification of the genetic stability of the organisms and factors affecting that stability.

Adenoviruses are known to be genetically stable (Bett et al 1993). Synchronous infection results in a single amplification cycle which, when combined with the replication efficiency of a replication- competent vector, offers little or no opportunity for the culture to be overrun by an undesirable mutant. Additionally, because the virus is capable of replication in human cells without viral genes provided in Trans, the virus can be produced in A549 cells.

As part of the qualification of the Master Viral Seed (MVS) stock, the complete Ad4-EnvCN54 viral genome was sequenced (under GXP conditions) and expression of the transgene confirmed at the MVS stage. Also, partial DNA sequencing is performed at the drug substance and drug product stages to confirm that the Env transgene is present and of the correct DNA sequence.

To evaluate genetic stability of the MVS under production conditions, Pre-MVS virus was serially passaged multiple times such that the total number of passages (Pre-MVS plus 2 passages) approximates the number of passages envisioned for full-scale clinical manufacture. The pre-MVS virus was transferred for GMP MVS production at passage 6 of the virus and the virus was passaged 2 additional times at the pre-MVS stage, i.e. 8 passages total. Each intermediate passage was then screened for DNA sequences using a research-grade PCR assay that amplifies across the E3 region and includes the transgenes and flanking Ad4 sequences. The serial passages were performed using an infectious titer that matches that used in the propagation of the virus in GMP manufacture, 2×10^8 vp/ml The PCR results demonstrated a single PCR species of the correct size across all passages up to and including passage 8.

In addition, the PCR product at passage 8 was sequenced to confirm that no point mutations had occurred in the transgene region and a restriction mapping of the viral genome was completed to confirm no gross changes in the genome structure. A confirmatory test was a Western blot to reaffirm the correct transgene protein expression. These tests demonstrated the genetic stability of the MVS under production conditions to passage 8.

12. The following pathological, ecological and physiological traits:

a. The classification of hazard according to existing Community rules concerning the protection of human health and the environment

In terms of classification of hazard, the human adenovirus serotype 4 is classified as a group 2 biological agent according to the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC).

b. The generation time in natural ecosystems, and the sexual and asexual reproductive cycle

Adenovirus serotype 4 has no known natural animal reservoirs and exclusively replicates in human cells. Adenovirus causes a transient infection in susceptible hosts, with elimination of viral components over several weeks. Host cells infected with adenovirus are short lived (days) and are cleared by induced immunity. Adenovirus replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA. Latent infection of humans with adenovirus serotype 4 has not been observed.

c. Information on survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia

Ad4-EnvCN54 is a live virus and does not form structures. Survivability is dependent upon the ability to replicate within a host cell. The virus is relatively stable when stored frozen or lyophilized under carefully controlled conditions. However stability decreases significantly as temperature is increased. Under normal environmental conditions, Ad4-EnvCN54 is expected to lose viability within days to weeks.

d. Pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms

Adenovirus serotype 4 only replicates in humans and there is no other host capable of supporting infection.

The “parent wild-type” Ad4 vaccine is currently commercially available and widely distributed in the United States. Considering the lack of human experience with Ad4-EnvCN54 and the absence of relevant animal models of Ad4 or Ad4-EnvCN54-associated pathology, potential side effects may best be predicted by review of the experience with the US military Ad4 vaccine virus and by the symptoms associated with naturally-occurring Ad4 infections. The US military live oral Ad4 virus vaccine was safely administered orally to approximately 10 million US military recruits between 1971 and 1999 and was recently re-licensed in 2011. Early studies with the Ad4 vaccine, which was often administered in combination with the analogous Ad7 vaccine, reported remarkably few adverse reactions (Gutekunst 1967; Peckinpaugh 1968; van der Veen et al 1968; Gaydos 1995).

The more recent studies completed by Teva Pharmaceuticals to support relicensing of the military Ad4/Ad7 vaccine (Lyons 2008, Kuschner et al 2013, Teva Pharmaceuticals USA, Inc., 2014) provide a detailed description of what might be expected in the Phase 1 clinical study of Ad4-EnvCN54.

The most common (≥ 5%) systemic adverse reactions observed in clinical trials supporting the recent licensure of the Teva Ad4/Ad7 vaccine were upper respiratory tract infections, headache, nasal congestion, pharyngolaryngeal pain, cough, arthralgia, nausea, abdominal pain, diarrhea, and vomiting.

Less common (less than 5%) adverse reactions reported in the Phase 3 clinical trial in military recruits receiving Teva Ad4/Ad7 vaccine versus placebo, respectively,

included rhinorrhea (128 [4.22%] vs. 25 [2.48%]), pain in extremity (130 [4.29%] vs. 37 [3.67%]), and pyrexia (fever greater than or equal to 100.5° F) (126 [4.16%] vs. 49 [4.86%]) (Kuschner 2013).

Serious adverse events in the Teva Ad4/Ad7 Phase 3 trial considered possibly related to vaccination included hematuria, gastroenteritis, febrile gastroenteritis, gastritis, pneumonia, and hematochezia. The rate of serious adverse events was similar in the vaccine and placebo groups.

e. Antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;
Not applicable.

f. Involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration.
There is no evidence of Ad4 having any involvement in environmental processes.

13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.
Not applicable. Infection of a cell with Ad4-EnvCN54 results in tropism of mucosal surfaces of the GI tract, initiating replication in submucosal tissues, thus maximizing effective expression of native HIV envelope trimers on the surface of infected human cells only.

14. The history of previous genetic modifications.
The PaxVax Ad4 vector design is based on the Ad4 military live virus vaccine. The Ad4-EnvCN54 vectored vaccine is replication-competent and based on the Ad4 delivery vehicle where the HIV-1 envelope (Env) gene is inserted downstream of the intact E3 region between the E3B poly A sequence and the L5 fiber gene. The HIV-1 Env is a viral surface glycoprotein and the appropriate Env-specific immune responses generated following administration of the vaccine may provide protection against the HIV-1 virus.

The HIV1-CN54-Env glycoprotein-150 strain 97CN001 (GenBank AX149771.1) was synthesized by GeneArt and codon optimized which includes MPER and terminates 118 amino acids upstream of the carboxyl end of the Env glycoprotein. The expression cassette consists of HIV1-CN54 Env glycoprotein-150 transgene followed by BGH polyA allowing for robust processing of the RNA, and is driven by the Ad4 virus endogenous E3 promoter.

The current vector is based on the wild type adenovirus vaccine strain with no E3 deletions, whereas Ad4 vector constructs generated thus far for the influenza, anthrax, and HIV vaccine development programs have had a range of E3 gene deletions.

Characteristics of The Vector

15. The nature and source of the vector.

The Ad4 “parent wild type” virus used to generate recombinant Ad4-EnvCN54 vaccine was obtained from the Wyeth Ad4 wild type virus vaccine tablet and is described in item 5, “Phenotypic and Genetic Markers”.

As described more fully in Items 19 and 20 below, the insertion of foreign genes into the Ad4 genome was accomplished using a plasmid transfer vector (pUC-Ad4-FLE3-HIV1-CN54-gp150env) that contains the EnvCN54 expression cassette flanked by additional adenovirus sequences that direct recombination within a specified genome region. The starting plasmid used to generate the plasmid transfer vector was derived from the plasmid vector pUC18 (ThermoFisher Scientific).

16. The sequence of transposons, vectors and other non-coding genetic segments used to construct the genetically modified organisms and to make the introduced vector and insert function in those organisms.

As described more fully in Items 19 and 20 below, HIV envelope gene inserted into the parental adenovirus genome by homologous DNA recombination within transformed bacterial cells using pUC18 plasmid transfer vectors that contain the EnvCN54 expression cassette flanked by additional adenovirus sequences that direct recombination within a specified genome region. The genetic structures of the plasmid transfer vector used in the generation of Ad4-EnvCN54, as well as the origin of the Ad4 vector component is described in detail in Item 20 below.

17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector.

Not applicable. Homologous recombination between adenovirus DNA and the encoded gene of interest (EnvCN54 gp150) results in insertion of the heterologous genes into the adenoviral genome; the original plasmid transfer vector is not part of the GMO. Evaluation of EnvCN54 insertion event into the correct position is confirmed by partial genetic sequencing of the EnvCN54 gene and flanking Ad4 sequences.

18. The degree to which the vector is limited to the DNA required to perform the intended function.

The Ad4-EnvCN54 vectored vaccine is limited to the Ad4 genes needed for Ad4 virus replication and additionally, the inserted HIV-1 EnvCN54 gene required to induce an immune response to HIV-1 virus.

The pUC transfer shuttle plasmid used in the generation of Ad4-EnvCN54 recombinant virus contains DNA sequences encoding the CN54 envelope gene, flanking Ad4 sequences, and pUC sequences. The EnvCN54 gene sequence is flanked by Ad4 genomic regions that allow homologous recombination between the transfer plasmid and the adenovirus genome, respectively. The backbone of the transfer plasmid includes a bacterial origin of replication and the ampicillin resistance

gene, which allow the selection and propagation of the plasmids in bacterial cells; however, only HIV envelope genes sequences is present in the final recombinant virus, Ad4-EnvCN54.

Characteristics of the Modified Organisms

19. The methods used for the modification.

As described briefly in Items 17 and 18 above, the generation of recombinant Ad4-EnvCN54 vaccine is accomplished via homologous recombination, within a transformed bacterial cell, between adenovirus DNA and a plasmid shuttle vector that carries the heterologous EnvCN54 sequences to be inserted. The plasmid shuttle vector encodes EnvCN54, flanking Ad4 sequences, and pUC sequences (i.e., ampicillin resistance gene and origin of replication of the plasmid in bacterial cells. The plasmid is transformed into BJ5183 bacterial cells together with the parental Ad4 virus which encodes a bacterial origin of replication and a kanamycin resistance gene. Recombination between Ad4 sequences on the shuttle plasmid and the corresponding DNA in the Ad4 viral genome results in the insertion into the Ad4 viral genome of the HIV-1 EnvCN54 gene. Recombinant DNA from bacterial colonies (Ad4 encoding the EnvCN54, a bacterial origin of replication, and a kanamycin resistance gene) are subsequently transformed into DH10B bacterial cells which are capable of producing high quality DNA at high levels. The recombinant DNA is digested with appropriate restriction enzymes to obtain the Ad4-EnvCN54 linear fragment (without the bacteria origin of replication and kanamycin resistance gene). The linear fragment is used for transfection of A549 mammalian cells to generate the Ad4-EnvCN54 virus vaccine.

20. The methods used:

A. To construct inserts and introduce them into the recipient organism:

The generation of the pUC18 shuttle vector encoding EnvCN54 and Ad4 flanking regions and generation of final Ad4-EnvCN54 virus occurred in the following major steps:

1. Generation of the human HIV-1 CN54Env (gp160) transgene: The HIV-1 strain GenBank AX149771.1 was synthesized by GeneArt and codon-optimized using their proprietary algorithm.

2. Generation of EnvCN54 gp150 PCR fragment: Sense and antisense primers were used to truncate the Env gene (gp160 to gp150) and introduce cloning sites.

3. Generation of the shuttle plasmid containing pUC18 genes, EnvCN54 gene, and flanking Ad4 genes: the 2.24 kb PCR fragment (EnvCN54 gp150) was cloned into the Spe1-Xho fragment of the shuttle vector to generate: Ad4 E3-intact (9 genes) region - [EnvCN54] - Ad4 fiber gene - pUC ampicillin resistance gene-and pUC bacterial origin of replication.

4. Generation of plasmid Ad4-EnvCN54: Having completed the construction of the shuttle plasmid the next step was to move the insert to the large Ad4 plasmid pPV-Ad4-partial deleted E3-EM7-GFP by homologous recombination in bacteria. The shuttle plasmid was linearized with HindIII and pPV-Ad4-PDE3-EM7-GFP was linearized with Spe1 generating 11.2kb and 35.8kb fragments respectively. Using a 1:10 vector: insert molar ratio the homologous recombination was executed in *E. coli* BJ5183 electrocompetent cells following the manufacturer's protocol and plated in kanamycin LB plates. After an overnight incubation, clones were picked, miniprepped,and screened with EcoR1/HindIII restriction enzymes. Due to the combination of a low copy number origin and the BJ5183 cells, the DNA from these bacterial cells are of low quality and quantity. Therefore plasmids from 2 positive clones from the original BJ5183 screening were transformed into DH10B chemically competent cells which are capable of producing high quality DNA at high levels. Sub-clones from DH10B were re-screened with the same restriction enzymes and two positive clones were picked for large scale DNA preparation and sequenced. The recombination event generates plasmid Ad4-EnvCN54.

5. Generation of linear Ad4-EnvCN54 for transfection of A549 cells: The plasmid Ad4-EnvCN54 DNA is digested with PacI restriction enzyme to obtain the Ad4-EnvCN54 linear fragment (without the bacteria origin of replication and kanamycin resistance gene). The linear Ad4-EnvCN54 DNA fragment was used to transfect A549 cells to generate Ad4-EnvCN54 virus.

B. To delete a sequence:

No sequence was deleted in the Ad4 virus; the sequence encoding an HIV-1 envelope glycoprotein CN54 strain (GenBank AX149771.1) was inserted downstream of the intact E3 region between the E3B poly A sequence and the L5 fiber gene. This is the only manipulation of the parental vaccine strain.

21. The description of any insert and/or vector construction

Detailed in items 19 and 20 above

22. The purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function.

For Ad4-EnvCN54 the DNA sequence of the inserted transgene was determined. Ad4-EnvCN54 exhibited the expected nucleotide sequence; the coding sequence of the inserted gene is identical to the predicted sequence. The transgene is inserted at the expected site in the viral genome. No unexpected rearrangements were detected. Portions of the vector manipulated during its derivation conformed to predicted sequence. The inserted sequence is limited to the inserted gene.

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23. The methods and criteria used for selection

The methods and criteria used to select the recombinant Ad4-EnvCN54 are detailed in items 19 and 20, above. Confirmation of identity, genomic structure, and protein expression were performed as described below.

Confirmation of the identity and genomic structure of Ad4-EnvCN54 viral DNA was accomplished by restriction enzyme digestion using two different enzymes and visualized on an agarose gel using ethidium bromide staining. Analysis of the recombinant virus was conducted in parallel with the viral plasmid DNA used for homologous recombination.

In addition, sequence analysis was performed on the inserted foreign gene, on the recombination junctions, and on the regions 200 base pairs upstream and downstream of the gene insert. Recombinant virus DNA sequences were compared to the sequence of the plasmid vector used to generate the recombinant.

Western blot analysis, using antibodies specific for EnvCN54 protein was used to examine the molecular weight and identity of the protein expressed by the recombinant virus.

24. The sequence, functional identity and location of the altered, inserted or deleted nucleic acid segments in question and, in particular, any known harmful sequence.

There are no harmful traits associated with the EnvCN54 rgp150 inserted into Ad4-EnvCN54. In Ad4-EnvCN54, the HIV-1 CN54 envelope gene of interest (GenBank AX149771.1) is inserted downstream of the intact E3 region between the E3B poly A sequence and the L5 fiber gene. No adenovirus genes are interrupted by this insertion.

The identity and function of the inserted genes is described in detail in Item 23, above.

Characteristics of the Genetically Modified Organisms in their Final Form

25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.

Ad4-EnvCN54 is an adenovirus serotype 4 into which has been inserted a gene encoding CN54 HIV envelope gp150. No adenoviral genes are interrupted by the insertion of gene expressing CN54 gp150. The inserted gene encodes for the envelope glycoprotein of HIV-1, although this has potential to bind to human CD4 on the surface of immune cells, there have been no adverse events seen it the wide range of clinical vaccine studies using viral vectors expressing the HIV envelop glycoprotein or with recombinant versions used at concentrate that would far exceed those expressed following Ad4-EnvCN54 infection.

There is no evidence to suggest that the inserted gene CN54gp150 can be transferred to related organisms. The encoded version of the HIV envelope glycoprotein is not functional and would provide no advantage or change in pathogenicity, thus in the unlikely event that it was transferred, it would render the organism less fit than any wild type progenitor. Thus there are no perceived hazards related to potential sequences being transferred to related organisms. Thus, with the exception of its ability to express the CN54 rgp150 gene, the genetic traits or phenotypic characteristics of Ad4-EnvCN54 are essentially those of parental Ad4 vaccine strain.

26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms.

The only exogenous gene present in Ad4-EnvCN54 is the gene CN54 HIV envelope rgp150.

27. The stability of the organism in terms of genetic traits.

The entire genome of the Master Viral Seed (MVS) has been sequenced. In addition, for the Bulk Drug Substance (BDS) and production lot, identity is demonstrated by partial genomic DNA sequencing which includes the EnvCN54 and flanking Ad4 regions. Also, for the MVS, BDS, and production lot, Western blot analysis is performed to confirm EnvCN54 protein expression. In aggregate this testing provides verification of genetic stability.

28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level.

This has not been measured

29. The activity of the gene product.

The presumed mode of action of Ad4-EnvCN54 involves the induction of anti-envelope immune responses against HIV-1. Ad4-EnvCN54 is a prophylactic vaccine designed to deliver the HIV envelope antigen gp150 to B cells and associated immune cells required to trigger an immune response. The expression and antigenicity of the EnvCN54 gene product has been determined by western blot and FACS analysis of EnvCN54 cell-surface expressed glycoprotein using broadly neutralizing Env-specific antibodies.

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector.

Confirmation of the identity and genomic structure of the recombinant viruses is accomplished by (1) PCR amplification of the inserted genes and flanking regions followed by DNA sequencing; (2) FACS assay using antibodies specific for CN54 gp150 protein to detect cell-surface expression of the Env protein; and (3) Western blot analysis using antibodies specific for CN54 gp150 to examine the molecular weight and identity of the polypeptide expressed by the recombinant virus in cell lines. Analysis is conducted in parallel with analysis of Ad4 reference standard.

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

Descriptions of the PCR and DNA sequencing, Western blot, AE-HPLC and TCID(50) assays are provided in item 8, above.

32. The history of previous releases or uses of the organisms.

The “parent wild-type” Ad4 vector is the live Ad4 vaccine administered to US Military recruits. In a large phase 3 study (3031 received vaccine; (1009 received placebo) one placebo recipient developed ARD caused by type 4 vaccine virus (Lyons et al 2008). This was not unexpected because person-to-person transmission of the Ad4 vaccines was previously documented. Such transmission was rare and facilitated by close contact, such as between married couples and within families (Mueller et al 1969; Stanley et al 1969). However, this was defined by serology as no molecular typing was available at the time, thus it is hard to distinguish transmission from naturally circulating strains. Intra-family spread was more common when young children received the Ad4 vaccines rather than their parents, suggesting faecal contamination as the possible mechanism. Recruit life may be particularly conducive to transmission, with individuals living, eating, and sleeping in close quarters. In addition, training necessitates working in close proximity and physical contact (e.g., combative training). Under these conditions, it is encouraging that only one case of transmission (0.1%) was identified (upper 95% CI, 0.6%). Furthermore, the trial design likely overestimates actual future risk of transmission between recruits.

Three types of replication-competent Ad4 vector vaccine candidates have also been assessed in Phase 1 clinical trials. These differ from the current vaccine (Ad4-EnvCN54) in that the vectors had deletions in the E3 gene associated with immune evasion. However these studies provide supporting information. The first and most extensively studied was an Ad4 vector expressing the hemagglutinin of H5N1 influenza – Ad4-H5-HA (Gurwith et al 2013). The Ad4-H5-HA vector in this study most resembles the proposed Ad4-CN54 vector, as it has only a partial E3 deletion (3 of 9 E3 genes) deleted and used the endogenous promoter. The Ad4-CN54-Env construct has no E3 deletion and with the CN54 clade C envelope gene driven by an Ad4 virus endogenous promoter.

Additionally, Ad4 vector vaccine candidates expressing HIV-Env and the protective antigen (PA) of B anthracis, or PA modified to include a GPI tail have been constructed and evaluated in Phase 1 trials. However, these vectors differ

substantially from the proposed Ad4-CN54 by having "full" deletions (8 of 9 E3 genes deleted). The initial results of these of the Phase 1 studies of these latter vector suggest that the full E3 deletion has substantially impaired there *in vivo* replication. Shedding of the vectors, seroconversion to Ad4, or seroconversion to the anthrax or HIV transgenes has been minimal in study subjects, and there has been no evidence at all Ad4 seroconversion, shedding, or seroconversion to the PA, PA-GPI, or HIV-Env transgenes in the household contacts. Thus, the clinical experience with the Ad4-H5-HA vector seems most relevant to the design of the Ad4-EnvCN54 phase 1 study.

The Ad4-H5-HA vector was extensively studied in a Phase 1 study. This was an ascending dosage, double-blind placebo-controlled study in which 5 cohorts of at least 32 subjects were enrolled. Subjects were recruited into each cohort in a dose-escalating manner and were randomly assigned to vaccine or placebo (vaccine:placebo = 3:1) within each cohort. Subjects in each cohort received up to 3 doses of Ad4-H5-HA (also known as Ad4-H5-Vtn) (10^7, $10^{8,}$ 10^9, 10^{10}, or 10^{11} vp/dose) or placebo, each dose given approximately 56 days apart. Enrolment was restricted to healthy men and non-pregnant women, ages 18 to 40. Based on experience with the US Military Ad4 vaccine which is the "parent" virus, intestinal replication and shedding of the Ad4-H5-Vtn virus and possible transmission of the vaccine virus was anticipated and therefore enrolment was restricted to subjects who either lived alone or had no more than two healthy, adult (ages 18-65) household contacts who were also willing to be enrolled in the study in order to evaluate potential transmission of Ad4-H5-HA virus.

33. In relation to human health, animal health and plant health

A. The toxic or allergenic effects of the non-viable organisms and/or their metabolic products,

The "parental wild type" Ad4 virus vaccine has been orally administered to more than 10 million U.S. recruits and no toxic or allergenic effects were reported. HIV envelope proteins encoded in non-replicative vectors have been administered to thousands of volunteers and again no toxic or allergenic effects have been reported.

B. The comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity,

As described in Item 5, Ad4-EnvCN54 recombinant virus is comparable to the parental vaccine strain with respect to pathogenicity.

C. The capacity of the organisms for colonization

Replication and transcription of members of the adenovirus family of viruses occurs in the cytosol of infected cells, with virally encoded enzymes driving these processes. Ad4-EnvCN54 DNA is extra-chromosomal and is not integrated.

Adenoviruses are cleared from the host within several weeks. Thus, colonization by Ad4-EnvCN54 does not occur.

D. If the organisms are pathogenic to humans who are immunocompetent -

i. Diseases caused and mechanisms of pathogenicity including invasiveness and virulence,

Adenovirus serotypes 4 has been associated with outbreaks of acute respiratory disease among military recruits (Gaydos et al 1995; Gurwith et al 1989). These outbreaks have resulted in hospitalization and some mortality (Gray et al 2000). The mode of transmission is commonly respiratory. However the parental Ad4 vaccine is attenuated by route of delivery (oral) and has been administered to more than 10 million US Military recruits without significant toxicity (Gaydos et al 1995). No significant vaccine associated illness has been reported.

ii. Communicability

Adenovirus 4 transmission predominantly occurs via short-range droplets and aerosols, and via direct contact with contaminated fomites with self-inoculation onto mucous membranes. Transmission can also be mediated by faecal-oral and occasionally water transmission. In a recent Phase 1 re-licensure study Ad4 vaccine virus shedding was not detected in throat swab specimens from any vaccinee; faecal shedding of the Ad4 vaccine was detected between 7 - 21 days post vaccination in 27% of vaccinees.

In a previous large phase 3 study (3031 received vaccine; (1009 received placebo) one placebo recipient developed ARD caused by type 4 vaccine virus (Lyons et al 2008). This was not unexpected because person-to-person transmission of the Ad4 vaccines was previously documented. Such transmission was rare and facilitated by close contact, such as between married couples and within families (Mueller et al 1969; Stanley et al 1969). However this was defined by serology as no molecular typing was available at the time, thus it is hard to distinguish transmission from naturally circulating strains.

Intra-family spread was more common when young children received the Ad4 vaccines rather than their parents, suggesting faecal contamination as the possible mechanism. Recruit life may be particularly conducive to transmission, with individuals living, eating, and sleeping in close quarters. In addition, training necessitates working in close proximity and physical contact (e.g., combative training). Under these conditions, it is encouraging that only one case of transmission (0.1%) was identified (upper 95% CI, 0.6%). Furthermore, the trial design likely overestimates actual future risk of transmission between recruits.

iii. Infective dose

The minimum infectious dose of Ad4 is unknown.

Under the proposed study protocol release, 36 volunteers will receive oral immunization with 1 x 10^{10} viral particles of Ad4-EnvCN54 at month 0, 24 volunteers will receive additional oral doses in month 3, and 12 in month 6.

iv. Host range and possibility of alteration,

Adenovirus 4 exclusively replicates in human cells, there is no other non-human host capable of supporting replication of this virus.

v. Possibility of survival outside of human host,

The Ad4 vector can only replicate in human cells and there is no other known host capable of supporting the replication of Ad4. Adenovirus 4 has the capacity to survive for considerable periods in dried material such as a lyophilized powder within a capsule. The “parent wild-type” lyophilized oral Ad4 vaccine has been administered to more than 10 million U.S. military recruits for over 45 years without environmental concerns. Moreover, stability decreases significantly as temperature is increased. Under normal environmental conditions, Ad4-EnvCN54 is expected to lose viability within days to weeks. In addition, adenoviruses are readily inactivated by ultraviolet light and bleach-based cleaning agents (Birmpa et al 2016; Magri et al 2015).

vi. Presence of vectors or means of dissemination

The Ad4 vector can only replicate in human cells and there is no other known host capable of supporting the replication of Ad4. The primary means of dissemination of Ad4 transmission is predominantly via short-range droplets and aerosols, and direct contact with contaminated fomites with self-inoculation onto mucous membranes.

Studies of the “parental wild-type” oral replication competent Ad4 vector have detected rectal, but not respiratory, shedding for up to 28 days after administration. The Ad4 virus can only replicate in humans and there are no other reservoirs of infection. Transmission of the vaccine strain to secondary contact is extremely rare and reported to only occur in less than 0.1% of close household contacts.

Viral shedding in humans via the faecal route after administration of the Ad4 “parent wild-type” vaccine was detected between 7 - 21 days post vaccination in 27% of study vaccinees. Viral shedding of Ad4 cannot be contained, but instead minimized by educating volunteers on the importance of hand washing procedures after using the bathroom.

vii. Biological stability

The evaluation of genetic stability of Ad4-EnvCN54 at several stages of the production process is described in Item 11. In terms of stability *in vivo*, oral delivery of vaccine strains causes a transient gastrointestinal infection in susceptible hosts, with elimination of viral components over 7-28 days. Host cells infected with

adenovirus are short lived (days) and die by either through apoptosis/necrosis and / or elimination by immune effector cells.

viii. Antibiotic-resistance patterns,

Not applicable

ix. Allergenicity

Adenovirus 4 vaccines have not been shown to be allergenic in any clinical studies to date. However, as with all vaccination studies, patients should remain in the clinic for at least 60 minutes following administration of the Ad4-EnvCN54 vaccine for observation for signs of adverse reactions.

x. Availability of appropriate therapies

Currently, there is no approved, specific antiviral treatment for Adenovirus infections. Cidofovir, approved for CMV infections, has shown clinical activity against adenovirus infections, though its use may be limited by toxicity. Brincidofovir, an antiviral in development by Chimerix, has been reported as showing efficacy in adenovirus infections.

e. Other product hazards

No other product hazards are known from studies to date.

Part III: Information relating to the conditions of release

The Release

34. The description of the proposed deliberate release, including the initial purpose or purposes of the release and any intention to use the genetically modified organisms as or in a product in the future.

The proposed clinical study is a Phase 1, single blind, randomised trial with placebo controls. The overall aim of this 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-CN54) to identify the most immunogenic strategy for the induction of durable mucosal and systemic protective antibody responses.

The Ad4-EnvCN54 study vaccine will be administered orally at the dose of 10^{10}VP to volunteers to optimize immune responses to the HIV-1 envelope glycoprotein, the primary target for protective antibodies.

APPLICATION FOR CONSENT TO RELEASE A GMO – ORGANISMS OTHER THAN HIGHER PLANTS

Study volunteers will be randomised with equal probability and dosed using sentinel methods to one of the eight groups listed in Table 1 below:

Table 1: STUDY DESIGN AND VACCINATION SCHEDULE – PART I & PART II

PART I							**PART II**	
	Group	**N**	**Month 0**	**Month 3**	**Month 6**		**N**	**Vaccinations 1,2,&3**
	A	**6**	Oral placebo	CN54 rgp140 +Oral placebo +I.M placebo	CN54 rgp140 +Oral placebo +I.M placebo	**Select best dosage from B -D to initiate Part IIdependent on meeting pass-criterion**	**6**	Selected dosage from Part I
	B	**6**	Ad4-EnvCN54	CN54 rgp140 + Oral placebo +I.M placebo	CN54 rgp140 + Oral placebo +I.M placebo			
	C	**6**	Ad4-EnvCN54	CN54 rgp140 Ad4-EnvCN54 +I.M placebo	CN54 rgp140 + Oral placebo +I.M placebo			
	D	**6**	Ad4-EnvCN54	CN54 rgp140 Ad4-EnvCN54 +I.M placebo	CN54 rgp140 Ad4-EnvCN54 +I.M placebo			
	E	**6**	Oral placebo	CN54 rgp140 + MVA-CN54 +Oral placebo	CN54 rgp140 +MVA-CN54 +Oral placebo	**Select best dosage from F -H to initiate Part IIdependent on meeting pass-criterion**	**6**	Selected dosage from Part I
	F	**6**	Ad4-EnvCN54	CN54 rgp140 +MVA-CN54	CN54 rgp140 +MVA-CN54			

				+Oral placebo	+Oral placebo			
	G	**6**	Ad4-EnvCN54	CN54 rgp140 +MVA-CN54 +Ad4-EnvCN54	CN54 rgp140 +MVA-CN54 +Oral placebo			
	H	**6**	Ad4-EnvCN54	CN54 rgp140 +MVA-CN54 +Ad4-EnvCN54	CN54 rgp140 +MVA-CN54 + Ad4-EnvCN54			
		48					**max 12**	

The study interventions will consist of:

Arm 1 (Groups A-D): Depending on the allocation of groups, volunteers will receive 6 Ad4-EnvCN54 or 6 placebo oral administrations, 8 CN54rgp140/MPLA, and 8 intramuscular placebo injections at month 0 (Week 0), 3 (Week 12) & 6 (Week 24).

Arm 2 (Groups E-H): Depending on the allocation of groups, volunteers will receive 6 Ad4-EnvCN54 or 6 placebo oral administrations, 8 CN54rgp140/MPLA, and 8 MVA-CN54 intramuscular 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.

The treatment phase of this clinical study is six months excluding follow up visits. In total, volunteers will be enrolled onto the clinical study for twelve months in both Part I and Part II.

The Primary Objective of the Phase I Clinical Trial is outlined below:
Is to evaluate the immunogenicity, safety and tolerability of oral Ad4 vector expressing HIV-1 CN54 (Ad4-EnvCN54) when combined with different boosting options (MVA-CN54 and CN54rgp140/MPLA) designed to optimise systemic and mucosal immune responses.

The Secondary Objective of the Phase I Clinical Trial is outlined below:

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))

The Exploratory Objective of the Phase I Clinical Trial is outlined below:

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 volunteers only)
- PBMC ex-vivo HIV suceptibility assay

35. The intended dates of the release and time planning of the experiment including frequency and duration of releases.

We propose enrolment of the Ad4HIV Phase I clinical trial will commence in the EU on 06_APR_2017, and it is anticipated to be completed by MAY_2020.

Release, timing and frequency are dependent on our rate of recruitment to enrol volunteers onto the trial. Volunteers will be vaccinated, observed and then released to carry out their normal duties until the next study visit 7 and 28 days later.

36. The preparation of the site before the release.

The principal investigator and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on Harmonisation E6 - Good Clinical Practices.

The clinical site where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection and storage of human specimens.

The vaccinations of clinical trial volunteers will take place at the NIHR/Wellcome Trust Imperial Clinical Research Facility (ICRF). This is a purpose-built facility dedicated to clinical research comprising clinic rooms, wards, laboratories and support facilities. Further detail is available at http://imperial.crf.nihr.ac.uk/facility/

The ICRF workforce includes doctors, nurses, laboratory and operational staff who have substantial clinical research training and experience. No specific preparation is required for this trial. The entire facility has restricted access.

All clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. A thorough study-specific training session will occur prior to the initiation of the study via a formal investigator meeting and/or on-site study initiation visit.

Vaccinated individuals will be released to return to their normal residencies, mostly in Greater London, no preparation is required.

37. The size of the site.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study volunteers.

The ICRF occupies the ground floor of the Imperial Centre for Translational and Experimental Medicine, Hammersmith Hospital, Du cane Road, London W12 0HS, at UK grid reference TQ225812. The footprint of the ICRF is approximately 1,200 m^2. Most volunteers will be recruited from the Greater London area, approximately 1,600 km^2.

Security at the ICRF is maintained by swipe card access such that there is no public access. Volunteers will be vaccinated and subsequent sampling carried out in single occupancy rooms with en suite toilet facilities.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings. No risk related to Ad4-EncCN54-related waste is anticipated. The clinical study sites will be instructed to follow normal site procedures for disposal of biomedical or infectious waste.

The study is not expected to have any effect on the local population other than for those volunteers enrolled on the study and those individuals with close contact with

the study subjects. There will be 1 clinical site in England. It is also projected that approximately 54 volunteers will be enrolled over the duration of two years.

The following clinical study site in England where study vaccine will be administered is listed below:

Address
NIHR / Wellcome Trust Imperial CRF Imperial Centre for Translational and Experimental Medicine, Imperial College Healthcare NHS Trust, Hammersmith Hospital, Du Cane Road, London, W12 0HS

The national grid appendices for this building is TQ225812. The population of Greater London where the study volunteers will be recruited from is approximately 8.63 million inhabitants.

38. The method or methods to be used for the release.

The Ad4-EnvCN54 study vaccine has been formulated as live lyophilized enteric-coated capsules for oral administration. Each capsule contains $1x10^{10}$ viral particles per administration. The study vaccine contains no adjuvants or preservatives.

39. The quantity of organisms to be released.

A central storage and distribution depot for the study vaccine and oral placebo will be located at PCI, Bridgend, Wales, United Kingdom.

Company Name: PCI Pharma Services
Address: Biotec House
Western Avenue
Bridgend Industrial Estate
Bridgend
CF31 3RT

The study vaccine will be packaged in bottles by the manufacturer Paxvax (555 Twin Dolphin Drive, 360, Redwood City, California 94065, USA). Each bottle will contain approximately 10 capsules. Nineteen bottles will be shipped by the manufacturer under temperature controlled conditions to the central storage facility (PCI Pharma Services). The clinical study vaccine bottles will be labelled, packaged in labelled cartons and all 18 bottles will be distributed from PCI to the clinical site. From the central storage and distribution depot, the clinical site will be supplied with enough IMP to service the enrolled volunteer population for the entire dosing period. It is anticipated that multiple shipments will not be necessary for this clinical study.

40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities.

Not applicable. The Ad4HIV protocol is not an agricultural release study.

41. The worker protection measures taken during the release.

The principal investigator and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on Harmonisation E6 - Good Clinical Practices.

The clinical site where the study is to being conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection and storage of human specimens.

Additionally, all clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. A thorough study-specific training will occur prior to the initiation of the study via a formal local investigator meeting and an on-site study initiation visit.

Study staff at the ICRF will wear appropriate personal protective equipment (e.g. gloves, aprons and eyewear) when at risk of exposure to the adenovirus. All members of the staffing team at the ICRF regularly receive GMO specific training. The risk of transmission of attenuated viruses to exposed healthcare workers is very low. There have been no cases of transmission to healthcare personnel in any of the studies with Ad4-related vaccine studies. If appropriate infection-control precautions are observed, healthcare workers will be at less risk of infection than research laboratory workers because of the smaller volume of lower titer of virus in clinical specimens when compared with research laboratory material.

Paxvax the manufacturer of the clinical study vaccine does not recommend prophylactic Ad4 vaccinations for healthcare workers, like that carried out on US military personnel conducting the study, since vaccinations would put such workers at a real risk of post-vaccination complications if susceptible. The most critical preventive measure is proper handling of the vaccine and thorough hand hygiene. In fact, there were no cases of contact transmission in health care settings or from civilian vaccinees to their contacts reported during the US vaccination campaign with the “parent wild-type” vaccine.

Procedures for preparation of the vaccine are described in the clinical protocol. Ad4-EnvCN54 is classified as a group 2 biological agent according to the European Economic Community (EEC) classification for the protection of workers with biological agents {Directive 2000/54/EC}. Local procedures state the routine use of standard universal precautions when directly handling the vaccine, including the wearing of a lab coat, eye protection, and gloves.

In case of spills, Ad4 is readily inactivated by a number of detergents and bleach-based agents and can easily be contained. Material Safety Data sheets will be provided with the product and clinical study staff will be provided with specific instructions to address spills, including information on containment, personal protective equipment, disinfection, and disposal procedures.

42. The post-release treatment of the site.

Transport of Ad4-EnvCN54 will be done according to EU guidelines for the transport of GMOs and IATA Transportation Regulations. The Ad4-EnvCN54 vaccine will be transported to the clinical study site as cartons of 18 individual, frozen bottles. The study vaccine will be stored at -20^{0}C or below in the original outer package and will be stored in a secure location with limited access to ICRF personnel only.

Following administration, used study vaccine bottles will be placed immediately into sealed bags and retained for accountability. Upon reconciliation and accountability, used study materials will be destroyed by the clinical site following institutional procedures for the disposal of biohazardous material. All unused study vaccine will be disposed of at the clinical site upon authorization from the sponsor of the clinical study and IMP manufacturer.

43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment or other purposes of the release.

Waste disposal and cleaning will be according to the ICRF SOP for work with GMOs, and will be done on an ongoing basis throughout the study – thus no special post-release treatment is planned.

As a consequence of shedding through faecal material the GMO will be released into the sewerage system in England and primarily within the Greater London area. Under normal circumstances GMO shed in this manner will be inactivated through the normal sewerage system processes. However, were there to be a failure of sewerage treatment, infectious adenoviruses could enter the aquatic environment and persist for some time (although possibly in a non-infectious state) until degraded by solar radiation and other natural processes. Group E Adenoviruses typically cause respiratory infections and are attenuated naturally for oral transmission.

The concentration of GMO viral particles in recreational bodies of water would be extremely low because of repeated dilutions, and the probability of a susceptible human ingesting ~1010 viral particles (the predicted immunogenic dose) would be remote. Aerosolisation of environmental water may theoretically increase infectiousness but would further reduce the concentration by mixing. Chlorination of drinking water would inactivate the GMO were contamination with sewerage to take place. As adenoviruses cannot replicate outside of humans, there are no environmental processes that could increase the concentration of viral particles.

44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems.

This is a first in man trial within the EU, we therefore do not have data on any previous releases of the study vaccine.

The Environment (Both on the Site and in the Wider Environment)

45. The geographical location and national grid reference of the site or sites onto which the release will be made, or the foreseen areas of use of the product.

A table of the principal investigator and address of the clinical site in England to be used in the study is provided below:

Address	National Grid Reference
NIHR / Wellcome Trust Imperial CRF Imperial Centre for Translational and Experimental Medicine, Imperial College Healthcare NHS Trust, Hammersmith Hospital, Du Cane Road, London, W12 0HS	TQ225812

46. The physical or biological proximity of the site to humans and other significant biota.

The study vaccine will be administered at a licensed National Healthcare facility where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g.*, the National Health Trust Standard Precautions.

It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem. There are no significant biota located in close proximity of the ICRF or Hammersmith Hospital.

47. The proximity to significant biotopes, protected areas or drinking water supplies.

The study vaccine will be administered at a licensed National Healthcare facility where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g*., the National Health Trust Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem. There are no significant biota located in close proximity of the ICRF or Hammersmith Hospital.

48. The climatic characteristics of the region or regions likely to be affected.
Adenovirus 4 is globally prevalent in humans and there is no specific regional distribution. As there is not an environmental reservoir this situation is not applicable.

49. The geographical, geological and pedological characteristics.
A list of the study site and its location is provided in Item 37. The study vaccine will be administered at a licensed National Healthcare facility where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g*., the National Health Trust Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

50. The flora and fauna, including crops, livestock and migratory species.
The study vaccine will be administered at a licensed National Healthcare facility where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g*., the National Health Trust Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

Ad4-EncCN54 cannot infect plant or insect cells. Adenovirus 4 host range is limited to human hosts only.

51. The description of target and non-target ecosystems likely to be affected.
The study vaccine will be administered at a licensed National Healthcare facility where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g*., the National Health Trust Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

52. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release.
Regarding natural habitats of the parental wild-type organism, the Ad4 virus has no known natural habitat and the origins in nature and as a vaccine are unknown. Adenovirus 4 infections are distributed in our nature worldwide. It is not anticipated that the study vaccine would have any advantage with respect to growth or survivability as compared to parental vaccines in the natural habitat.

53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.
Not applicable.
No developments or changes in land use in the region are anticipated.

Part IV: Information Relating to the Interactions Between the Organisms and the Environment

Characteristics affecting survival, multiplication and dissemination

54. The biological features which affect survival, multiplication and dispersal.

The potential for escape, dispersal, or establishment of Ad4 in the environment is low. Adenovirus 4 cannot reproduce in the absence of a susceptible human host GI cell, and has no known natural animal reservoirs. Adenovirus serotype 4 is a non-enveloped, icosahedral virion, 70-90nm in diameter containing a double-stranded linear DNA genome, and is rapidly inactivated by ultraviolet light and bleach-based agents. See item 56.

Adenovirus 4 transmission predominantly occurs via short-range droplets and aerosols, and via direct contact with contaminated fomites with self-inoculation onto mucous membranes. Transmission can also be mediated by faecal-oral and occasionally water transmission. However the “parental wild-type” Ad4 vaccine is attenuated by route of delivery (oral) and has been administered to more than 10 million US Military recruits without significant toxicity (Gaydos et al 1995).

No significant vaccine associated illness has been reported. In a recent Phase 1 re-licensure study Ad4 vaccine virus shedding was not detected in throat swab specimens from any vaccinee; faecal shedding of the Ad4 vaccine was detected between 7 - 21 days post vaccination in 27% of vaccinees.

The Ad4 virus is not capable of forming spores or generating other specialist structures to enhance environmental survival.

Adenovirus 4 is stable at sub-freezing temperatures but loses viability at higher temperatures. Additionally, Ad4 is readily inactivated by a number of detergents; thus, accidental spills can be contained and are not likely to result in spread of Ad4-EnvCN54 in the environment. The general environment is not likely to support propagation of this, which requires specific eukaryotic cells for replication.

In brief, there are no perceived hazards to the environment. The "parent wild-type" Ad4 oral vaccine has been administered orally to U.S. military recruits for over 45 years without environmental concerns (Radin 2014).

Adenovirus 4 is only able to replicate in humans and has no other host. The encoded transgene is non-functional and provides no advantage or change in pathogenicity

55. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature and pH.

There are no published studies on the persistence of Ad4 viruses in the environment. However, persistence in the environment, adverse sequelae, and other environmental issues have not been reported as a result of the use of licensed recombinant Ad4 virus-based products. As part of the licensure procedure, the USDA announced Findings of No Significant Impact with respect to the likelihood of an adverse environmental event using this vaccine.

Specific stability studies have been performed on the "parent wild-type" Ad4 virus and the study vaccine Ad4-EnvCN54. Replication of human adenoviruses are generally restricted to humans. No single animal or environmental model has emerged that adequately supports viral replication and the associated range of pathological lesions and clinical disease; this is particularly true for Ad4 virus. The lack of an animal and environmental models supporting Ad4 viral replication negates the value of conducting *in vivo* pharmacokinetic, bio-distribution, safety or toxicology studies in support of an initial CTA. Therefore, no such studies have been conducted.

In vitro growth characteristics of the Ad4-EnvCN54 vaccine were compared to the parent Ad4 wild type virus as a surrogate for evaluating enhanced virulence. The parent virus is used by the U.S. military to prevent adenovirus-associated respiratory disease and has been administered to over 10 million recruits. Importantly, the Ad4-EnvCN54 vaccine had comparable growth kinetics vs. the parent Ad4 wild type virus in both A549 (human lung alveolar basal epithelial) and MRC-5 (human foetal lung fibroblast) cells.

56. The sensitivity to specific agents.

Adenoviruses 4 is rapidly inactivated by exposure to UV light and bleach-based products. Adenovirus 4 and Ad4-based vaccine vectors are susceptible to 1% sodium hypochlorite (approximately 10% household bleach), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate, and ≥0.1N sodium hydroxide. The "parent wild-type" virus can lose viability over a period of days when treated with the agents listed above. Furthermore, Ad4 viruses are generally sensitive to heat >56°C; unusually stable at low concentrations of ethyl alcohol and pH conditions between pH 5 and pH 9. The disinfectant of choice is that we will be using this the clinical study will be Virkon, commonly used in the ICRF.

It has also been shown that no specific antiviral therapy has been approved for use during an Ad4 infection. There is *in vitro* experimental evidence that antiviral drugs including ribavirin, acyclovir, gangcyclovir and cidofovir have activity against Ad4 and Ad4-based vaccine vectors.

Interactions with the Environment

57. The predicted habitat of the organism.

Humans are the only host able to support replication of Ad4, and it is commonly known to not have any known natural animal reservoirs. The study vaccine Ad4-EnvCN54 has been genetically engineered specifically for the Phase 1 clinical trial, and there does not exist in nature. The habitat of the parental virus has been previously discussed (see Item 6 & 9). The wild-type Ad4 vaccine has been orally administered to over 10 million U.S. recruits between 1971 and 1999 and shown to be very well tolerated and safe. Due to the positive results observed in the US military, the vaccine was re-introduced in October 2011.

58. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.

No studies have been conducted on the ecological impact of Ad4-EnvCN54 on simulated natural environments.

59. The capability of post-release transfer of genetic material-

A. From the genetically modified organisms into organisms in affected ecosystems,

There is minimal potential for gene transfer to other species under the proposed release of the Phase 1 clinical trial GMO. Ad4-EnvCN54 cannot infect microbes, insects, cold-blooded vertebrates, or plant cells. The GMO will be released in a Clinical Research Facility (located on NHS hospital grounds) examination room and is unlikely to come in contact with other animal species. Furthermore, dissemination of Ad4-EnvCN54 outside the attenuated oral route has been shown.

Recombination between the DNA genome of Ad4-EnvCN54 with other DNA genomes, such as the human genome or other viral genomes in the infected host, is improbable for the following reasons. Adenovirus replication takes place entirely in the human gastrointestinal tract and is not integrated into the human hosts DNA. Adenovirus 4 tropism for mucosal surfaces of the GI tract initiates replication in submucosal tissues, thus maximizing effective expression of native HIV envelope trimers on the surface of infected cells (Alexander et al 2013). This localized mucosal expression of native envelope has the strongest potential to induce potent immunity to strengthen defences. As a result, it is not subject to events that could lead to rearrangement or recombination in volunteers participating in the clinical study. The Ad4 virus is cleared from the host within several days of administration of the

attenuated virus. Therefore, the risk of Ad4 virus persistence by integration into the host chromosome is very low to non-existent.

Recombination with other viral genomes is unlikely due to the lack of homology between different families of viruses.

Adenovirus serotype 4 is comparable to its corresponding wild-type non-recombinant parental virus, with respect to growth characteristics and stability in the environment. The added HIV-1 DNA has not fundamentally altered the inherent properties of the recombinant virus. Therefore, Ad4-EnvCN54 has not acquired any known phenotypic properties that would increase their risk to the environment beyond those associated with the use of the corresponding non-recombinant parental virus.

Recombination between Ad4-EnvCN54 and the “parental wild-type” Ad4 virus in an infected host organism is impossible. Also, the likelihood of recombination between Ad4-EnvCN54 and the “parental wild-type” Ad4 virus *in vivo* is extremely low because Ad4 viruses are not found in nature.

B. From indigenous organisms to the genetically modified organisms.
Recombination between the DNA genome of indigenous organisms to the genetically modified organisms is unlikely for reasons described in 61a, above. The Ad4 life cycle is carried out on the mucosal surfaces of the human gastrointestinal tract and is not integrated into the human hosts DNA. Adenovirus 4 tropism for mucosal surfaces of the GI tract initiates replication in submucosal tissues only. Therefore, the physical segregation between host and viral genome renders recombination with Ad4-EnvCN54 improbable.

60. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms.
There is no evidence that selection leading to the expression of unexpected or undesirable traits in Ad4-EnvCN54 would occur under the conditions of release.

61. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.

Genetic stability of Ad4-EnvCN54 following administration under the conditions of this release will not be monitored. As stated above the DNA does not incorporate into host cell genome and is cleared along with infected host cells within 7-21 days.

The GMO is limited in its ability to disperse in the environment. Under normal environmental conditions adenoviruses lose viability within days to weeks.

62. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact and burrowing.

Adenovirus 4 transmission occurs via short-range droplets and aerosols, and via direct contact with contaminated fomites with self-inoculation onto mucous membranes. Transmission is mediated by faecal-oral and occasionally water transmission. The "parental wild-type" Ad4 vaccine is attenuated by route of delivery (oral only) and has been administered to 10 million US Military recruits without significant toxicity (Gaydos et al 1995). No significant vaccine associated illnesses have been reported.

Administration of Ad4 orally attenuates the virus. Risk of transmission is reduced by use of universal precautions by healthcare workers and educating of volunteers in proper hygiene and proper care during the course of the clinical study. In a recent Phase 1 re-licensure study Ad4 vaccine virus shedding was not detected in throat swab specimens from any vaccine. However, faecal shedding of the Ad4 vaccine was detected between 7-21 days post vaccination in 27% of vaccinees. Viral shedding of Ad4 cannot be contained, but instead minimized by educating volunteers on the importance of hand washing procedures after using the bathroom.

63. The description of ecosystems to which the organisms could be disseminated.

The "parental wild-type" Ad4 virus can be disseminated through short-range droplets and aerosols, and via direct contact with contaminated fomites with self-inoculation onto mucous membranes. Transmission is mediated by faecal-oral and occasionally water transmission. The GMO product for the clinical trial does not have the potential to disseminate and impact ecosystems, because dissemination requires close contact with a susceptible human host or indirect contact with contaminated surfaces or objects. The study will be conducted at standard healthcare facility, and it is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

Potential Environmental Impact

64. The potential for excessive population increase of the organisms in the environment.

Ad4-EnvCN54 is a virus that cannot replicate outside permissive human cells. Therefore, it cannot increase in number without contact with permissive cells or host.

65. The competitive advantage of the organisms in relation to the unmodified recipient or parental organism or organisms.

Ad4-EnvCN54 has not been shown to display a competitive advantage over its unmodified parental virus with respect to replication *in vitro*.

66. The identification and description of the target organisms if applicable.

APPLICATION FOR CONSENT TO RELEASE A GMO – ORGANISMS OTHER THAN HIGHER PLANTS

Ad4-EnvCN54 will be administered to healthy men and women aged 18 to 50 years old at low risk for HIV infection, who are available for the duration of the trial and provide written informed consent to participate in the clinical trial.

67. The anticipated mechanism and result of interaction between the released organisms and the target organisms if applicable.

The study vaccine will be administered orally in three vaccinations, over a six month period. The vaccine is intended to induce an anti-envelope immune response against HIV-1.
Ad4-EnvCN54 is a prophylactic vaccine designed to deliver the HIV envelope antigen gp150 to B cells and associated immune cells required to trigger an immune response in naïve volunteers.

Ad4-EnvCN54 is a vector-based product that causes a transient gastrointestinal infection in susceptible hosts, with elimination of viral components over 7-28 days. Host cells infected with adenovirus are short lived (days) and die by either through apoptosis/necrosis and/or elimination by immune effector cells.

68. The identification and description of non-target organisms which may be adversely affected by the release of the genetically modified organisms, and the anticipated mechanisms of any identified adverse reaction.

The potential for Ad4-EnvCN54 interaction with other organisms in the environment would be limited to viral shedding and indirect contact with contaminated surfaces or objects in the volunteer's possession. Humans are the only hosts which support replication of Ad4, and the most likely non-target organisms that would include health care workers or contacts of vaccinees; thus, there is no known risk to aquatic animals from any potential environmental release. Ad4-EnvCN54 cannot infect microbes, insects, cold-blooded vertebrates, or plant cells. The GMO will be released in a Clinical Research Facility (located on NHS hospital grounds) examination room and is unlikely to come in contact with other animal species. Furthermore, dissemination of Ad4-EnvCN54 outside the attenuated oral route has been shown.

The extent of exposure to non-target species is expected to be limited by the fact that vaccine administration occurs in a clinical site under controlled conditions. The administration of Ad4-EnvCN54 orally, the use of hand sanitizer gels, volunteer hand washing instructions, and comprehensive education of healthcare providers and volunteers serve to minimize exposure to non-target species.

To date there has never been a published report of transmission of the "parent wild-type" vaccine to clinical study volunteer's health-care personnel (*CDC, 2015*). Furthermore, 10 million individuals have been vaccinated with "parent wild-type" vaccine, and no evidence of contact transmission has been noted in any clinical trial to date.

69. The likelihood of post-release shifts in biological interactions or in the host range.

Adenovirus serotype 4 can only infect human cells and there is not another host that can support an infection.

There is no reason to believe that the host range of Ad4-EnvCN54 would be altered under the conditions of this release. As indicated above, the likelihood of release into the general environment is remote.

The intrinsic mutation rate of adenoviruses has not been precisely determined but is probably similar to that of other systems with proofreading DNA polymerases. Shifts in biological interactions or in the host range would require rapid genetic changes in the GMO; as this is not expected to occur, the likelihood of post-release shifts in biological interactions or host range is negligible.

70. The known or predicted interactions with non-target organisms in the environment, including competitors, prey, hosts, symbionts, predators, parasites and pathogens.

The extent of exposure to non-target organisms is expected to be limited by the fact that vaccine administration occurs in a clinical site under controlled conditions. Ad4-EnvCN54 exclusively replicates in human cells, there is no other host that can support replication of the virus. Ad4-EnvCN54 cannot infect microbes, insects, prey, pathogens, parasites, symbiont's, cold-blooded vertebrates, or plant cells. The GMO will be released in a Clinical Research Facility (located on NHS hospital grounds) examination room and is unlikely to come in contact with other animal species. Furthermore, dissemination of Ad4-EnvCN54 outside the attenuated oral route has been shown.

71. The known or predicted involvement in biogeochemical processes.

Not applicable. Adenovirus serotype 4 is not anticipated to have any involvement in biogeochemical processes.

72. Any other potentially significant interactions with the environment.

Ad4-EnvCN54 exclusively replicates in human cells, there is no other host that can support replication of the virus. Ad4-EnvCN54 is a live virus that requires a host cell to replicate. It can only remain viable following infection and proliferation in an appropriate host organism. Other than potential host organisms, no environmental niches or habitats would be affected, either directly or indirectly, by exposure to Ad4-EnvCN54.

Part V: Information on Monitoring, Control, Waste Treatment and Emergency Response Plans

Monitoring techniques

73. Methods for tracing the organisms and for monitoring their effects.

The study will be monitored by the study management team and the ICRF operational team on a regular basis throughout the study period in accordance with general monitoring principles set forth in ICH E5. All study documents (subject files, signed informed consent forms, copies of case report forms, study vaccine accountability records, etc.) must be kept secured until disposal is authorized by the Sponsor, which will when all study volunteers have been vaccinated and the data collected throughout the trial have been analysed by a medical statistician.

Primary endpoints

The primary endpoints include the following:
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:

- Any grade of adverse event that occurs in a participant that has received at least one immunisation
- Proportion of participants with moderate or greater (Grades 2-4) reactogenicity (i.e., solicited adverse events) up to Day 7 follow-up visit after each vaccination
- Proportion of participants with moderate or greater (Grades 2-4) and/or vaccine-related unsolicited adverse events (AEs), including safety laboratory (biochemical, haematological) parameters, post each vaccination
- Proportion of participants with vaccine-related serious adverse events (SAEs) throughout the study period

Secondary endpoints

The secondary endpoints include the following:

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

- Frequency of serum and mucosal binding antibodies to HIV CN54rgp140 antigen measured by binding ELISA.

Safety Assessment and Monitoring

Study volunteers will be carefully monitored during the study protocol for any signs of symptoms of treatment-emergent toxicity by means of a focused physical exam, haematology, serum chemistry panels, recording adverse events, concomitant

medications and viral shedding. All SAEs will be immediately reviewed by the Sponsor and Principal Investigator. In addition, this study will employ a Data Monitoring Committee.

Study volunteers will also be monitoring for their immune responses following administration of the vaccines through the collection of blood samples and pre-defined time points throughout the clinical study. Volunteers are required to attend safety visits where safety assessments are carried out. Monitoring of study volunteers will be conducted approximately 1 hour following each immunisation. Trained personnel at the site will also train volunteers to conduct self-monitor of any adverse events they experience for 7 - 21 days following each immunisation. All adverse events will be documented on a diary card, and assessed by the Principal Investigator of the clinical trial. Volunteers are advised to call the clinic staff if they have any concerns.

Immune Response Assessment and Monitoring
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 SAP. Titres of antigen specific antibodies will be described by time-point and group, and compared using rank tests where appropriate.

Viral Shedding Assessment and Monitoring
The Ad4 parental vaccine protects against respiratory disease and is attenuated by the oral route of delivery. In a recent Phase 1 re-licensure study faecal shedding of the Ad4 vaccine was detected between 7 - 21 days post vaccination in 27% of vaccinees. However viral shedding did not occur at any other site including urine and upper respiratory tract.

Viral shedding of Ad4 cannot be contained, but instead minimized by educating volunteers and clinical staff on the importance of hand washing procedures after using the bathroom. Volunteers will also be educated on good hygiene towards house hold contacts. For this reason, we have included the exclusion criteria points listed below excluding volunteers from participating in the clinical trial if they have

children under 5, are living with immunocompromised individuals or are planning on starting a family whilst enrolled on the trial.

Participant Exclusion Criteria (Please note two points have been listed only):

1. Planning to get pregnant or father a baby in the next 6 months, Pregnant, Breast Feeding, or living with young dependants under the age of 5 years old
2. Has close contact with an immunocompromised individual thought to be at clinical

Additional viral shedding assessment and monitoring visits have also been incorporated into the existing study protocol on days 28, 112 and 196 of the vaccine schedule. Rectal swabs will be taken on the days stated above and viral particles measured using PCR methods to determine if volunteers are still shedding Ad4-EnvCN54 at these time points.

74. Specificity (to identify the organisms and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques.

Identity tests may be performed to confirm that the isolated virus is Ad4-EnvCN54. A partial DNA sequence provides the most specific way to confirm identity. The reference sequence used for comparison is based on the known Ad4 vector backbone and EnvCN54 DNA sequences.

75. Techniques for detecting transfer of the donated genetic material to other organisms.

As noted previously, there is minimal risk of gene exchange between the GMO and other organisms. Therefore, no monitoring of other organisms is planned.

76. Duration and frequency of the monitoring.

We will be monitoring Ad4 viral shedding, adverse events and serious adverse events to the vaccine.

Adverse events and serious adverse events will be assessed during the enrolment visit before and after immunisations and at every visit thereafter.

Ad4-EnvCN54 viral shedding analysed from rectal swabs using PCR methods will be assessed at weeks 0, 1, 4, 12, 16, 25, 26, 28 and 52.

Control of the Release

77. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use.

Procedures are in place to avoid and/or minimize the spread of the GMO by controlled containment during transport and at the clinical site and by minimizing the

potential of secondary transmission to vulnerable populations through exclusion criteria defined in the study protocol.

The principal investigator and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on harmonization E6-Good Clinical Practices. The clinical site where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection, processing and storage of human specimens. All clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. Thorough study-specific training will occur prior to the initiation of the study via a formal local investigator meeting and an on-site study initiation visit.

Clinical site staff responsible for administering Ad4-EnvCN54, collecting clinical samples, or conducting clinical evaluation of study subjects will be instructed to follow local NHS precautions for the prevention of transmission of infectious agents in healthcare settings. Methods and procedures to avoid and / or minimise the spread of the organism will be carried out on site in the ICRF by trained members of staffing team. Internal standing operating procedures will ensure that the spread of the organism is minimised on site, however, the responsibility of minimising the spread of the organism beyond the site will be the delegated to the recently immunised volunteer.

Volunteers will be immunised, observed and then released to carry out their normal duties until their next study visit. A leaflet describing the importance of hand washing procedures was designed to be disseminated to study volunteers. The ICRF team will discuss the importance of hand washing procedures with the volunteer after using the lavatory. This type of training has shown to greatly reduce the risk of close contact cross reactivity between household contacts and volunteers enrolled onto the clinical trial.

Strict accountability of Ad4-EnvCN54 imported into the UK will be maintained at all times. The Ad4-EnvCN54 study vaccine will be shipped from the manufacturer in the USA directly to PCI Pharma Services, Bridgend, Wales, United Kingdom, who will distribute to the clinical site. Upon receipt at the clinical site, the shipment will be promptly inspected and processed immediately. The study vaccine will be stored at -20^{0}C or below in the original outer package in a secure location with limited access.

All unused study vaccine will be disposed of at the clinical site upon authorization from the sponsor according to the site's standard destruction policies for infectious medical waste, or sent to a licensed infectious medical waste destruction facility according to site SOPs. Accurate study vaccine accountability records will be maintained by site personnel. Following administration, the used study vaccine bottles will be placed immediately into locked containers or sealed bags and according to site SOPs, either retained for accountability or sent for immediate

destruction. All sample collections will be performed by study site personnel who have been trained appropriately.

The other possible route of release of Ad4-EnvCN54 is via viral shedding during the clinical trials. Studies of the "parental wild-type" oral replication competent Ad4 vector have detected rectal, but not respiratory, shedding for up to 28 days after administration. The Ad4 virus can only replicate in humans and there are no other reservoirs of infection. Transmission of the vaccine strain to secondary contact is extremely rare and reported to only occur in less than 0.1% of close household contacts. Viral shedding in humans via the faecal route after administration of the Ad4 "parent wild-type" vaccine was detected between 7 - 21 days post vaccination in 27% of study vaccinees. Viral shedding of Ad4 cannot be contained, but instead minimized by educating volunteers on the importance of hand washing procedures after using the bathroom.

The study exclusion criteria outlined in the protocol excludes subjects from participation if they have the potential to come into contact with individuals considered to be at risk for secondary transmission of Ad4 should a subject be shedding the vaccine virus. These exclusion criteria provide a guideline for the extent of contact that should be avoided to minimize the risk of transmission to these populations. The exclusion criteria, together with education of vaccinees in proper care for hand hygiene, and avoidance of contact with at-risk individuals, offer reasonable and effective prophylactic against accidental contact with the study vaccine.

78. Methods and procedures to protect the site from intrusion by unauthorised individuals.

Access to the ICRF is restricted, by swipe card access, to authorised personnel only. Study vaccine and subject data will be stored in a secure location with limited access to clinical study staff. The clinical site will otherwise follow standard daily operating procedures for security measures, and national regulations regarding the maintenance of confidential patient information during the conduct of a clinical trial.

79. Methods and procedures to prevent other organisms from entering the site.

All staff working in the facility will use personal protective equipment (laboratory coat, apron, safety glasses, and disposable gloves) as appropriate and will follow local documented procedures for Infection Control, which should minimise the risk of other organisms entering the facility.

Waste Treatment

80. Type of waste generated.

Clinical waste (including but limited to: faecal/urine/blood/saliva/nasal swab samples, tissues, sharps, syringes, disposable gloves and aprons) and empty bottles that held the GMO vaccine).

81. Expected amount of waste.

The amount of waste will be typical for the clinical site amounting to a few clinical waste bags and bins per day waste. The amount of expected waste will be managed by standard operating procedures currently in place at the site.

The Principal Investigator or her designee must maintain accurate records of dates, quantities and the lot number of all study drugs received, to whom dispensed (volunteer-by-volunteer accounting), and accounts of any product accidentally wasted or intentionally destroyed. The Investigator or designee must retain all used, unused, partially used, wasted, or expired study drug until the study monitor has confirmed accountability unless the institution has a policy of immediate disposal/destruction for used experimental products.

At the conclusion of the Treatment phase of the study, all unused vaccine will be destroyed on site or by a licensed facility contracted by the site. At the conclusion of the Treatment phase of the study, an overall summary of all study drug received, unused, partially used, and destroyed will be prepared.

Any investigational GMO product that is not destroyed at the clinical site upon authorization from the sponsor according to the sites standard destruction policies for infectious medical waste, or sent to a licensed infectious medical waste destruction facility according to site SOPs, will be returned to the PCI Pharma Services listed in item 39.

82. Description of treatment envisaged.
The clinical study site will be instructed to follow normal site procedures for disposal of infectious biomedical waste. All waste that is known or suspected to contain the GMO will be autoclaved.

Emergency Response Plans

83. Methods and procedures for controlling the organisms in case of unexpected spread.

In the event that the contents of the vaccine bottles are accidentally released and come in contact with shipping materials, exposed skin, clothing or laboratory surfaces, standard safety precautions will be used. Adenovirus serotype 4 is a non-enveloped, icosahedral virion, 70-90nm in diameter containing a double-stranded linear DNA genome. In case of spills, Ad4 is readily inactivated by a number of detergents and bleach-based agents and can easily be contained. Material Safety Data sheets will be provided with the product and clinical study staff will be provided with specific instructions to address spills, including information on containment, personal protective equipment, disinfection, and disposal procedures (see item 56).

Contaminated materials should be placed in biohazard safety bags and disposed of as biohazard waste. Surfaces in contact with Ad4-EnvCN54 should be thoroughly cleaned with an appropriate products and cleaning materials should be disposed of as biohazard. Sites of skin contact should be cleaned with standard detergents appropriate for hand washing. Accidental exposure to the eyes should be dealt with by rinsing eyes in an eyewash for at least 15 minutes.

Accidental release could also potentially occur by direct household contact through shedding of the study vaccine. Therefore, educating volunteers on the importance of hand washing procedures after using the bathroom whilst enrolled onto the trial will be adopted. We have also adapted the clinical study protocol to state that the vaccine should not be given to subjects who are primary care-givers for children ≤ 3 years old or those that are immunocompromised.

Accidental transmission of the study vaccine to a clinic staff member or a member of the volunteers family or friends will be reported on a modified SAE form and the event will be followed by the Principal Investigator until resolved. All such events will be summarized in the annual safety update to the appropriate regulatory authorities. Any accidental or suspected secondary transmission will be reported immediately to the Sponsor. All such events will be summarized for the Data Monitoring Committee and forwarded prior to the next scheduled meeting.

Adenovirus serotype 4 has no known natural animal reservoirs and exclusively replicates in human cells. Host cells infected with adenovirus are short lived (days) and are cleared by induced immunity. Adenovirus replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA. Therefore the risk of horizontal transmission is extremely low. This risk will be further mitigated through the use standard biosafety precautions such as those that are used when handling human blood or tissue samples. In addition, it should be noted that in extensive clinical experience with related viral vaccines, horizontal transmission has not been reported.

84. Methods, such as eradication of the organisms, for decontamination of the areas affected.

In case of accidental release, the source of the spill or leak should be contained. Allow powder to settle; wearing protective clothing gently cover the spill with absorbent paper towel and apply copious amounts of a fresh 1% sodium hypochlorite solution starting at the perimeter and working towards the centre taking care to soak the powder thoroughly; allow sufficient contact time (10 min) before clean up.

Absorbent and cleaning materials must be disposed of in biohazard bags. Individuals involved in clean up should wear protective clothing including gloves, eye protection and laboratory coat.

85. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread.

Administration of Ad4-EnvCN54 will occur only within contained clinical site. It is therefore not anticipated that Ad4-EnvCN54 will come into direct contact with any plants, animals or soils. Additionally, Ad4-EnvCN54 is not capable of infecting microbes or plants.

86. Methods for the isolation of the areas affected by the spread.

The Ad4-EnvCN54 vaccine is formulated as white enteric-coated capsules for oral administration containing $1x10^{10}$ viral particles/capsule. The capsules are designed

to pass through the stomach and release the active recombinant vaccine virus into the intestine, where the virus would replicate and induce an HIV-1 envelope-specific immune response.

Bottles for the clinical study vaccine will be labelled, packaged in labelled cartons and all 18 bottles will be distributed from PCI to the clinical site. From the central storage and distribution depot, the clinical site will be supplied with enough IMP to service the enrolled volunteer population for the entire dosing period. Therefore, the amount of vaccine stored at each clinical study site and resultant waste will be quite low. Additionally, any unexpected release or spills can be decontaminated quickly using detergent-based cleaners or items listed in item 56. It is therefore not anticipated that isolation procedures will be required.

87. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.
As described, extensive procedural controls are in place for the transport, storage, administration, disposal, and monitoring of Ad4-EnvCN54 treatment for the duration of the clinical study. Should any unexpected undesirable effect occur, the Sponsor and Principal Investigator will follow standard procedures of assessment of the effect and decisions regarding study continuance.

Part VI: A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this schedule, and the name of the body or bodies responsible for carrying out the studies.

The Ad4HIV protocol will not be implemented until approvals have been obtained from all the necessary regulatory and ethical review bodies.

Clinical Trial Authorisation from the Medicines and Healthcare product Regulatory Agency (MHRA) will be obtained before we can proceed recruiting onto the study protocol. The MHRA regulates investigational medicines, medical devices and blood components for transfusion in the UK, and is an independent executive agency, sponsored by the Department of Health.

“Favourable Opinion” (Directive 2001/20/EC) from an ethics committee (an independent body in a member state of the European Union, consisting of healthcare professionals and non-medical members, whose responsibility is to protect the rights, safety and wellbeing of human subjects involved in a clinical trial that uses an investigational medicinal product) will also be obtained before the trial proceeds to recruit.

Local institutional sponsorship approval was obtained from Imperial College London (Joint Research Compliance Office), whose responsibility is to take ultimate responsibility for the initiation and management of the clinical research project. It is a legal requirement that clinical trials of an investigational medicinal product is

sponsored. The sponsor takes primary responsibility for ensuring that the design of the study meets appropriate standards and that arrangements are in place to ensure appropriate conduct and reporting.

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