EML-Vac

A first-in-human clinical trial to assess the safety and immunogenicity of three self-amplifying ribonucleic acid (saRNA) vaccines encoding the surface glycoprotein of Ebola virus, Marburg virus and Lassa virus (EML-Vac)

Version:

2.0, 1st September 2025

MAIN SPONSOR: Imperial College London

FUNDERS: The trial is funded by a grant from Innovate UK, part of UKRI with additional funding provided by philanthropic donation from Partners of Citadel and Citadel Securities.

STUDY COORDINATION CENTRE: Imperial College London

IRAS Project ID: 1012266

REC reference: 25/LO/0596

Protocol authorised by:

Name & Role	Date	Signature
Dr Marta Boffito Chief Investigator		

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1.5 Clinical Queries

Clinical queries should be directed to 02033155601 who will direct the query to the appropriate person

1.6 Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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1.7 Funder

This trial is funded by a grant from Innovate UK, part of UKRI with additional funding provided by philanthropic donation from Partners of Citadel and Citadel Securities.

This protocol describes the EML-Vac study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, when entering participants for the first time contact the trials centre to confirm you have the most recent version.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

RANDOMISATIONS

Randomisation to LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01vaccine, LNP-LASSAsaRNA-01 or combined vaccination will be via a web based randomisation software (Sealed Envelope) at site

SAE REPORTING

Serious AR/AEs should be reported on the same day as site is aware of the event

SAEs and Notable Events should be reported within 24 hours of the site becoming aware of the event.

Contact details for reporting SAEs and SUSARs

RGIT.ctimp.team@imperial.ac.uk

If you have any issues with reporting Serious AR/AE, SAE and NE or have any questions please email CI, Dr Marta Boffito (marta.boffito@nhs.net) or telephone : 020 3315 5601

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	EXPANSION
A&E	Accident and Emergency
AE	Adverse event
AIDS	Acquired Immune Deficiency Syndrome
ANA	Antinuclear antibody
AR	Adverse reaction
ART	Antiretroviral therapy
BMI	Body mass index
CF	Consent Form
CI	Chief Investigator
CI	Confidence interval
CLRN	Comprehensive Local Research Network
COM	Clinical Operations Manager
CPM	Clinical Project Manager
CRF	Case Report Form
CRN	Clinical Research Network
CTA	Clinical Trials Authorisation
CTAAC	Clinical Trials Awards and Advisory Committee
CTIMP	Clinical trial of an investigational medicinal product
CTL	Cytotoxic T-lymphocyte
DCF	Data Clarification Form
DH	Department of Health
DM	Data Manager
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DPA	(UK) Data Protection Act
DSUR	Developmental Safety Update Report
EBOV	Ebola Virus
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic Data Capture
EFGCP	European Forum for Good Clinical Practice

ABBREVIATION	EXPANSION
ELISA	Enzyme-linked Immunosorbent Assay
ELISPOT	Enzyme-linked immunosorbent spot
EMA	European Medicines Agency
EML	Ebola-Marburg-Lassa
EU	European Union
EudraCT	European Union Drug Regulatory Agency Clinical Trial
FDA	(US) Food and Drug Administration
GCP	Good Clinical Practice
GP	General Practitioner
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
IB	Investigator Brochure
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IDMC	Independent Data Monitoring Committee
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
IM	Intramuscular
IMP	Investigational medicinal product
IRAS	Integrated Research Application System
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention-to-treat
IUD	Intrauterine device
LASSA	Lassa Virus
LNP	Lipid nanoparticles
LNP-MARVsaRNA	Lipid nanoparticle Marburg self-amplifying ribonucleic acid
LNP-EBOVsaRNA	Lipid nanoparticle Ebola self-amplifying ribonucleic acid
LNP-LASSAsaRNA	Lipid nanoparticle Lassa self-amplifying ribonucleic acid

ABBREVIATION	EXPANSION
MARV	Marburg virus
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
mL	Millilitre
MREC	Multi-centre Research Ethics Committee
mRNA	Messenger ribonucleic acid
NCRN	National Cancer Research Network
NHP	Non-human primate
NHS	National Health Service
NHSCR	National Health Service Central Register
NHS-IC	National Health Service Information Centre
NIHR	National Institute for Health Research
NIHR CSP	National Institute for Health Research Co-ordinated System for gaining NHS Permission
NOAEL	No Observed Adverse Effect Level
NRES	National Research Ethics Service
PEG	Polyethylene glycol
PI	Principal Investigator
PIS	Participant Information Sheet
QMAG	Quality Management Advisory Group
QP	Qualified Person
R&D	Research and Development
REC	Research Ethics Committee
RGC	Research Governance Committee
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious adverse reaction
saRNA	Self-amplifying ribonucleic acid
SAS	Safety analysis set
SD	Standard deviation
SGP	Subgenomic promoter
SIR	Suspected infected and recovered

ABBREVIATION	EXPANSION
siRNA	Small interfering RNA
SPC	Summary of Product Characteristics
SSA	Site-specific approval
SSG	Scientific Strategy Group
SSI	Site-specific information
SUSAR	Suspected unexpected serious adverse reaction
TM	Trial Manager
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
TSC	Trial Steering Committee
UAR	Unexpected adverse reaction
UKCRN	UK Clinical Research Network (now the NIHR CRN)
UN	United Nations
VEEV	Venezuelan equine encephalitis virus
VHF	Viral Haemorrhagic Fever
VLP	Virus-like particle
WBC	White blood cells
WHO	World Health Organization
WOCP	women of childbearing potential
µg	microgram

2. SUMMARY OF TRIAL

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Acronym	EML-Vac
Long Title of Trial	A first-in-human clinical trial to assess the safety and immunogenicity of a 3 self-amplifying ribonucleic acid (saRNA) vaccines encoding the surface glycoprotein of Marburg virus, Ebola Virus and Lassa Virus
Version	2.0
Date	1st September 2025
IRAS ID	1012266
Study Design	A cohort will be recruited to assess: 1. A self-amplifying ribonucleic acid (saRNA) vaccine; **LNP-MARVsaRNA-01** given at 5 µg at 0 and 12 weeks apart in 8 individuals aged 18-50 years enrolled through a single centre. Participants and laboratory staff will be blind to allocation (see Table 1). 2. A self-amplifying ribonucleic acid (saRNA) vaccine **LNP-EBOVsaRNA-01** given at 4 µg at 0 and 12 weeks in 8 individuals aged 18-50 years enrolled through a single centre (see Table 1). 3. A self-amplifying ribonucleic acid (saRNA) vaccine **LNP-LASSAsaRNA-01** given at 4 µg at 0 and 12 weeks in 8 individuals aged 18-50 years enrolled through a single centre (see Table 1). 4. Two self-amplifying ribonucleic acid (saRNA) vaccines: **LNP-MARVsaRNA-01** (5 µg) **and LNP-EBOVsaRNA-01** (4 µg), 1 dose of each vaccine given at 0 and 12 weeks apart to 8 individuals aged 18-50 years enrolled through a single centre (see Table 1). 5. Three self-amplifying ribonucleic acid (saRNA) vaccines: **LNP-MARVsaRNA-01** (5 µg) **LNP-EBOVsaRNA-01** (4 µg) **and LNP-LASSAsaRNA-01** (4 µg) **–** 1 dose of each vaccine

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	given at 0 and 12 weeks apart to 8 individuals aged 18-50 years enrolled through a single centre (see Table 1).
Aims/Objectives	➢ To evaluate the safety and immunogenicity of two immunisations with LNP-MARVsaRNA-01 administered IM 12 weeks apart at a 5 µg dose in 8 participants age 18-50 years. ➢ To evaluate the safety and immunogenicity of two immunisations with LNP-EBOVsaRNA-01 administered IM 12 weeks apart at one dose levels 4 µg in 8 participants age 18-50 years. ➢ To evaluate the safety and immunogenicity of two immunisations with LNP-LASSAsaRNA-01 administered IM 12 weeks apart at one dose levels 4 µg in 8 participants age 18-50 years. ➢ To evaluate the safety and immunogenicity of two immunisations with a combination of LNP-MARVsaRNA-01 (5 µg) and LNP-EBOVsaRNA-01 (4 µg) administered IM 12 weeks apart in 8 participants age 18-50 years ➢ To evaluate the safety and immunogenicity of two immunisations with a combination of LNP-MARVsaRNA-01 (5 µg), LNP-EBOVsaRNA-01 (4 µg) and LNP-LASSAsaRNA-01 (4 µg) administered IM 12 weeks apart in 8 participants age 18-50 years
Outcome Measures	➢ Solicited local injection site reactions starting within 7 days of administration of the vaccine: pain, tenderness, erythema, swelling ➢ Solicited systemic reactions starting within 7 days of administration of the vaccine: pyrexia, fatigue, myalgia, headache, chills, arthralgia ➢ Unsolicited adverse reactions (ARs) throughout the trial period (including serious ARs) ➢ Serious Adverse Events ➢ Unsolicited adverse events throughout the trial period ➢ The titre of vaccine-induced serum IgG binding antibody responses to the Marburg, Ebola and Lassa fever virus surface glycoproteins 2 weeks after the second vaccination

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Exploratory Aims/Objectives	➢ To characterise the humoral and cellular immune responses to LNP-MARVsaRNA-01 administered at one dose ➢ To characterise the humoral and cellular immune responses to LNP-EBOVsaRNA-01 administered at one dose ➢ To characterise the humoral and cellular immune responses to LNP-LASSAsaRNA-01 administered at one dose ➢ To characterise the humoral and cellular immune responses to LNP-MARVsaRNA-01 and LNP-EBOVsaRNA-01 administered together at one dose ➢ To characterise the humoral and cellular immune responses to LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 administered together at one dose
Exploratory Outcome Measures	➢ Cell-mediated vaccine-induced immune responses measured by T and B cell ELISpot in participants ➢ Cell-mediated vaccine-induced immune responses measured by flow cytometry and intracellular cytokine staining ➢ Serum neutralising antibodies in a Marburg, Ebola or Lassa pseudovirus-based neutralisation assay ➢ The profile of class and sub-class of antibody response ➢ Serum markers of innate immune response ➢ Purification of antigen-specific B cells to isolate neutralising monoclonal antibodies to enhance understanding of targeted epitopes
Randomisation	➢ 40 participants across the five evaluation cohorts (See Figure 1)
Population: Type of Participants to be Studied and Justification	Healthy adults aged 18-50 years who do not have active conditions that require investigation or a change in treatment. The upper age limit will be 50 years as there is greater variability of immune responses in those aged over 50 years. 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 trial 8 participants will have been exposed to each vaccine alone (4/5 µg dose), 8 participants to two combined vaccines (5+4 µg, i.e. a total dose of 9 µg of saRNA) and 8 participants to all three vaccines combined (5+4+4 µg, i.e. a total dose of 13 µg of saRNA) and this provides

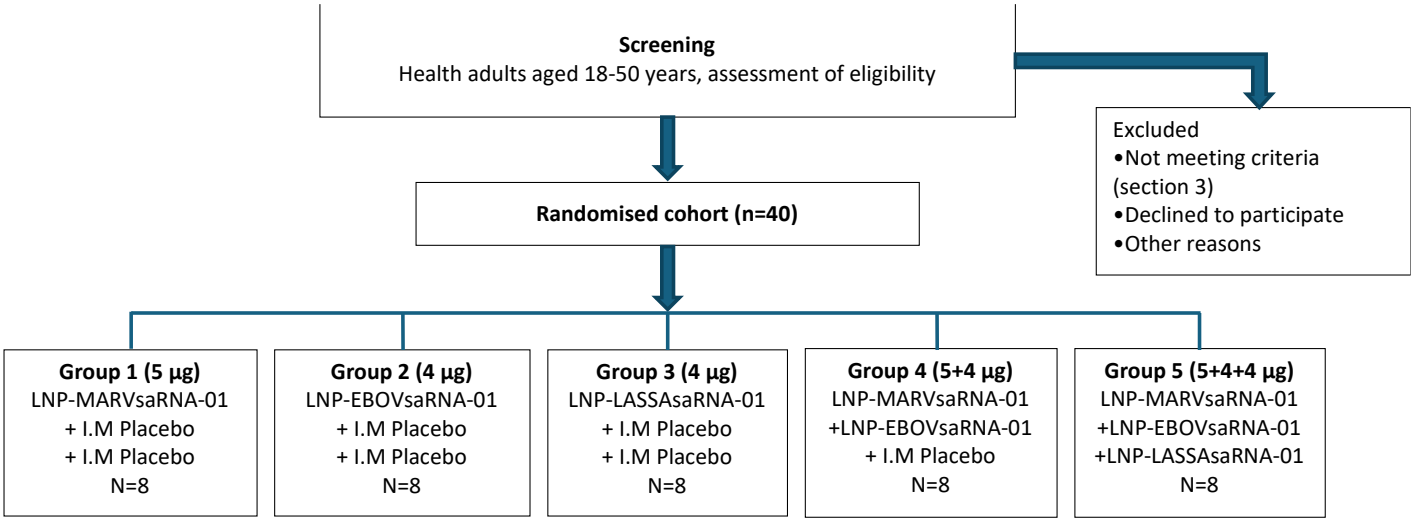
SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	confidence around the response/event proportions of 0–100% in table 6.
Eligibility	1. Healthy adults, aged 18-50 years on the day of screening 2. Willing and able to provide written informed consent 3. If female and of childbearing potential, willing to use a highly effective method of contraception from screening until 18 weeks after last injection 4. If male and not sterilised, willing to avoid impregnating female partners from screening until 18 weeks after last injection 5. Willing to avoid all other vaccines from within 4 weeks before and after the first and second injections 6. Willing and able to comply with visit schedule, complete online diaries and provide samples 7. Willing to abstain from donating blood for three months after the end of their participation in the trial or longer, if necessary 8. Willing to grant authorised persons access to his/her trial-related medical record and GP records
Treatments to be Compared **(See Table 1)**	1. LNP-MARVsaRNA-01 vaccine (5 µg dose) at 0 and 12 weeks 2. LNP-EBOVsaRNA-01 vaccine (4 µg dose) at 0 and 12 weeks 3. LNP-LASSAsaRNA-01 vaccine (4 µg dose) at 0 and 12 weeks 4. LNP-MARVsaRNA-01 vaccine (5 µg) plus LNP-EBOVsaRNA-01 (4 µg) at 0 and 12 weeks 5. LNP-MARVsaRNA-01 vaccine (5 µg) plus LNP-EBOVsaRNA-01 (4 µg) plus LNP-LASSAsaRNA-01 (4 µg) at 0 and 12 weeks
Trial Assessments	See Table 2
Duration	Each participant who received either LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01, LNP-LASSAsaRNA-01 or a combination of the vaccines will be followed for one year. The trial is anticipated to start in September 2025 and complete enrolment for within 3 months. The total duration of the trial is anticipated to be 14 months.

2.1 Trial Schema

TABLE 1: TRIAL GROUPS

Group	Description	Active Dose (µg) Prime & boost	Interval between doses	Age (years)	N
Group 1	LNP-MARVsaRNA-01, plus two buffered saline placebo	5 µg	12 weeks	18-50	8
Group 2	LNP-EBOVsaRNA-01, plus two buffered saline placebo	4 µg	12 weeks	18-50	8
Group 3	LNP-LASSAsaRNA-01, plus two buffered saline placebo	4 µg	12 weeks	18-50	8
Group 4	LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01, plus one buffered saline placebo	MARV 5 µg plus EBOV 4 µg (9 µg in total)	12 weeks	18-50	8
Group 5	LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01, LNP-LASSAsaRNA-01	MARV 5 µg plus EBOV 4 µg plus LASSA 4 µg (13 µg in total)	12 weeks	18-50	8
Total					40

Figure 1: Trial Entry



(n=40)

1. To evaluate the safety and immunogenicity of two immunisations with LNP-MARVsaRNA-01 administered IM 12 weeks apart at a 5 µg dose in 8 participants age 18-50 years.

2. To evaluate the safety and immunogenicity of two immunisations with LNP-EBOVsaRNA-01 administered IM 12 weeks apart at a 4 µg dose in 8 participants age 18-50 years

3. To evaluate the safety and immunogenicity of two immunisations with LNP-LASSAsaRNA-01 administered IM 12 weeks apart at a 4 µg dose in 8 participants age 18-50 years

4. To evaluate the safety and immunogenicity of two immunisations with a combination of LNP-MARVsaRNA-01 (5 µg) and LNP-EBOVsaRNA-01 (4 µg) administered IM 12 weeks apart (total 9 µg) in 8 participants age 18-50 years

5. To evaluate the safety and immunogenicity of two immunisations with a combination of LNP-MARVsaRNA-01 (5 µg), LNP-EBOVsaRNA-01 (4 µg) and LNP-LASSAsaRNA-01 (4 µg) administered IM 12 weeks apart (total 13 µg) in 8 participants age 18-50 years

TRIAL ASSESSMENT SCHEDULES

Table 2: Trial Assessment Schedule for all groups

Trial Visit	V1	V2	V2a	V3	V4	V5	V5a	V6	V7	V8	V9	V10	V11
Visit Type (site or telephone)	Site	Site	Site	Site	Site	Site	Site	Site	Site	Site	Site	Site	Site
Trial Week	–8	0	0	1	4	12	12	13	14	16	24	36	52
Trial Day	–	0	1	7	28	84	85	91	98	112	168	252	364
Windows (days)	–56 to –1	–	none	0 to +2	–2 to +2	–4 to +7	none	0 to +2	–2 to +2	–4 to +4	–7 to +7	–20 to +7	–36 to +7
Informed consent	X												
Medical history demographics	X												
Eligibility assessment	X	X											
Physical examination	X	X		X		X		X					
ECG	X	X				X							
Weight/height (BMI)	X												
Vital signs (BP, HR, O_2 saturation and oral temperature)	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomisation		X											
Vaccination		X				X							
Issue diary card for AEs	X	X				X							
Review diary card for AEs	X	X	X	X			X	X					
Symptom-directed physical examination as required			X		X		X		X	X	X	X	X

Trial Visit	V1	V2	V2a	V3	V4	V5	V5a	V6	V7	V8	V9	V10	V11
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV and HCV screen	~5 mL												
Laboratory safety tests[1]	~10 mL	~10 mL		~10 mL	~10 mL	~10 mL		~10 mL	~10 mL	~10 mL	~10 mL	~10 mL	~10 mL
Urine dipstick	X												
Urinary pregnancy test (WOCP only)	X	X				X						X	
Blood for central serum immunogenicity assays		6 mL		6 mL	6 mL	6 mL		6 mL	6 mL	6 mL	6 mL	6 mL	6 mL
Blood for central cellular immunogenicity assays		60 mL				42 mL			60 mL	42 mL			
Blood for innate cytokines		6 mL	6 mL			6 mL	6 mL						
Blood volume (approx.) [2]	15 mL	82 mL	6 mL	16 mL	16 mL	64 mL	6 mL	16 mL	76 mL	58 mL	16 mL	16 mL	16 mL

1 Haemoglobin, lymphocytes, neutrophils, platelets, creatinine, AST/ALT, ALP, total bilirubin, non-fasting glucose throughout. GGT at screening only.

2. Total blood draw across 52 weeks is 403 mL

3. LAY SUMMARY

EML-Vac is a trial that is looking at new RNA vaccines against the Marburg, Ebola and Lassa Fever viruses which are the major causes of viral blood (heamorrhagic) fever. The aim of the trial is to assess the safety of these vaccines alone and in combination, since this will be the first time that these have been used in humans. But this trial is NOT looking at whether or not the vaccines are effective in terms of protection. It is just assessing the safety of these vaccines and how well the immune system responds to the vaccine.

EML-Vac saRNA Vaccines

Since this is the first time these vaccines have been used in humans, the safety will be assessed in healthy young adults. 40 participants aged 18-50 years will be randomised to one of five different groups receiving either Ebola, Marburg or Lassa self-amplifying RNA (saRNA) vaccines individually or in combination by injection into the muscle. Participants will receive prime and boosting immunisations 12 weeks apart. They will be careful monitoring for any reactions to the vaccine.

There are likely to be mild side-effects near to the injection site. There may also be more general side-effects such as headache, temperature and chills. Participants will be asked to record any symptoms in an online diary. In order to see how well the immune system is responding, participants will need to give blood samples several times during the first 4 weeks; then for 24 weeks following their boosted immunisation and finally at 12 months to assess durability of response. This will happen at one trial centre. If any part of the trial shows that either dose is unsafe or poorly tolerated, it will be omitted from further trial.

Although there have been no clinical studies using these three saRNA vaccines, a previous first-in-human trial (COVAC-1) evaluated the safety and immunogenicity of an saRNA vaccine encoding the spike glycoprotein of SARS-CoV-2, the causative agent of COVID-19 in over 200 participants (phase I/IIa). The saRNA vaccine was administered as prime and boost intramuscular injections, 4 and 14 weeks apart at dose levels of 0.1, 0.3, 1, 2.5, 5 and 10 µg and participants were followed up for an additional 48 weeks. Safety data have shown that this vaccine was safe and well tolerated and the majority of reactions following vaccinations were mild or moderate. The majority of solicited adverse events reported within 7 days after the prime and boost vaccination were mild.

3. BACKGROUND

3.1 Introduction

3.1.1 VIRAL HAEMORRHAGIC FEVERS

Viral haemorrhagic fevers (VHFs) are a group of diseases that are caused by several distinct families of viruses. The viruses are single stranded RNA virus that have a lipid envelope that make them susceptible to low pH environments and detergents, however they are stable in blood and at cold temperatures [1]. Marburg, Ebola and Lassa viruses are classed as VHFs. Marburg and Ebola viruses belong to the family of filoviruses and Lassa virus belong to the family of arenaviruses. Outbreaks of these VHFs are common, and infection results in high fatality rates, so cost effective, widely available vaccines, where many doses can be rapidly manufactured, is believed to be a vitally important tool in tackling these viruses and preventing future pandemics.

3.1.2 MARBURG VIRUS

The Marburg virus is a highly virulent haemorrhagic fever virus which can lead to Marburg Virus Disease. The Marburg virus spreads though human-to-human transmission following direct contact with blood and other bodily fluids of infected people through broken skin or mucous membranes. The virus can also be transmitted through surfaces and materials such as bedding, and clothes contaminated with these fluids from infected individuals [2].

The disease was first identified following two large outbreaks in Serbia and Germany in 1967. These outbreaks started in laboratories that were carrying out work on African Green Monkeys which had been imported from Uganda. Since 1967 further significant outbreaks of Marburg Virus Disease have been reported in Central and Southern Africa [2].

Between days 5-7, many patients who have been infected by the Marburg Virus develop severe haemorrhagic manifestations, and in cases that are fatal, bleeding from the nose, gums and vagina (in women) is observed. Death occurs between 5-8 days after the onset of symptoms and is preceded by extreme blood loss and shock [2].

3.1.3 EBOLA VIRUS

The Ebola virus first appeared in 1976, in two simultaneous outbreaks in the Democratic Republic of Congo and South Sudan. The virus takes its name from the Ebola River, a tributary of the Congo River in the Democratic Republic of Congo, where it was first identified [3].

The Ebola virus is a highly virulent haemorrhagic fever virus which can lead to Ebola Virus Disease and Ebola Haemorrhagic Fever in humans and primates. It is thought that the virus may be carried by fruit bats who are unable to become infected. Spread of the virus in humans is through body fluids or through contact with surfaces and materials that have been contaminated with infectious body fluids.

Symptoms manifest between 2 days and 3 weeks post infection of the virus and present as fever, sore throat and muscle pain, followed by vomiting and diarrhoea and reduced liver and kidney function. In some instances, patients will bleed both internally and externally leading to death. Up to 90% of people that will be infected with the Ebola virus will not survive [3].

Currently, there are 2 licensed vaccines to prevent Ebola virus, the 1-dose rVSVΔG-ZEBOV-GP (ERVEBO [Merck]) and the 2-dose regimen of Ad26.ZEBOV and MVA-BN-Filo (Zabdeno/Mvabea [Johnson & Johnson]).

3.1.4 LASSA VIRUS

The Lassa virus is a highly virulent haemorrhagic fever virus which can lead to Lassa Haemorrhagic Fever and is endemic in many West African countries. The virus is spread through contact with the urine or faeces of a multimammate mouse [4], and onward transmission through human-to-human contact. Many people infected with this virus remain asymptomatic. When symptoms present, they include headache, muscle ache, vomiting and chills. The mortality rate of this virus is around 1%, although thought to be 15% in hospitalised patients and death can occur within 2 weeks of infection.

There is no licensed vaccine for Lassa virus, however new vaccine candidates against this virus are currently being developed and tested.

3.1.5 CURRENT THERAPEUTICS

The development and manufacture of new vaccines is a costly, lengthy process. When disease outbreaks arise, a major issue is the scale and the time frame in which new vaccines need to be developed. A self-amplifying RNA (saRNA) vaccine provides a novel, feasible, and time-sensitive solution to develop vaccines against Haemorrhagic Fever viruses.

A number of studies have demonstrated that nucleic acid-based vaccination can protect against viral infections in non-human primate (NHP) studies [6-10], providing proof of concept that gene-based vaccination can induce protective antibodies. However, DNA vaccines have thus far failed to live up to their full potential as a standalone vaccine technology and require multiple immunisations with the use of electroporation to induce significant immune response in humans [11]. Non-replicating mRNA-based therapeutics have been widely explored in the field of oncology [12] and more recently against viral infections [13-14] but typically use high doses of RNA (100-600 µg) [14]. The requirement for high doses, and associated cost, suggest non-replicating mRNA may struggle to produce the potentially hundreds of millions of doses required to rapidly respond to a pandemic. In contrast, small animal and NHP experiments suggest that saRNA induces significantly enhanced responses in comparison to either DNA vaccines delivered with electroporation or mRNA. Indeed, a single immunisation with an saRNA vaccine has shown protection against Ebola virus in animal models [5]. Should a 4 or 5 µg dose of saRNA provide protection from Marburg, Ebola and Lassa viruses, this would provide critical advantages for manufacturing where a million doses can by synthesised in a one litre reaction volume [15] with a 300-400 fold development window over mRNA vaccines. In this respect, the delivery of our vaccines using saRNA offers significant advantages over more conventional vaccine platforms (viral vectors, recombinant proteins and attenuated pathogens) in terms of cost, speed and reactogenicity. In contrast to viral vectors, lack of anti-vector immunity provides the opportunity for repeat immunisations with multiple RNA-encoded immunogens.

3.1.6 PREVIOUS STUDIES

3.1.6.1 Pre-clinical

The 3 vaccines, LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 that will be tested in the EML-Vac clinic trial have all shown to induce robust immune responses in pre-clinical models administered at a 5 µg dose alone or together via the intramuscular route (see Investigators brochure). In addition, in the pig model, cross-reactive Ebola-specific IgG was detected in animals immunised with LNP-

saRNA Marburg vaccine. The protective effect of all 3 vaccines was shown in efficacy studies in guinea pigs, whereby 100% of animals vaccinated with either 1 µg/dose of LNP-MARVsaRNA-01, 1 µg/dose of LNP-EBOVsaRNA-01 or 1 µg/dose of LNP-LASSAsaRNA-01 were protected from disease progression respectively following challenge with 1,000 TCID50 of virus 4 weeks post vaccination.

In a pivotal nonclinical toxicology study (Labcorp Study no. 8494899) conducted to Good Laboratory practice (GLP) in rats, the toxicity of 5 µg/dose of LNP-MARVsaRNA-01 and 15 µg/dose EML-saRNA (three VEEV-saRNAs encoding the glycoproteins of Ebola, Lassa, and Marburg viruses) was evaluated when administered on Days 1, 8, and 15 via intramuscular to rats. Intramuscular administration of 15 µg/occasion EML-saRNA on three occasions over 2 weeks (7 days between each administration) resulted in mild transient loss of function in the dosed leg followed by swelling of the thigh for 2 to 3 days resulting in minimally to slightly increased incidence of localised inflammatory infiltrates at the dose sites and was well tolerated. Based on the absence of any adverse findings and under the conditions of this study, the no observed adverse effect level (NOAEL) is 5 µg/dose for the LNP-MARVsaRNA-01 vaccine, the no observed adverse effect level (NOAEL) is 15 µg/occasion for EML-saRNA. For further information please refer to the Investigators brochure (IB).

3.1.6.2 Clinical studies

Although no clinical studies have been carried out with LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01, a first in man Phase I/IIa trial has evaluated the safety and immunogenicity of an LNP-nCoVsaRNA vaccine, a self-amplifying RNA which encodes a modified, codon-optimised SARS-CoV-2 surface (S) glycoprotein encapsulated in lipid nanoparticles (LNPs) (COVAC-1) [16,17].

In COVAC-1 there were no safety concerns in the period up to 28 days following two IM injections of LNP-nCoVsaRNA at doses in the range of 0.1 to 10·0 µg in the 192 adults aged 18-45 years in the dose-ranging cohort. Two participants had adverse events that led to a delay in their second vaccination, which were administered with no recurrence. Reactogenicity was dose dependent, with the highest proportion of grade 3 reactions (11%) in those receiving a 10·0 µg dose [16, 17].

The reactogenicity profile of LNP-nCoVsaRNA appears similar to other mRNA COVID-19 vaccines, where systemic and local reactions of grade 2 and above were common, particularly in younger adults. There was no evidence of clinically significant potentiation after the second dose, beyond a slight increase in grade 2 headaches. No allergic events were considered related to the saRNA vaccine, although this may reflect exclusion of subjects with a significant allergy history.

Based on the findings from vaccination with LNP-nCoVsaRNA, we will now conduct a Phase I, first-in-human clinical trial (EML-Vac) to evaluate the safety and immunogenicity of the saRNA vaccine to Marburg, Ebola and/or Lassa virus; LNP-MARVsaRNA-01, LNP-EBOV-saRNA-01 and/or LNP-LASSA-saRNA-01. The vaccine will be administered as a prime and boost regimen as two IM injections given at a dose of 5 µg (LNP-MARVsaRNA-01) and/or 4 µg (LNP-EBOV-saRNA-01 and/or LNP-LASSA-saRNA-01) at 12 weeks apart.

3.1.7 SELF-AMPLIFYING RNA VACCINES

The active pharmaceutical ingredient for each vaccine is an optimised saRNA vector that encodes the spike glycoprotein of Marburg, Ebola or Lassa viruses (i.e. MARVsaRNA-01, EBOV-saRNA-01 and LASSA-saRNA-01, respectively). Each of these glycoproteins are stabilised in a prefusion conformation that facilitates induction of neutralising antibodies (for full sequence details refer to the IB). Each saRNA construct is based on a non-

infectious Venezuelan equine encephalitis virus (VEEV) replicon backbone encoding non-structural proteins (nsP1–4) required for self-amplification [18, 24]. The codon-optimised spike glycoprotein gene of either Marburg, Ebola or Lassa viruses' have been inserted in place of structural genes downstream of a sub-genomic promoter (SGP) that drives their transcription and surface expression on cells that have taken up the RNA vector. To ensure efficient uptake following intramuscular administration the naked saRNA is formulated in lipid nanoparticles (LNPs). These particles are made from a mixture of ionizable cationic lipid (C12-200), phosphatidylcholine, cholesterol, and polyethylene glycol (PEG)-lipid. The saRNA is encapsulated within the LNPs protecting the RNA from degradation and delivering the payload to the cytoplasm of cells following endocytocytic uptake of the LNP. This approach builds on the successful use of LNPs for mRNA and saRNA COVID-19 vaccines including those licensed by Moderna and Pfizer [25, 26].

Following intramuscular injection, the formulated saRNA is taken up into the cytoplasm of target cells. This leads to intracellular amplification of the saRNA by the encoded replicase machinery and very high expression levels of the gene of interest (Figure 2). This process in turn induces strong immune-stimulatory potency against the expressed immunogen due to its intrinsic adjuvant activity [27, 28]. However, this does not generate viral particles, the amplification process is self-limiting (days), and there is no onward transmission of the saRNA to other cells. The saRNA is strictly confined to the cytoplasm of the cell and does not enter the nucleus. It is unable to affect chromosomal DNA and as a result causes no changes of the cells genetic makeup.

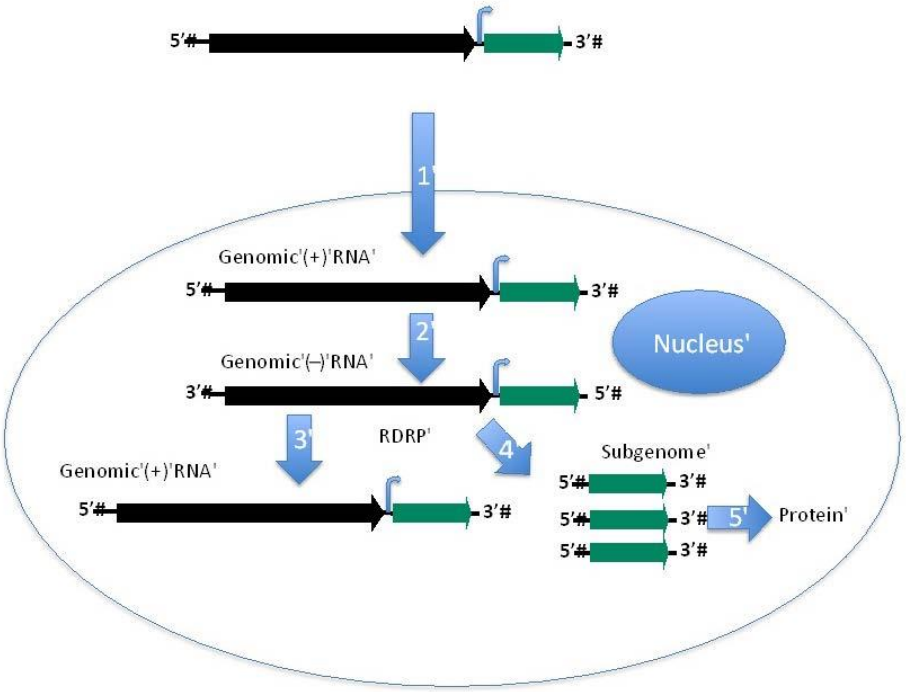


Figure 2. *On delivery into cells (1), the polymerase machinery of the VEEV backbone is preferentially expressed. This in turns leads to amplification of the RNA through production of negative (2) and positive copies of the saRNA vector (3). Within a few hours, sequential cleavage of the polymerase machinery leads to a shift to preferential transcription of the subgenomic sequence encoding the viral spike glycoprotein of interest (e.g. that of Marburg, Ebola or Lassa virus) (4). This is then translated to express high level of the encoded glycoprotein antigen (5).*

3.1.8 MANUFACTURING PROCESS

The manufacturing process for the generation of our saRNA drug substance consists of three main steps [15]: (1) in vitro saRNA transcription from the linear DNA template using bacteriophage T7 RNA polymerase followed by hydrolysis of the DNA template; (2) purification of the saRNA; and (3) the incorporation of saRNA LNPs. The saRNA is encapsulated within LNPs with a unique lipid composition, using a self-assembly in-line process in which >90% of saRNA in aqueous solution is encapsulated, generating an ionizable cationic LNP of approximately 100 nm in diameter. This provides protection of the nucleic acid payload against nucleases while facilitating cellular uptake and endosomal escape (for further detail refer to IB).

3.2 Potential Risks and Mitigations

3.2.1 ESTABLISHED USE OF VEEV AS THE SARNA BACKBONE

Our use of VEEV as the saRNA backbone builds on a long history in the use of VEEV for vaccination. A live-attenuated VEEV (TC-83) has been used to protect at-risk laboratory human personnel since the 1960s with no adverse effects [18]. As a vector, alphavirus-based recombinant RNA replicons were initially packaged into viral like particles (VLPs). A number of previous clinical trials have demonstrated the safety of alphavirus-based recombinant RNA replicon viral like particles (VLPs) in humans [19-23], and NCT00439803, NCT00063778, NCT00440362, NCT01890213. More recently, with the development of formulations able to facilitate RNA uptake into cells, naked VEEV RNA has been used on its own. This still retains the same capacity for self-amplification within cells but does not require any recombinant protein elements. This builds on the wider experience of RNA-based vaccines and pharmaceuticals that have been shown to be safe in non-clinical and clinical tests. The saRNA vaccine manufacturing process is devoid of any virus particle forming genes. In addition to our own clinical trials of saRNA vaccines against COVID-19, a number of additional COVID-19 saRNA vaccine studies have been performed including phase III efficacy trials [29-33] and one study of a rabies saRNA vaccine [34].

3.2.2 LIPID NANOPARTICLES (LNPS) DELIVERY SYSTEM

To ensure efficient uptake of the saRNA, it will be administered intramuscularly formulated in LNPs. These particles are made from a mixture of ionizable cationic lipid (C12-200), phosphatidylcholine, cholesterol, and polyethylene glycol (PEG)-lipid. The saRNA is encapsulated within the LNPs protecting the RNA from degradation and delivering the payload to the cytoplasm of cells following endocytosis of the LNP. This approach builds on the successful use of LNPs for siRNA, exemplified by the granting of a license for Onpattro (patisiran) delivered in LNPs [35]. This approach has been recently applied to mRNA and saRNA vaccines against COVID-19, including those lincesed by Moderna and Pfizer [25, 26]. Building on these studies we have developed an LNP formulation optimised for saRNA delivery [36].

3.2.3 EXPECTED SIDE EFFECTS

Based on other vaccines against different diseases, including those that use synthetic mRNA, the side effects are expected to be mild to moderate, short-lived reactions at the injection site such as discomfort, warmth, redness and swelling. Short-lived systemic symptoms such as fatigue, general malaise and headache are expected very commonly (more than 1 in 10 people). Uncommon side effects (1 in 100 people) include abdominal pain, diarrhoea, sore throat, enlarged lymph nodes, insomnia and allergic reactions such as rash or itching. These are also anticipated to resolve within a few days (<7 days). Rare reactions associated with mRNA vaccine, that may also be associated with saRNA vaccines include (less than 1 in 1,000 people, but more than 1 in 10,000) enlarged lymph nodes, high fever (≥40 °C), hypersensitivity (exaggerated reaction to the vaccine), urticaria (raised, itchy skin rash), and granuloma (area of inflammation in the skin) or sterile

abscess (lump) at the injection site. Non-severe allergic reactions (1 in 1,000) such as hives or swelling of the face may occur and severe very rare allergic reactions (1 in 1 million) may occur, however those with a history of allergy will not be eligible. Very rarely (less than 1 in 10,000 people) vaccines may cause convulsions with fever, drowsiness, and macrophagic myofasciitis (a rare muscle disease). Myocarditis (inflammation of the heart muscle), and Pericarditis (inflammation of the lining outside the heart) are also very rare potential serious side effects associated with mRNA vaccine occurring most commonly in adolescent males 12 through 17 years of age (1 in 37,000). Those with any previous history of either of these conditions will not be eligible. These risks will be carefully explained so that participants know what is involved and what to expect in the way of side effects.

3.3 Potential Benefits

A theoretical benefit of participating in this clinical safety trial is that the vaccine may provide protection against Marburg virus, Ebola virus and/or Lassa virus infection, viruses that causes outbreaks in West Africa and has pandemic potential. Despite promising pre-clinical data, at this stage equipoise remains as to relative risks and benefits.

On the basis of the background risks and mitigations described above, it is considered safe and appropriate to enter a clinical early phase immunogenicity and safety trial with Marburg, Ebola and Lassa saRNA vaccines.

3.4 Rationale for current trial

Marburg, Ebola and Lassa viruses are the major causes of sporadic, but common, outbreaks of viral haemorrhagic fevers (VHFs), where infection results in high fatality rates. Clinical trials of antivirals and other drug therapies are ongoing but the intervention most likely to mitigate the long-term medical, social and economic impact of VHF in countries at risk of outbreaks remains population-wide immunisation. Furthermore, cost effective, widely available vaccines, where many doses can be rapidly manufactured, is believed to be a vitally important tool in tackling these viruses and preventing future pandemics.

The pre-clinical data described above (and in greater detail within the IB) suggested that LNP-MARVsaRNA, LNP-EBOVsaRNA and LNP-LASSAsaRNA will protection against VHFs and development of serious disease.

3.5 Rationale for dose selection

The choice of dose is supported by the pivotal toxicology study, showing no observed adverse effect level (NOAEL) for the LNP-MARVsaRNA-01 vaccine is 5 µg/dose when given alone, and 13 µg when administered at the same time as LNP-EBOV-saRNA-01 (4 µg) and LNP-LASSA-saRNA-01 (4 µg). In addition, data from immunogenicity studies showing that that 5 µg/dose of LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 were well tolerated and induced robust immune responses Although there is no toxicology data for vaccination with just Ebola or Lassa saRNA vaccines, the absence of adverse effects when all vaccines are administered on one occasion, provides evidence that these vaccines will be safe when given alone. The LNP-EBOV-saRNA-01 and LNP-LASSAsaRNA-01 will be assessed a 4 µg/dose to accommodate differences in manufactured concentrations and avoiding volume adjustment in the pharmacy of clinic. From an immunological standpoint the difference between 4 and 5 µg is unlikely to be significant.

4. STUDY OBJECTIVES

EML-Vac is a trial that is looking at new RNA vaccines against the Marburg, Ebola and Lassa viruses which are the major causes of viral haemorrhagic fever. The aim of the trial is to assess the safety of these vaccines alone and in combination, since this will be the first time that these have been used in humans. As this is a first-in-human trial, accrual will be limited to healthy adults between 18-50 years. Safety data will be assessed as an event rate and confidence interval with clear thresholds for pausing vaccinations in an individual and the trial (see **Section 7.1.5**). Provided the threshold for pausing vaccines in the trial is not crossed, immunogenicity will be determined by the quantity of binding antibodies 2 and 4 weeks after the last injection. As placebo does not inform the analysis for either of these endpoints, there will be no allocation to a placebo group. However, placebo doses of buffered saline will be administered I.M to ensure the participants remain blinded as to their group randomisation.

This trial is NOT looking at whether the vaccines are effective in terms of protection. It is just assessing whether and how well the immune system responds to the vaccine.

As exploratory endpoints, we will assess participants' neutralising antibody titres, B cell responses, and T cell responses to explore any associations with the induction of neutralising titres. We will also assess serum markers of innate response to both immunisations. Peripheral blood mononuclear cells (PBMCs) from participants will be processed for the isolation of antigen-specific B cells to isolate neutralising monoclonal antibodies to enhance understanding of targeted epitopes, with priority given to those showing higher serum neutralising antibody titres.

Objectives	➢ To evaluate the safety and immunogenicity of two immunisations with LNP-MARVsaRNA-01 administered IM 12 weeks apart at a 5 µg dose in 8 participants age 18-50 years. ➢ To evaluate the safety and immunogenicity of two immunisations with LNP-EBOVsaRNA-01 administered IM 12 weeks apart at one dose levels 4 µg in 8 participants age 18-50 years. ➢ To evaluate the safety and immunogenicity of two immunisations with LNP-LASSAsaRNA-01 administered IM 12 weeks apart at one dose levels 4 µg in 8 participants age 18-50 years. ➢ To evaluate the safety and immunogenicity of two immunisations with a combination of LNP-MARVsaRNA-01 (5 µg) and LNP-EBOVsaRNA-01 (4 µg) administered IM 12 weeks apart in 8 participants age 18-50 years ➢ To evaluate the safety and immunogenicity of two immunisations with a combination of LNP-MARVsaRNA-01 (5 µg), LNP-EBOVsaRNA-01 (4 µg) and LNP-LASSAsaRNA-01 (4 µg)

	administered IM 12 weeks apart in 8 participants age 18-50 years

4.1 Study Design

This is the first-in-human trial of the LNP-MARVsaRNA, LNP-EBOVsaRNA and LNP-LASSAsaRNA vaccine conducted in 18-50 year olds in a single centre with sentinel individuals for each group. Previous studies of our self-amplifying RNA vaccine demonstrated that doses up to 10 µg were well tolerated [16, 17] and indicated that a 12-week interval between doses was better than a four-week interval with respect to the magnitude of elicited responses [17].

4.1.1 LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 AND LNP-LASSAsaRNA-01 EVALUATION

The initial dosing of the sentinel participant for each of the five groups will proceed through groups 1-3 administering either LNP-MARVsaRNA-01 (5 µg), LNP-EBOVsaRNA-01 (4 µg), or LNP-LASSAsaRNA-01 (4 µg), group 4 administering LNP-MARVsaRNA-01 (5 µg) plus LNP-EBOVsaRNA-01 (4 µg) (9 µg total RNA) and group 5 administering LNP-MARVsaRNA-01 (5 µg), LNP-EBOVsaRNA-01 (4 µg), or LNP-LASSAsaRNA-01 (4 µg), (13 µg total RNA). Participants and laboratory staff will be blinded to the administered dose:

1. The first sentinel participant will receive 5 µg of LNP-MARVsaRNA, plus two buffered saline placebo injections and be invited to enter information on local and systemic reactions, into an online diary, that evening and daily thereafter for 6 days.
2. At 1 day post immunisation, the participant will attend the trial site to review AEs and donate blood to measure innate immune responses.
3. At 48 (–/+5) hours post-vaccination the team will call the participant and go through his/her diary. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the second participant may receive 4 µg of LNP-EBOVsaRNA, plus two buffered saline placebo injections.
4. At 1 day post immunisation, the second participant will attend the trial site to review AEs and donate blood to measure innate immune responses.
5. At 48 (–/+5) hours post-vaccination the team will call the second participant and go through their diaries. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the third participant may receive 4 µg of LNP-LASSAsaRNA, plus two buffered saline placebo injections.
6. At 1 day post immunisation, the third participant will attend the trial site to review AEs and donate blood to measure innate immune responses.
7. At 48 (–/+5) hours post-vaccination the team will call the third participant and go through their diaries. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the fourth participant may receive 5 µg of LNP-MARVsaRNA plus 4 µg of LNP-EBOVsaRNA, plus one buffered saline placebo injection.
8. At 1 day post immunisation, the fourth participant will attend the trial site to review AEs and donate blood to measure innate immune responses.
9. At 48 (–/+5) hours post-vaccination the team will call the fourth participant and go through their diaries. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the fifth participant may receive 5 µg of LNP-MARVsaRNA plus 4 µg of LNP-EBOVsaRNA plus 4 µg of LNP-LASSAsaRNA

10. At 1 day post immunisation, the fifth participant will attend the trial site to review AEs and donate blood to measure innate immune responses.
11. At 48 (–/+5) hours post-vaccination the team will call the fifth participant and go through their diaries. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the dose evaluation cohort of remaining 35 participants may start to be enrolled and randomised into one of the 5 groups to receive their first immunisation.
12. Sentinel participants will be evaluated again as above after receiving their second dose. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the cohort of remaining 35 participants may receive their second dose

The steps above describe the fastest plan to enrol into the 5 groups and is justified based on previous experience with this self-amplifying RNA platform. However, if there are persistent Grade 3 reactions observed in any of the sentinels, the team will invite the affected participant to attend the trial site in order to evaluate these and will proceed to vaccinate a second sentinel within the same group. The group will not be changed until at least 1 participant has provided acceptable safety data to at least 48 (–/+5) hours post-vaccination. If 2 or more of the participants at any dose level in this part of the trial have persistent Grade 3 (or worse) reactions, or if 1 or more has a serious adverse reaction, the dose will not be expanded, and the trial stopping rules described below (7.1.5) will be triggered.

4.2 Study Outcome Measures

Outcome Measures	➢ Solicited local injection site reactions starting within 7 days of administration of the vaccine: pain, tenderness, erythema, swelling ➢ Solicited systemic reactions starting within 7 days of administration of the vaccine: pyrexia, fatigue, myalgia, headache, chills, arthralgia ➢ Unsolicited adverse reactions (ARs) throughout the trial period (including serious ARs) ➢ Serious Adverse Events throughout the trial period ➢ Unsolicited adverse events throughout the trial period ➢ The titre of vaccine-induced serum IgG binding antibody responses to the Marburg virus, Ebola virus and Lassa virus fever virus surface glycoproteins 2 weeks after the second vaccinations
Exploratory Aims/Objectives	➢ To characterise the humoral and cellular immune responses to LNP-MARVsaRNA-01 administered twice at one dose ➢ To characterise the humoral and cellular immune responses to LNP-EBOVsaRNA-01 administered twice at one dose ➢ To characterise the humoral and cellular immune responses to LNP-LASSAsaRNA-01 administered twice at one dose

	➢ To characterise the humoral and cellular immune responses to LNP-MARVsaRNA-01 and LNP-EBOVsaRNA-01 administered together twice at one dose ➢ To characterise the humoral and cellular immune responses to LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 administered together twice at one dose
Exploratory Outcome Measures	➢ Cell-mediated vaccine-induced immune responses measured by T and B cell ELISpot in participants ➢ Cell-mediated vaccine-induced immune responses measured by flow cytometry and intracellular cytokine staining ➢ Serum neutralising antibodies in a Marburg, Ebola or Lassa pseudovirus-based neutralisation assay ➢ The profile of class and sub-class of antibody response ➢ Serum markers of innate immune response ➢ Purification of antigen-specific B cells to isolate neutralising monoclonal antibodies to enhance understanding of targeted epitopes

5. PARTICIPANT ENTRY

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

The eligibility criteria are the standards used to ensure that only medically appropriate participants are considered for this trial. Participants not meeting the criteria should not join the trial. For the safety of the participants, as well as to ensure that the results of this trial can be useful for making treatment decisions regarding other patients with similar health statuses, it is important that no exceptions be made to these criteria for admission to the trial.

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.

5.1 Pre-Randomisation Investigations

Informed consent to enter into the trial must be obtained from participants after explanation of the aims, methods, benefits and potential hazards of the trial and *before* any trial-specific procedures are performed or any blood is taken for the trial. Participants must be willing and able to provide informed consent (as detailed in **Section 5.2**). This therefore excludes: persons deprived of their liberty by a judicial or administrative decision, persons under psychiatric care and persons admitted to a health or social institution for purposes other than research; and persons who are the subject of a legal protection measure or who are unable to express their consent.

It must be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment.

Signed consent forms must be kept by the investigator and documented on the case report form (CRF) and a copy given to the participant. With consent, the participant's GP will be sent a letter informing them of their patient's intention to participate in the trial and requesting that they corroborate their patient's medical history. Corroboration may also be obtained via the trial team accessing patient's electronic care summaries, GP and other medical records from local systems, or via participants bringing their medical care summaries from their GP to the trial team. However, participants may be enrolled based on the medical history given at the screening visit only, at the investigator's discretion.

Investigator sites registered with The Overvolunteering Protection System (TOPS) should run checks for the purposes of assessing exclusion criterion 13.

Screening procedures and investigations are listed in **Table 2** and covered in more detail in **Section 5.2** below**.**

5.2 Participant Inclusion Criteria

1. Healthy adults, aged 18-50 years on the day of screening
2. Willing and able to provide written informed consent
3. If female and of childbearing[i] potential, willing to use a highly effective method[ii] of contraception from screening until 18 weeks[iii] after last injection
4. If male and not sterilised, willing to avoid impregnating female partners[iv] from screening until 18 weeks[iii] after last injection
5. Willing to avoid all other vaccines from within 4 weeks before and after the first and second injection
6. Willing and able to comply with visit schedule, complete online diaries and provide samples
7. Willing to abstain from donating blood for three months after the end of their participation in the trial or longer, if necessary
8. Willing to grant authorised persons access to his/her trial-related medical record and GP records either directly or indirectly

i A woman will be considered of childbearing potential following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A post-menopausal state is defined as no menses for 18 months without an alternative medical cause.

ii The following methods are considered highly effective:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation – oral, intravaginal or transdermal;
- progestogen-only hormonal contraception associated with inhibition of ovulation – oral, injectable or implantable
- intrauterine device (IUD);
- intrauterine hormone-releasing system (IUS);
- bilateral tubal occlusion;
- vasectomised partner, where the vasectomised partner has received medical assessment of the surgical success; and
- sexual abstinence, defined as refraining from heterosexual intercourse – must be the preferred and usual lifestyle of the participant.

iii Nonclinical studies of saRNAs [37] showed maximal expression of the vaccine immunogen at 7 days post-immunisation, approaching baseline by 3 weeks post-immunisation, with some residual very low expression seen out to 9 weeks. Biodistribution studies with LNP-MARVsaRNA, LNP-EBOVsaRNA and LNP-LASSAsaRNA are planned, but in the absence of data we wish to take a conservative approach to the contraception period and require an 18-week washout period.

iv Through the use of condoms or sexual abstinence (see definition in footnote ii above)

It is recommended that participants have an up to date vaccination status for any required immunisations.

5.3 Participant Exclusion Criteria

1. Pregnant or lactating
2. Has a significant clinical history, physical finding on clinical examination during screening, or presence of a disease that is active or requires treatment to control it, including cardiac, respiratory, endocrine, metabolic, autoimmune, liver, neurological, oncological, psychiatric, immunosuppresive/immunodeficient or other disorders which in the opinion of the investigator is not compatible with healthy status, may compromise the volunteer's safety, preclude vaccination or compromise interpretation of the immune response to vaccine. Individuals with mild/moderate, well-controlled comorbidities are allowed.
3. History of Marburg virus, Ebola virus and/or Lassa virus infection
4. History of anaphylaxis or angioedema
5. History of severe or multiple allergies to drugs or pharmaceutical agents
6. History of severe local or general reaction to vaccination defined as:
    a. **local**: extensive, indurated redness and swelling involving most of the arm, not resolving within 72 hours
    b. **general**: fever ≥39.5 °C within 48 hours; bronchospasm; laryngeal oedema; collapse; convulsions or encephalopathy within 72 hours
7. Ever received an experimental or authorised vaccine against Ebola, Marburg, or Lassa fever viruses
8. Receipt of any immunosuppressive agents within 18 weeks of screening by any route other than topical
9. Detection of antibodies to hepatitis C
10. Detection of antibodies to HIV
11. ALT/AST exceeding the upper limits of normal ≤1.0
12. Abnormal urine analysis result (3 x repeats >24 hours apart are allowed)
13. History or current diagnosis of myocarditis, pericarditis, or other significant inflammatory heart disease, or unexplained chest pain, palpitations or dyspnoea with ECG changes at screening. Grade 1 and above abnormalities in routine laboratory parameters (see **Table 4**) using the FDA toxicity table Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, taking account of local laboratory reference ranges. https://www.fda.gov/media/73679/download
14. Participating in another clinical trial with an investigational drug or device or treated with an investigational drug within 28 days of screening.

15. Has received an immunisation within 28 days of screening

5.4 Withdrawal criteria

Participants are free to withdraw from the trial at any time without giving a reason. Those who withdraw will be encouraged to attend a final visit, primarily for safety reasons.

The investigator may at any time withdraw a participant if his/her participation is no longer considered safe or relevant.

The date and reason (if given) for withdrawal must be recorded in the CRF.

If a participant for any reason withdraws or is withdrawn from the trial or has a significant protocol deviation, he/she will be replaced to ensure the per-protocol targets are achieved for the primary endpoints.

In consenting to the trial, participants are consenting to trial treatment, trial follow-up and data collection.

An individual participant may stop injections early or be stopped early for any of the following reasons:

- Pregnancy in the participant
- Unacceptable toxicity that precludes further injections
- Intercurrent illness that prevents further injections including emergent conditions that meet the exclusion criteria
- Withdrawal of consent for injections by the participant

A decision by the Medical Delegate to discontinue further injections should be discussed with the local PI, and the Chief Investigator/Medical Delegate should be informed using the expedited reporting method described in **Section 8**. They may recommend additional investigations and/or referral for a specialist opinion.

As participation in the trial is entirely voluntary, a participant may choose to discontinue the trial treatment at any time without penalty or loss of benefits to which they are otherwise entitled. Although the participant is not required to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason while fully respecting the participant's rights. The implications of withdrawing and how this may impact on the results and interpretation of the trial, will be explained to the participant.

Participants should remain in the trial for the purpose of follow-up and data analysis (unless they withdraw their consent from all stages of the trial, in which case refer to **Section 9.8**).

Data that are already collected from participants who stop follow-up early will be included in the analysis.

6. RANDOMISATION AND ENROLMENT PROCEDURES

6.1 Randomisation and Registration Practicalities

Investigator site staff will know the allocated dose, but participants and laboratory staff will not. Online randomisation software (Sealed Envelope) will be used at the site to randomise participants in the EML-Vac trial.

6.2 Unblinding

There will be no unblinding of the participants or laboratory staff during the course of the trial. Unblinding will only take place in an emergency situation.

In case of an emergency, the Investigator has the sole responsibility for determining if unblinding of a participant's study intervention assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the Investigator decides that unblinding is warranted, the Investigator may contact the Sponsor to discuss the situation prior to unblinding a participant's study intervention assignment unless this could delay emergency treatment for the participant. If a participant's study intervention assignment is unblinded, the Sponsor must be notified within 24 hours of this occurrence. The date and reason for the unblinding must be recorded.

The trial EDC system will include an automated unblinding facility, in case unblinding is required. In the event that emergency unblinding of an individual participant is required, authorised staff (as documented on the delegation log) will follow trial procedures to unblind the participant in question and proceed with expedited reporting if required.

6.3 Co-enrolment Guidelines and Reporting

Co-enrolment in other clinical trials with an investigational or non-investigational drug or device is not permitted, and individuals who have been treated with an investigational drug within 56 days before the first vaccination will be excluded.

All other co-enrolments should be discussed with the Chief Investigator and will be decided on a case-by-case basis.

7. TREATMENT OF PARTICIPANTS

7.1 Treatment Arms

Participants will each receive two IM doses of either LNP-MARVsaRNA (5 µg) (Group 1), LNP-EBOVsaRNA (4 µg) (Group 2), LNP-LASSAsaRNA (4 µg) (Group 3), LNP-MARVsaRNA (5 µg) plus LNP-EBOVsaRNA (4 µg) (Group 4) or LNP-MARVsaRNA (5 µg), plus LNP-EBOVsaRNA (4 µg) plus LNP-LASSA saRNA (4 µg) (Group 5) into the deltoid muscle at Week 0 and week 12 (see **Table 3** below). The volume of each injection will be 0.5 mL.

Storage, dispensing, reconstitution and dilution of IMP, the volume for injection and method of administration for each dose level will be described in the EML-Vac Pharmacy Manual.

The saRNA (drug substance) and LNP-saRNA bulk drug product have been manufactured to GMP grade by the Centre for Process Innovation, Darlington, UK and fill finish and labelling has been performed by Nova Laboratories, Wigston, UK. Release of vaccine vials and shipping to the clinical site will be carried out by Nova Laboratories, Wigston, UK.

Table 3: LNP-MARVsaRNA, LNP-EBOVsaRNA and LNP-LASSAsaRNA candidate and dispensing schedule

Study component	**Description**	**Route**	**Dose**	**Visit 2 (day/week)** **Prime**	**Visit 5(day/week)** **Boost**
Group 1	LNP-MARVsaRNA-01, plus two buffered saline placebo	IM	5 µg (0.5 mL), plus 2 x 0.5 mL buffered saline	D0/W0	D84/W12
Group 2	LNP-EBOVsaRNA-01, plus two buffered saline placebo	IM	4 µg (0.5 mL), plus 2 x 0.5 mL buffered saline	D0/W0	D84/W12
Group 3	LNP-LASSAsaRNA-01, plus two buffered saline placebo	IM	4 µg (0.5 mL), plus 2 x 0.5 mL buffered saline	D0/W0	D84/W12
Group 4	LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01, plus one buffered saline placebo	IM	5 µg (0.5 mL) + 4 µg (0.5 mL) (9 µg per dose), plus 1 x 0.5 mL buffered saline	D0/W0	D84/W12
Group 5	LNP-MARVsaRNA-01, LNP-EBOVsaRNA-	IM	5 µg (0.5 mL) + 4 (0.5 mL) µg +	D0/W0	D84/W12

	01, LNP-LASSA saRNA-01		4 µg (0.5 mL) (13 µg per dose)		

7.1.1 LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 VACCINES

LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 are aqueous formulations of RNA encapsulated in Lipid Nano Particles (LNP) which are provided at a target concentrations of 10 µg /mL (LNP-MARVsaRNA-01) or 8 µg/mL (LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01) for IM injection.

LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 vaccines are manufactured by Centre for Process Innovation (CPI) in accordance to GMP standard on behalf of the sponsor, who is also responsible for the product development.

LNP-MARVsaRNA-01, LNP-EBOVsaRNA-01 and LNP-LASSAsaRNA-01 are fill finished by Nova Laboratories and shipped directly to the trial site. Nova Laboratories will supply the IMPs with EML-Vac-specific labels, according to GMP.

The drug products are presented as a white to off-white suspension; in Type I glass vials, with a butyl rubber stopper and aluminium crimp seal. A 0.65 mL fill is provided to allow for 0.5 mL for administration as per instructions in the EML-Vac pharmacy manual.

These vaccine candidates are not classified as a genetically modified organism (GMO).

7.1.2 Placebo Injections

While there is no placebo group planned within this trial, to ensure all participants remain blinded to their group allocation, three injections will be given at each dosing timepoint, irrespective of group. Two placebo doses will be given to participants who are randomised to groups 1-3 and one placebo dose will be given to participants who are randomised to group 4. The placebo dose will consist of 0.5 mL buffered saline.

7.1.3 Administration

Vaccines and placebo should be administered intramuscularly in the deltoid muscle of the upper arm using a 23G 1-inch needle. The participant may choose which arm. All injections should be in the same arm.

To keep the participants blinded, syringes containing the saline and the active drug substance will be covered with a blinding label before entering the room to ensure the participants cannot observe the contents of the syringe.

Participants will be observed for at least 60 minutes after the injection.

7.1.4 Storage

The vaccines will be stored in a secure, limited access storage area under the specified storage requirements. The vaccines will be stored according to the Pharmacy Manual and the arrangements will be reviewed at site initiation.

7.1.5 Dose Modifications For Toxicity & Schedule Interruptions

Target visit dates (based on the enrolment date) should be adhered to as far as possible, based on the allowable window (detailed in Table 2). The schedule may be interrupted if a participant has symptoms or signs on the day of scheduled injection, and the investigator considers it best to defer the injection. A temperature over 37.5°C would prevent injection on the day. Clinicians should consult the PI/Medical Delegate if there are any Grade 1 (mild) symptoms or signs listed in **Table 4**. The participant will be asked to return for review within the ideal window period of the scheduled injection. Provided the injection is administered during the window period outlined in the paragraph below for missed visits, this will not be a protocol deviation.

The PI or Medical Delegate should interrupt the vaccine schedule and inform the Chief Investigator within 24 hours using the safety email if there is a confirmed:

- Grade 3 (severe) or worse solicited adverse event that has persisted for more than 72 hours regardless of relationship

  or

- other Grade 3 (severe) or worse adverse event that is possibly, probably or definitely related to vaccine or
- serious adverse reaction regardless of grade

The Chief Investigator/Delegate may recommend further investigations or referral to an independent expert to support the clinical management of the participant. Such events are highly likely to result in discontinuation, but a decision to resume will be taken by the PI with the participant, and only with the approval of the independent members of the TSC.

Injections for all participants will be paused pending a review of all safety data by the Trial Steering Committee if two participants out of 20, and the equivalent (10%) thereafter develop persisting Grade 3 (severe) adverse reactions within 7 days of immunisation, or any participant experiences a serious adverse reaction at any time. Approval of an amendment from the competent authorities is required before the trial can be resumed.

If two participants in any Group experience a Grade 3 reaction the trial will be paused and only resumed after approval of a substantial amendment.

Participant visits will continue during a pause. Missed injections will be rescheduled and the remaining trial visits rescheduled/repeated accordingly. There are no known important risks associated with the LNP-MARVsaRNA, LNP-EBOVsaRNA or LNP-LASSAsaRNA vaccine. There is no known antidote. Participants who are overdosed should be closely monitored and provided with medical support per the investigator's judgment.

7.1.6 Discontinuation Of Injections in All Participants

Protocol planned interruptions and discontinuations will be reported to the Chief Investigator/Medical Delegate who will make a recommendation regarding immediate reporting to the TSC. The Chief Investigator and ICL staff responsible for preparing reports for the TSC will forward the clinical report and the dose allocation. All serious adverse reactions will be unexpected and reported on to the authorities (see **section**

8.3) by the Chief Investigator. The TSC may recommend to the Sponsor that injections are discontinued in all participants.

7.2 Treatment Data Collection

Study staff will collect vaccination information on the worksheets and enter data in the Electronic Data Capture (EDC) system. Pharmacy staff, or other staff delegated by the PI, will maintain the IMP accountability logs which will be securely stored.

7.2.1 Interactions with other Drugs and Vaccines

Non-trial treatments will be reviewed before each injection to ensure that the participant remains eligible and able to receive the vaccine. There are no known interactions between the LNP-MARVsaRNA, LNP-EBOVsaRNA or LNP-LASSAsaRNA vaccine and other drugs. All medications taken by participants from screening to the final visit will be recorded in the EDC system.

Participants should avoid taking systemic immunosuppressants, which might reduce their immune response to the vaccine.

Vaccination with authorised vaccines should be avoided from 28 days before screening until 28 days after the second injection because they may impact on the assessment of immune response to trial vaccine, and it is plausible that the self-amplification process is ongoing for this period of time.

7.2.2 Concomitant Therapies

Systemic immunosuppressive agents, such as corticosteroids, may not be administered during the trial. Dermal steroids are permitted, but not if applied to the IM injection site.

Vaccination with authorised vaccines should be avoided from 28 days before screening until 28 days after the second injection, as they may affect the assessment of the immune response to the trial vaccine. It is plausible that the self-amplification process continues during this period.

7.3 Dispensing and Accountability

7.3.1 Dispensing

The pharmacist, or other person delegated by the PI, will ensure that the vaccines are dispensed in accordance with the protocol and Pharmacy Manual and local procedures as appropriate. Local working instructions will be reviewed at site initiation, if applicable.

A vaccine accountability log will be kept to record the identification of the participant to whom the vaccine was given and the date they received it. Any damaged or unused vials that are returned will also be documented. The log will be checked during the monitoring visits and at the end of the trial.

7.3.2 ACCOUNTABILITY

The Pharmacist, or other person delegated by the PI, will ensure that all injection products are dispensed in accordance with the protocol, Pharmacy Manual and local procedures, and that records are maintained of receipt, dispensing and destruction of all supplies.

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 monitors. The Sponsor and the PI will retain copies of the complete IMP accountability records and copies will be provided to the supplier of the vaccines.

All used vials of injections will be destroyed immediately after use in compliance with the instruction manual.

The site will be instructed to either return unused vaccine to the supplier, or to destroy it at site. Following IMP destruction, the pharmacist, or other person delegated by the PI, at the site must complete a certificate of IMP destruction and send it to the PI with copies to the Sponsor.

All injections will be administered by site staff and recorded on worksheets and in the Electronic Data Capture (EDC) system. If an injection is not given within the ideal window (see **Table 2**), this will be recorded in the EDC system together with the reason. Compliance with the schedule will be reviewed each week and reported to the TMG monthly.

8. PHARMACOVIGILANCE

The principles of GCP require that both investigators and Sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials. These procedures are described in this section of the protocol. **Section 8.1** lists definitions, **Section 8.3** gives details of reporting procedures. Collection of adverse event data must begin from the time that informed consent if given by the participant.

8.1 Definitions

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

Table 4. Definitions

TERM	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
Adverse Reaction (AR)	All untoward and unintended responses to an IMP related to any dose administered.
Unexpected Adverse Reaction (UAR)	an AR, the nature or severity of which is not listed in the reference safety information (RSI) e.g. list of expected medical events within investigator's brochure for an unapproved investigational product or section 4.8 of the summary of product characteristics (SmPC) for an authorised product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)	Any untoward medical occurrence or effect that at any dose: • **Results in death.** • **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.* • **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation.** • **Results in persistent or significant disability or incapacity.** • **Is a congenital anomaly or birth defect.**
Suspected Unexpected Serious Adverse Reaction (SUSAR):	any suspected adverse reaction related to an IMP that is both unexpected and serious.

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

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation.

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

8.1.1 Adverse Events

Adverse Events include:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the trial treatment

8.1.2 Expected Adverse Events

The safety and reactogenicity of the IMP is expected to be comparable to the safety observed in previous clinical trials conducted where saRNA have been tested with other vaccine antigens in Phase I clinical trials (COVAC1, COVAC Uganda). See summary table 5 below for systemic and local reactions reported in individuals who received a 5 µg dose of LNP-nCoVsaRNA vaccine.

Up to 100% of participants are expected to experience grade 1-2 systemic reactions including fatigue, myalgia, headaches, chills/shivering and arthralgia. Similarly, close to 100% of participants are expected to experience grade 1-2 local reactions, with pain and tenderness/discomfort likely to be the most common local reactions.

Uncommon reactions include high temperature (≥ 38ºC), nausea and vomiting, and erythema and induration at site of injection.

Vasovagal syncope (i.e. fainting), may occur in this population of subjects. It will be attempted to minimise the occurrence of this reaction, by allowing the subjects to lie down during administration of vaccine and blood drawings.

Table 5. Adverse Events in participants the COVAC1 clinical trial who received a 5 µg dose of LNP-nCoV-saRNA

		LNP-nCoVsaRNA dose
		5.0 µg N=24
Systemic	**Any**	
	Normal	0 (0.0%)
	Grade 1	8 (33.3%)
	Grade 2	14 (58.3%)
	Grade 3	2 (8.3%)
	Temperature	
	Normal	21 (87.5%)
	Grade 1	2 (8.3%)
	Grade 2	0 (0.0%)
	Grade 3	1 (4.2%)
	Chills/shivering	
	Normal	10 (41.7%)
	Grade 1	11 (45.8%)
	Grade 2	3 (12.5%)
	Myalgia (flu-like general muscle aches)	
	Normal	5 (20.8%)
	Grade 1	13 (54.2%)
	Grade 2	6 (25.0%)
	Grade 3	0 (0.0%)
	Arthralgia	
	Normal	11 (45.8%)
	Grade 1	9 (37.5%)
	Grade 2	4 (16.7%)
	Grade 3	0 (0.0%)
	Fatigue	
	Normal	3 (12.5%)
	Grade 1	11 (45.8%)
	Grade 2	10 (41.7%)
	Grade 3	0 (0.0%)
	Headache	
	Normal	2 (8.3%)
	Grade 1	14 (58.3%)
	Grade 2	8 (33.3%)
	Nausea	
	Normal	19 (79.2%)
	Grade 1	5 (20.8%)
	Grade 2	0 (0.0%)
	Grade 3	0 (0.0%)
	Vomiting	
	Normal	24 (100.0%)
	Grade 3	0 (0.0%)
Local	**Any**	
	Normal	1 (4.2%)
	Grade 1	15 (62.5%)
	Grade 2	8 (33.3%)
	Pain	
	Normal	6 (25.0%)
	Grade 1	16 (66.7%)
	Grade 2	2 (8.3%)
	Tenderness/discomfort	
	Normal	2 (8.3%)
	Grade 1	14 (58.3%)
	Grade 2	8 (33.3%)
	Erythema/redness	
	Normal	23 (95.8%)
	Grade 1	1 (4.2%)
	Grade 2	0 (0.0%)
	Induration/swelling	
	Normal	23 (95.8%)
	Grade 1	1 (4.2%)

8.1.3 Exempted Adverse Events

Adverse Events do not include:

- Medical or surgical procedures; the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen

8.1.4 Other Notable Events

Notable adverse events which impact on the injection schedule and therefore require expedited (within 24 hours of the investigator becoming aware of the event) reporting whether or not they meet the serious criteria include:

- Grade 3 (severe) and above solicited adverse events which last more than 72 hours and Grade 3 and above laboratory adverse events that are confirmed on repeat testing if possible
- Any adverse event leading to a clinical decision to interrupt or discontinue the injection schedule
- Pregnancy within 18 weeks of an injection

8.1.5 Pregnancy

Pregnancy is not an adverse event. However, any pregnancy that occurs during the conduct of the study and for 18 weeks after the last vaccination in a female participant or the partner of a male participant must be reported and followed to outcome and for six months after delivery to determine if a serious adverse event is observed. It should be reported to the Sponsor (Imperial College London) within 24 hours of the investigator becoming aware of a pregnancy. The pregnancy should be reported using the Notable Event form and Pregnancy form in the EDC system. Collecting information about the outcome from partners is subject to consent from the partner.

In the event of a pregnancy in a female participant, injections will be discontinued.

All Notable events, including pregnancy must be reported immediately using the EDC system. Under no circumstances should this exceed 24 hours following knowledge of the event.

8.2 Causality

All non-serious AEs and ARs, whether expected or not, should be recorded in the participants' medical notes and reported on the AE form in the EDC system on the day of the visit from screening through to the last trial visit for each subject.

SAEs and SARs should be notified to the sponsor (Imperial College London) within 24 hours of the investigator becoming aware of the event via the EDC system Under no circumstance should this exceed 24 hours following knowledge of the SAE or SAR. Immediate reporting should allow the sponsor to take the appropriate measures to address any potential new risks in the trial.

Participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow-up should continue after completion of the protocol if necessary.

8.2.1 SERIOUSNESS

When an AE or AR occurs, the investigator responsible for the care of the patient must first assess whether or not the event is serious using the definition given in **Table 4**. If the event is serious, then an SAE details must be entered in the EDC system within 24 hours. If the event is not an SAE but meets the notable event criteria (see **section 8.1**) complete a Notable Event Form and forward the report within 24 hours via the same mechanism.

8.2.2 SEVERITY OR GRADING OF ADVERSE EVENTS

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded using the FDA: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

8.2.3 CAUSALITY

The investigator must assess the causality of all events in relation to the trial therapy using the definitions in **Table 6**. There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an unrelated AE. If the causality is assessed as possibly, probably or definitely related, then the event is classified as an AR.

The investigator will also be asked to record concomitant medications and to assess the relationship of the event to each of these. If a serious adverse event is considered related to a concomitant medication, the investigator should report this to the MHRA via a yellow card.

8.2.4 EXPECTEDNESS

If there is at least a possible involvement of any trial treatment given to the participants, the Chief Investigator, on behalf of the Sponsor, will make an initial assessment of the expectedness of each SAR. An unexpected serious adverse reaction is one not previously reported in the current Reference Safety Information, which is in the LNP-MARVsaRNA/LNP-EBOVsaRNA/LNP-LASSAsaRNA Investigator's Brochure. A SAR that is more frequent or more severe than stated in the reference safety information would also be considered to be unexpected. If an SAR is assessed as being unexpected, it becomes a SUSAR.

Table 6: Assigning Type of AE Through Causality

RELATIONSHIP	DESCRIPTION	TYPE OF EVENT
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	AR
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	AR
Possible	There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (for example, the patient's clinical condition, other concomitant treatments).	AR

Unlikely	There is little evidence to suggest that there is a causal relationship (for example, the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (for example, the patient's clinical condition, other concomitant treatment).	Unrelated AE
Unrelated	There is no evidence of any causal relationship	Unrelated AE

If an AE is considered to be related to trial treatment and the injections are interrupted please refer to **Section 7.1.4.**

8.3 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the trial coordination centre in the first instance. A flowchart is given below to aid in the reporting procedures (Figure 3).

8.3.1 NON-SERIOUS AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the trial coordination centre within one month of the form being due.

8.3.2 SERIOUS AR/AEs

Fatal or life-threatening SAEs and SUSARs should be reported on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

8.3.3 SAEs

An SAE form should be completed and faxed to the trial coordination centre for all SAEs within 24 hours. However, relapse, death and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

8.3.4 SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

Complete the SAE case report form & send it immediately (within 24 hours,), signed and dated to the trial coordination centre together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

Contact the trial coordination centre by phone and then send the completed SAE form to the trial coordination centre within the following 24 hours as above.

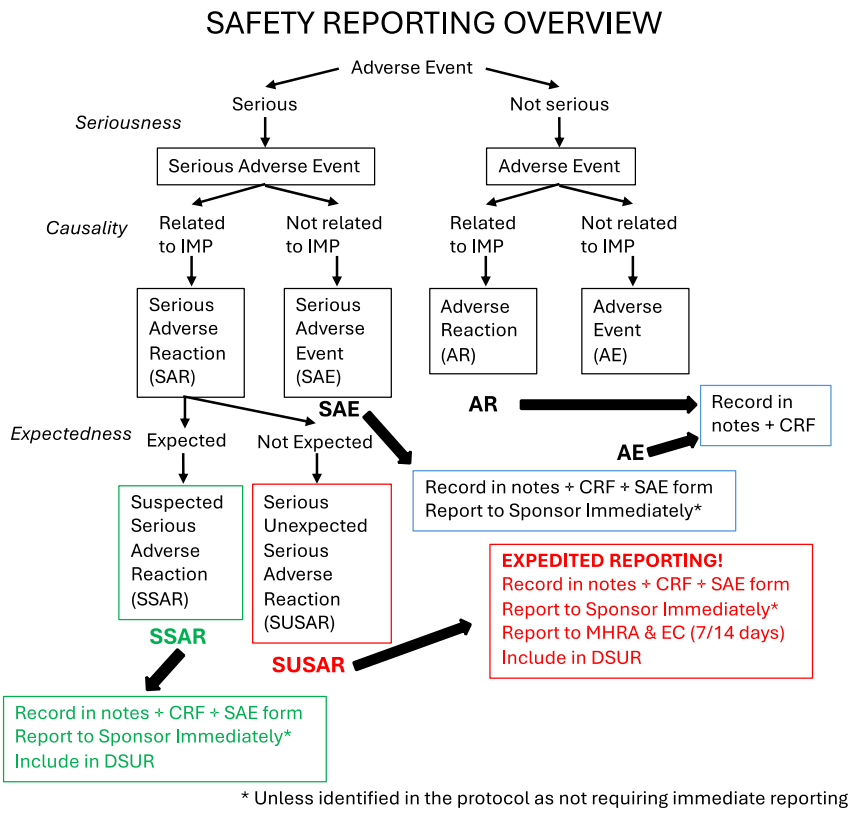
The trial coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the trial according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the trial.

The minimum criteria required for reporting an SAE are the participant's trial number and date of birth, name of investigator reporting, the event, and why it is considered serious.

The SAE details should be entered in the EDC system by an investigator (a clinician named on the Signature List and Delegation of Responsibilities Log, who is responsible for the participant's care), with due care being paid to the grading and causality, as outlined above. In the absence of the responsible investigator, the data should be entered by a member of the site trial team. The responsible investigator should subsequently check the SAE details on the EDC system, make changes as appropriate, as soon as possible.

Follow up details on the SAE should be entered in the EDC system as they become available. Extra, annotated information and/or copies of test results may be provided separately and can be emailed securely. The participant must be identified by trial number, month and year of birth and initials only. The participant's name should not be used on any correspondence and should be deleted from any test results.

Figure 3. Safety reporting flowchart



SAE REPORTING

Contact details for reporting SAEs and SUSARs

RGIT.ctimp.team@imperial.ac.uk

CI email marta.boffito@nhs.net

Please send SAE forms to: xxx

Tel: xxx (Mon to Fri 09.00 – 17.00)

8.3.5 Developmental Safety Update Reports (DSUR)

Developmental Safety Update Reports (DSUR) will be submitted to the Sponsor and Regulatory Authority in accordance with local (MHRA) regulatory requirements.

9. ASSESSMENTS & FOLLOW-UP

Potentially eligible participants will be identified through adverts, mainstream, community and social media. They will be able to request written information and be able to make an appointment for a screening visit for further discussion should they prefer to do this in person.

The Delegation Log will determine which members of the trial team are authorised to conduct the assessments and procedures described in this section.

9.1 Trial Assessment Schedule

The assessments in clinic and online, samples and volumes to be collected are outlined in: **Table 2**:

Collection of adverse event data must begin from the time that informed consent if given by the participant.

The maximum blood volume drawn from a participant in the EML-Vac trial, who completes 52 weeks of follow-up in the trial period, is approximately 403 mL. These volumes do not include the blood volume that would be required if additional diagnostic tests are needed, or safety tests have to be repeated. The required volumes per visit together with sample collection and processing guidelines are described in detail in the Laboratory Manual.

The visits in the trial assessment schedule are: a screening visit (Visit 1); an enrolment visit which must take place within 8 weeks of the screening visit and is defined as Week 0 (Visit 2); injection visits at Weeks 0 and 12 (Visits 2 and 5); safety visits at Weeks 1, 4, 13, 14, 16, 24 and 36 (Visits 3, 4, 6, 7, 8, 9 and 10) and a final visit at Week 52 (Visit 11). The visit windows are provided in **Table 2**.

Information on vaccine reactions will be solicited directly from participants for one week following each injection using the participant facing tools in the EDC system. Participants will attend a site visit 1 day after each injection.

9.2 Procedures During the Screening Period

Screening will take place as close to the planned enrolment as possible, no longer than 8 weeks before enrolment but allowing time for all relevant laboratory assessment to check participant eligibility.

9.2.1 INFORMED CONSENT

Participants will be provided with information about the product, trial design and data collection in writing. They will have the opportunity to ask questions in person or on the phone.

Key points to communicate during the informed consent process:

- That we do not know if the vaccine will prevent Marburg virus, Ebola virus or Lassa virus infection
- That the vaccine does not contain any infectious component and cannot cause symptoms associated with Marburg virus, Ebola virus or Lassa virus infection
- That pregnancy is to be avoided until 18 weeks after the second injection as the safety of the vaccine is not known

If they are happy to proceed, they will be asked to indicate their informed consent in writing prior to answering questions about their health and providing samples for the screening investigations. Laboratory investigations or other screening procedures defined in this protocol that have been performed at the local clinical sites(s) for some other purpose (routine NHS visit, healthy volunteer database screening, other research) may be used for the purpose of screening so long as the date they were performed is within the window period defined in this protocol.

A copy will be provided to the participant and one copy kept in the trial file according to local procedures.

9.2.2 ELIGIBILITY

To assess eligibility, demographic information, a past and current medical history, and details of all current medication will be collected on worksheets and transcribed into the EDC system. Details of contraception to assess the risk of pregnancy arising in the participant/their partner will also be collected.

The screening examination will include weight (kg), height (cm), temperature, blood pressure, pulse, oxygen saturation, inspection of the skin to exclude severe eczema and respiratory, cardio-vascular, abdominal and neurological examination. An assessment of cervical, axillary and inguinal lymph nodes will also be undertaken.

Clinical Investigators should carefully assess participants who have pre-existing conditions and consider the maximum severity in the past, the extent of treatment needed to control the condition at the time of screening, and how long the participant has been stable for on their current treatment. If further investigation is required, including an additional ECG, or the Clinical Investigator suspects the need for a change in treatment, the participant should be considered ineligible under exclusion criterion number 2 (see **Section 5.3**).

9.2.3 INVESTIGATIONS

Blood will be collected for analysis of routine parameters and processed in the local NHS laboratories for full blood count and biochemistry. The parameters are listed in a footnote to **Table 2** but additional tests to be conducted at screening are:

- Hepatitis C antibody
- HIV antibody
- Gamma glutaryl transferase
- Urine dipstick for glucose, blood, WBC, nitrite and protein

Volunteers with Grade 1 abnormalities in haematology, biochemistry or urinalysis parameters at the initial screening visit may have the tests repeated once and may enter the trial if the repeat result is normal, at the investigator's discretion. In the event the repeat reveals a new Grade 1 abnormality the volunteer will be considered ineligible.

For female participants of childbearing potential, a pregnancy test will be performed by analysis of a urine sample for Human Chorionic Gonadotrophin (HCG).

9.3 Procedures at Enrolment

9.3.1 ELIGIBILITY

Study staff will review any new medical conditions or new medications since the screening data were collected and, for female participants, any changes in contraception. Temperature, blood pressure, pulse, oxygen saturation and inspection of the skin will be repeated at the enrolment visit in case anything has changed. Study staff will repeat the other aspects of the physical examination done at the screening visit if symptoms indicate the need for this. All this information will be recorded on the EDC system.

A temperature over 37.5°C would prevent injection on the day. Clinicians should consult the PI/Medical Delegate if there are any Grade 1 (mild) symptoms or signs listed in **Table 4.**

Blood for immunogenicity testing will be collected. Samples will be sent to the central laboratory at Imperial College London for analysis.

For female participants of childbearing potential, a pregnancy test will be performed by analysis of a urine sample for Human Chorionic Gonadotrophin (HCG) and a negative result will be confirmed before proceeding to enrolment.

9.3.2 ENROLMENT/RANDOMISATION

Confirmation of eligibility will depend on entering screening data in the EDC system.

9.3.3 INJECTION

Study staff will draw up the vaccine product (0.5 mL) or placebo (0.5 mL) from the vial and administer it into the deltoid muscle of the participant’s choice and record this in the EDC system including the time of injection. All injections should be administered to the same arm.

Following injection participants should remain in clinic for at least 60 minutes, in order to assess and record solicited adverse events within 25-60 minutes following injection. Study staff will go through the detail to be added in the online vaccine diary cards with participants and explain how and when to complete these over the following 7 days.

9.4 Procedures for Assessing Safety

Vaccines are associated with a number of well-characterised local, systemic and laboratory reactions referred to as solicited adverse events (**Table 4**). These adverse events will be purposively collected.

Local and systemic assessments will take place on the day of each injection before the injection, and 25-60 minutes after the injection. **Participants should remain in the clinic for at least one hour after each injection.**

Participants will be asked to complete vaccine diary cards on the EDC system to assist collection and grading of local and systemic adverse events that start within 7 days of the injection. They will be advised to contact trial staff if any events are Grade 3 (severe), and these will be flagged in the EDC system for immediate attention with a view to organising an early visit. Information entered will be checked by staff at site 1 day after each injection and at the next visit.

Blood (~10 mL) for routine safety parameters will be collected at all trial visits. If the total bilirubin is elevated, trial staff will request a result for conjugated bilirubin in order to grade the abnormality and determine any action to be taken with respect to further investigation and interruption to the vaccine schedule.

Vital signs (BP, HR, oxygen saturation and oral temperature) will be measured at every trial visit.

Physical examinations of the injection site, and other body systems if indicated, will be performed on the day of each vaccination, and 1 week after. Symptom-directed physical examinations will be performed at all other follow-up visits.

9.5 Procedures for Assessing Immune Responses

Vaccine immunogenicity will be assessed through collection of blood samples at every post-screening visit and sent to a central laboratory to evaluate serum binding and neutralising antibodies according to the Laboratory Analytic Plan. Neutralisation titre will be determined by Marburg, Ebola and Lassa pseudovirus-based neutralisation assay.

9.5.1 CELLULAR IMMUNE RESPONSES

In addition, blood samples will be collected and sent to a central laboratory for processing and to assess B and T cellular responses in gamma interferon ELISpot assays and through flow cytometry using intracellular cytokine staining, according to the Laboratory Analytic Plan.

9.5.2 INNATE IMMUNE RESPONSE

Additional blood samples will be collected to assess serum analysis of the innate response to vaccination. Blood will be taken on the day of each vaccination and 24 hours post each vaccination and processed for serum. These samples will be sent to a central laboratory for processing. Serum will be used to determine soluble markers of innate immune activation such as CXCL10 (IP10) and interferon alpha.

9.6 Other Adverse Events

Other adverse events will be collected through an open question about health at every trial visit. Study staff will record the diagnosis or the symptoms if a diagnosis is not apparent, the date of onset and the date of resolution, if appropriate. If the event is ongoing, it may be appropriate to conduct a symptom-directed examination.

Events should be graded according to the FDA: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, taking account of local laboratory reference ranges. https://www.fda.gov/media/73679/download

9.7 Incidental findings

An incidental finding is one that has potential health or reproductive importance which is discovered unexpectedly in the course of conducting research but is unrelated to the purpose or aims of the trial – e.g. an abnormal laboratory safety test result. Depending on the nature of the finding, the subject might have to be withdrawn, or vaccinations discontinued, per **Section 5.4**, and his/her GP informed if consent to do so is received from the participant.

9.8 Early Stopping of Follow-up

If a participant chooses to discontinue injections, they should always be followed up for safety and pregnancy events providing they are willing. If they do not wish to remain on trial follow-up, however, their decision must be respected, and the participant will be withdrawn from the trial completely. The Chief Investigator should be informed of this in writing using the appropriate documentation.

Medical data collected in the trial will be kept for research and analysis purposes are pseudo-anonymised. Consent for future use of stored samples already collected can be refused when leaving the trial early (but this should be discouraged and should follow a discussion).

9.9 Loss to Follow-up

Study staff will make every effort to contact participants who do not attend their scheduled visits. At least three attempts will be made to contact the participant during the period from enrolment through to Week 52.

Participants who are lost to follow-up after at least three attempts to contact them will be able to return to follow-up if they make contact at a later date before trial closure.

Participants will be followed up in the long-term through usual mechanisms and with the appropriate consent, which may include flagging via NHS Digital, or similar approaches.

9.10 Trial Closure

The trial will be closed when all participants have made their final follow-up visit and assessments are completed including those to determine resolution of any adverse events, the data entered into the database, checked and the database locked.

10. STATISTICAL CONSIDERATIONS

10.1 Method of Randomisation

Participants will be randomised to receive LNP-MARVsaRNA, LNP-EBOVsaRNA or LNP-LASSAsaRNA vaccines either singly or in combination, with equal allocation ratio (see Figure 1). This is a single-blind trial, in which participants and laboratory staff will be blinded to the dose allocation, while clinic staff will remain unblinded.

10.2 Outcome Measures

The outcome measures are:

- Solicited local injection site reactions starting within 7 days of administration of the vaccine: pain, tenderness, erythema, swelling
- Solicited systemic reactions starting within 7 days of administration of the vaccine: pyrexia, fatigue, myalgia, headache, chills, arthralgia
- Unsolicited adverse reactions (ARs) throughout the trial period (including serious ARs)
- Serious Adverse Events
- Unsolicited adverse events throughout the trial period
- The titre of vaccine-induced serum IgG binding antibody responses to the Marburg virus, Ebola virus and Lassa virus surface glycoprotein 2 weeks after the second vaccinations

The exploratory outcome measures are:

- The titre of serum neutralising antibodies 2 weeks after the second vaccination in the Marburg, Ebola and Lassa pseudovirus-based neutralisation assay
- Cell-mediated vaccine-induced immune responses measured by T and B cell ELISpot in participants in the dose escalation and evaluation parts
- Cell-mediated vaccine-induced immune responses measured by flow cytometry and intracellular cytokine staining in participants in the dose escalation and evaluation parts
- Serum markers of innate immune response

10.3 Sample Size

10.3.1 SAFETY

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, and thus no formal hypothesis testing will be carried out for safety. By the end of this trial 8 participants will have been exposed to each vaccine alone or in combinations and this provides confidence around the response/event proportions of 0–100% in **Table 7**.

Table 7: confidence around the response/event proportions per dose irrespective of schedule

Number of "responders"	Proportion if n=8	95% confidence interval[1]
0	0%	0 – 32%
1	12.5%	1 – 47%
2	25%	7 – 59%
3	37.5%	14 – 69%
4	50%	22 – 78%
5	62.5%	31 – 86%
6	75%	41 – 93%
7	87.5%	53 – 99%
8	100%	68 - 100%

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

10.3.2 IMMUNOGENICITY

A total of 40 participants will be included in the randomised evaluation of immunogenicity, 8 participants per group. The primary analysis will compare binding antibody responses to Ebola virus, Lassa virus and Marburg virus glycoproteins at 14 weeks (2 weeks after the booster at 12 weeks). 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, and the large number of experimental parameters in this experimental trial. The main objective of groups 1-5 is to assess whether overall immune responses to individual Ebola, Lassa and Marburg LNP saRNA vaccines given alone are similar to those administered in combination. No formal hypothesis testing will be performed for immunogenicity endpoints; all comparisons will be descriptive. Ideally, binding and neutralisation antibody should be observed in 100% of participants. With a sample size of 8 participants per group, the lower 5% CI for the response rate is 68-100% if there are no non-responders, and 41-93% if there are two non-responders in any group. The number of 'responders' in each assay will be presented for each timepoint and group as a proportion with a 95% confidence interval. A 'responder' will be defined as a participant in whom a response was detected in two weeks after the final vaccination immunogenicity sample. A positive result will be defined relative to a pre-defined cut-off threshold value and assays will be validated using predefined thresholds based on the responses to positive and negative control stimuli. More information on the assay and definition of positive results will be supplied

in the SAP. Titres of antigen specific antibodies will be described by timepoint and group and compared using rank tests, where appropriate.

10.4 Analysis Plan

A full analysis plan will be provided in a separate Statistical Analysis Plan.

Primary safety analyses will be based on all participants who receive at least one dose of vaccine. Safety endpoints will be compared between the different dose groups. The frequency of adverse events will be tabulated by grade and MedDRA System Organ Class and treatment group, and MedDRA Preferred Term and treatment group. Groups will be compared using Fisher's exact test in terms of the proportion ever experiencing an event in each MedDRA System Organ Class.

11. MONITORING

The trial will be overseen by a Trial Steering Committee (TSC), which will include membership independent of the Trial Management Group (TMG). The reason for this is the need for real-time assessment of the safety data. The TSC will be informed by the Chief Investigator when the first participant receives the first injection of 5 µg, and a report of 7-day reactogenicity will be provided when the first 4 individuals have completed their Week 1 visit (see **Section 4.1.1**). The TSC will receive weekly progress reports. Weekly reports will continue whilst day 7 data are accumulating; after which point the frequency will be determined by the TSC (in accordance with the TSC Charter). Any adverse reactions that lead to interruption in the schedule for an individual or plan for enrolments described in **Section 7.1.4** will be immediately reported to the TSC. The site will be requested to notify the Chief Investigator within 24 hours of any adverse reaction that is a cause for concern as described in **Section 8.** These reports will be shared with the Trial Steering Committee immediately. If there are 2 out of 8 participants (25% thereafter) who experience similarly severe adverse reactions, vaccinations will be paused in all individuals and the competent authority informed. The TSC will be asked to review the accumulating safety data from all participants and make a recommendation about resuming vaccinations.

11.1 Risk Assessment

A trial-specific risk assessment will be performed by the Sponsor, in collaboration with the Chief Investigator and the PI prior to the start of the trial.

The three parties above 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.

This assessment will be used to guide the development of procedures with respect to informed consent, confidentiality, trial monitoring and audit, and lead to the development of a Data Management Plan (DMP) and Safety Reporting Plan and Monitoring Plan. This risk assessment will be updated as required during the course of the trial.

11.1.1 Safety and Rights of Partcipants

The pre-clinical data available for LNP-MARVsaRNA, LNP-EBOVsaRNA and LNP-LASSAsaRNA for four species (mice, guinea pigs, rats and pigs) suggest that this product will be similar to licensed RNA vaccines and cause mild-moderate reactions that are transient. However, this is a first-in-human trial and therefore there will be a sentinel cohort with one individual allocated to each group. There will be immediate review of notable and serious adverse events with onward reporting to the TSC, and clear indications for pausing injections in individuals and the trial.

It will be necessary to hold personal contact details in order to collect the data on reactogenicity. The justification for this will be explained in the PIS as well as the storage and destruction of these data after the trial.

11.1.2 Project Design and Reliability

There is considerable interest in participating, and retention in previous early phase studies at the CRF has always been good. For the purposes of reporting, immune responses will be assessed in a single laboratory, although samples are likely to be analysed in other laboratories too. They will be couriered using a reliable courier.

11.1.3 Project Management and Governance

Imperial College London has worked with the Clinical Research Facility at Chealsea and Westminster hospital on different trials including vaccines 2 previous clinical trials.

11.2 Monitoring

The Clinical Trial manager will review electronic data for errors and missing data points.

Other essential trial issues, events and outputs will be detailed in the Monitoring Plan that is based on the trial-specific Risk Assessment.

The trial will be monitored periodically by the Sponsor (Research Governance and Integrity Team (RGIT)), according to the monitoring plan, to assess the progress of the trial, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy and consistency of the data.

Monitoring procedures and requirements will be documented in a Monitoring Plan, developed in accordance with the risk assessment.

11.3 On-site and Remote Monitoring

The frequency, type and intensity for routine monitoring and the requirements for triggered monitoring will be detailed in the Monitoring Plan. This plan will also detail the procedures for review and sign-off. Remote or self-monitoring will be utilised through the course of the trial. Site staff may be asked to scan and send anonymised sections of a participant's medical record or in the case of consent forms remote review by videoconferencing

or transfer via secure portals may be utilised to enable complete remote verification. Site staff may also be asked to complete a form to confirm compliance with protocol procedures.

11.3.1 Direct Access to Participant Records

Participating investigators should agree to allow trial-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required. Participants' consent for this must be obtained.

11.3.2 Confidentiality

Investigator site, and Sponsor must follow the principles of the UK Data Protection Act.

All personal data leaving the investigator site will be pseudonymised in that it will bear the participants' trial ID and not readily identifiable information such as name or contact details. The investigator site will maintain a participant identification list which links trial IDs to participants' names and NHS numbers (NHS sites only).

The exceptions to the above are:

- Details such as first name, telephone number and email address will be entered by investigator site staff into the EDC system, so that the system can communicate directly with participants, to send reminder messages for example. These details will be encrypted and stored in the EDC system such that only the relevant investigator site staff can view them. Sponsor will be unable to view them.

12. REGULATORY & ETHICAL ISSUE

12.1 Clinical Trials Authorisation

This trial has CTA from the UK Competent Authority; MHRA. Reference: xxx

12.1.1 Regulatory Compliance

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki 1996, the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (R2), Commission Clinical Trials Directive 2005/28/EC* with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, General Data Protection Regulation and the UK Data Protection Act 2018, and the UK Policy Framework for Health and Social Care Research.

*Until the Clinical Trials Regulation EU No 536/2014 becomes applicable, the trial will be conducted in accordance with the Clinical Trials Directive as implemented in the UK statutory instrument. When the directive is repealed on the day of entry into application of the Clinical Trial Regulation the trial will work towards implementation of the Regulation (536/2014) following any transition period.

12.1.2 Data Collection & Retention

Worksheets, clinical notes and administrative documentation should be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for a minimum of 10 years after the end of the trial. During this period, all data should be accessible, with suitable notice, to the competent or equivalent authorities, the Sponsor, and other relevant parties in accordance with the applicable regulations. The data may be subject to an audit by the competent authorities. Medical files of trial participants should be retained in accordance with the maximum period of time permitted by the hospital, institution or private practice.

12.2 Ethical Approval and Conduct

The Study Coordination Centre has obtained approval from the xxx Research Ethics Committee (REC) and Health Research Authority (HRA). The trial will also receive confirmation of capacity and capability from the participating NHS Trust before accepting participants into the trial or any research activity is carried out. The trial will be conducted in accordance with the recommendations for physicians involved in research on human subjects as detailed under **Section 12.1.1** above.

12.2.1 Ethical Considerations

Please see **Section 8.1** for the risks identified for the safety and rights of participants, which include the risk of unexpected serious adverse reactions and the need to collect and hold personal data. The main ethical considerations, and mitigations, are described below:

- The trial is in healthy volunteers, so all visits required by the trial are in addition to their usual lifestyle and therefore are a burden. Vaccine trials are (necessarily) relatively lengthy, so participation

represents a significant time commitment. The requirement to attend all trial visits within specified windows might be an inconvenience to some volunteers if their circumstances change during the trial.

- Fainting may occur around the time of vaccine injection or blood sampling, particularly in those who strongly dislike needles. To minimise this risk, participants will be asked to recline or lie down during those procedures.
- Blood tests can sometimes cause bruising and soreness of the arms or, very rarely, a blockage of a vein or a small nerve injury which can cause numbness and pain.
- The vaccine has not been tested for safety in pregnancy and might harm an unborn child. The contraception requirements for women participants of child-bearing potential might be a burden. Male participants will also be required to use contraception with female partners capable of becoming pregnant.
- The collection of sensitive or personal data will be undertaken only by staff trained in GCP and in the trial protocol.
- Participants will be informed of the results either at a seminar, or by email or on the phone. The PIS explains the hope to publish the results in medical journals, and present them at international conferences, and clarifies that participants will not be named in any of these or identified in any other way.
- Participants might believe that they are entitled to a share of potential future profits from commercialisation of the vaccine. The PIS explains that this is not the case.
- The confidentiality of participants' personal information is described in **Section 12.4**.
- The trial has some medication restrictions: non-trial vaccines received within 28 days before or after any trial vaccination are not allowed; systemic immunosuppressive agents, such as a corticosteroid, may not be administered during the trial; dermal steroids are allowed but not if applied to the IM injection site.
- Based on other vaccines against different diseases, including those that use synthetic mRNA, the side effects are expected to be mild to moderate, short-lived reactions at the injection site such as discomfort, warmth, redness and swelling. Short-lived systemic symptoms such as fatigue, general malaise and headache are expected very commonly (more than 1 in 10 people). Less common reactions (expected in fewer than 1 in 10 people, but in more than 1 in 100) include chills, muscle pain, rash, and injection site itching.

- Uncommon side effects (1 in 100 people) include abdominal pain, diarrhoea, sore throat, enlarged lymph nodes, insomnia and allergic reactions such as rash or itching. These are also anticipated to resolve within a few days (<7 days).

- Rare reactions associated with mRNA vaccine, that may also be associated with saRNA vaccines include (less than 1 in 1,000 people, but more than 1 in 10,000) enlarged lymph nodes, high fever (≥40 °C), hypersensitivity (exaggerated reaction to the vaccine), urticaria (raised, itchy skin rash), and granuloma (area of inflammation in the skin) or sterile abscess (lump) at the injection site. Non-severe allergic reactions (1 in 1,000) such as hives or swelling of the face may occur and severe very rare allergic

reactions (1 in 1 million) may occur, however those with a history of allergy will not be eligible. Very rarely (less than 1 in 10,000 people) vaccines may cause convulsions with fever, drowsiness, and macrophagic myofasciitis (a rare muscle disease).

- Myocarditis (inflammation of the heart muscle), and Pericarditis (inflammation of the lining outside the heart) are also very rare potential serious side effects associated with mRNA vaccine occurring most commonly in adolescent males 12 through 17 years of age (1 in 37,000). Those with any previous history of either of these conditions will not be eligible. These risks will be carefully explained so that participants know what is involved and what to expect in the way of side effects.

These risks will be carefully explained so that participants know what is involved and what to expect in the way of side effects.

Participants will be monitored closely during the trial, to identify as early as possible any problems so that they can be handled appropriately. They will be given 24-hour phone numbers in case they wish to speak to a doctor.

Because this is a first-in-human trial, there will be sentinel participants in each dose group, who will be vaccinated 2 days before any others. The dose evaluation plan is detailed in **Section 4.1.1**.

The rules for pausing and discontinuing vaccinations in individuals and all participants in the trial have been clearly defined.

Participants will be paid for their time, inconvenience and travel expenses: £200 per scheduled visit, paid as a lump sum at the end of participation. This includes any travel expenses they might incur. Volunteers who attend screening but who are not enrolled will not receive payment. If their participation ends early, participants will be paid for the number of visits they've attended.

12.3 Consent

Consent to enter the trial must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the trial for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

12.4 Confidentiality

Data will be pseudonymised. Pseudonymised data is data that can be linked back to a person (e.g. coded data). It is considered both personal and identifiable data. Anonymised data is data that has no code and cannot be

linked back to a person (e.g. aggregated data for publication, data without a code that cannot be linked back to a person).

The Chief Investigator will preserve the confidentiality of participants taking part in the trial and is registered under the Data Protection Act.

12.5 Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this trial.

12.6 Sponsor

Imperial College London will act as the main Sponsor for this trial. Delegated responsibilities will be assigned to the NHS trusts taking part in this trial.

12.7 Funding

The trial is funded by a grant from Innovate UK, part of UKRI with additional funding provided by philanthropic donation from Partners of Citadel and Citadel Securities.

12.8 Audits and Inspections

The trial may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

13. TRIAL MANAGEMENT

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

13.1 Trial Management Team (TMT)

A Trial Management Team (TMT) will be formed comprising the Chief Investigator, the Scientific Lead, and members of the Clinical Research Facility (CRF) who have a coordinating role. The TMT will be responsible for preparing and reviewing the central data monitoring reports including the safety reports, and for onward reporting to the TMG and TSC. Safety reports will be weekly during vaccination weeks to capture the day 7 reactogenicity, and 2-4 weekly otherwise.

13.2 Trial Management Group (TMG)

A Trial Management Group (TMG) will be formed comprising the Chief Investigator, other lead investigators (clinical and non-clinical), and members of Clinical Research Facility (CRF). The TMG will be responsible for the day-to-day running and management of the trial. It will convene approximately every two weeks in the first instance and usually by tele/video conference. The full details can be found in the TMG Charter.

13.3 Trial Steering Committee (TSC)

The Trial Steering Committee (TSC) has membership from the TMG plus independent members, including the Chair. The role of the TSC is to provide overall supervision for the trial, in particular to provide advice on safety and immune responses. The ultimate decision for the continuation of the trial lies with the TSC. Further details of TSC functioning are presented in the TSC Charter.

13.4 Independent Data Monitoring Committee (IDMC)

There is no plan to form an IDMC for this trial because there is no placebo group and a need for real-time monitoring of safety data. Only participants and laboratory staff are blind to the dose administered in the dose evaluation cohorts. Therefore, we propose to submit regular reports of safety to the independent members of the TSC, and to forward any safety concerns should they emerge (see **Sections 11** and **13.1-3** above).

13.5 Patient and Public Involvement Advisory Groups

PPI contributors will be individuals that are identified to reflect the wider community from which participants are drawn.

Any issues identified by the PPI contributors will be forwarded to the TMT as they arise and onward to TMG if this is necessary in order to address the issue. If there are issues that the TMT or TMG identify that require consultation with the PPI contributors, this may be done via a survey or web forum. The associated PIS and consent form has been evaluated by our PPI representatives at Chelsea and Westminster Hospital NHS Foundation Trust.

14. PUBLICATION AND DISSEMINATION OF RESULTS

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 by the TMG and with the PPI Advisory Group. Details of dissemination can be found in the trial specific communication plan.

Authorship will reflect work done by the investigators and other personnel involved in the analysis and interpretation of the data, in accordance with generally recognised principles of scientific collaboration. These will include at least the trial's Chief Investigator, Statistician and Trial Coordinator.

Details regarding the roles and responsibilities and timelines are contained in the Clinical Trial Agreement.

15. DATA AND/OR SAMPLE SHARING

Data will be shared according to the controlled access approach, based on the following principles:

- No data should be released that would compromise an ongoing trial or trial.
- There must be a strong scientific or other legitimate rationale for the data to be used for the requested purpose.
- Investigators who have invested time and effort into developing a trial should have a period of exclusivity in which to pursue their aims with the data, before key trial data are made available to other researchers.
- The resources required to process requests should not be under-estimated, particularly successful requests which lead to preparing data for release. Therefore, adequate resources must be available in order to comply in a timely manner or at all, and the scientific aims of the trial must justify the use of such resources.
- Data exchange complies with Information Governance and Data Security Policies in the UK.

Data will be available for sharing. Researchers wishing to access EML-Vac data should contact the Trial Management Group in the first instance.

16. PROTOCOL AMENDMENTS

The protocol current at the start of the trial was v2.0, dated 1st September 2025. Any subsequent amendments will be recorded below.

Version	**Date**	**Reason for change**
1.0	20th May 2025	New protocol
2.0	1st September 2025	Updated in accordance with MHRA suggestions/requests

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