CUTHIVAC 002

Statistical Analysis Plan

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Contents

1. Trial overview

CUTHIVAC002 is an open-label 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 (IM) and intradermal (ID) methods with and without electroporation (EP), 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.

Participants are randomised 1:1:1 to ID/EP, IM/EP or ID/IM/EP, the randomisation is unblinded. Following the immunisation schedule of table 1, participants return at weeks 4, 8 and 20. Primary immunogenicity assessment is at week 22.

Table 1: Immunisation schedules

Group	**Immunization: Dose of DNA**			**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 needle – into upper arm with/without electroporation (EP)

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

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

1.1. Primary Objectives

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 identifying optimal conditions capable of promoting enhanced antibody responses to HIV.

1.2. Secondary Objectives

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

1.3. Exploratory Objectives

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.

2. General Considerations

2.1. Randomisation

Participants will be block-randomised using a computer-generated algorithm with a back-up manual procedure and randomisation will be stratified on the basis of gender. The randomisation lists are generated and checked by the trial statistician, and stored securely in the trial database.

Participants are randomised 1:1:1 to ID/EP, IM/EP or ID/IM/EP. Randomisation is unblinded.

2.2. 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 randomised to each method of delivery.

2.3. Analysis datasets

The statistical analysis will be conducted on randomised participants on both an Intention-to-Treat (ITT) and a Per-Protocol (PP) basis. This is a phase I trial concerned with both safety and efficacy, ITT provides estimates of effectiveness in a 'real-world` setting where participants missed doses or became lost-to-follow-up, PP estimates efficacy under ideal conditions.

- Intention-To-Treat (ITT) dataset: all participants randomised and given at least one immunisation in the trial.
- Per-Protocol (PP) dataset: all participants randomised and immunised with all scheduled immunisations, completing the trial with no major protocol deviations.

A safety analysis population is also defined as all participants receiving at least one dose of trial medication.

2.4. Missing Data

The level and pattern of missing data on the primary immunogenicity outcome will be described in tables 1 to 3 of the statistical report.

3. Endpoint Definitions

3.1. Primary Endpoints

3.1.1. Safety

Any of the following events occurring between week 0 and week 22 will be included in the analysis:

- 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

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

3.1.2. Immunogenicity

The primary immunogenicity endpoint is the titre of systemic CN54rgp140-specific IgG antibodies measured 2 weeks ± 3-day window after a participant's scheduled final vaccination (any exception to the 3-day window to be discussed by the TMG). Responses are measured using a semi-quantitative sandwich ELISA assay and defined as positive when exceeding mean OD at 450nm plus 3 SDs at a 1/100 dilution for a panel of serum samples obtained from 20 HIV negative volunteers. Samples deemed "response positive" are analysed further in a titration assay where samples are subsequently diluted to achieve at

least two OD at 450nm values that fall on the linear part of the relevant human IgG or IgA standard curve of known concentration. These values are interpolated automatically by the ELISA software (SoftMax Pro v 5.4) to give two extrapolated values in µg/ml. The mean of these 2 values is then reported as the "titre" of antigen specific IgG or IgA for response detected samples.

3.2. Secondary Endpoint

3.2.1. Safety

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

3.3. Exploratory Endpoints

3.3.1. 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.

3.3.2. Tolerability

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

4. Statistical Analyses

Analyses of safety and tolerability endpoints for randomized participants will be performed at the MRC CTU at UCL. Immunogenicity data are not held at the CTU, and will be analysed elsewhere.

Adverse events and vaccine responses will be described as frequencies with 95% confidence intervals. Confidence intervals will be calculated using Wilson intervals, suitable for small sample sizes (table 3).

Table 3. Wilson intervals

	N=8		N=7		N=6		N=5	
Number of events	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
0	0%	0,32%	0%	0,35%	0%	0,39%	0%	0,43%
1	13%	2,47%	14%	3,51%	17%	3,56%	20%	4,62%
2	25%	7,59%	29%	8,64%	33%	10,70%	40%	12,77%
3	38%	14,69%	43%	16,75%	50%	19,81%	60%	23,88%
4	50%	22,78%	57%	25,84%	67%	30,90%	80%	38,96%
5	63%	31,86%	71%	36,92%	83%	44,97%	100%	57,100%
6	75%	41,93%	86%	49,97%	100%	61,100%		
7	87%	53,98%	100%	65,100%				
8	100%	68,100%						

4.1. Enrolment Description

The following numbers will be reported:

- Total volunteers screened
- Volunteers screened and
  - not randomised (with reason)
  - randomised
- Participants randomised with at least one immunisation by randomisation group.

A graphical representation (trial profile) will be prepared according to the CONSORT statement on publication of randomised clinical trials.

4.2. Baseline Characteristics

The baseline characteristics of the trial participants will be summarised by treatment group using appropriate summary statistics (mean and standard deviation for continuous normal variables, median and interquartile range for continuous non-normal variables, percentages for binary and categorical variables).

Baseline is defined as the date of the week 0 visit (day of randomisation), and for all variables the measurement is defined as the measurement on this day. If a measurement is not available on this day, the nearest measurement within 42 days prior to the week 0 visit will be used.

4.3. Follow-up Description

- The duration of follow-up, defined as the difference in weeks between the date of last contact with the volunteer and the date of randomisation, will be described in terms of median, IQR and range.
- The number of visits attended vs. the expected number of visits according to the number of participants randomised, by randomisation group.
- Loss to follow-up rate: the number (%) of participants not reaching 20 weeks will be described, indicating week last seen and the reason of loss to follow up.

Visit windows are defined as follows:
Immunisation visits at weeks 4 and 8 are compliant with the protocol if they take place ±3 days from the scheduled date. Follow-up visits at weeks 1, 5 and 9 are compliant if ±3 days, while follow-up at week 22 is compliant if ±7 days from the scheduled date.

4.4. Interim Analysis

There is no planned interim analysis.

4.5. Primary Analysis

4.5.1. Safety

For each arm the proportion and its 95% CI will be reported for participants with a primary safety endpoint as defined in 3.1.1. The proportion with events will be compared in a pairwise manner between the three randomised groups, using Fisher's exact tests.

Grade 3 or above safety endpoints and any endpoints that lead to discontinuation of the vaccine regimen will be described, and a full listing of all events for each participant will be filed in the statistical master file.

4.5.2. Immunogenicity

Endpoint titres (concentration) of systemic CN54rgp140-specific IgG antibodies at 4 weeks from the final vaccinations will be described (mean and 95% C.I. – log10 scale - or median and IQR – original scale) by group for the responders, and compared using the Wilcoxon rank-sum test.

4.6. Secondary Analysis

4.6.1. Safety

For each arm the proportion and its 95% CI will be reported for participants with a secondary safety endpoint as defined in 3.2.1. CIs and significance tests will be reported similarly to the primary safety endpoint.

4.7. Exploratory Analysis

Proportions and their 95% CIs will be reported for the categorical exploratory outcomes defined in 3.3, with arms compared using Fisher's exact test. For continuous outcomes means or medians will be presented as appropriate, and arms compared using Welch tests.

4.8. Subgroup Analysis

No subgroup analysis has been planned for this study.

A. Tables

Table 1 Doses received

Week	Group ID/EP N=	 IM/EP N=	 IM/ID/EP N=
0	n (%)	n (%)	n (%)
4	n (%)	n (%)	n (%)
8	n (%)	n (%)	n (%)
20	n (%)	n (%)	n (%)

Table 2 Primary safety outcome, grade 3+ local or systemic solicited adverse event, or any event leading to treatment discontinuation.

	ID/EP (N=)	IM/EP (N=)	IM/ID/EP (N=)	Total (N=)
Participants with ≥1 event	n(%)	n(%)	n(%)	n(%)
Events per participant **0** **1** **2** **3** **4** **≥5**	 n n n n n n			
Total number of events	N	N	N	N
Type of event **solicited local** **solicited systemic** **systemic laboratory**	 n(%) n(%) n(%)			
Grade of event **1** **2** **3** **4**	 n(%) n(%) n(%) n(%)			
Relationship to vaccine **Definite** **Probable** **Possible** **Unlikely** **None**	 n(%) n(%) n(%) n(%) n(%)			

Description of any grade of local safety endpoints and any that lead to discontinuation of the vaccine regimen.

Table 3 Secondary safety outcome, AE local to injection site, within 7 days

	ID/EP			IM/EP			IM/ID/EP		
	Vaccine number								
	1	2	3	1	2	3	1	2	3
Total events									
Participants with ≥1 event									
Grade of event **1** **2** **3** **4**									

Table 4 Number of Solicited Adverse Events, by vaccination number

		ID/EP				IM/EP				IM/ID/EP			
		Vaccine number											
		1	2	3	4	1	2	3	4	1	2	3	4
Total													
Per participant	**0**												
	1												
	2												
	3												
	4												
	≥5												
Per grade	1												
	2												
	3												
	4												

Table 5 Solicited adverse events, by type, grade and vaccination number

a) Local adverse event

Symptom	Maximum Grade	Group Vaccination Number n = number at risk											
		1 N=	2 N=	3 N=	4 N=	1 N=	2 N=	3 N=	4 N=	1 N=	2 N=	3 N=	4 N=
Discomfort	1												
	2												
	3												
	4												
Redness	1												
	2												
	3												
	4												
Swelling (soft)	1												
	2												
	3												
	4												
Induration (hard)	1												
	2												
	3												
	4												
Blisters	1												
	2												
	3												
	4												

b) Systemic adverse event

Symptom	Maximum Grade	Group Vaccination Number n = number at risk											
		1 N=	2 N=	3 N=	4 N=	1 N=	2 N=	3 N=	4 N=	1 N=	2 N=	3 N=	4 N=
Temperature	1												
	2												
	3												
	4												
Chills	1												
	2												
	3												
	4												
Myalgia	1												
	2												
	3												
	4												
Malaise	1												
	2												
	3												
	4												
Headache	1												
	2												
	3												
	4												
Nausea	1												
	2												
	3												
	4												

c) Systemic laboratory AEs

Symptom	Maximum Grade	Group Vaccination Number n = number at risk											
		1 N=	2 N=	3 N=	4 N=	1 N=	2 N=	3 N=	4 N=	1 N=	2 N=	3 N=	4 N=
Creatinine	1												
	2												
	3												
	4												
AST	1												
	2												
	3												
	4												
ALT	1												
	2												
	3												
	4												
ALP	1												
	2												
	3												
	4												
Tot Bilirubin	1												
	2												
	3												
	4												
Glucose	1												
	2												
	3												
	4												
Hb	1												
	2												
	3												
	4												
White cells	1												
	2												
	3												
	4												
Neutrophils	1												
	2												
	3												
	4												
Lymphocytes	1												
	2												
	3												
	4												
Platelets	1												
	2												
	3												
	4												

Table 6. Duration in days of solicited clinical adverse events

	ID/EP	IM/EP	ID/IM/EP
Local solicited Systemic solicited			

N, median (IQR) [range]

Table 7. Solicited events lasting more than 7 days

Group	event	onset after last vaccination (days)	max grade	duration (days)	relationship

Table 8. Non-solicited adverse events

	ID/EP (N=)	IM/EP (N=)	ID/IM/EP (N=)	Total (N=)
Participants with ≥1 event	*n(%)*	n(%)	n(%)	n(%)
Events per participant				
0	*n*			
1	*n*			
2	*n*			
3	*n*			
4	*n*			
≥5	*n*			
Total number of events	*N*	N	N	N
Grade of event				
1	*n(%)*			
2	*n(%)*			
3	*n(%)*			
4	*n(%)*			
Relationship to vaccine				
Definite	*n(%)*			
Probable	*n(%)*			
Possible	*n(%)*			
Unlikely	*n(%)*			
None	*n(%)*			

Table 9. Summary of non-solicited adverse events

	ID/EP	IM/EP	ID/IM/EP	Total
Description of Event				
Type				
Type				

Table 10. Tolerability of electroporation

	ID/EP (N=)	IM/EP (N=)	ID/IM/EP (N=)
Pain at vaccination 1			
before EP	median (range)	x (x,x)	x (x,x)
during EP	x (x,x)		
10 mins after	x (x,x)		
30 mins after	x (x,x)		
Pain at vaccination 2			
before EP	x (x,x)		
during EP	x (x,x)		
10 mins after	x (x,x)		
30 mins after	x (x,x)		
Pain at vaccination 3			
before EP	x (x,x)		
during EP	x (x,x)		
10 mins after	x (x,x)		
30 mins after	x (x,x)		

Pain scores are median (range), 0 (best) to 10 (worst)