CUTHIVAC
CUTANEOUS HIV VACCINATION

Imperial College London

Imperial College Healthcare NHS Trust

CUTHIVAC002

A Phase I clinical trial to assess the safety and immunogenicity of HIV DNA-C CN54ENV immunisations administered via the Intramuscular and Intradermal methods with and without electroporation followed by boosting with recombinant HIV CN54gp140 in healthy male and female volunteers

EUDRACT: 2015-001023-23

Protocol version number 4.0

Protocol date: 10_APR_2017

Authorised by:			
Name:	Dr David Lewis	**Role:**	Chief Investigator NIHR/Wellcome Trust Imperial Clinical Research Facility Hammersmith Hospital
Signature:		**Date:**	
Name:	Mr Gary Roper	**Role:**	Head of Regulatory Compliance Joint Research Compliance Office Imperial College London (Sponsor)
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Name:	Mr Gary Roper	**Role:**	Head of Regulatory Compliance Joint Research Compliance Office Imperial College London (Sponsor)
Signature:		**Date:**	28 APR 2017.

GENERAL INFORMATION

This document describes the CUTHIVAC002 trial and provides information about procedures for entering participants into it. Every care was taken in drafting the protocol, but corrections or amendments may be necessary.

Compliance

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki (1994) the principles of Good Clinical Practice (GCP), Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act (DPA number: Z5886415), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

Sponsor

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IMP Manufacturer and Supply of the Vaccines for the Trial

Vaccines will be supplied by Imperial College London and Manufactured as below :

DNA-C CN54ENV Althea, 11040 Roselle Street, San Diego, CA 92121, USA

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

GMP Storage, Labelling, Packaging, QP release and Distribution of Vaccine to Sites

PCI
Biotech House
Central Park
Western Avenue
Bridgend Industrial Estate, Bridgend
CF31 3RT; UK

SAE AND IMPORTANT AE NOTIFICATION

Within 24 hours of becoming aware of an SAE, please email the Imperial College London Joint Research Compliance Office at jrco.ctimp.team@imperial.ac.uk and the MRC CTU at UCL at mrcctu.cuthivac002safety@ucl.ac.uk using the template provided

POSSIBLE SERIOUS BREACH NOTIFICATION

Within 24 hours of becoming aware of a serious breach, please e-mail details to the Imperial College London Joint Research Compliance Office at jrco.ctimp.team@imperial.ac.uk and the MRC CTU at UCL on mrcctu.cuthivac002safety@ucl.ac.uk

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ABBREVIATIONS AND GLOSSARY

Ab	Antibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
AE	Adverse event
Ag	Antigen
APC	Antigen presenting cells
AR	Adverse reaction
ASC	Ab secreting cells
CF	Consent form
CFSE	Carboxyfluorescein succinimidyl ester
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTL	Cytotoxic T-lymphocyte
dDC	Dermal dendritic cell
DC	Dendritic cell
EP	Electroporation
EUDRACT	European Union Drug Regulatory Agency Clinical Trial
GTU	Gene Transfer Unit
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICL	Imperial College London
ICS	Intracellular cytokine staining
ID	Intradermal
IM	Intramuscular
JRCO	Joint Research Compliance Office
LC	Langerhans cell
MHRA	Medicines and Healthcare Regulatory Agency
MRC	Medical Research Council
MRC CTU at UCL	Medical Research Council Clinical Trials Unit at University College London (UCL)
NHS	National Health Service
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PIS	Participant Information Sheet
R&D	Research and Development
REC	Research Ethics Committee
SAE	Serious adverse event
SAR	Serious adverse reaction
SFC	Spot forming cell
SIV	Simian immunodeficiency virus
SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TDS	TriGrid Delivery System
Tfh	T follicular helper
Th	T-helper
TLR	Toll-like receptor
TMG	Trial Management Group
TOPS	The Overvolunteering Prevention System
UAR	Unexpected adverse reaction

1. SUMMARY

1.1 Summary of trial design

1.1.1 Type of design

CUTHIVAC002 is an open-labelled randomised Phase I study aimed at exploring the safety and immunogenicity of two different modes of delivery of a deoxyribonucleic acid (DNA) vaccine (DNA-C CN54ENV) via combined intramuscular and intradermal methods with and without electroporation, and boosted with recombinant HIV CN54gp140 administered by intradermal injection in healthy volunteers.

The aim of this study is to identify optimal DNA delivery conditions for promoting enhanced antibody responses to boosting with recombinant protein by the intradermal method.

1.1.2 Disease/participants studied

Healthy male and female volunteers 18 to 50 years old who are at low risk of HIV infection are to be recruited. For more details refer to **Section 4.**

1.1.3 Trial interventions

The trial interventions are:

• 2.6mg DNA/dose: plasmid encoding clade C gp140 envelope derived from HIV-1 isolate 97CN54

• 50ug CN54gp140/dose: trimeric recombinant clade C envelope protein derived from 97CN54

DNA immunisations will be administered by the combined intramuscular (IM) and intradermal (ID) methods as described in **Table 1** below. The CN54gp140 will be delivered by ID injection.

The doses, methods and schedule of immunisation are described in **Table 1.**

Table 1: Schedule of Doses and Methods of Immunisation

Group	Method of Immunization; Dose of DNA			Method of Immunization; Dose of CN54gp140
	Dose 1 at WK 0	Dose 2 at WK 4	Dose 3 at WK 8	Dose 4 at WK 20
1 **N=8**	0.6mg ID*/ EP 2mg IM**	0.6mg ID*/ EP 2mg IM**	0.6mg ID*/ EP 2mg IM**	50ug ID***
2 **N=8**	0.6mg ID* 2mg IM**/ EP	0.6mg ID* 2mg IM**/ EP	0.6mg ID* 2mg IM**/ EP	50ug ID***
3 **N=8**	0.6mg ID*/ EP 2mg IM**/ EP	0.6mg ID*/ EP 2mg IM**/ EP	0.6mg ID*/ EP 2mg IM**/ EP	50ug ID***

* 1 x 0.15ml injections ID via a needle – into the upper arm with or without electroporation (EP)

** 1 x 0.5ml injections IM – into the upper thigh with or without electroporation (EP)

*** 1 x 0.1ml injections ID via a needle - into the upper arm

1.1.4 Aim

This study will test the hypothesis that DNA-C CN54ENV vaccination administered via the combined intramuscular and intradermal methods with and without electroporation can maximise responses on subsequent intradermal boosting with recombinant CN54gp140 protein.

1.1.5 Objectives

Primary

- To evaluate the safety and immunogenicity of an HIV-1 DNA vaccine delivered via combined intramuscular and intradermal methods with and without electroporation, and boosted with CN54gp140 administered by intradermal injection with the aim of indentifying optimal conditions capable of promoting enhanced antibody responses to HIV.

Secondary

- To compare the safety of the electroporation devices to standard intramuscular or intradermal injections

Exploratory:

- To describe qualitative differences between the different methods of delivery in terms of changes from baseline in overall magnitude and functionality of antibody

responses to the vaccines, and quantitative differences in cellular responses (CD4+ and CD8+ T cells)
- To evaluate the tolerability of vaccination using the electroporation devices

1.1.6 Endpoints

Primary

Safety

- Grade 3 or above local solicited adverse event **(Table 2)**
- Grade 3 or above systemic clinical and laboratory solicited adverse event **(Table 2)**
- Any grade of adverse event that results in a clinical decision to discontinue further immunisations
- Any grade of adverse event within 7 days of receiving intradermal and standard intramuscular vaccinations with or without electroporation

Immunogenicity

- The magnitude of antigen specific systemic IgG antibody binding responses (µg/ml) 2 weeks after the final vaccination.

Secondary

Safety

- Any grade of adverse event, local to the ID and IM injection sites that start within 7 days after Doses 1-3.

Exploratory: Immunogenicity

- Frequency and magnitude of HIV-gp140 specific B-cell-mediated responses in the systemic compartment measured by B-cell ELISPOT
- The magnitude of vaccine specific systemic T cell responses by T cell ELISpot assay
- The magnitude of antigen specific systemic IgA antibody responses (ug/ml)
- Frequency, titre and avidity of serum binding antibodies to other HIV Env antigens (alternative clades) by ELISA or other assays.
- Mapping of serum binding antibodies using Env subunit constructs (e.g., V2 scaffolds and hotspots) by ELISA.
- Frequency and magnitude of mucosal IgG and IgA antibody responses to CN54gp140 measured four weeks after the final immunisation.
- Frequency and titre of serum neutralising antibodies to homologous virus, and, if warranted a wider panel of viruses representing different clades.
- Frequency and magnitude of HIV-specific T-cell mediated responses measured by T-cell CFSE, and ICS (Intracellular Cytokine Staining).
- Frequency and magnitude of T-cell chemokine and cytokine release following ex-vivo antigen stimulation quantified by Luminex.
- Isolation and characterization of Env-specific monoclonal antibodies (IgG) from memory B cells in the systemic compartments (dependent upon elicited specific memory B-cell numbers).
- Characterisation of non-neutralising antibody function using ADCC/ADCVI, viral capture and aggregation assays.
- Epitope mapping of B- and T-cell responses.

Exploratory: Tolerability

- Pain scores at 0, 10 and 30 minutes following vaccination with EP

Table 2 Solicited Adverse Events

Type	Event
Local AEs (immunisation site)	Discomfort Redness Swelling (soft) Induration (hard) Blisters
Systemic Clinical AEs	Abnormally raised temperature Chills Myalgia/flu-like general muscle aches Malaise (excess fatigue) Headache Nausea Vomiting
Systemic Laboratory AEs	Abnormalities in: Creatinine, AST, ALT, alkaline phosphatase, total bilirubin, glucose Hb, total WCC, neutrophils, lymphocytes, platelets

1.1.7 Duration

It is anticipated that enrolment will take 12 months. Each subject's participation in the trial, from screening until final visit (a total of 12 visits), will be approximately 12 months. Therefore the end of the trial, defined here as the final visit of the last participant, should be achieved approximately 24 months after the start.

1.1.8 Data collection for the study

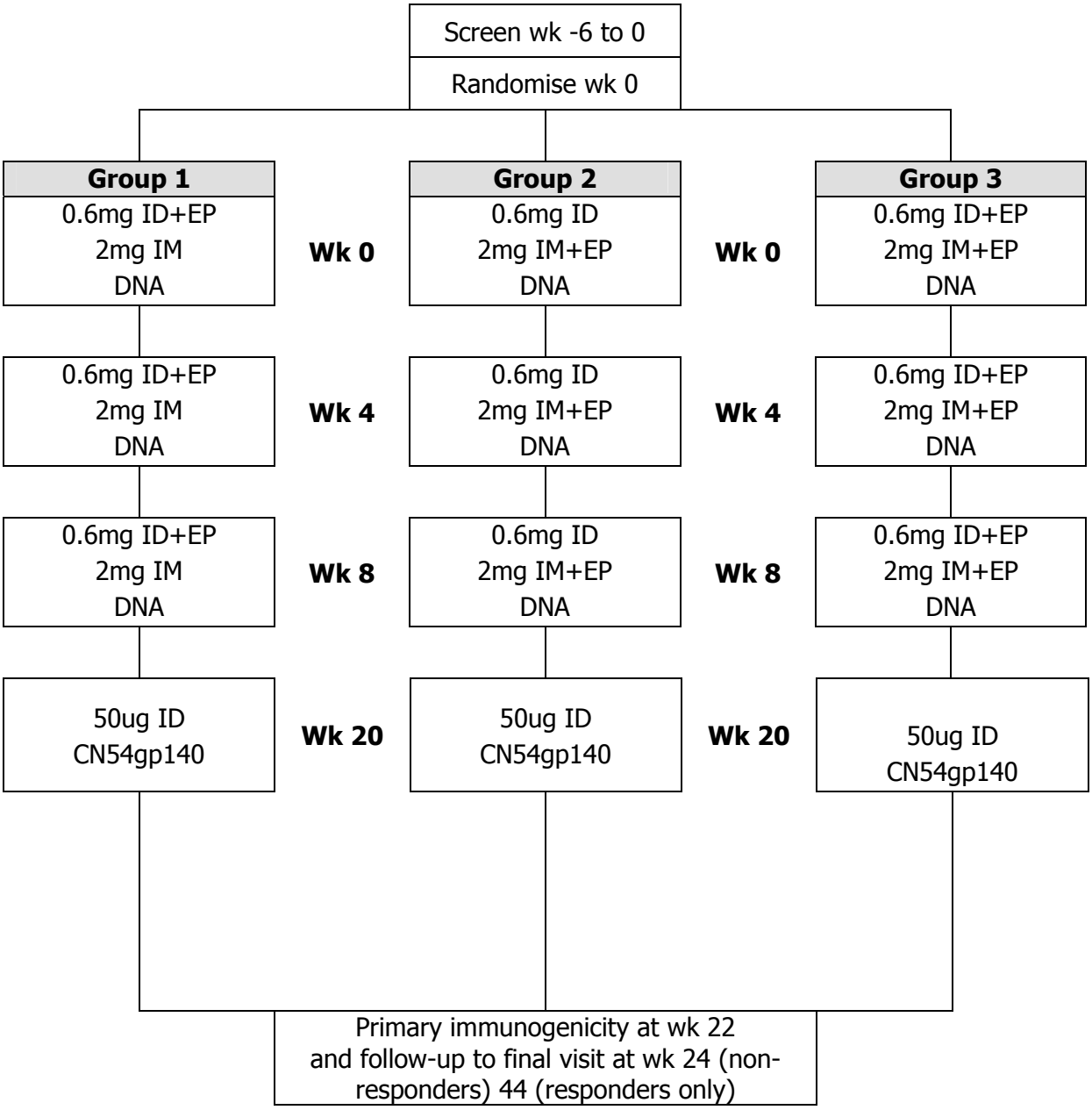
All participants will be registered as patients at Hammersmith Hospital. Data will be recorded in the medical notes which will contain the handwritten clinical notes and additional information such as the results of pregnancy tests and urinalysis. Data will be entered onto the case report form (CRF) and (randomised subjects only) sent to MRC CTU at UCL for data entry into a secure database. The CRF data will include medical history related to eligibility, dates of visits including immunisation, results of pregnancy tests, solicited adverse events, a description of non-solicited adverse events, and concomitant medication. Participants will record information on solicited adverse events in a diary card which will act primarily as an aide memoire to be checked at the next clinic visit and (randomised subjects only) sent to MRC CTU at UCL for data entry when complete.

1.1.9 Organisation

Imperial College London (ICL) will act as sponsor for the CUTHIVAC002 trial and the MRC CTU at UCL will manage the data (randomised subjects only) on their behalf. The clinical site will be the Hammersmith Hospital (Imperial College Healthcare NHS Trust). The project is funded by the European Commission FP7.

1.2 Flow Diagram

Figure 1 Screening, Randomisation, Immunisations and Follow-up Flow Diagram



2. BACKGROUND

2.1 Background

2.1.1 The global HIV-1 situation and the need for a vaccine

The global AIDS epidemic continues to grow. In its *2013 Report on the global AIDS epidemic*, UNAIDS published the following estimates for 2012.

- 35.3 million people living with HIV
- There were 6301 new infections per day
- Gains in expanding access to HIV treatment cannot be sustained without a reduction in the rate of new HIV infections

2.1.2 The status of the field

A prophylactic HIV vaccine is considered to be the most effective and sustainable way of reducing the rate of new infections. The International AIDS Vaccine Initiative (IAVI) has estimated that a vaccine has the potential to prevent over 70 million infections in 15 years [1]. The six different HIV vaccine efficacy trials which have been conducted to date have suggested an important role for both humoral and cellular immune responses and both are considered central to vaccine design, but to date there has been no robust correlate of protection against infection. The first vaccine efficacy trials (Vax 003 and Vax 004) focussed on the humoral response to the viral envelope and when they failed to show efficacy [2] [3] the focus of design shifted to T-cell responses. There was no envelope immunogen included in the vaccine used in the Step and Phambili trials which both made use of an adenovirus (Ad5) derived vaccine encoding *gag*, *pol* and *nef*. These two trials were both stopped early for futility and there was also a worrying suggestion of increased infection risk in vaccinated individuals [4, 5]. The HVTN505 trial also included an attenuated Ad 5 vaccine – this time given as a single boost after 3 priming immunisations with DNA encoding matched HIV surface and internal proteins (VRC-HIVDNA016-00VP). Although this trial was also stopped early for futility, there was no evidence of increased risk of infection in those who had received the vaccine, providing some reassurance about the platform [6]. RV144 is the only Phase III trial carried out to date to demonstrate (partial) efficacy, where IgG antibodies to the V1/V2 region of gp120 correlated with decreased risk of infection. Nevertheless, both non-efficacious vaccine and partially effective vaccine trials have provided immune correlates of infection risk. Three have described correlates of infection risk/incidence per se (VAX004, Step and RV144) and two have identified potential sites of selective immune pressure on the virus (Step and RV144), reviewed in [7].

2.1.3 The immune response to HIV

2.1.3.1 The role of Antibodies

Neutralizing antibodies (NAb) are the dominant correlate of protection in most prophylactic vaccines, and a successful vaccine against HIV-1 will probably be no exception. Studies in animals have suggested that pre-existing neutralising antibodies can prevent HIV infection, but it is not clear how to induce such responses with a vaccine. A significant minority of HIV infections result in heterogeneous mixtures of polyclonal broadly neutralizing antibodies (bNAbs) with broad specificity for the virus envelope [8]. Passive transfer studies in macaques using cloned bNAbs have shown that realistically achievable levels of such antibodies are able to block infection following low dose intravaginal challenge with SHIV - providing proof of concept for the strategy [9, 10]. Four of the completed efficacy trials induced envelope-specific antibody responses and whilst conducted in very different populations with varying risks of infection, some lessons can be drawn. The Vax 003 trial which was conducted in high risk injecting drug users showed that antibodies to pre-specified and vunerable regions on the viral envelope (V2, V3 and gp120) were not correlates of infection risk and neither were those which blocked CD4 binding or neutralised certain strains of HIV-1 (MN).

These results contrasted with those reported in Vax 004 which enrolled high risk men (predominantly MSM) and women. Follow-up studies in this trial reported that higher neutralising antibody responses to tier 1 viruses, those that blocked CD4 binding and those with antibody-dependent cellular virus inhibitory functions correlated with lower levels of HIV infection. An ideal HIV vaccine should induce potent and sustained bNAb responses. It is assumed that the breadth and magnitude of responses induced in Vax 004 were not sufficient to be protective. The RV144 trial reported an efficacy of 31% and so provided the first ever opportunity to look for correlates of protection. An international consortium of scientists worked together on a retrospective case control study. Six primary immunological variables were selected and analysis revealed that only two correlated with the risk of HIV-1 infection. IgG antibodies to the V1/V2 region of gp120 correlated with decreased risk and levels of envelope-specific IgA in plasma correlated with increased risk of infection in vaccinated individuals (ie decreased vaccine efficacy). Although neutralising antibodies were one of the six primary variables included in the primary analysis, levels were not correlated with risk of infection. Nevertheless, this does not exclude the possibility that the low but detectable neutralising responses may have had an impact on the risk of transmission [11].

The envelope protein immunogen used (A244 gp120) had an 11 amino acid deletion leading to better exposure of certain epitopes. This protein was antigenic for both linear V2 epitopes as well as for V1V2-glycan epitopes bound by V1V2 broadly neutralising antibodies. Understanding why the immune responses in this population were so dominated by antibodies to non-glycan epitopes remains an area of intense interest.

2.1.3.2 The role of T cells

A direct role for CD8+ T-cells in the control of viral replication in humans has been demonstrated during acute infection and also in those rare individuals who are able to naturally control infection. Non-human primate (NHP) models have provided compelling evidence that Class I restricted CD8+ T-cells can be sufficient for highly effective control of viral replication and eventual viral clearance. More recently Picker and colleagues have described a protective role for a novel subset of Class II restricted CD8+ T-cells with broad specificity for HIV-1 which develop following vaccination with this CMV derived vaccine [12, 13]. Step and Phambili were the first two Phase III trials which focussed solely on the CD8+ T-cell response. The trials were both designed to test the concept that a replication

defective adenovirus serotype 5 virus could decrease viral load in those who became infected- a hypothesis also tested in the HVTN505 trial which used the same Ad5 vaccine but this time as a single boost after priming three times with DNA. All three trials were stopped prematurely for lack of efficacy and there were increased infections seen in those who received vaccine relative to placebo in the Step trial – which was not seen in HVTN505. Post-hoc analysis revealed that the increased susceptibility correlated with levels of pre-existing antibody to Ad5 in uncircumcised individuals, although the effect could not be disentangled from other confounders [14]. Nevertheless, "sieve analysis" of the viruses circulating in infected vaccinated individuals revealed that CD8+ T-cells were exerting a direct impact on viral replication -arguing the case for continued investment in this strategy. However, proof of concept that CD8 responses can provide protection for infection in human is still needed.

2.1.4 Current Vaccine Strategies

In an effort to induce balanced immune responses several groups are focussed on the optimisation of heterologous prime boost vaccination regimens with DNA and modified viral vectors [15] [16] [17]. In light of the results of the RV144 trial, there is also an interest in combining recombinant proteins with viral vaccines. The rationale for these strategies initially stemmed from the results of pre-clinical studies which showed that priming with DNA followed by boosting with modified viruses induced stronger cellular immunity than either DNA or virus alone. The first HIV-based DNA vaccines have been somewhat disappointing in man in terms of their immunogenicity- but DNA has repeatedly been shown to play a significant role in priming B and T-cell responses. The approach has proved partially protective in animal models and early phase trials in humans have reported particularly strong immunogenicity within the T-cell compartment with varying levels of success within the B-cell compartment [15] [16]. However, importantly for this study DNA prime-protein boost strategies have been shown to increase antibody functional activity and avidity [18, 19] [20]. A vaccine able to induce neutralising antibodies remains elusive- but the antibody responses in RV144 which correlated with risk of infection has brought the role of binding antibodies and those with other non-neutralising functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) into the spotlight.

2.1.5 DNA Vaccines

DNA plasmid vaccines showed great promise in small animals, resulting in the licensing of three veterinary DNA-based vaccines but these successes failed to translate in human trials and this is probably because the uptake of DNA is very inefficient with as much as 91%-99% degraded within 90 minutes [21] [22]. DNA is particularly well suited to priming T-cell responses (CD4+ & CD8+) and does not carry the disadvantages associated with modified viral vectors and so is an attractive candidate for HIV vaccines [23-25]. Preclinical studies suggested a clear dose dependency in immune responses to DNA, but there is relatively limited clinical data available. When comparisons have been made, results have actually been somewhat inconsistent with some showing dose dependency [19] [26] and others not [27] [28]. In the study of Bansal and colleagues 100% (n=6) of healthy volunteers given 7.2mg DNA (5 plasmids encoding gp120 and 1 encoding gag) three times over 12 wks made CD4+ ELISPOT responses to Env peptides, whereas only ~30% responded to 6-fold lower dose of vaccine [26]. Interestingly, CD8+ T-cell responses to gag and poly [26] functional CD4+ T-cell responses (2 or more cytokines) were seen only in response to the higher dose of DNA. Although early approaches to DNA vaccination failed to induce significant antibody responses in humans, more recent trials have shown that DNA vaccines can induce neutralizing antibodies against a number of viruses [29-31] and detectable antibody responses to HIV-1 [32]. A dose relationship for DNA delivered with EP in humans has recently been shown for an immunotherapeutic vaccine against HPV16/18 in humans

[33]. This demonstrated a dose response trend where mid dose (1mg) appeared to be marginally better in inducing antibody responses than low (0.3mg) or high (3mg) doses. Various strategies can now be used to improve and augment the immunogenicity of DNA vaccines including: promoter selection and codon optimization; the use of electroporation (EP); and the method of administration (intramuscular (IM) or intradermal [34, 35].

2.1.6 Increasing the potency of DNA vaccination

2.1.6.1 Electroporation

When administered via a needle, relatively high doses of DNA are required, typically in the range of milligrams per dose. Physical methods of delivering the DNA such as via *in vivo* electroporation (EP) or gene gun have been shown to enhance immunogenicity [36] [37, 38]. In addition to increasing uptake directly, EP also results in increased local inflammation and recruitment of antigen-presenting cells (APC) which has been shown to augment immunogenicity and increase the duration and levels of antigen expression. EP has been shown to increase the potency of HIV DNA vaccines in animal models [39-41]. T-cell responses to the GTU®MultiHIV vaccine were enhanced when tweezer electrodes were used in association with ID immunisation of macaques and mice [42]. The same TriGrid device from Ichor which is advocated for use has been used to deliver EP with IM for administration of the ADVAX DNA vaccine to rabbits and mice. In an immunogenicity study carried out in mice, the magnitude of ELISPOT and intracellular cytokine responses was increased by EP without increased risks of integration of the DNA [43]. The first use of EP with an HIV DNA vaccine has been published and showed that the method was safe and T-cell immunogenicity was enhanced [44]. More recently EP has been used to augment the potency of HIV vaccines in early phase trials and has shown to be well tolerated and effective and is being assessed for feasibility in larger Phase II trials by several different networks [45].

2.1.6.2 DNA

Volunteers will receive 3 injections of 0.6 mg, ID with or without electroporation in a volume of 0.15ml at 0, 4 and 8 weeks together with 2mg of DNA in 0.5ml delivered via IM with or without electroporation. The DNA-C CN54ENV plasmid encodes clade C envelope derived from the HIV-1 97CN54 coding sequences (Geneart). Sequenced optimised inserts were introduced into the VRC8400 CMV/R vector (NIAID/NIH) for GMP manufacture. This vaccine has been manufactured by Althea Technology, Inc (USA). The DNA is formulated in 1 x phosphate buffered saline (PBS; pH 7.2) and will be supplied at a concentration of 4.0 mg/mL in vials containing 1.2 mL.

2.1.6.3 Recombinant CN54gp140

CN54gp140 is a recombinant GP140 derived from the HIV-1 97CN54 coding sequence and has been manufactured by Polymun, Austria. CN54gp140, is a trimeric recombinant C-clade ENV protein, derived from the 97CN54 Chinese viral isolate. The protein comprises a sequence of 670 amino acids, and has been shown to be immunogenic in humans. MucoVac 1 (EudraCT number 2007-000781-20) was the first human clinical trial to have used the trimeric CN54gp140 although the protein was not administered systemically but topically. Mucovac 2 generated the first safety data on the systemic administration of the CN54gp140 in healthy volunteers (**see section 2.8.3**). In this trial the protein will be supplied at 0.5mg/ml in a volume of 0.3mls. 50ug of protein will be administered ID at Week 20 (see below).

2.1.7 The relevance of the method of vaccination

Vaccine success depends upon the targeting and activation of dendritic cells (DCs). Vaccines are typically injected into the skeletal muscle (IM) or subcutaneous (SC) tissues for reasons of convenience. They are also typically given with adjuvants which augment recruitment of immature DC, stimulating their maturation and migration to the draining lymph nodes where they interact with naive T-cells. There is increasing interest in cutaneous vaccination (ID, TC and epidermal) because in comparison to muscle and SC tissues, the skin has relatively high numbers of resident APC. The relatively polarised location and function of the two major subsets of DC found in skin (Langerhans and dermal DC) has provided biological insights into the outcomes of dermal vaccination. The human epidermis is particularly rich in LC whereas dermal DC (dDC) are found primarily in the dermis. Both these cell types are able to migrate to the draining lymph nodes or the spleen via lymphatic drainage where they initiate immune responses following interaction with T- and B-cells [45]. Klechevsky et al., purified these two subsets from human skin and analysed their functions *in vitro* [46]. dDC (CD14+) interacted preferentially with CD4+ T follicular helper (Tfh) cells, inducing naive T- and B-cells to make antibody, and were relatively poor at stimulating CD8+ T-cell responses, primarily because they were unable to induce granzyme A/B or perforin in those cells.

By contrast, epithelial LC were not able to induce Tfh development or induce naive B-cells to make antibody but were efficient at processing antigens via the Class I pathway and were relatively good at stimulating lytic CD8+ T-cells. These findings might explain the immunological effects of scarification as used with small pox vaccination. This results in injury to both the epidermis and dermis thereby mobilising both dDC and epidermal LC - resulting in the stimulation of both humoral and cellular immune responses. The two cell subsets have also been shown to differ in their expression of Toll-like receptor (TLR), with dDC expressing a broader repertoire than LC, which might account for the relative unresponsiveness of LC to (commensal) bacteria [47] [48] . In a study directly supporting the design of this trial, concomitant DNA priming ID and IM followed by boosting SC was shown to optimise antibody responses in the mouse. EP delivered by tweezer electrodes IM and ID was also shown to significantly augment T-cell and B-cell responses and to increase antibody avidity [41].

(i) Dermal Immune Responses

It thought that cells activated in the dermis are able to home back both to the skin and the mucosa linking these two immunological compartments and thus making dermal vaccination attractive for targeting mucosal immune responses [49] [50]. The volumes which can be delivered ID are much smaller than can be delivered IM/SC which is a practical constraint when considering this route. The dose sparing effects of ID vaccination has been shown using a variety of vaccines including rabies, hepatitis and influenza, typically, sparing 80-90% of the dose which would be required via IM/SC routes [16, 45, 51]. Using the hepatitis B vaccine, the ID route is also able to overcome the lack of responsiveness seen in patients on hemodialysis to IM vaccination [52]. Several explanations have been suggested for the relatively increased efficacy of ID vaccination; ID may result in increased local inflammation which, in turn, might result in the recruitment of APC such as LC and DC, resulting in the increased cross presentation of antigens. ID vaccination has also been shown to favour the direct migration of antigens through the lymphatic ducts. Soluble proteins injected this way are able to reach the lymph nodes within 2 hrs which is ~12 hrs before the arrival DCs. This reduced transit time is thought to be purely a consequence of the mechanics of fluid injection. The increase in interstitial pressure caused by the injected fluid probably results in increased permeability in the capillaries and so also increased lymphatic flow.

2.1.8 Research Leading up to the Proposed Trial

2.1.8.1 The vaccine

DNA-C CN54ENV

The DNA vaccine will utilize the pVRC8400 plasmid expression vector containing the optimized pCMVR promotor [53]encoding a codon-optimized sequence for clade C gp140. The sequence was derived from a Chinese viral isolate, 97CN54. The C-clade HIV subtype is believed to cause more than 50% of worldwide HIV-1 infections, and is predominant in southern and eastern Africa and India [54]. Previous clinical studies using this plasmid backbone to express similar HIV-1 Env sequences generated low but detectable antibody responses to HIV-1 Env and achieved 70% seroconversion [32] following intramuscular injection without EP. Transgene codon optimization for CN54gp140 and insertion into the pVRC8400 vector have been performed by GeneArt and GMP product has been manufactured by Althea Technology, Inc (USA). There are no specific nonclinical pharmacology studies with the DNA-C CN54ENV vaccine alone. A murine study carried out to inform the design UKHVC_003 trial included a group which received the CN54ENV DNA alone. Whilst a relatively much higher concentration of DNA was used, the DNA was well tolerated and was clearly serving to prime and influence immune responses to the other boosting vaccines used. The env encoding plasmid vaccine (CN54ENV) is very similar in structure and HIV insert sequence to the equivalent plasmid previously tested, in combination with a NYVAC-C boost, in clinical trials EV02 and EV03 [55, 56]. In these studies, priming with DNA at a dose of 4mg on two or three occasions was shown to be well-tolerated and effective in contributing to overall immunogenicity. Identical CN54EENV DNA plasmid is currently in use in the UKHVC spoke 3 (EUDRACT 2012-003277-26) trial initiated in July 2013, which is exploring priming with DNA followed by boosting with MVA combined with CN54gp140 or the same immunogens given sequentially.

CN54gp140

The CN54gp140 is a recombinant gp140 derived from the HIV-1 97CN54 coding sequence and will be manufactured by Polymun, Austria. CN54rGP140, is a trimeric recombinant C-clade envelope protein. The protein comprises a sequence of 670 amino acids, and has been shown to be immunogenic in humans. To date Mucovac 2 (EUDRACT 2010-019103-27) is the only completed trial to have generated safety data on the systemic administration of the CN54gp140 in GLA-AF in healthy volunteers. 100μg of protein was administered IM after bedside mixing with GLA-AF (an aqueous formulation of synthetic MPLA). Analysis of the (presumed) peak antibody responses measured after the last immunisation demonstrated induction of robust antibody responses, with moderate neutralization activity against easy to neutralize tier 1 viral isolates.

Preclinical Studies

A Single Dose Biodistribution Study (No 525574) of CN54ENV DNA delivered intramuscularly with electroporation has been performed in rats. The results from this study show that biodistribution of the plasmid is restricted to the site of administration and that there is no persistence of the plasmid in these tissues beyond 60 days. A good laboratory practice (GLP) rabbit tolerance and toxicity study (CR 525569) has been performed to directly support this study, where animals received vaccinations with a 2mg dose of CN54DNA given intramuscularly three times at 3 weekly intervals with electroporation, followed by intradermal or intramuscular boosting with CN54gp140 (100ug). There were no adverse

clinical signs that were considered to be related to treatment with DNA-C-CN54ENV, DNA-C-ZM96GPN or CN54 gp140. There was transient very slight local irritation noted after intradermal injection of CN54gp140. There were no worrying local side effects or indications of systemic toxicity. Analysis of the (presumed) peak antibody responses measured 2 weeks after the last immunisation corroborated what had been seen previously in mouse and rabbit studies.

Clinical Studies

In the TaMoVac 1 trial which was carried out in two sites in Tanzania, 40 subjects who had previously been given three doses of HIVIS-DNA followed by two with MVA-CMDR were given two further boosts with 100μg CN54gp140, adjuvanted with 5μg GLA-AF. The rationale for boosting with CN54gp140 in TaMoVac 1 was informed by the results of RV144 and other trials using recombinant protein vaccines and a desire to complement the T-cell response with a strong B-cell response to HIV envelope. The adjuvanted protein was well tolerated and systemic CN54gp140-specific antibody responses significantly boosted in those primed with DNA and MVA to levels in the range seen in the RV144 trial (A.Bauer personal communication). The neutralising antibody responses were, however disappointing (A.Joachim personal communication) CN54gp140 has also been given to human subjects in two completed trials: MUCOVAC 1 and MUCOVAC 2 and is part of the regimen in 3 ongoing trials: UKHVC-003 which is a two centre Phase I trial running in the UK; TaMoVac 02, a two centre Phase II trial which is running in two sites in Tanzania and one in Mozambique; and X001 which is a single centre trial in the UK.

In MUCOVAC 1, which was conducted in the UK in 2008, CN54gp140 formulated without adjuvant was administered to healthy female volunteers by the IVAG route. Subjects received nine doses over a 3-week period over the course of one menstrual cycle. The vaccine was well tolerated and there were no serious adverse events. The immune responses were disappointing and there were no specific antibody responses detected. One possible explanation given was that the vaccinations were given too close together for optimal priming but there was no adjuvant included in the formulation which may also have contributed [57].

MUCOVAC 2 (EudraCT 2010-019103-27) built directly on MUCOVAC 1 with a continued focus on mucosal immune responses. The trial was conducted in 36 healthy women in two centres in the UK who were randomised to one of four groups to receive CN54gp140 with or without GLA-AF/Chitosan and by a variety of methods. The first (reference) group received three doses of 100μg CN54gp10 adjuvanted with 5μg GLA-AF (IM high dose), the second received three doses at a lower dose of 20μg also adjuvanted with GLA-AF (IM low dose). A third group received a single priming dose of 100μg CN54gp140/GLA-AF IM followed by two doses of 500 μg given intra-vaginally formulated in carbopol gel (IM IVAG) and a fourth received three doses of 100ug intranasally formulated in chitosan (IN). Following a protocol amendment, a group of 8 individuals (5 from the high dose IM group and 3 from the IN group) went on to receive 2 further systemic immunisations with GLA-AF adjuvanted CN54gp140 IM. The lower dose (20μg) of CN54gp140 was as potent as the higher dose (100μg) when given IM with GLA-AF- eliciting systemic binding IgG responses in the majority of individuals in both groups (9/11 and 9/9 respectively). 4/11 and 4/9 of the same women also had cervico-vaginal IgG responses. By contrast, there were no mucosal immune responses detected in the groups of women who received mucosal immunisations (IN or IVAG). Only 1/11 and 0/5 women in the IM IVAG or IN groups made systemic IgG. Interestingly after a single IM boost of the IN group this increased to 3/3, suggestive of amnestic responses and some effect of priming via the IN route. There were

no vaccine related serious adverse events although participants experienced at least one mild or moderate solicited adverse event but there were no safety concerns of note attributable to the study products and all participants completed all their allocated immunisations.

Studies directly supporting the design of CUTHIVAC002

Preclinical studies

To directly support the design of this trial Mann and colleagues carried out studies in mice and rabbits using a similar DNA plasmid encoding env for priming and the same CN54gp140 protein for boosting. The aims of the studies were (i) to assess the impact of DNA priming via different methods on T-cell and B-cell responses and (ii) to assess the impact of EP on each route after boosting with CN54gp140 [41]. The first part of the murine study was focussed on the optimisation of DNA priming. Vaccine specific antibody and cellular responses could be seen after DNA priming alone, which is one advantage of the model. Results showed that priming via the ID route favoured T-cell responses whilst the IM route favoured B-cell responses and that both were significantly augmented by EP. They also clearly showed that in addition to increasing the magnitude of the antibody response to CN54gp140, when given with both ID and IM priming , EP also increased the avidity of the antibody response. The greatest impact upon the avidity of the response was seen only when EP was given with both ID and IM priming suggesting that both the T-cell and B-cell responses were required for optimal avidity.

The same study also examined the optimal route for boosting with CN54gp140 DNA priming ID/IM with or without EP. The effect of DNA priming was striking and antibody responses were generally poor without it. Of the methods tested, subcutaneous (SC) and intranasal (IN) boosting was the best for generating systemic IgG responses, with IM boosting performing almost as well. IN boosting was significantly better than any other method in terms of IgA responses followed by the SC and IM methods. The transcutaneous route (TC) was able to boost systemic antibody responses but IgA responses were disappointing. In contrast to what was seen for antibody responses, the route of boosting with CN54gp140 did not have much impact on the magnitude of cellular responses seen after DNA priming (ID and IM) in the absence of EP but without priming, the responses were negligible. Studies in rabbits also clearly showed that priming by combined ID and IM immunization delivered with electroporation, followed by boosting SC resulted in antibody responses with neutralising activity (Tier 1) and that both were required for optimal responses. Increasing antibody affinity could also be seen after repeated protein boosts. Additional animal studies have shown that the route of DNA administration (IM vs. ID) can also impact on the quality of antibody induction. Recent studies in macaques suggest that both ID/EP and IM/EP DNA delivery promoted robust antibody responses to SIV Envs (reciprocal endpoint titre log 4.5–5) but that ID/EP administration induced higher cross-reactive neutralizing responses than IM/EP administration [58].This is further supported by previous studies using DNA encoding H5 hemaglutinin where concomitant IM/EP plus ID/EP induced significantly stronger responses than either route individually [59].These data suggest that split dose vaccination when given at different anatomical sites may have a positive impact on the magnitude of induced antibody responses. While these preclinical data are promising, differences in skin structure and resident immune cell populations between species mean the relative merits of ID/EP and IM/EP in the context of Env DNA can only be accurately determined in humans. Therefore we will also assess the impact of concurrent ID and IM administration in this study.

Clinical studies: CUT*HIVAC_001

This Phase I trial, which is fully recruited and volunteers are currently in follow up in London, is part of the same programme of research and is also exploring different methods of vaccination with a similar HIV DNA plasmid vaccine developed by FitBiotech and encoding a polypeptide containing peptides derived from envelope and core proteins.

The primary objective of this study is the induction of CD8 T cell responses to the vaccine immunogen. 30 healthy volunteers were randomised to receive 13.2 mg of GTU® MultiHIV B clade DNA plasmid given in three immunisations over 12 weeks via (i) IM and ID (ii) IM and Transcutaneously (TC) or (iii) IM with EP (Ichor TriGrid device). The transcutaneous route has been shown to favour the development of CD8+ T-cell responses in animal models and also in one Phase I clinical trial of a licensed influenza vaccine.

2.2 Rationale and Objective

2.2.1 Study Rationale

We will explore three combination regimens with and without EP with the overall aim of (i) optimising humoral and cellular immune responses (ii) developing safe and well tolerated vaccination strategies. We propose to combine the previously used IM and ID methods because clinical and preclinical data suggest that the combination will favour the development of balanced immune responses. All groups will receive 3 doses of 2.0mg of the CN54ENV plasmid DNA (CN54ENV) vaccine in 0.5ml volume IM together with 3 doses of 0.6mg of the CN54ENV vaccine in 0.15ml ID each given at 4 weekly intervals over 8 weeks followed by 1 dose of 50μg CN54gp140 ID at Week 20. A directly supportive murine study using a similar DNA vaccine, the same CN54gp140 protein and following the same design (albeit using a different EP device) demonstrated that priming via the combined IM+ID route favoured the development of high avidity humoral immune responses. This is further supported by the preclinical toxicology study (CR 525569) performed in rabbits using one of the Ichor devices that will be used in this study.

We are interested to see whether such concomitant priming performs similarly well in man resulting in optimal balanced immune responses. DNA vaccines have been relatively disappointing in man but there is still great interest in this platform because of its intrinsic flexibility, the relatively low cost of production of GMP material and the speed at which immunogens can be modified. We will be able to assess the effect of EP on each different route of vaccination and also to make a direct assessment of the translation of the very clear results seen in the murine study to the human which would be of general benefit to the field [34]. The trial is not powered to carry out formal statistical comparisons between the three groups and the immunological component is intended to be primarily descriptive.

2.2.2 Investigational Product / Intervention(s)

2.2.2.1 Investigational product DNA

The plasmid (DNA-C CN54ENV) encodes the HIV-1 clade C gp140 (env) derived from 97CN54. The IM dose will be 2mg which will be given in 0.5ml and the ID dose 600ug given in 150ul. The codon-optimised HIV-1 insert was introduced into an *E coli* VRC8400 CMV/R vector for manufacture. For the proposed clinical study, CN54ENV DNA will be manufactured in accordance with GMP by Ajinomoto Althea Technologies. Manufacture of the plasmid at Ajinomoto Althea is based on starting material containing synthetic genes

assembled by GENEART AG. The manufacturing process for CN54ENV DNA is based on established procedures as follows: fermentation and harvest of *E coli* host, cell lysis and diafiltration; purification by multiple steps (including endotoxin removal, anion exchange chromatography, ethanol precipitation, sterile filtration); formulation and filling. The plasmid is formulated in phosphate buffered saline, pH 7.2 and supplied as a sterile solution for injection at a concentration of 4.0 mg/mL in a 1.2ml volume. The DNA preparation is presented as a clear to opalescent, colourless liquid in 2 mL glass vials with rubber stoppers and flip-off seals.

2.2.2.2 Recombinant CN54gp140

For the proposed clinical study, the CN54gp140 will be manufactured by Polymun Scientific. CN54gp140 is a trimeric recombinant HIV-1 clade C envelope glycoprotein derived from a Chinese viral isolate 97CN54 [60] [61] . CN54gp140 solution comprises CN54gp140 recombinant glycoprotein formulated in an aqueous dilution buffer. The HIV-derived amino acid sequence of CN54gp140, as predicted from the primary DNA sequence of the clone, comprises 634 residues. The molecular mass predicted by the polypeptide sequence alone is approximately 70 kD. However, the protein is heavily glycosylated and has a mass of approximately 140 kD as determined by SDS-PAGE and size-exclusion chromatography. Furthermore, the CN54gp140 secreted by CHO cells is oligomeric, and following purification is essentially trimeric, with a projected mass of 420 kD. CN54gp140 solution is provided at a concentration of 0.50 mg/mL, in a volume of 0.3ml, as a clear, colourless, sterile liquid, presented in translucent polypropylene vials.

2.2.2.3 Device Specific Interventions

(i) Electroporation: The Ichor TriGrid™ Delivery System for Intramuscular Delivery (TDS-IM)

Background

Ichor developed the first integrated, automated application system called the TriGrid Delivery System (TDS) for the intramuscular administration of DNA vaccines with EP**.** A comprehensive technical dossier providing detailed descriptions of the device, its function, manufacturing procedures, as well as relevant verification, validation, and safety studies will be submitted by Ichor in the form of a Clinical Investigation Application for a Medical device submitted to the MHRA which references the corresponding Medicinal product application. The TDS-IM device has not been used to deliver this DNA plasmid vaccine, but has been used for the delivery of several other similar HIV DNA vaccines (summarised in tables 3 and 4)

The TriGrid^{TM} array consists of four electrodes arranged in two equilateral triangles arranged to form a diamond shape around a central injection needle. Technical details of the device can be found in **Appendix 1.** This ensures effective and reliable "co-localisation" of the electrical fields and the agent to be delivered. Over 600 individuals have now received a variety of DNA vaccines in the clinic including ten clinical trials which have been completed and ten more which are ongoing. Extensive non-clinical studies of plasmid biodistribution, persistence, and integration have shown that, consistent with conventional IM injection, EP based DNA delivery using the TDS-IM device is associated with localised uptake of the DNA at the injection site in surrounding tissues and draining lymph nodes within one week of administration. By 30-90 days there is substantial reduction in the levels

of DNA present in the injection site tissues and no evidence of significant integration of the vaccine candidates into genomic DNA. A single dose biodistribution study (Charles River Laboratories, UK Study No 525574) to directly support the proposed trial, demonstrated that the distribution of CN54 ENV expression plasmid when delivered using the TDS IM device was restricted to the site of administration and that there is no persistence of the plasmid in these tissues beyond 60 days.

Preclinical assessment of the device: Safety

The TDS IM device has been shown to enhance cellular and humoral immune responses to a viral, parasitic and tumor associated antigens in a wide variety of animal species including rodent, rabbit, non-human primate and cattle. The device has been assessed in multiple GLP compliant repeat dose/toxicity studies.

Formal safety studies have been conducted with nine individual DNA vaccines:

- Melanoma DNA vaccine encoding a xenogenic form of the tyrosinase antigen.
- Multi-antigen DNA vaccine encoding the gag pol nef tat vif and env antigens of HIV subtype C
- A malaria DNA vaccine encoding multiple epitopes isolated from malaria antigens.
- An epitope based melanoma DNA vaccine encoding epitopes isolated from the TRP-2 and gp-100 antigens.
- HIV-1 DNA vaccine encoding the subtype B gag, pol, nef, tat vif, env antigens coformulated with plasmid DNA encoding the human IL12 cytokine.
- HBV DNA vaccine coformulated with plasmid DNA encoding the human IL-12 cytokine.
- DNA vaccines encoding the M antigen of the Hantaan and Puumala hantaviruses.
- A DNA vaccine encoding HPV E7 antigen with calrecticulin based adjuvant.
- A DNA vaccine encoding multiple antigens from the Venezuelan equine encephalitis virus.
- A single dose Biodistribution Study (N0 525574) of CN54ENV DNA delivered intramuscularly with electroporation preformed in rats
- A rabbit tolerance and toxicity study (CR 525569) where animals received vaccinations with a 2mg dose of CN54DNA given intramuscularly three times at 3 weekly intervals with electroporation, followed by intradermal or intramuscular boosting with CN54gp140 (100ug).

The evaluations included repeated-dose safety/toxicology studies with as many as 5 immunisations of doses of DNA up to 4.0 mg as well as biodistribution and persistence/integration studies which were conducted in rabbits- with the exception of the epitope based melanoma vaccine (which was conducted in HLA-A2 transgenic mice) and the HPV E7 vaccine (which was conducted in C57Bl6 mice). The data from the studies of ADVAX have been published [43] . Extensive analysis of tissues at necroscopy reveal EP delivery of DNA to be limited to localised inflammatory responses of mild to moderate severity at the site of administration which resolves over a few weeks. Extensive biodistribution and integration studies conducted after 7 and 30-90 days showed negligible systemic uptake of the DNA. The low levels of residual plasmid (i.e <1000 copies/mg genomic DNA) for all DNA vaccines suggested a low level risk of potential integration. The only difference between EP based delivery and conventional IM delivery was the detection of DNA in the draining lymph node.

Clinical Experience to Date

Ten clinical trials of the TDS-IM device have been completed and are summarised below:

Table 3: Summary of completed trials using the TDS-IM device

Clinical trial.gov or EudraCT study reference	Vaccine candidate	Subject population
NCT00545987	Multigene HIV-1 DNA vaccine (ADVAX)	Healthy, HIV uninfected adult volunteers
NCT00471133	Xenogeneic tyrosinase DNA vaccine (pINGmuTYR)	Patients with Stage IIB-IV melanoma
NCT01169077	Multi-epitope malaria DNA vaccine (EP-1300)	Healthy adult volunteers
NCT01502345	Multi-antigen hantavirus DNA vaccine (pWRG/HTN-M9x) and pWRG/PUUVM9s2))	Healthy adult volunteers
NCT01641536	Multi-antigen HBVDNA vaccine with DNA-based IL12 adjuvant (HB-110)	HBV infected adult volunteers
NCT01496989	Multi-antigen HIV DNA vaccine (HIV-MAG) with or without DNA based IL-12 (GENEVAX) prior to adenovirus vector (Ad35GRIN)	Healthy, HIV uninfected volunteers
NCT01634503	Multi-antigen HPV DNA vaccine administered with a DNA-based human FLT3 adjuvant (GX-188E)	Patients with HPV16 or 18 associated Grade 3 Cervical Intraepithelial Neoplasia
NCT01266616	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX)	HIV infected adult volunteers
NCT01578889	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to administration of an vesicular stomatitis virus vector (rVSVgag)	Healthy adult volunteers
NCT01493154	HPV DNA vaccine administered with a DNA-based calreticulin adjuvant (pNGVL-4a-CRT/E7 (detox))	HPV-associated squamous cell carcinoma of the head and neck

The TDS-IM device is currently being evaluated in ten clinical trials which are summarised below:

Table 4: Summary of trials in which the TDS-IM device is currently being evaluated

Clinical trial.gov or EudraCT study reference	Vaccine candidate	Subject population
NCT01138410	Epitope-based TRP-2 melanoma vaccine (SCIB-1)	NCT01138410
NCT01859325	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to administration of an vesicular stomatitis virus vector (rVSVgag)	HIV infected adult volunteers
NCT02075983	GTU®-multiHIV B clade DNA vaccine	Healthy adult volunteers
NCT01984983	VEEV DNA vaccine candidate	Healthy adult volunteers
NCT02099994	HIV DNA vaccine (pSG2.HIVconsv DNA) administered prior to administration with an adenovirus vector (Ad35GRIN) and a vaccinia virus vector (MVA.HIVconsv)	Healthy adult volunteers
NCT02116205	Multi-antigen hantavirus DNA vaccine (pWRG/HTN-M(x) and pWRG/PUUV-M(s2))	Healthy adult volunteers
NCT02139267	Multi-antigen HPV DNA vaccine administered with a DNA-based human FLT3 adjuvant (GX-188E)	Patients with HPV16 or 18 associated Grade 3 Cervical Intraepithelial Neoplasia
NCT02204098	Mammaglobin DNA vaccine	Breast cancer patients undergoing neoadjuvant endocrine therapy
2011-003171-11	CUTHIVAC_001	Healthy adult volunteers
2014-001997-33	DNAVAC	Healthy adult volunteers

Safety

To date, the 20 clinical trials that have been completed or are currently ongoing have enrolled over 600 subjects in the electroporation arms of the studies (including subjects receiving either the DNA vaccine candidate or placebo). The device has been used for administration of DNA injections of up to 1.0 ml volume and 4.0 mg DNA dose per injection site. Subjects have received the vaccine candidate either as a single injection in one muscle site (total DNA dose up to 4.0 mg per administration time point) or as 2 injections in 2 separate muscle sites (total DNA dose up to 8.0 mg per administration time point). Subjects given the DNA dose as a single injection have received up to 5 TDS-IM injections and up to 4.0 mg DNA, and subjects administered with the DNA dose in 2 injections received up to 10 administrations (i.e., 20 total TDS-IM injections).

Acute adverse responses associated with the use of the device include transient pain associated with electroporation-induced localised muscle contractions reported by virtually all subjects. This has occasionally been accompanied by mild paresthesia in the administered limb lasting for several seconds to minutes after application of electroporation. Mild, transient bleeding at the sites of electrode/needle penetration is commonly observed following removal of the device from the administration site. Local site reactogenicity, including injection site soreness, erythema, and/or induration of mild to moderate severity, has been commonly reported following electroporation-mediated DNA delivery. Mild to moderate bruising at the administration site has been observed occasionally. The local site reactions typically resolve within 24-72 hours following administration, but, in rare instances, local site soreness has been reported to persist for up to one week.

Transient local site tenderness of Grade 3 has been reported in several subjects. Vasovagal reactions comprising mild dizziness, light-headedness, and/or hypotension occurring immediately after procedure application have been reported in approximately 1% of subjects. A more pronounced vasovagal reaction including a brief syncopic episode (of approximately 30 seconds duration) following procedure application was observed in one subject in the tyrosinase study. Systemic adverse events reported during the studies and judged to be possibly related to the study product and/or delivery device have been generally mild to moderate in severity and include headache, fatigue, fever, dizziness, malaise, , arthralgia, myalgia, and aphthous stomatits. Minor serological and hematological abnormalities observed in these studies have included transient Grade 1-3 elevation in serum creatine phosphokinase. Two subjects (one in the malaria vaccine study, one in an HIV study) have reported a single instance of severe fatigue within 24 hours of dosing that was resolved by the following day. No serious events attributed to the device or administration procedure have been observed to date.

Immunogenicity

The completed study of the multi-antigen HIV DNA candidate was a randomised placebo-controlled comparison of EP relative to conventional IM. There were no cellular immune responses after two immunisations IM with 4.0mg of DNA given without EP- but there was a dose dependent increase in both the frequency of responders and the magnitude of the response seen with EP [44]. 6/8 individuals made a cellular immune response after 2 immunisations with 4.0mg DNA given with EP and this increased to 87% after a third dose. The completed study of the tyrosinase DNA vaccine was a single arm dose escalation study (0.2, 0.5 and 1.5 mg DNA) of the vaccine in HLA-A1 and A24 positive individuals with Stage IIB-IV melanoma. The immune responses of 21/24 were assessed and positive anti-tyronsinase responses were observed in 6/15 patients who received 1.5mg doses of DNA.

(ii) The Ichor TriGrid™ Delivery System: For Intradermal Delivery (TDS-ID)

Background

The TDS-ID device has not been used to deliver this HIV DNA vaccine to healthy volunteers in man before, and there is not as much clinical data available as there is for the TDS-IM device. To date, the device has been used in 20 healthy volunteers. Building on the initial data generated with the TDS-IM device, Ichor has adapted the TDS platform to enable assessment of intradermal DNA delivery. The objective of this development effort is to provide the means to evaluate both intramuscular and intradermal delivery for a given vaccine candidate, thereby allowing identification of the route of administration best suited for further clinical development. The TriGrid Delivery System for intradermal administration (TDS-ID) is a device developed for EP mediated intradermal DNA delivery in the setting of early phase clinical trials. To accommodate testing of multiple product candidates with the platform as well as the range of parameters likely to be tested in these early phase clinical studies, the TDS-ID has been designed to utilize an "off-the-shelf" needle free injector (the Medi-Jector Vision [Antares Pharma]) that utilizes a standard vial interface for loading the syringe and is capable of administering a range of injection volumes. If warranted by interim clinical trial results, a refined TDS-ID design incorporating an integrated needle free injection apparatus suitable for use in late stage clinical studies and commercial deployment will be implemented.

The procedure for EP mediated intradermal DNA delivery with the TDS-ID comprises three principal steps: electrode insertion, agent administration, and EP application. Briefly, the procedure is initiated with the placement of the device against the skin at the target site of administration. User activation of the device results in the automated insertion of an array of four conductive electrodes positioned around the injection orifice of the Medi-Jector Vision needle-free injection device. The electrode insertion step is concluded with the application of a brief electrical signal to the electrodes in order to determine the impedance between the electrodes. If the impedance value indicates an ineffective insertion into the tissue or the potential for a safety hazard, the procedure is halted and the array removed from the tissue prior to the administration of DNA. If the impedance is within the range consistent with an acceptable insertion, the procedure proceeds with the injection of the agent of interest through the central injection orifice. The injection results in the formation of a circular bleb at the site of injection and within the skin tissue circumscribed by the deployed electrodes. Immediately following distribution of the agent to the tissue, the procedure concludes with the propagation of the electroporation inducing electrical fields at the site of agent administration. Once the electroporation sequence is completed, the electrodes and injection needle are immediately removed from the recipient. The entire procedure requires approximately ten seconds to complete from the time of device placement against the recipient's skin until removal of the device. Technical details of the device can be found in **Appendix 1.**

Summary of Experience with Device

The TDS-ID has been authorized for use as the means of DNA vaccine administration in a human clinical study of a DNA vaccine candidate for Venezuelan equine encephalitis virus. The study has enrolled 20 subjects in the TDS-ID arm of the study. The device was used to administer a series of three injections at DNA doses of up to 0.3 mg. Adverse responses reported in association with use of the device include acute discomfort / pain during EP application and minor cutaneous bleeding at the site of injection. Pinpoint eschar formation at the sites of needle formation has been commonly observed as well as transient injection site reactogenicity (erythema, induration, and/or soreness) of mild to moderate severity,

typically resolving within 24-72 hours following administration. Systemic adverse events reported during the study and judged to be possibly related to the study product and/or delivery device have been generally mild in severity and have included fatigue, headache, sore throat, and flushing. The findings are largely consistent with the adverse events observed with the TDS-IM device which has been utilized for the delivery of a wide range of DNA vaccines intended for use in oncology and infectious disease applications.

The TDS-IM has been used as the means for administration in 20 completed or ongoing clinical studies totalling over 600 subjects. Adverse reactions associated with the use of the device include acute pain with localized muscle contractions in almost all subjects. Occasionally this is accompanied by mild, transient paresthesia. Mild, transient bleeding at the sites of electrode/needle penetration is commonly observed following removal of the device. Soreness, erythema, and/or induration of mild to moderate severity are commonly reported at the administration site. Transient vasovagal reactions, comprising mild dizziness, skin pallor, diapheresis, light-headedness, and/or hypotension occurring immediately after device application, have been reported in approximately 1% of subjects. A more pronounced vasovagal reaction including a brief syncopic episode (of approximately 30 seconds duration) following device application was observed in one subject. Systemic adverse events reported during the studies and judged to be possibly related to the study product and/or delivery device have been generally mild to moderate in severity and include headache, fever, dizziness, malaise, fatigue, arthralgia, myalgia, influenza-like symptoms, and aphthous stomatitis. Transient elevation in serum creatinine phophokinase of mild to moderate severity and judged to have at least a potential association with candidate administration have been reported in a small minority of subjects. Two subjects have reported a single instance of severe fatigue within 24 hours of dosing that was resolved by the following day.

To date, TDS-ID associated malfunctions observed in clinical trials to date have been limited to reports of device initiated interruption of the procedure prior to its completion. As described above, the device performs an impedance evaluation following electrode array insertion but prior to agent administration. In the event of an improper insertion of the electrodes (due to, for example, an inadequate depth of penetration into the skin or theoretically, distortion of the electrodes) is detected, the TDS-ID system will interrupt the administration procedure prior to the injection of the agent and notify the user. At that point, the device is withdrawn from the subject. The overall design of the TDS-ID system and the specific implementation of the impedance check are intended to minimize the occurrence of DNA administration without the subsequent application of EP.

Subject to the specific procedures described in the clinical protocol, following an incomplete procedure application in which the DNA was not delivered to the subject, the user may obtain a new Application Cartridge and agent dose to attempt re-administration of the procedure. In its investigation of these occurrences, Ichor has identified several causes for the interruption of the procedure application.

These include: Improper loading, placement, and/or activation of the device. In several cases, investigation indicates that user error has contributed to the occurrence of an interrupted procedure applications. Specific circumstances have included improper device set up and suboptimal positioning of the device relative to the target skin site. Since their identification, these issues have been addressed as points of emphasis during the conduct of the training seminar used to qualify clinical site personnel prior to use of the TDS-ID device.

2.2.3 Rationale for Interventions

DNA Doses

There are very few published studies of dose response to DNA vaccination delivered with electroporation in humans, thus it is hard to directly extrapolate the results above to the human setting. A dose relationship for DNA delivered with EP in humans has recently been shown for an immunotherapeutic vaccine against HPV16/18 in humans [33]. This demonstrated a dose response trend where mid dose (1mg) appeared to be marginally better in inducing antibody responses than low (0.3mg) or high (3mg) doses.

To the best of our knowledge the dose relationship for a single plasmid expressing an Env transgene delivered with electroporation has not been defined in humans. Using a similar design to that performed for the Human Papilloma Virus (HPV), and taking into account our own preclinical studies, we propose to assess a mid-dose 2mg (500ul) for intramuscular injection. As we are constrained by the volume that can be administered we will use a low dose 600ug (150ul) for intrademal immunization. The difference in DNA concentrations between IM (2mg) and ID (0.6mg) doses mirrors that modelled in supportive preclinical studies [41]. Vaccinations will be delivered by IM or ID electroporation using the Ichor TriGrid™ Delivery System (http://www.ichorms.com) - see **appendix 1** for additional detail.

All volunteers will receive 3 immunisations of 2.0mg DNA IM and 0.6mg DNA ID and the immunisations will be administered with or without EP as described in table 1. In a supportive rabbit GLP toxicity study which was carried out with the same vaccine, the individual IM dose was 2mg, this was given for up to six administrations was shown to be well tolerated in rabbits (CR 525569) and lack of DNA persistence or distribution beyond the site of administration was demonstrated in rats (CR525574). Furthermore, a previous Phase I trial to use electroporation (EP) for the delivery of a DNA vaccine for HIV demonstrated increased immunogenicity in the absence of associated safety concerns [44] [62].

CN54gp140 Doses

We will give 1 immunisation of 50μg CN54gp140. In MucoVac 2 there was no significant impact on the magnitude of the responses after reducing the dose from 100μg to 20μg although this was in the presence of adjuvant.

2.2.4 Possible Next Steps

The following hypotheses will be explored with a view to informing the design of future trials both in terms of size and selection of methods:

- That each of the methods and combination regimens delivered with and without EP will be safe and acceptable
    - If any of the methods or regimens have unacceptable safety defined in terms of the proportion of participants that experience a grade 3 or above solicited adverse event or an event that leads to an investigator decision to discontinue immunisations, then there will be no further exploration of the route with the product/regimen.
- That immunisation via the combined IM+ID methods will elicit balanced immune responses.
- That EP will augment antibody responses to both ID and IM.

- That the greatest proportion of individuals will respond and make significantly greater antibody responses in group 3 (ID/EP + IM/EP) relative to the other groups. If EP is shown to impact upon the proportion of individuals making systemic IgG without compromising safety/tolerability, the strategy would be taken forward into future trials.

2.2.5 Risks and Benefits

This is a Phase I exploratory study in healthy male and female volunteers.

HIV DNA plasmid-based vaccines are widely used alone and in combination regimens, including the multicentre EuroVacc trials EV01-3 in which we have been involved and which volunteers were given up to 12mg DNA over 12 weeks with no safety concerns. There is currently limited data available on the systemic use of CN54ENV DNA but our group is directly involved in the only trial using CN54ENV and CN54gp140 delivered IM in DNA-MVA-protein strategy (UKHVC SPOKE 003; EUDRACT: 2012-003277-26) initiated in July 2013 and now completed, for which our group have direct access to safety data as it becomes available.

While electroporation has been used in multiple studies, it has not been used to deliver this paricular vaccine in humans before, but the supporting preclinical study (CR 525569), was shown to be well tolerated in rabbits. Furthermore, a previous Phase I trial to use electroporation (EP) for the delivery of a DNA vaccine for HIV demonstrated increased immunogenicity in the absence of associated safety concerns [44] [62]. A more recent study (HVTN 080) assessed a mixture of 3 expression plasmids encoding HIV-1 Clade B Env, Gag, and Pol delivered together with a DNA expressing IL-12 followed by EP with no associated safety concerns [63]). The vaccination schedules in this study are complex and on each visit volunteers will receive at least one vaccination with EP. There is an increased risk that retention to the trial will suffer as a result. The first Phase I trial to use EP for the delivery of a DNA vaccine for HIV has recently reported and observed an increase in immunogenicity. All the volunteers completed their immunisations, and adverse reactions were in line with licensed vaccines. In the ongoing healthy volunteer study (CUTHIVAC 001**,** EUDRACT 2011-003171-11), one participant elected to discontinue further immunisations due to pain and eight participants were subsequently randomised to EP and preceded without a problem.

There is no direct benefit to the volunteers. They will be reimbursed for their time and travel. Volunteers also derive the benefits of clinical screening and any follow up care for the time that they are enrolled in the study and afterwards where appropriate. As there is no placebo group, there is a risk that adverse events will be over-reported, but this should not influence grade and is not a concern at this stage of evaluation.

The laboratory endpoints will be analysed in laboratories at Imperial College London, and the staff blinded to the regimen, although not the time point. Samples may also be shipped to specialist laboratories in Europe and the USA for wider exploratory immunological measurements of interest. Procedures will be put in place to ensure the chain of custody of samples when transporting from clinic to laboratory and between laboratories; therefore we consider that the risk of loss or compromise of samples is low.

3. SELECTION OF CENTRES/CLINICIANS

The Principal Investigator, Sonya Abraham (Imperial Clinical Research Facility, Hammersmith Hospital) is an experienced clinical trialist. Volunteers will be seen by clinical staff from the Imperial Clinical Research Facility, Imperial College Healthcare NHS Trust, who have experience of running Phase 1 clinical trials.

4. SELECTION OF PARTICIPANTS

There will be **no exceptions** to eligibility requirements at the time of randomisation. Questions about eligibility should be addressed prior to attempting to randomise the participant.

The eligibility criteria for this trial have been carefully considered. The eligibility criteria are the standards used to ensure that only appropriate participants are considered for this study. Participants not meeting the criteria should not join the study. For the safety of the participants it is important that no exceptions be made to these criteria for admission to the study.

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

4.1 Participant Inclusion Criteria

1. Men and women aged between 18 and 50 years (inclusive) on the day of screening
2. BMI between 18-30 kg/m^2 (inclusive)
3. Available for follow-up for the duration of the study (up to ~12 months from screening)
4. Willing and able to give written informed consent
5. At low risk of HIV and willing to remain so for the duration of the study defined as:
    - no history of injecting drug use in the previous ten years
    - no gonorrhoea or syphilis in the last six months
    - no high risk partner (e.g. injecting drug use, HIV positive partner) either currently or within the past six months
    - no unprotected anal intercourse in the last six months, outside a relationship with a regular partner known to be HIV negative
    - no unprotected vaginal intercourse in the last six months outside a relationship with a regular known/presumed HIV negative partner
6. Willing to undergo a HIV test
7. Willing to undergo a genital infection screen
8. Must agree to require male sexual partner to use condoms, from at least 14 days before the first vaccination until at least 4 months after the last
9. If heterosexually active female capable of becoming pregnant, must (in addition to requiring male partner to use condoms) agree to use hormonal contraception, or to

complete abstinence, from at least 14 days before the first vaccination until at least 4 months after the last. [Note: Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal, and IUD/IUS, are not acceptable methods of contraception.] If sexually active male, must agree to use condoms from the day of first vaccination until at least 4 months after the last. [Note: Additional use of an effective method of contraception is recommended for any non-pregnant female partner over the same period.]

10. Agree to abstain from donating blood for three months after the end of their participation in the trial, or longer if necessary
11. Registered with a GP for at least the past three months
12. Entered and clearance obtained from The Over-volunteering Prevention System (TOPS) database.

4.2 Participant Exclusion Criteria

1. Pregnant or lactating
2. History of cardiac arrhythmia or palpitations [e.g., supraventricular tachycardia, atrial fibrillation, frequent ectopy, or sinus bradycardia prior to study entry (sinus arrhythmia is not excluded)
3. History of syncope or fainting episodes within 1 year of study entry
4. History of grand-mal epilepsy, seizure disorder or any history of prior seizure
5. Individuals in which a skin-fold measurement (cutaneous and subcutaneous tissue) of the upper right and left thigh exceeds 40 mm
6. Clinically relevant abnormality on history or examination
7. Known hypersensitivity to any component of the vaccine formulations used in this trial, or have severe or multiple allergies to drugs or pharmaceutical agents
8. History of severe local or general reaction to vaccination defined as
    - local: extensive, indurated redness and swelling involving most of the antero-lateral thigh or the major circumference of the arm, not resolving within 72 hours
    - general: fever ≥39.5^{o}C within 48 hours; anaphylaxis; bronchospasm; laryngeal oedema; collapse; convulsions or encephalopathy within 72 hours
9. Receipt of live attenuated vaccine within 60 days or other vaccines within 14 days of enrolment
10. Receipt of an experimental vaccine containing HIV envelope components at any time in the past
11. Receipt of blood products or immunoglobulin within 4 months of screening
12.
13. Has received treatment with immunosuppressive agents within 3 months of screening e.g. oral, inhaled, nasal or injected corticosteroids. (Topical steroids are allowed, unless applied to the IM or ID injection sites.)Participation in another trial of a medicinal product, completed less than 30 days prior to enrolment.
14. HIV 1 or 2 positive or indeterminate on screening.

15. Positive for hepatitis B surface antigen, hepatitis C antibody or serology indicating active syphilis requiring treatment
16. Grade 1 (or above) clinically significant routine laboratory parameters (see appendix 2 for definitions). Hyperbilirubinaemia to be considered an exclusion criterion only when confirmed to be conjugated bilirubinaemia
17. Current use of any electronic stimulation device, such as cardiac demand pacemakers, automatic implantable cardiac defibrillator, nerve stimulators, or deep brain stimulators.
18. Presence of any surgical or traumatic metal implants at the sites of administration
19. Unable to read and speak English to a fluency level adequate for the full comprehension of procedures required in participation and consent.
20. Women with a history of toxic shock syndrome.
21. Women using an intrauterine device for contraception (as incompatible with softcup sampling)
22. Unlikely to comply with protocol.

4.3 Number and Source of Participants

Healthy volunteers will be recruited through advertising. They will be provided with further information about the study and asked to complete a short interview (by telephone or in person) to assess their suitability. They will be given or sent an information sheet. The target is for 24 participants to complete the trial.

4.4 Screening Procedures and Pre-Randomisation Investigations

At the screening visit the volunteer will be allocated a number from the register. The trial will be discussed in detail, and a brief check of eligibility conducted. Any questions about the study will be answered. If volunteers appear potentially eligible, and willing and interested, they will be asked to sign the informed consent form.

After written informed consent has been collected, assessments and investigations will be undertaken according to the schedule in **Table 5 , section 7**. These include demographic, sexual and medical histories and general examination, and collection of urine and blood samples for routine laboratory investigations. Samples for screening sexually transmitted infections including HIV will be collected **(see section 7.2).** An ECG will be performed.

As soon as all required test results are available:

- Data will be entered onto the screening CRF and the results of the screening investigations will be reviewed and eligibility signed off by a physician.

5. RANDOMISATION & ENROLMENT PROCEDURE

5.1 Randomisation Practicalities

The enrolment visit will take place within 6 weeks (42 days) of the screening visit and will be the same day as the first vaccination. Assessments and procedures will be undertaken according to the schedules (**Section 7** and **Table 5**), and data entered on the case report forms.

Volunteers who are eligible and willing will be randomised when all inclusion/exclusion criteria have been met and the appropriate CRF completed. The PI or team member will log into a web-based system managed by MRC CTU at UCL to obtain the regimen allocated to the participant.

Further details on the process of randomisation can be found in **Section 10**.

5.2 Randomisation Codes and Un-blinding

The master randomisation list linking subject numbers to allocation will be generated by the trial statistician. Randomisation will be carried out on the day of the first dosing visit.

Randomisation arm will be known to participants and clinical staff.

The laboratory staff conducting the assays will be blind to the regimen throughout.

The trial is open-label so no unblinding will be necessary.

5.3 Co-Enrolment Guidelines

Participants will be advised that they cannot enrol in any other trials that would interfere with the study endpoints during the period from screening to the final visit in CUTHIVAC002. All participants will be entered onto the TOPS (The Overvolunteering Prevention System) database as a measure to prevent over volunteering.

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

6. TREATMENT OF PARTICIPANTS

6.1 Vaccine Products

Participants will be vaccinated in a schedule of doses and methods as described below. Each participant will receive three DNA immunisations, at wk 0, 4 and 8, boosted with recombinant HIV CN54gp140 administered by intradermal injection at wk 20.

A vaccine accountability log will be kept throughout the study. This should be used to record the Trial ID of the subject to whom the study vaccine was dispensed. This will be verified by the study monitor. The date and time of administration will be recorded on the CRF.

6.1.1 Products and Administration

The individual IMP vials will be labelled, and packaged in cartons which will also be labelled. The Principal Investigator will ensure that staff administering the vaccines have been appropriately trained in the written procedures for ID, IM and EP.

All individuals will be given one IM injection of 2.0mg CN54ENV DNA and one ID injection of 0.6mg of CN54ENV DNA at Weeks 0, 4 and 8, and one ID injection of 50 µg CN54gp140 at Week 20. See table 1, section 6.1.3.

6.1.2 Accountability for Used and Unused Supplies

The PI will ensure that the IMPs are dispensed in accordance with the protocol and local procedures, and that records are maintained of receipt, dispensing and return/destruction of all supplies.

The PI must ensure that all IMP supplies are kept in a secure area accessible only to authorised individuals, and maintained in storage that guarantees the following temperatures:

- CN54ENV DNA at -25 to -15^{o}C
- CN54gp140 at 2-8^{o}C

Upon receipt of supplies, a designated member of staff will conduct an inventory and acknowledge receipt to the supplier.

A record must be kept of all CN54ENV DNA and CN54gp140 used during the trial. This will include the description (lot numbers and expiry dates) and quantity of IMP received at the trial site and date of receipt, as well as a record of when (date of administration) and to whom (Trial ID) it was dispensed.

At the end of the trial, IMP accountability will be checked by the designated member of staff responsible for the inventory, and by the trial monitor. The Sponsor and the PI will retain copies of the complete IMP accountability.

All supplies (used and unused) will be retained at the trial site until the Sponsor gives instructions for their return or destruction.

6.1.3 Immunisation schedule

The doses, methods and schedule of immunisation are described in **Table 1** below.

Table 1: Schedule of Doses and Methods of Immunisation

Group	Method of immunization; Dose of DNA			Method of immunization; Dose of CN54gp140
	Dose 1 at WK 0	**Dose 2 at WK 4**	**Dose 3 at WK 8**	**Dose 4 at WK 20**
1 **N=8**	0.6mg ID*/ EP 2mg IM**	0.6mg ID*/ EP 2mg IM**	0.6mg ID*/ EP 2mg IM**	50ug ID***
2 **N=8**	0.6mg ID* 2mg IM**/ EP	0.6mg ID* 2mg IM**/ EP	0.6mg ID* 2mg IM**/ EP	50ug ID***
3 **N=8**	0.6mg ID*/ EP 2mg IM**/ EP	0.6mg ID*/ EP 2mg IM**/ EP	0.6mg ID*/ EP 2mg IM**/ EP	50ug ID***

* 1 x 0.15ml injections ID via a needle - into the upper arm with or without electroporation (EP).

** 1 x 0.5ml injections IM – into the upper thigh with or without electroporation (EP).

*** 1 x 0.1ml injections ID via a needle - into the upper arm.

6.1.4 Compliance and Adherence

All immunisations will be administered by site staff according to written procedures.

6.1.5 Dose Modifications and Discontinuation

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

The schedule may be modified if a participant has symptoms or signs on the day of scheduled immunisation, and the investigator considers it best to defer the immunisation. The participant will be asked to return for review within the window period of the scheduled immunisations (-3/+7 days from week 4, 8 and 20 for the second, third and fourth immunisations respectively).

A clinical investigator may decide to permanently discontinue dosing in a participant who has received one or more immunisations, if the investigator deems that continuing might compromise participant wellbeing or interfere with the achievement of the trial's objectives. Such a decision should only be taken in consultation with the Chief Investigator. Participants will be encouraged to continue to attend trial visits for sampling and safety monitoring.

Discontinuation of dosing is essential following a grade 3 or 4 clinical or laboratory event (confirmed on examination or repeat testing respectively) which is considered possibly, probably or definitely related to the vaccine and which did not resolve within 72 hours.

Provided the participant agrees they will continue to be followed up for the duration of the study and samples will be collected at the allotted times.

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

Participants may decide to discontinue dosing. They will be encouraged to provide a reason, and to remain in follow-up. The appropriate CRF should be completed as soon as possible if a patient chooses to stop receiving vaccines or to withdraw from the trial, and within 24 hours of a decision being taken to discontinue a participant when that decision is informed by an adverse event (**section 8.1**).

6.2 Clinical Management of Adverse Events

Events will be managed by the clinical trial team who will assess and treat the event as appropriate, including referral to an independent physician and/or the participant's General Practitioner if required.

See also **section 7.4**

6.3 Non-Trial Treatment

As stated in the exclusion criteria in **Section 4.2**, participants should not have received other immunisations, immunosuppressive agents, blood products, immunoglobulin or other trial medication within specified periods prior to enrolment. This applies during the trial through to the final safety visit 22 weeks after enrolment, 2 weeks after the last scheduled immunisation, unless the treatment is required for an emergency.

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

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

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

6.4 Issues Related to HIV

Only volunteers with a negative 4th generation HIV antibody/antigen result will be enrolled onto the study.

A 4th generation HIV antibody/antigen assay, the standard laboratory method for diagnosing HIV infection, will be used to screen volunteers after they have received appropriate counselling. An HIV test will be performed at two time-points in the study: the screening visit, and the visit 4 weeks after the final vaccination, unless clinically indicated at other times.

At each immunisation visit a risk assessment will be conducted and an additional HIV test will be performed if there has been a change in risk status. Participants will be counselled by study personnel about the importance of condoms and reminded on the day of each immunisation. Hypo-allergenic condoms will be provided free of charge.

In the event that any blood specimen is positive, participants will be invited for confirmatory tests to distinguish between vaccine-induced seropositivity and genuine HIV infection. Participants with vaccine-induced seropositivity will be provided with appropriate certification of their HIV status, and retested at the visit 6 months after the final vaccination. If that test is positive also, they will be invited to return to the clinical centre for re-testing until such time as the test becomes negative.

6.4.1 Verification of HIV status of participants

In the event of an equivocal or positive result, a specimen will be processed through a range of assays according to the local laboratory operating procedures to establish the HIV status of the individuals. A confirmatory specimen will be collected at a later date, if the first result suggests that the participant is HIV infected.

If a participant requires certification independent of the local laboratory, then this can be arranged through the clinic team.

6.4.2 HIV infection

In the unexpected circumstances that a participant in the trial acquires HIV infection, they will be referred for clinical care and counselling.

7. ASSESSMENTS AND FOLLOW-UP

7.1 Duration of Follow-up and Schedule

The assessments that will be performed at each visit are described in **Table 5.**

Participants will be required to make a minimum of 11 scheduled outpatient visits over the course of up to 30 weeks. Time 0 will start on the day of enrolment, which is also the day of randomisation and first immunisation.

Screening can take place up to 6 weeks (42 days) before time 0.

Immunisations will take place at weeks 0, 4, 8 and 20.

A 12th scheduled outpatient visit, at Week 44, will only take place for participants known to have responded to the vaccine.

Serum will be collected for immunogenicity at enrolment, and weeks 4, 5, 8, 9, 20, 21, 22 (the primary endpoint), 24 and 44. Peripheral blood mononuclear cells (PBMC) will be collected at enrolment, and weeks 4, 8, 20, 21, 22, 24 and 44.

Adverse events will be assessed during the enrolment visit before and after immunisation and at every visit thereafter up to (and including) Week 22. Routine laboratory safety parameters will be collected at screening, enrolment, and weeks 1, 4, 5, 8, 9, 20, 21 and 22, and at any other visit if clinically indicated.

7.1.1 Additional Visits

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

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

7.1.2 Visit Windows

The second, third and fourth immunisation visits scheduled for weeks 4, 8 and 20 will be compliant with the protocol if they take place ±3 days either side of the target date determined by the date of the previous vaccination. If there is a delay >4 weeks a decision will be made by the trial management group (TMG see **section 16.1**) as to whether immunisation will continue.

The post-immunisation safety visits scheduled for weeks 1, 5 and 9 will be compliant with the protocol if they take place -3 to +7 days of the target date determined by the date of the previous vaccination. The safety visit at Week 21 will be compliant with the protocol if it takes place -3 to +3 days of the target date.

The primary endpoint and follow-up visits scheduled for weeks 22 and 24 will be compliant with the protocol if they take place ±3 days either side of the target date determined by the date of the fourth vaccination.

The follow-up visit scheduled for week 44 will be compliant with the protocol if it takes place ±14 days either side of the target date determined by the date of the fourth vaccination.

7.2 Screening Assessments

7.2.1 Demographics, Medical History and Examination

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

A past and current medical history will be collected at screening during a face to face structured interview using a case report form, including details of any previous reaction to vaccination, allergies, history of epileptic fit, reproductive and respiratory symptoms, contraceptive practices (current method and the length of time using the method), and smoking, alcohol, steroid use and illicit drug history.

The general examination will include weight (kg), height (cm), calculation of BMI, temperature, arm and thigh circumference, blood pressure, inspection of the skin to exclude severe eczema and respiratory, cardio-vascular, abdominal, and neurological examination. Skin fold thickness will be assessed within the upper right and left thigh. An assessment of cervical, axillary and inguinal lymph nodes will also be undertaken.

An ECG will be done to exclude cardiac arrhythmia or palpitations [e.g., supraventricular tachycardia, atrial fibrillation, frequent ectopy, or sinus bradycardia (i.e., <50 beats per minute on exam)]

7.2.2 Sexual History and Genital Infection Screen

A sexual history will be taken for the preceding 6 month period, and participants will be asked whether they have ever been tested for sexually transmitted infections in the past and if so, whether any were found.

The following will be collected in all participants

- serology for syphilis
- serology for markers of hepatitis B surface antigen and hepatitis C antibody
- urine for Chlamydia and gonorrhoea at screening.

Other gonorrhoea and chlamydia tests will be collected if indicated on account of risk and symptoms, at the discretion of the PI.

7.2.3 Routine Laboratory Parameters

Peripheral blood will be collected by clinical staff experienced in phlebotomy into the appropriate containers and transported to the local laboratory.

Mid-stream urine will be collected into a sterile container and either tested on site (urine dipstick and pregnancy tests) or transported to the local laboratory. All laboratory assessments will be performed by Imperial College Healthcare NHS Trust.

The following parameters will be collected in all participants:

- Hb, total WBC, neutrophils, lymphocytes and platelets
- Creatinine, total and conjugated bilirubin (conjugated only if indicated) , alkaline phosphatase, AST, ALT and glucose
- Urinalysis using a dipstick for protein, ketones, blood, leukocyte esterase, and nitrites

and for women of child-bearing potential only

- Urine test to exclude pregnancy

A urine specimen will be sent to the laboratory if the level of protein, blood, leukocyte esterase or nitrites is considered clinically relevant.

7.3 Immunogenicity Assessments

Samples will be collected at the timepoints specified in **Table 5** and transferred to the Immunology Core Laboratory for analysis.

7.3.1 Mucosal Specimens

Rectal secretions will be collected from male and female volunteers using an ophthalmic sponge. The sponge will be pre-moistened with sterile normal saline and inserted into the rectum using a proctoscope. Sponges will be held against the rectal wall for up to for 2 min. Should a participant not wish to have proctoscopy the sponge may be inserted by the nurse or doctor 3 cm into the anal canal for 2 minutes.

Vaginal secretions will be collected from female volunteers using the INSTEAD Softcup, a commercially available, self-inserted menstrual cup made of polyethylene. Mucosal secretion collection will occur at the time points indicated in Table 5, except at visits when female volunteers are menstruating in which case vaginal secretions will not be collected.

7.3.2 Blood and Serology Specimens

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

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

7.4 Procedures for Assessing Safety and Tolerability

7.4.1 Adverse Event Assessment

Information on adverse events will be collected through a structured interview at scheduled visits as indicated in **Table 5**.

The investigator will record the diagnosis or the symptoms if a diagnosis is not apparent, the date of onset and the date of resolution if appropriate. For events not specified in the tables in study specific toxicity grading table (**Appendix 2**) the severity will be determined according to the FDA guidance (2007) http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf

The seriousness of the event and its relationship to vaccine will be determined by the investigator according to the definitions provided in **section 8**. All of this information will be recorded in the adverse event CRF.

7.4.2 General Examination and Vital Signs

General examinations will be done at screening and on dosing days, as per **section 7.2.1** (height and weight at screening only). General examinations at other visits per **Table 5** will be done only if clinically indicated. Assessment of vital signs (blood pressure, pulse, respiratory rate and oral temperature) will be performed at all visits up to Week 22.

7.4.3 Routine Laboratory Parameters and Urinalysis

A pregnancy test will be performed by analysis of a urine sample for Human Chorionic Gonadotrophin (HCG) collected from women of child-bearing potential at screening, the day of each immunisation and at the final safety visit (Week 22). The analysis will be conducted by a member of the study team.

Peripheral blood and urine will be collected and analysed, as described in **section 7.2.3**, for the following parameters at the time points specified in **Table 5**, and at additional time points if indicated to further evaluate or follow up adverse events:

7.4.3.1 Blood

- Creatinine, AST, ALT, alkaline phosphatase, total bilirubin, glucose
- Hb, total WCC, neutrophils, lymphocytes, platelets

7.4.3.2 Urine

- protein, ketones, blood, leukocyte esterase, and nitrites – by dipstick

A urine specimen will be sent to the laboratory if the level of protein, blood, leukocyte esterase or nitrites is considered clinically relevant.

7.4.4 Solicited Local and Systemic Clinical and Laboratory Adverse Events

Various local and systemic adverse events are known to be associated with licensed vaccines, and are referred to as '**solicited adverse events'**. These include disturbances in routine laboratory parameters, and are described in **table 2** below.

Information on solicited adverse events will be collected on the day of each immunisation (pre-immunisation, and at 10 and 60 minutes post-immunisation), at a safety call the day after each immunisation and for the following 7 days through a structured interview and examination according to the schedule.

In addition systemic laboratory adverse events will be collected through routine laboratory testing according to the schedule. These will be recorded on the appropriate CRF page.

Participants will also be asked to complete a diary card recording solicited adverse events that start within 7 days of each immunisation.

Table 2: Solicited Adverse Events

Type	Event
Local AEs (immunisation site)	Discomfort Redness Swelling (soft) Induration (hard) Blisters
Systemic Clinical AEs	Abnormally raised temperature Chills Myalgia/flu-like general muscle aches Malaise (excess fatigue) Headache Nausea Vomiting
Systemic Laboratory AEs	Abnormalities in: Creatinine, AST, ALT, alkaline phosphatase, total bilirubin, glucose Hb, total WCC, neutrophils, lymphocytes, platelets

The events will be **graded** according to the study specific toxicity table (see **Appendix 2**).

Relationship to study product, which is defined in **section 8**, will be recorded in the immunisation CRF but not on the diary card, on the assumption that any of these events starting within 7 days of an immunisation are at least possibly related. If the onset is beyond 7 days, the event will be recorded on the adverse event CRF and a relationship determined by the investigator reviewing the event.

7.4.5 Non-solicited Adverse Events

Non-solicited AEs starting from the first vaccination until the final safety visit (Week 22) will be captured and reported on the adverse event CRF. When making a non-solicited AE enquiry, staff should ask a non-leading question such as 'how have you been feeling?'

7.4.6 Follow-up of Adverse Events and Pregnancy

The Investigator will make every effort to monitor all adverse events, regardless of severity, until resolution or stabilisation, and to obtain documentary evidence of the outcome of pregnancy, in order to report this on the CRF during the trial.

7.4.7 Serious adverse events

A serious adverse event is defined in **section 8**.

All serious adverse events should be reviewed by the Principal Investigator for the clinical centre, and discussed at the next Trial Management Group call **(section 16.1)**.

The assessment should include consideration of whether or not to discontinue dosing or to withdraw a participant from the trial.

7.4.8 Tolerability of vaccination using the electroporation devices

A tolerability questionnaire will be given to subjects to complete after each vaccination with EP. They will score pain at the time of vaccine injection, the time of electrical stimulation, and 10 and 30 min afterwards, using a 10-point scale. They will also be asked general questions about acceptability, and asked for their comments.

7.5 Criteria for Stopping Treatment Groups or Whole Trial

In the event of a SUSAR, the Chief Investigator should notify the sponsor within 24 hours of the event taking place, and the Chief Investigator will arrange for any necessary expert reviews to take place within 3 working days.

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

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

If 3 or more participants experience a grade 3 or 4 clinical or laboratory event (confirmed on attendance or repeat testing) not resolved within 72 hours and considered possibly, probably or definitely related to vaccine product, further immunisations will be put on hold and the Chief Investigator will call a meeting with the Trial Management Group (TMG) to review the safety data. If upon review of the safety data it is deemed appropriate to restart dosing, a substantial amendment with relevant data has to be submitted to the MHRA for approval. If an unscheduled TMG meeting is warranted, the Sponsor will be informed and the TMG asked to make a recommendation to the Chief Investigator and the Sponsor about continuing further immunisations.

At any time if the study is put on hold (for example following the occurrence of a SUSAR) the Regulatory authority has to be informed of the temporary halt and a substantial amendment with relevant data has to be submitted to the MHRA for approval in case a

decision to resume dosing is taken. The Sponsor reserves the right to stop the whole trial at any time.

7.6 Procedures at the end of the trial

The trial will be closed when all participants have made their final follow-up visit (visit 12), the data collected on randomised participants entered into the database and the database locked. There will be a final monitoring/closeout visit to the clinical site between the last visit and the database lock.

Table 5: Schedule of visits, Immunisations and Assessments

Visit number	**1**	**2**		**3**	**4**		**5**	**6**		**7**	**8**		**9**	**10**	**11**	**12**
Nature of visit	**Screen**	**Dosing visit**	**Safety call**	**Safety visit**	**Dosing visit**	**Safety call**	**Safety visit**	**Dosing visit**	**Safety call**	**Safety visit**	**Dosing visit**	**Safety call**	**Safety visit**	**Final safety visit**	**Follow up to assess response**	**Follow up to assess response**
Week[6]	**-6**	**0**	**0+1d**	**1**	**4**	**4+1d**	**5**	**8**	**8+1d**	**9**	**20**	**20+1d**	**21**	**22**	**24**	**44**
Visit window (days)	**N/A**	**N/A**	**Vaccine 1+3d**	**-3d+7d**	**±3d**	**Vaccine 2+3d**	**-3d+7d**	**±3d**	**Vaccine 3+3d**	**-3d+7d**	**±3d**	**V4+3d**	**-3d+3d**	**-3d+3d**	**-3d+3d**	**–14d to +14d**
Informed consent	X															
Demographics/ Medical history/ screening exam	X															
General exam	X	X		(X)	X		(X)	X		(X)	X		(X)	(X)		
ECG	X															
Vital signs	X	X[7]		X	X[7]		X	X[7]		X	X[7]		X	X		
Inspection of administration sites	X	X[7]	X[8]	X	X[7]	X[8]	X	X[7]	X[8]	X	X[7]	X[8]	X	(X)		
Adverse event and conmeds assessment		X[7]	X	X	X[7]	X	X	X[7]	X	X	X[7]	X	X	X		
Urine pregnancy test[4]	X	X			X			X			X			X		
Routine safety bloods[1]	X	X		X	X		X	X		X	X		X	X		
Urinalysis	X	X		X	X		X	X		X	X		X	X		
HIV test[2]	X	(X)			(X)			(X)			(X)				X	(X)
HBV, HCV, syphilis, urine for chlamydia, and gonnorhoea[4]	X															
Other gonnorhoea and chlamydia tests[4]	(X)															
Blood for serum – Immunogenicity		X			X		X	X		X	X		X	X	X	X
Mucosal sampling		X									X			X		

Blood for PBMC – Immunogenicity		X			X			X			X		X	X	X	X
Immunisation[5]		**V1**			**V2**			**V3**			**V4**					
Tolerability questionnaire[9]		X			X			X								
Diary card[3]				X			X			X			X			

X means mandatory, (X) means if clinically indicated

1 The parameters are detailed in section 7.2.3

2 At each dosing visit clinic staff will determine whether the risk status for HIV has changed and repeat the test if necessary

3 Diary card given to subjects at each vaccination visit, and reviewed with subject at time-points indicated

4 Additional HIV/STI and pregnancy tests will be performed if indicated by a change in risk status or menstrual history respectively

5 Immunisation should be after completion of the other procedures scheduled for that visit except for the AE assessment and injection site inspection which will be conducted before and after each immunisation

6 After the first vaccination the timing of all visits is determined by the date of the preceding vaccination

7 Pre-dose and again at 10 (+/–2) min and 60 (+/–10) min post-dose; subjects are free to leave after the 60 min post-dose assessment unless otherwise indicated by AEs etc.

8 Volunteer invited back to clinic for assessment if reporting grade 3 AE or above

9 The tolerability questionnaire should be completed post-vaccinations 1–3 only

8. SAFETY REPORTING

UK clinical trials regulations require that both investigators and sponsors follow specific procedures when notifying and reporting adverse events/reactions in clinical trials. These procedures are described in this section of the protocol. **Section 8.1** lists definitions, **section 8.2** describes details of the responsibilities of the institutions/investigators.

8.1 Definitions

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

Adverse event

Any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

The investigator will use the following criteria when deciding whether to report a laboratory parameter that falls outside the normal range according to the local laboratory guidelines as an adverse event:

- The test result is associated with relevant accompanying symptoms
- Additional diagnostic tests or medication are indicated
- As a consequence of the test result, an immunisation is delayed or further immunisations are discontinued
- The investigator considers the result to constitute an adverse event for any other reason

Adverse reaction

Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

'Related' means possibly, probably or definitely as defined below.

All solicited adverse events which start within 7 days of a vaccination will be automatically classified as an adverse reaction.

Unexpected adverse reaction

An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Investigator's Brochure.

Serious Adverse Events (SAEs), Serious Adverse Reaction (SARs), Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any adverse event, adverse reaction, or unexpected adverse reaction is considered to be a "serious" if it:

- Results in death
- Is life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect
- Any other medically important event

Notes:

- '**A threat to life**' refers to an event or reaction in which the patient was at risk of death at the time of the event; it does not refer to an event or reaction which hypothetically might have caused death had it been more severe.

If an AE is serious and unexpected and considered possibly, probably or definitely related to study product according to the classification below then it meets the criteria for a SUSAR and should be reported accordingly.

Relationship to study product

This can be classified as:

Unrelated adverse events that can be clearly explained by extraneous causes and for which there is no plausible association with study product, or adverse events for which there is no temporal relationship

Unlikely adverse events that may be temporally linked, but which are much more likely to be due to other causes than study product and which do not get worse with continuing use of product

Possibly adverse events that could equally well be explained by study product or other causes, which are usually temporally linked and may improve when not using study product but do not reappear when using study product

Probably adverse events that are temporally linked and for which the study product is more likely to be the explanation than other causes, which may improve when not using study product

Definitely adverse events that are temporally linked and for which the study product is the most likely explanation, which disappear or decrease when not using study product and reappear when using study product

8.2 Reporting Adverse Events

Adverse events should be recorded on the appropriate CRF.

All **SAEs** should be reported to the sponsor within 24 hours of the Clinical Investigator becoming aware of the event fulfilling the criteria. The SAE form should be completed and e-mailed to the Project Manager/Monitor and the Joint Research Compliance Office. The minimum criteria required in reporting a SAE are the participant identifiers (trial number/date of birth), reporting source (name of Investigator), and why the adverse event is identifiable as serious.

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

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

SAE AND IMPORTANT AE NOTIFICATION

Within 24 hours of becoming aware of an SAE or Important AE, please enter onto the SAE CRF and indicate as serious and email the MRC CTU at UCL mrcctu.cuthivac002safety@ucl.ac.uk and the Imperial College London Joint Research Compliance Office at jrco.ctimp.team@imperial.ac.uk

SAE AND IMPORTANT AE NOTIFICATION

Faxes and emails should be acknowledged by the MRC CTU immediately on receipt. If the Clinical Investigator does not receive an acknowledgement they will assume that MRC CTU is not aware of the event and use an alternative method of notification.

The Principal Investigator will ensure that the SAE form is sent within 24 hours of an event. The Chief Investigator will assess whether the event qualifies in seriousness and relationship as a **Suspected Unexpected Serious Adverse Reaction (SUSAR**). Fatal and life-threatening SUSARs must be reported to the MHRA within 7 days of day 0 which is defined as the day the sponsor became aware of the event. Relevant follow-up information should be sought and a further report completed as soon as possible and submitted within 8 additional days. SUSARs which do not result in death or a threat to life should be reported within 15 days of day 0. Ultimate responsibility for classification resides with the study Sponsor.

Investigators should notify the JRCO of all SAEs occurring from the time of randomisation until 30 days after the last protocol treatment administration. SARs and SUSARs must be notified to the JRCO until trial closure.

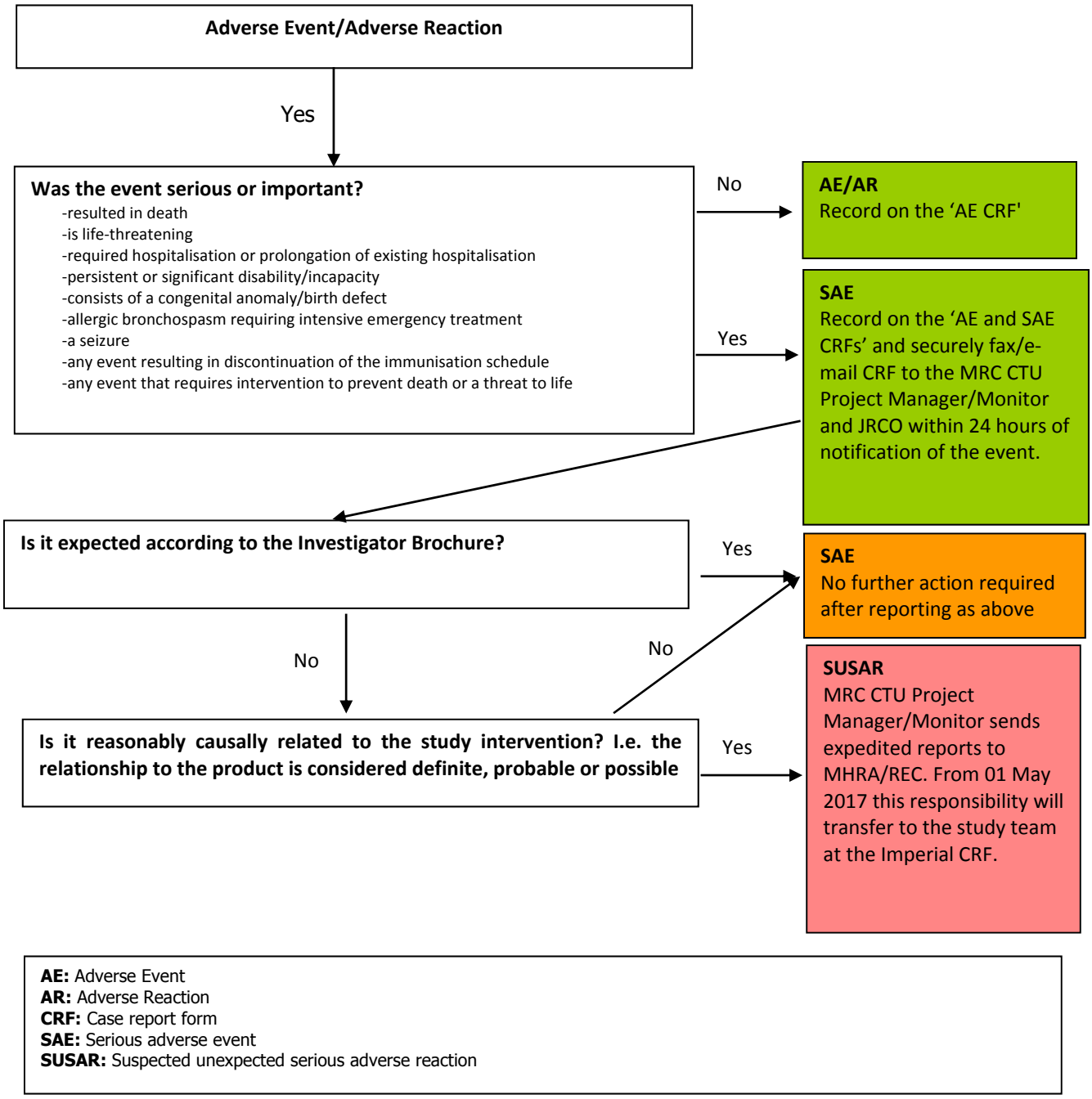
The MRC CTU Project Manager/Monitor will be responsible for coordination of the report review and filing with the JRCO at Imperial College London, and for ensuring the REC and MHRA are informed. From 01 May 2017 this responsibility will transfer to the study team at the Imperial CRF.

8.3 Pregnancy

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

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

Figure 2: Safety Reporting Flowchart



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

9. WITHDRAWAL OF PARTICIPANTS

Withdrawal from the trial means stopping all further visits.

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

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

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

Withdrawn participants who have received any immunisations should be asked to undergo the procedures scheduled for the primary endpoint visit (Visit 10, week 22).

Policy for replacing withdrawals
Additional volunteers will be enrolled to replace early withdrawals or those who have discontinued their immunisations early, with the aim of having 24 subjects complete the study with no major protocol deviations. However, no more than 30 individuals in total will be exposed to the vaccine.

10. STATISTICAL CONSIDERATIONS

10.1 Method of Randomisation

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

10.2 Endpoints

10.2.1 Primary Endpoints

10.2.1.1 Safety

- Grade 3 or above local solicited adverse event **(Table 2)**
- Grade 3 or above systemic clinical and laboratory solicited adverse event **(Table 2)**
- Any grade of adverse event that results in a clinical decision to discontinue further immunisations
- Any grade of adverse event within 7 days of receiving intradermal and standard intramuscular vaccinations with or without electroporation

10.2.1.2 Immunogenicity

- The magnitude of antigen specific systemic IgG antibody binding responses (µg/ml) 2 weeks after the final vaccination.

10.2.2 Secondary Endpoints

10.2.2.1 Safety

- Any grade of adverse event, local to the ID and IM injection sites, that starts within 7 days after Doses 1-3.

10.2.3 Exploratory immunogenicity endpoints

- Frequency and magnitude of HIV-gp140 specific B-cell-mediated responses in the systemic compartment measured by B-cell ELISPOT
- The magnitude of vaccine specific systemic T cell responses by T cell ELISpot assay
- The magnitude of antigen specific systemic IgA antibody responses (ug/ml)
- Frequency, titre and avidity of serum binding antibodies to other HIV Env antigens (alternative clades) by ELISA or other assays.
- Mapping of serum binding antibodies using Env subunit constructs (e.g., V2 scaffolds and hotspots) by ELISA.
- Frequency and magnitude of mucosal IgG and IgA antibody responses to CN54gp140 measured four weeks after the final immunisation.
- Frequency and titre of serum neutralising antibodies to homologous virus, and, if warranted a wider panel of viruses representing different clades.
- Frequency and magnitude of HIV-specific T-cell mediated responses measured by T-cell CFSE, and ICS (Intracellular Cytokine Staining).
- Frequency and magnitude of T-cell chemokine and cytokine release following ex-vivo antigen stimulation quantified by Luminex.
- Isolation and characterization of Env-specific monoclonal antibodies (IgG) from memory B cells in the systemic compartments (dependent upon elicited specific memory B-cell numbers).
- Characterisation of non-neutralising antibody function using ADCC/ADCVI, viral capture and aggregation assays.
- Epitope mapping of B- and T-cell responses.

10.2.4 Exploratory tolerability endpoints

- Pain scores at 0, 10 and 30 minutes following vaccination with EP

10.3 Sample Size

It is not the remit of this Phase I trial to recruit a sufficient number of participants to be statistically confident about the differences between groups. By the end of this study 8 participants will have been exposed to each schedule in groups 1, 2, and 3 and this provides confidence around the response/event proportions of 0–60% in table 6.

Table 6: Sample Size

Number of "responders"	Proportion if n=8	95% confidence interval[1]
0	0%	0 – 28%
1	10%	2 – 40%
2	20%	6 – 51%
3	30%	11 – 60%
4	40%	17 – 69%
5	50%	24 – 76%
6	60%	31 – 83%

[1] **Wilson interval (suitable for small sample sizes)**

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

10.4 Data analyses and Presentations

A full statistical analysis plan will be developed before the final analysis. It will be based on the following summary:

10.4.1 Participant Populations

- Intention-to-treat (ITT) population: all participants randomised and given at least one immunisation in the trial.
- Replacement participant population: participants who were not randomised but did receive at least one immunisation.
- Per-protocol (PP) population: all participants randomised and non-randomised who were immunised with all scheduled immunisations, and who complete the trial.

10.4.2 Immunogenicity

10.4.2.1 Primary Immunogenicity Outcomes

All 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 at least one post treatment immunogenicity sample. Titres of antigen specific antibodies will be described by time-point and group, and compared using rank tests where appropriate. Although all samples will be processed, the analyses will be for the per-protocol population and conducted in Robin Shattock's laboratory.

10.4.3 Exploratory Immunogenicity Outcomes

The proportion of individuals mounting a B cell response using frozen PBMC will be determined by B cell Elispot assay for antigen induced IgG production from antibody secreting cells (ASC's). CN54gp140 specific responses will be analysed as well as total IgG and compared between vaccine groups.
In addition vaccine induced T cell responses will be analysed by T cell Elispot assay for IFN-γ production on frozen PBMC samples and the proportion of individuals mounting a response quantified as SFU (spot forming units) compared between vaccine groups. These exploratory analyses will use descriptive statistics and will be conducted in Robin Shattock's laboratory.

10.4.4 Safety Outcomes

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

All safety end-points will be graded by the Clinical Investigators.

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

For the primary ITT analysis of safety endpoints (as defined in **section 10.2**), results will be expressed as a proportion with confidence interval, and groups compared using Fisher's exact test.

Safety outcomes for the non-randomised participants will be reported separately.

10.4.5 Tolerability Outcomes

The tolerability data from the questionnaires will not be part of the SAP or reported in the Clinical Study Report.

11. DATA MANAGEMENT

Data management, analysis and reporting of trial data collected from all randomised subjects will be prepared by the MRC CTU at UCL according to the Data Management Plan and Statistical Analysis Plan.

11.1 Data management at the Clinical Centre

Staff at the clinical centre will be responsible for:

- Creating medical notes for each participant which includes paper copies of the consent documents and the blood results
- Entering information relevant to eligibility and emergent adverse events and documenting the result of any pregnancy tests and urinalysis in the medical notes
- The accurate completion of the CRFs
- Collection and review of the diary cards from participants
- Notification of SAEs within 24 hours of becoming aware of the event to the MRC CTU Project Manager/Monitor and the JRCO
- Notification of pregnancy within 24 hours of becoming aware of the pregnancy to the Chief Investigator
- From 1st May 2017, notification of SUSARs within the timelines defined in Section 8 and maintaining the Trial Master File.

The dates of visits including immunisation dates, and details of clinical management (description of

significant adverse events and concomitant medication) will be documented in the medical notes.

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

A member of the clinical trial team must sign the laboratory and ECG reports. In the event of an abnormality, an indication should be given whether or not the abnormality is clinically significant, the date of review and the signature of the clinician reviewing the result.

The database and medical notes should be kept in a secure location for 2 years after the last approval of a marketing application or until 2 years have elapsed since formal discontinuation of product development, and at least 15 years after the clinical trial has ended.

11.2 Data Management in the Immunology Laboratories

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

11.3 Data management at the MRC CTU at UCL

Staff at the MRC CTU will be responsible for:

- Designing the CRFs
- Creating a database
- Training staff at the clinical centre in data collection and overseeing data entry which may include data entry at the clinical centre directly into the database
- Notification of SUSARs within the timelines defined in **section 8**
- Drafting the Data Management and Statistical Analysis Plans and conducting the analyses
- Clinical site monitoring
- Maintaining the Trial Master File on behalf of the sponsor until 1st May 2017
- Coordination of the Trial Management Group

12. TRIAL MONITORING

12.1 Risk Assessment

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

The risk assessment will be reviewed by the Chief Investigator.

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

12.2 Monitoring by Medical Research Council Clinical Trials Unit at University College London (MRC CTU at UCL)

Monitoring will be performed according to ICH-GCP. A monitoring plan will be written based on the risk assessment.

On-site monitoring will be delegated to a trained monitor by the MRC CTU at UCL to ensure that the study is conducted in compliance with the protocol, is consistent with SOPs, the principles of GCP, applicable regulatory requirements and locally accepted practices. The investigators, as well as volunteers through consenting to the study, agree that the monitor may inspect study facilities and source records (e.g., informed consent forms, clinic and laboratory records, other source documents), as well as observe the performance of study procedures. Such information will be treated as strictly confidential and will under no circumstances be made publicly available.

The Principal Investigator will permit inspection of the facilities and all study-related documentation by authorised representatives of the Sponsor, and Regulatory Authorities responsible for this study.

Prior to the first volunteer being screened, a site initiation visit will be made by the MRC CTU and will consist of review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), review of recruitment strategy, review of site facilities and equipment, essential document receipt, collection and filing, and archiving and inspection. Copies of the trial specific documents will be given to the investigators. The approved version of the protocol should be followed at all times, and any significant protocol deviations will be documented on a Protocol Deviation Form and submitted by MRC CTU to the Sponsor as soon as possible. The investigators will allow the monitors to:

- Inspect the site, the facilities, IMP management and materials used for the trial
- Meet all members of the team involved in the trial, and ensure all staff working on the trial are experienced and appropriately trained, and have access to review all of the documents relevant to the trial
- Have access to the case report forms and source data
- Discuss with the investigator and site staff trial progress and any issues on a regular basis

12.3 Monitoring by the Imperial CRF

From 01 May 2017, MRC CTU at UCL will cease to perform monitoring and that responsibility will transfer to the Imperial CRF.

12.4 Clinical Site Monitoring

The trial site will be monitored to ensure that:

- All participant records exist; participants are eligible and informed consents signed
- There is adherence to the protocol, including consistency with inclusion/exclusion criteria

- There is compliance with the principles of GCP and regulatory requirements
- Trial Documentation is complete and up to date (e.g. correct versions of documents being used, source data captured) and relevant documents are collected for the Trial Master File (TMF)
- The CRFs have been completed correctly and accurately, and all entries correspond to data captured in source documents
- The IMP accountability records are in order (receipt, dispensing and return/destruction), storage is under appropriate conditions and secure, expiry dates are being checked and adhered to, and dispensing is according to the protocol and trial procedures.

All information dealt with during such visits will be treated as strictly confidential. At the end of the trial, a close out visit will be performed by the monitor after the final participant visit has been completed and prior to database lock. During this visit the monitor will verify that all trial close out activities are completed – all queries resolved, missing data completed, monitoring completed, archiving arrangements in place, IMP accountability complete and all used and unused IMP returned/destroyed, ISF (investigator site file) completed and TMF documents collected, and end of trial notified. Each investigator will also be notified that an audit or inspection may be carried out - by the sponsor; sponsor's representatives or the regulatory authorities - at any time, before, during or after the end of the trial. The investigator must allow the representatives of the audit or inspection team:

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

12.5 Monitoring by the Trial Management Group

The Trial Management Group (see **section 16.1**) will monitor the following:

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

12.6 Confidentiality

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

Each participant's GP will be notified of their patient's participation in the trial.

13. ETHICAL CONSIDERATIONS AND APPROVAL

13.1 Ethical Issues

There are three aspects of this trial that raise ethical issues

First, safety: DNA plasmid vaccines have been widely used in man at similar doses to those employed in this trial to vaccinate against a wide range of infectious diseases such as HIV as well as non infectious diseases such as cancer. To date, there have been no safety concerns. Also such vaccines have been administered via conventional methods such as IM and ID as well as using EP.

Second, issues particular to HIV: because the product under investigation is a **candidate HIV vaccine**, and HIV is transmitted sexually. The nature of the product may lead volunteers to erroneously conclude they are protected against HIV and to engage in riskier behaviour as a consequence. It is possible that following immunisations, participants may have equivocal results in the standard laboratory tests for HIV. However, any accredited laboratory would be able to distinguish between a response to the vaccine and the occurrence of a natural infection using routine assays.

Third, financial: **the reimbursement** to compensate for the intense follow-up schedule, which is a feature of healthy volunteer trials, could be sufficient incentive for individuals to take part against their better judgement. However, the rate of reimbursement in this trial is in line with those of similar healthy volunteer trials.

13.2 Ethical Considerations

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

The trial proposal including the trial specific information to be provided to volunteers will be reviewed by a recognised Research Ethics Committee (REC) and by the Medicines and Healthcare products Regulatory Authority (MHRA), The trial will not proceed unless the sponsor obtains approvals from these two authorities.

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

The safety assessments are intense. Participants will be asked to remain in clinic for about 1 hour following each immunisation, to complete a diary card for at least 7 days thereafter, respond to a 'safety [telephone] call' the day after each immunisation, and return for a safety visit a week (-3 days/+ 7 days) after each immunisation. They will be advised to call the clinic staff if they are concerned, and 24 hour cover will be available.

14. INDEMNITY

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

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

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

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

15. FINANCE

The clinical trial activities, acquisition of product, data management and analysis are funded by the EC under the FP7 framework.

Participants will receive recompense for their time and travel, £200 per vaccination visit and £100 per screening/safety/follow-up visit up to a total of £1600 over the course of the study. Only enrolled participants will be paid for the screening visit.

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

16. TRIAL COMMITTEES

16.1 Trial Management Group (TMG)

The Trial Management Group (TMG) will be formed of the Chief, Principal and other lead Investigators (clinical and non-clinical) from the clinical centre and immunology laboratory as well as members of MRC CTU.

The TMG will be responsible for the day-to-day running and management of the trial and will be accountable to the Sponsor.

The TMG will also be responsible for the composition of the expert panel to review any emergent SUSARs.

16.2 CUT'HIVAC Consortium Governing Board

This Governing Board has reviewed the design of the trial.

The Governing Board will be notified by the Project Lead of the progress of the trial, and provided with the final Clinical Study Report.

The CUT'HIVAC Consortium funding ended in December 2015. Subsequently the accountability has been to the Project Coordinator at INSERM, France.

16.3 Trial Management Team (TMT)

Weekly clinical TMT meetings are held at the Imperial CRF, Hammersmith Hospital.

17. PUBLICATIONS

The preparation of a manuscript for publication in a peer-reviewed professional journal or an abstract for presentation, oral or written, to a learned society or symposium will be discussed on the Trial Management Group calls. The Sponsor will be notified of this intention through the Chief Investigator and the TMG notes. Every effort will be made to allow the Sponsor and other relevant parties involved in the clinical trial and named in the clinical trial agreement prepared by the Sponsor, 30 days to comment before any results are submitted. This timeline will be strictly observed for peer-review journals, but may be more difficult to adhere to for conference presentations. Approval from the Chief Investigator, the clinical centre Principal Investigator and Project Lead must be obtained as a minimum before submission to a conference.

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

18. PROTOCOL AMENDMENTS

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

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

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

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APPENDICES

APPENDIX 1: ELECTROPORATION ICHOR TRIGRID™ DELIVERY SYSTEM FOR INTRAMUSCULAR (TDS-IM) AND INTRADERMAL (TDS-ID) DELIVERY

TDS Delivery Technology

EP is an efficient DNA delivery method that has been shown to significantly increase DNA vaccine potency by up to several orders of magnitude compared to delivery by conventional injection. EP is a technique for intracellular delivery based on the brief application of electrical fields in a target region of tissue. This process induces a transient state of membrane destabilization/permeability, during which time normally impermeant substances present in the interstitial space at the site of EP application can be taken up into the affected cells. Shortly after EP, the cell membrane stabilizes and the cells resume normal function. EP has been demonstrated to be a potent method for DNA delivery in a variety of tissues including skeletal muscle, liver, lung, skin, and various tumour types, enhancing intracellular DNA uptake and gene expression by 2-3 orders of magnitude compared to conventional methods of administration.

Properties of the TDS Technology

The TDS devices are designed for intramuscular or intradermal administration of DNA using EP. The TDS technology utilizes the in vivo application of electrical fields to enhance the intracellular delivery of agents of interest in a targeted region of tissue. Specifically, the device is designed applying electrical fields at the site of administration to induce the EP effect in the presence of the DNA to be delivered. The device has been designed for use in human clinical studies and complies with the applicable safety and electromagnetic compatibility requirements of the International Electrotechnical Commission (IEC) 60601-1.

TDS device components

Both the TDS-IM and TDS-ID consist of the following components:

- Pulse Stimulator
- Integrated Applicator
- Single use Application Cartridge

In addition, the TDS-ID device has a

- Medi-Jector Vision needle free injection device

The configuration of the TDS device is designed for the administration of investigational agents in the context of early phase human clinical studies.

Device Components

Pulse Stimulator

The Pulse Stimulator is an electronic device that controls the administration sequence, generates the electrical signals necessary to enhance the intracellular delivery of the agent, and monitors the administration sequence for safety hazards. The Pulse Stimulator performs a comprehensive self-diagnostic to ensure that the device and all internal safety systems are functioning properly before an administration procedure can be initiated. The device has been designed to comply with the applicable safety and electromagnetic compatibility requirements of IE.

Integrated Applicator

The Integrated Applicator is a reusable hand-held device that contains mechanisms to automatically deploy the electrodes into the tissue and administer the biologic agent. The device is configured so that the entire procedure is applied in an automated fashion following the activation the procedure initiation button. This ensures that the prescribed administration parameters (e.g. site of agent injection relative to EP application, rate of agent injection, and time interval between agent injection and EP application) will be implemented for every recipient in a uniform fashion, thereby minimizing variability in the application of the procedure arising as result of operator skill or level of training. A user activated mechanical safety switch reduces the possibility of an inadvertent discharge of the device during set up.

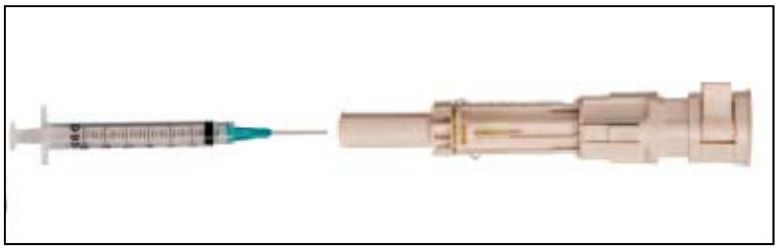
TDS-IM

TDS-ID

Application Cartridge

Each Application Cartridge is packaged sterile for single use and is the only component of the system that contacts the recipient

TDS-IM



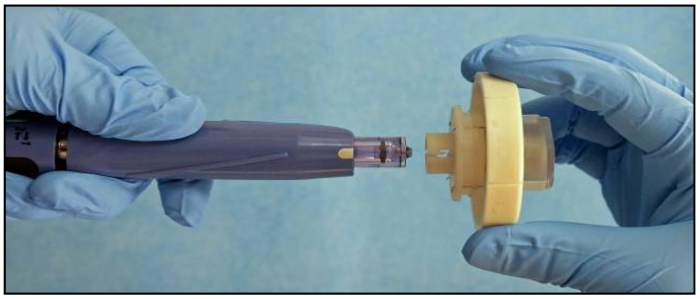
The TDS-IM Application Cartridge is used to house the agent to be delivered (in a standard syringe) and the electrodes used for EP application. It is comprised of a plastic, injection molded body that encloses the four electroporation electrodes.
The TDS electrode array consists of four electrodes arranged in two equilateral triangles to form a diamond configuration around a central injection needle. With the long axis of the diamond placed in parallel with the direction of the muscle fibres this configuration corresponds with the ellipsoid fluid distribution characteristic of an intramuscular injection.

TDS-ID

The TDS-ID Application Cartridge consists of a plastic body that encloses the four electrode TriGridTM array and interfaces with a Medi-Jector Vision needle free injection device containing the agent to be administered. It is attached to the Medi-Jector Vision syringe once it has been loaded with the dose to be administered.
Prior to administration, the electrodes remain recessed within the sterile Application Cartridge body. A plastic safety cap located on the tip of the cartridge protects the operator from accidental stick injury and ensures that the electrodes remain sterile prior to administration. Each Application Cartridge also incorporates a spring loaded stick shield that deploys over the electrodes as the device is retracted from the recipients skin. This facilitates safe and simple disposal of the single use Application Cartridge and ensures that the electrodes are never visible to the recipient

TDS-ID Medi-Jector Vision needle free injection device

The Medi-Jector Vision injection device and needle free syringe is filled to the prescribed dosage and inserted into rear aperture of the application cartridge. Once the syringe is inserted, tabs located on the cartridge body lock it into place to facilitate safe disposal.



APPENDIX 2: TOXICITY TABLE

Based on systems in use at the MRC CTU, IAVI and NIH Division of AIDS

Abbreviations:				
	ULN	Upper Limit of Normal	LLN	Lower Limit of Normal
	R_x	Therapy	Req	Required
	Mod	Moderate	IV	Intravenous
	ADL	Activities of Daily Living	Dec	Decreased

For other events not specified in the tables below the severity will be determined according to the US department of health and human services: Guidance for Industry – Toxicity grading scale for Healthy adult and adolescent volunteers enrolled in Preventative Vaccine Clinical trials.
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf

LABORATORY PARAMETERS

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 EXTREME
HAEMATOLOGY				
Hb	10.0-10.9 g/dL	9.0-9.9 g/dL	7.0-8.9 g/dL	<7.0 g/dL
White Blood Count	13.0 – 14.9 x10^9/l or 2 – 2.5 x10^9/l	15.0 – 19.9 x10^9/l or 1.5 – <2.0 x10^9/l	20.0 – 29.9 x10^9/l or 1 – <1.5 x10^9/l	≥30.0 or <1.0 x10^9/l
Absolute Neutrophils	1.3-1.0 x10^9/l	<1.0-≥0.75 x10^9/l	<0.75-≥0.5 x10^9/l	<0.5 x10^9/l
Percent neutrophils	>80%	90%	≥95%	---
Lymphocytes	0.7- 0.899 x10^9/l	0.5-0.699 x10^9/l	0.35-0.499 x10^9/l	<0.35 x10^9/l
Platelets	100 –124.999 x 10^9/l	50 – 99.999 x 10^9/l	25 – 49.999 x10^9/l	<25.0 x10^9/l
CD4 Count	300-400/mm^3	<300mm^3	<200/mm^3	<100/mm^3
BIOCHEMISTRY				
Potassium Hyperkalemia Hypokalemia	 5.6 – 6.0 meq/L 3.0 – 3.4 meq/L	 6.1-6.5 meq/L 2.5 – 2.9 meq/L	 6.6-7.0 meq/L 2.0– 2.4 meq/L	 >7.0 meq/L <2.0 meq/L
Bilirubin Hyperbilirubinemia	 >1.25 – 2.0 x ULN	 >2.0 – 2.5 x ULN	 >2.5 – 5 x ULN	 >5 x ULN
Glucose Hypoglycaemia Hyperglycaemia nonfasting; no prior diabetes	 2.3-2.4 mmol/l 7.0-10.0 mmol/l	 2.1-2.2 mmol/l 10.1-15.0 mmol/l	 1.5-2.0 mmol/l 15.1-25.0 mmol/l	 <1.5 mmol/l >25.0 mmol/l
Transaminases AST (SGOT) ALT (SGPT) GGT Alk Phos	 1.25 – 2.5 x ULN 1.25 – 2.5 x ULN 1.25 – 2.5 x ULN 1.25 – 2.5 x ULN	 >2.5 – 5.0 x ULN >2.5 – 5.0 x ULN >2.5 – 5.0 x ULN >2.5 – 5.0 x ULN	 >5.0 – 10.0 x ULN >5.0 – 10.0 x ULN >5.0 – 10.0 x ULN >5.0 – 10.0 x ULN	 > 10.0 x ULN > 10.0 x ULN > 10.0 x ULN > 10.0 x ULN
Amylase	>1.0 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0 – 5.0 x ULN	>5.0 x ULN
Creatinine	130-180μmol/l	181-360μmol/l	361-720μmol/l	>720μmol/l
URINALYSIS				
Proteinuria: 24 hour urine	200 mg - 1 g loss/day OR <0.3% OR <3 g/l	1 – 2 g loss/day OR 0.3 – 1.0% OR 3 - 10 g/l	2 – 3.5 g loss/day OR >1.0% OR > 10 g/l	Nephrotic syndrome OR >3.5 g loss/day
Haematuria	Microscopic only ≤10 RBC/HPF	>10 RBC/HPF	Gross, with or without clots OR RBC casts	Obstructive OR transfusion req

SOLICITED VACCINE REACTIONS

GENERAL				
Fever Oral>12 hours	37.7 - 38.9°C (100.0 – 101.5°F)	39.0 – 39.7°C (101.6 – 102.9°F)	39.8 – 40.5°C (103 - 105°F)	>40.5°C (105°F) OR max temp of >105°F
Chills/rigors	Mild hot/cold flush requires blanket or occasional over the counter treatment	Limiting daily activity >6 hours, or need regular over the counter treatment	Uncontrollable shaking, prescription treatment needed	Hospitalisation
Malaise/abnormal tiredness	Normal activity reduced – not bad enough to go to bed	Fatigue such that ½ day in bed for 1 or 2 days	Fatigue such that in bed all day or ½ day for more than 2 days	Hospitalisation
General (all over) muscle aches and pains	No limitation of activity	Muscle tenderness, aches/pains limiting activity e.g. difficulty climbing stairs	Severe limitation e.g. can't climb stairs	Hospitalisation
Headache	No treatment or responds to over the counter treatment	Regular over the counter or occasional prescription treatment needed	Regular prescription treatment needed	Hospitalisation
Nausea	Intake maintained	Intake reduced less than 3 days	Minimal intake 3 days or more	Hospitalisation
Nausea/vomiting	Less than 4 x a day or lasting less than 1 week	At least 4 x day or lasting 1 week or more	Unable to keep any food or fluids down	Hospitalisation
CUTANEOUS				
Discomfort/pain in injected muscle (including ache) or overlying skin	Mild itch or ache that responds to over the counter treatment, if needed	Pain requiring regular over the counter treatment or occasional prescription treatment	Pain requiring regular prescription treatment	Hospitalisation
Immediate reactions (within 6 hours of injection)	Symptoms of irritation locally (usually itching at the injection site) OR Erythema +/- swelling at the injection site		Laryngeal oedema insufficient to require intubation; diarrhoea insufficient to require IV fluids, or asthma nsufficient to require hospitalisation OR Urticaria, angio-oedema OR Generalised pruritus	Anaphylactic shock
Erythema at injection site	Erythema up to and including 50% of baseline arm circumference OR Symptoms of irritation that are easily tolerated and do not require repeated medication OR Both	Erythema greater than 50% of the arm circumference at baseline With or without Symptoms of irritation that do not require repeated medication OR Symptoms of irritation that require repeated medication AND erythema up to and including 50%	Erythema greater than 50% of the arm circumference at baseline AND symptoms of irritation requiring repeated medication	Hospitalisation
Blistering or ulceration at injection site	Fluid filled vesicles or superficial disruption of epithelium covering an area < 1cm	Fluid filled vesicles or superficial disruption of epithelium, area 1 - 2cm OR Blood filled vesicles OR Full thickness disruption of epithelium healed within 2 weeks	Full thickness disruption of epithelium not healed within 2 weeks	Necrosis
Soft swelling – local	Swelling <25% of arm	Swelling 25-50%of arm	Swelling >50% of arm Or Induration/hardened swelling (when considered by the	

			clinician to be associated with a process arising in the muscle)	