RAB-Vac

A phase I clinical trial to assess the reactogenicity, tolerability and immunogenicity of a self-amplifying ribonucleic acid (saRNA) vaccine encoding the surface glycoprotein of rabies virus (RAB-Vac)

Version:

1.0, 02 February 2026

MAIN SPONSOR: Imperial College London

FUNDERS: The trial is funded by a grant from the Coalition for Epidemic Preparedness Innovations (CEPI).

STUDY COORDINATION CENTRE: Imperial College London

IRAS Project ID: **1013204**

REC reference: xxx

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:

Research Governance and Integrity Team (RGIT)

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1.7 Funder

This trial is funded by a grant from Coalition for Epidemic Preparedness Innovations (CEPI).

This protocol describes the RAB-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 different trial groups 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 the 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

ABBREVIATION	EXPANSION
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
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic Data Capture
EFGCP	European Forum for Good Clinical Practice
ELISA	Enzyme-linked Immunosorbent Assay
ELISPOT	Enzyme-linked immunosorbent spot
EMA	European Medicines Agency
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

ABBREVIATION	EXPANSION
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
LNP	Lipid nanoparticles
LNP-RABsaRNA	Lipid nanoparticle Rabies self-amplifying ribonucleic acid
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

ABBREVIATION	EXPANSION
QP	Qualified Person
R&D	Research and Development
RAB-Vac	Rabies vaccine
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
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

ABBREVIATION	EXPANSION
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	RAB-Vac
Long Title of Trial	A phase I clinical trial to assess the reactogenicity, tolerability, and immunogenicity of a self-amplifying ribonucleic acid (saRNA) vaccine encoding the surface glycoprotein of the Rabies virus
Version	1.0
Date	10th November 2025
IRAS ID	1013204
Study Design	A cohort will be recruited to assess: A self-amplifying ribonucleic acid (saRNA) vaccine; **LNP-RABsaRNA-01** given at 0.2 µg, 1.0 µg, or 5.0 µg and administered three times at either 0, 4 and 24 weeks or 0, 12 and 24 weeks in 8 healthy individuals aged 18-50 years enrolled through a single centre. Participants and laboratory staff will be blind to dose (see Table 1).
Aims/Objectives	➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 0.2 µg dose administered IM at 0, 4and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 1.0 µg dose administered IM at 0, 4 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 5.0 µg dose administered IM at 0, 4 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	01 at a 0.2 µg dose administered IM at 0, 12 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 1.0 µg dose administered IM at 0, 12 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 5.0 µg dose administered IM at 0, 12 and 24 weeks 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 level of vaccine-induced serum neutralising antibody responses (FAVN assay, IU/ml) 4 weeks after the second vaccination
Exploratory Aims/Objectives	➢ To characterise the humoral and cellular immune responses to LNP-RABsaRNA-01 administered at 0.2 µg, 1.0 µg, or 5.0 µg dose administered IM at either 0, 4 and 24 weeks or 0, 12 and 24 weeks
Exploratory Outcome Measures	➢ Cell-mediated vaccine-induced immune responses measured by T and B cell ELISpot ➢ Cell-mediated vaccine-induced immune responses measured by flow cytometry and intracellular cytokine staining ➢ Kinetics and durability of serum neutralising antibodies (FAVN assay) ➢ Serum IgG binding antibodies by ELISA ➢ The profile of class and sub-class of antibody response

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	➢ Serum markers of innate immune response ➢ Purification of antigen-specific B cells to isolate neutralising monoclonal antibodies to enhance understanding of targeted epitopes
Randomisation	➢ 48 participants across the six 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 enough participants to be statistically confident about the differences between groups. By the end of this trial 16 participants will have been exposed to each vaccine dose (0.2 µg, 1.0 µg or 5.0 µg dose), administered at 0, 4 and 24 weeks or 0, 12 and 24 weeks; this provides 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-RABsaRNA-01 vaccine (0.2 µg dose) at 0, 4 and 24 weeks 2. LNP-RABsaRNA-01 vaccine (1.0 µg dose) at 0, 4 and 24 weeks

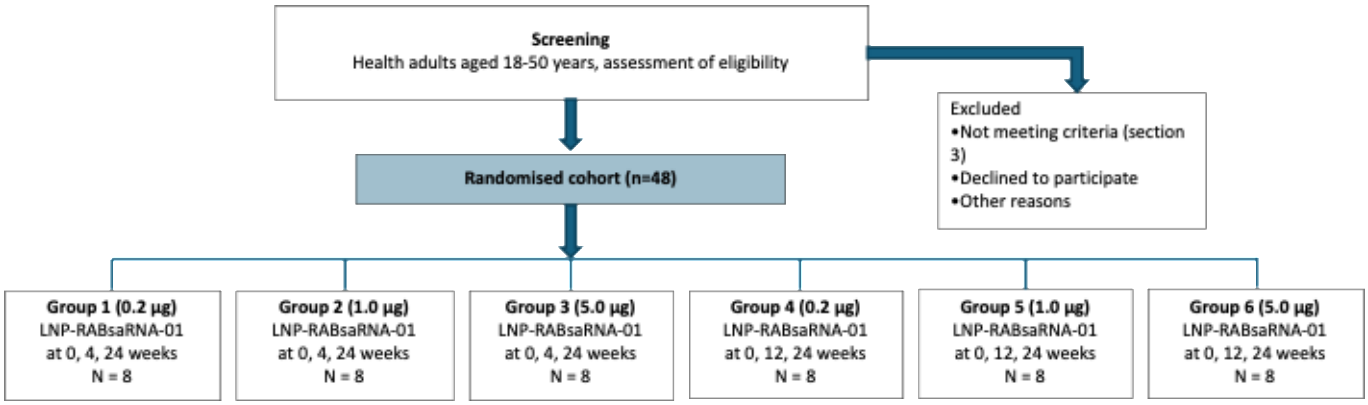
SUMMARY INFORMATION TYPE	SUMMARY DETAILS
	3. LNP-RABsaRNA-01 vaccine (5.0 µg dose) at 0, 4 and 24 weeks 4. LNP-RABsaRNA-01 vaccine (0.2 µg dose) at 0, 12 and 24 weeks 5. LNP-RABsaRNA-01 vaccine (1.0 µg dose) at 0, 12 and 24 weeks 6. LNP-RABsaRNA-01 vaccine (5.0 µg dose) at 0, 12 and 24 weeks
Trial Assessments	See Tables 2.1 and 2.2
Duration	Each participant who received the LNP-RABsaRNA vaccine will be followed for one year. The trial is anticipated to start in Q1 2026 and complete enrolment for within 3 months. The total duration of the trial is anticipated to be 21 months.

2.1 Trial Schema

TABLE 1: TRIAL GROUPS

Group	Description	Active Dose (µg) Prime & boost	Dose schedule	Age (years)	N
Group 1	LNP-RABsaRNA-01,	0.2 µg	0, 4, 24 weeks	18-50	8
Group 2	LNP-RABsaRNA-01,	1.0 µg	0, 4, 24 weeks	18-50	8
Group 3	LNP-RABsaRNA-01,	5.0 µg	0, 4, 24 weeks	18-50	8
Group 4	LNP-RABsaRNA-01,	0.2 µg	0, 12, 24 weeks	18-50	8
Group 5	LNP-RABsaRNA-01,	1.0 µg	0, 12, 24 weeks	18-50	8
Group 6	LNP-RABsaRNA-01,	5.0 µg	0, 12, 24 weeks	18-50	8
		Total			48

Figure 1: Trial Entry



(n=48)
1. To evaluate the safety and immunogenicity of three immunisations with LNP-RABsaRNA-01 administered IM 0, 4 and 24 weeks apart at a 0.2 µg dose in 8 participants age 18-50 years.
2. To evaluate the safety and immunogenicity of three immunisations with LNP-RABsaRNA-01 administered IM 0, 4 and 24 weeks apart at a 1.0 µg dose in 8 participants age 18-50 years.
3. To evaluate the safety and immunogenicity of three immunisations with LNP-RABsaRNA-01 administered IM 0, 4 and 24 weeks apart at a 5.0 µg dose in 8 participants age 18-50 years.
4. To evaluate the safety and immunogenicity of three immunisations with LNP-RABsaRNA-01 administered IM 0, 12 and 24 weeks apart at a 0.2 µg dose in 8 participants age 18-50 years.
5. To evaluate the safety and immunogenicity of three immunisations with LNP-RABsaRNA-01 administered IM 0, 12 and 24 weeks apart at a 1.0 µg dose in 8 participants age 18-50 years.
6. To evaluate the safety and immunogenicity of three immunisations with LNP-RABsaRNA-01 administered IM 0, 12 and 24 weeks apart at a 5.0 µg dose in 8 participants age 18-50 years.

TRIAL ASSESSMENT SCHEDULES

Table 2.1: Trial Assessment Schedule for groups 1-3

Trial Visit	V1	V2	V2a	V3	V4	V4a	V5	V6	V7	V8	V8a[3]	V9	V10	V11	V12
Visit Type (site or telephone)	Site	Site	Site	Site	Site	Site	Site	Site	Site	Site	Phone	Site	Site	Site	Site
Trial Week	−8	0	0	2	4	4	8	10	12	24	24	26	28	36	76
Trial Day	–	0	1	14	28	29	56	70	84	168	169	182	196	252	532
Windows (days)	−56 −1	–	none	−2 +2	−4 +4	none	−4 +4	−2 +2	−4 +4	−4 +4	none	−2 +2	−2 +2	−2 +2	−36 to +7
Informed consent	X														
Medical history/demographics	X														
Eligibility assessment	X	X													
Physical examination	X	X		X	X		X			X		X			
ECG	X	X			X					X					
Weight/height (BMI)	X														
Vital signs (BP, HR, O_2 saturation and temperature)	X	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	X	X
Randomisation		X													
Vaccination		X			X					X					
Issue diary card for AEs		X			X					X					
Review diary card for AEs			X	X		X	X					X			
Symptom-directed physical examination as required			X	X	X	X	X	X	X	X		X	X	X	X
Motor function test of vaccinated arm		X	X	X	X	X	X			X		X			
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV and HCV screen (X ml)	5 mL														
Rabies Screen	X														
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	10 mL
Urine dipstick	X														
Urinary pregnancy test (WOCP only)	X	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	6 mL
Blood for central cellular immunogenicity assays		60 mL			42mL		42 mL	42 mL	42 mL	42 mL		42 mL	42 mL		
Blood for innate cytokine analysis		6 mL	6 mL		6 mL	6mL									
Blood volume (approx.)[2]	15 mL	82 mL	6 mL	16 mL	64 mL	6 mL	58 mL	58 mL	58 mL	58 mL		58 mL	58 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 76 weeks is 569 mL. 3. Telephone visit 24 hours post vaccination for safety review

Table 2.2: Trial Assessment Schedule for groups 4-6

Trial Visit	V1	V2	V2a	V3	V4	V5	V5a	V6	V7	V8	V8a[2]	V9	V10	V11	V12
Visit Type (site or telephone)	Site	Site	Site	Site	Site	Site	Site	Site	Site	Site	Phone	Site	Site	Site	Site
Trial Week	−8	0	0	2	4	12	12	14	16	24	24	26	28	36	76
Trial Day	–	0	1	14	28	84	85	98	112	168	169	182	196	252	532
Windows (days)	−56 −1	–	none	−2 +2	−4 +4	−4 +4	none	−2 +2	−4 +4	−4 +4	none	−2 +2	−2 +2	−2 +2	−36 to +7
Informed consent	X														
Medical history/demographics	X														
Eligibility assessment	X	X													
Physical examination	X	X		X		X		X		X		X			
ECG	X	X				X				X					
Weight/height (BMI)	X														
Vital signs (BP, HR, O_2 saturation and temperature)	X	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	X	X
Randomisation		X													
Vaccination		X				X				X					
Issue diary card for AEs		X				X				X					
Review diary card for AEs			X	X			X	X				X			
Symptom-directed physical examination as required			X	X	X	X	X	X	X	X		X	X	X	X
Motor function test of vaccinated arm		X	X	X		X	X	X		X		X			
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV and HCV screen (X ml)	5 mL														
Rabies Screen	X														
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	10 mL
Urine dipstick	X														
Urinary pregnancy test (WOCP only)	X	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	6 mL
Blood for central cellular immunogenicity assays		60 mL			42mL	42 mL		42 mL	42 mL	42 mL		42 mL	42 mL		
Blood for innate cytokine analysis		6 mL	6 mL			6 mL	6 mL								
Blood volume (approx.)[3]	15 mL	82 mL	6 mL	16 mL	58 mL	64 mL	6 mL	58 mL	58 mL	58 mL		58 mL	58 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 76 weeks is 569 mL. 3. Telephone visit 24 hours post vaccination for safety review

3. LAY SUMMARY

RAB-Vac is a trial testing a new RNA vaccine against the Rabies virus, which causes rabies. The trial aims to evaluate the vaccine's reactogenicity and tolerability, as it will be the first time it has been used in humans. However, this trial does not assess whether the vaccine provides protection. It solely focuses on the safety of the vaccine and how the immune system responds to it.

RAB-Vac saRNA Vaccine

Since this is the first time this vaccine has been used in humans, the reactogenicity and tolerability of the vaccine will be assessed in healthy young adults. 48 participants aged 18-50 years will be randomly assigned to one of six different groups, receiving one of three doses of the self-amplifying RNA (saRNA) vaccine by injection into the muscle and administered at either 0, 4, and 24 weeks or 0, 12, and 24 weeks. Participants will be carefully monitored for any reactions to the vaccine.

There are likely to be mild side effects near the injection site. There may also be more general side effects, such as headaches, fever, and chills. Participants will be asked to record any symptoms in an online diary. To assess how well the immune system responds, participants will need to provide blood samples several times during the first 4 weeks, after the second primary and the booster immunisations, and finally at 12 months after the booster immunisation to evaluate the durability of the response. This will take place at one trial centre. If any part of the trial indicates that any of the three doses are unsafe or poorly tolerated, they will be excluded from further study.

Although there have been no clinical studies using this saRNA vaccine, 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 two prime 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 is safe and well-tolerated, with most reactions following vaccination being mild or moderate. Most solicited adverse events reported within 7 days after the two prime vaccinations were mild.

3. BACKGROUND

3.1 Introduction

3.1.1 RABIES

The rabies virus spreads to humans through the saliva of an infected mammal, typically via a bite or scratch, or through contact with mucous membranes or open wounds. Once symptoms develop, rabies is almost always fatal. The highest risk regions are in Africa and Asia (95% of infections), where rabies is endemic among dog populations. Still, it also poses a threat in other areas where wild or domestic animals may be infected [1]. There are approximately 60,000 cases of rabies each year worldwide due to the lack of accessible, effective, low-cost vaccines. Around 40% of deaths occur in children under 14 years of age [2]. The global cost of rabies is estimated at around US$8.6 billion annually, covering lost lives and livelihoods, medical expenses, and associated costs, as well as uncalculated psychological trauma. A worldwide plan has been formulated to eliminate human dog-mediated rabies deaths globally by 2030 [3].

3.1.2 RABIES VIRUS

The virus attaches to its cellular targets through the surface glycoprotein (RABV-G), rapidly accesses peripheral nerves, and then, following retrograde axonal transport and trans-synaptic spread, ultimately reaches the brain. Preventing rabies infection requires the immediate neutralisation of the virus by antibodies targeting the viral G protein upon entry into uninfected tissue, and/or the early elimination of infected cells by virus-specific cytotoxic T cells, when limited replication may occur in non-neuronal tissue at the entry site. This is most effective when neutralising antibodies and effective T cell responses have been induced by vaccination before exposure. In cases of post-exposure treatment, this involves the swift administration of rabies immunoglobulin (RIG) alongside immunisation as soon as possible after the exposure event [4,5].

3.1.3 CURRENT THERAPEUTICS

There are currently four effective inactivated rabies virus vaccines produced using cell cultures: Rabipur, by Bavarian Nordic, RABIVAX-S by Serum Institute of India Pvt. Ltd., VaxiRab N by Zydus Lifesciences Limited, and VERORAB by Sanofi Pasteur [6-8]. Nearly all recipients of either vaccine series develop a seroprotective titre of rabies viral neutralising antibodies (VNA), which is defined as a VNA titre of ≥0.5 IU/mL according to the World Health Organization's rapid fluorescent focus inhibition test (RFFIT). Despite the availability of effective vaccines, rabies infection still causes at least 55,000 human deaths annually. In fact, there is currently an insufficient supply of these vaccines for individuals living in high-risk areas and resource-poor settings [9-13]. This healthcare need underscores the importance of ongoing development of alternative vaccine technologies to help mitigate the high costs associated with existing egg-based or cell-culture-derived vaccines, prevent shortages in vaccine supply, and facilitate equitable distribution. Therefore, the global provision of safe, effective, and affordable rabies vaccines for human use remains a priority. A synthetic, low-dose, low-cost self-amplifying RNA rabies vaccine could provide significant improvements in vaccine manufacture and supply.

3.1.4 PREVIOUS STUDIES

3.1.4.1 Pre-clinical

The LNP-RABsaRNA-01, which will be tested in the RAB-Vac clinical study, has been shown to induce strong immune responses in pre-clinical models when administered at a 1.0 µg dose in mice via the intramuscular route (see Investigators brochure). Additionally, in the pig model, a 5.0 µg dose of LNP-RABsaRNA elicited a potent induction of neutralising antibodies, reaching levels of 1000 IU per dose, significantly surpassing the WHO correlate of protection (0.5 IU per ml) in the absence of any observed injection site reaction of loss of muscle function. This indicates the potential of this vaccine to be protective in humans.

In a pivotal nonclinical toxicology study (Labcorp Study no. 8553420) conducted to Good Laboratory practice (GLP) in rats, the toxicity of 5 µg/dose of LNP-RABsaRNA-01 was evaluated when administered on Days 1, 8, and 15 via intramuscular injection in rats. Intramuscular administration of 5 µg of RAB-saRNA in 500 µl (250 µl per thigh), the full human dose, on three occasions over 2 weeks (7 days between each administration) caused no clinical observations or test-article-related oedema or erythema at the injection site. Muscle degeneration and regeneration with localised inflammatory infiltrates, associated with the large volume immunisation (250 µl per thigh), were confined to the administration site and inversely correlated to animal weight. However, these changes, with a functional effect of reduced motor activity, had not fully resolved by four weeks after the final administration, the pre-specified time for euthanasia. We believe that these findings are due to the very large dose volume given to animals (250 µl to each thigh), as other LNP-saRNA vaccines (with identical formulations and manufacturing processes) administered at lower dose volumes (≥100ul) have not elicited this effect in previous toxicology studies. Furthermore, no impact on muscle function was observed when testing this vaccine in pigs and it has not been observed when testing a similar rabies saRNA vaccine in humans [16, 17]. As a precaution, we will assess motor function of the injected arm on the day of each injection and at 1- and 14-days post-injection for the two priming doses and at the day of vaccination and 14 days after for the boost.

For further information, please refer to the Investigators' Brochure (IB).

3.1.4.2 Clinical studies

Although no clinical studies have been carried out with LNP-RABsaRNA-01, a first in human 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) [14,15].

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. Common local reactions included tenderness/discomfort and pain (69%) and 73 (38%) respectively; erythema (5; 3%) and swelling (2; 1%) were uncommon. Common systemic reactions included fatigue (56%), headache (52%), myalgia (35%), arthralgia (24%), chills (23%) and nausea (18%). Two participants experienced adverse events that resulted in a delay in their second vaccination, which was administered without recurrence. Reactogenicity was dose-dependent, with the highest proportion of grade 3 reactions (11%) observed in those receiving a 10.0 µg dose [14, 15]. Laboratory safety parameters remained largely within normal limits seven days after each vaccination.

The reactogenicity profile of LNP-nCoVsaRNA administered at a 10µg dose 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 be due to the exclusion of subjects with a significant history of allergies.

An observed increase in seroconversion was noted when the second dose of the vaccine was administered 12 weeks rather than 4 weeks after the first dose. It is unclear if a 12-week interval is optimal; therefore, this study will compare a 4 and 12-week interval between the two priming doses.

Based on the findings from vaccination with LNP-nCoVsaRNA, we will now conduct a Phase I, first-in-human clinical trial (RAB-Vac) to evaluate the safety and immunogenicity of the saRNA vaccine to Rabies virus; LNP-RABsaRNA-01. The vaccine will be administered in three doses (0.2 µg, 1.0 µg, or 5.0 µg), with two priming doses given at 4- or 12-week intervals, and a third common boost at 24 weeks.

More recently, a different LNP-formulated saRNA vaccine (RBI-4000) [16, 17] was evaluated in a Phase I study (NCT06048770). RBI-4000 was able to elicit humoral and cellular responses in most healthy participants when administered at doses of 0.1, 1.0 , or 10.0 µg (71%, 94%, 100%, respectively) in a prime-prime schedule (0 and 8 weeks). No serious adverse events have been reported across all cohorts. Although similar to the vaccine to be evaluated in this trial, the RBI-4000 vaccine used a different proprietary LNP formulation and did not incorporate the RNA sequence optimisations present in our vaccine candidate. Our preclinical modelling suggests that responses to the RAB-Vac vaccine are likely to be improved compared to those observed with the RABI-4000 vaccine.

These findings support a clinical 5 µg/dose for the LNP-RABsaRNA-01 vaccine.

Self-amplifying RNA vaccines

The active pharmaceutical ingredient for the vaccine is an optimised saRNA vector that encodes the spike glycoprotein (G-protein) of rabies virus (i.e., RABsaRNA-01). This glycoprotein is the main target for generating neutralising antibodies. The rabies glycoprotein candidate contains an F318V mutation, chosen based on neutralisation titre, to prevent binding to the p75NTR receptor used by the virus. This adds a safety feature by preventing the vaccine from transiently mimicking rabies infection symptoms (for full sequence details, see the IB). The saRNA construct is made using a non-infectious Venezuelan equine encephalitis virus (VEEV) replicon backbone encoding non-structural proteins (nsP1–4) necessary for self-amplification [18, 24]. The codon-optimised rabies G spike glycoprotein gene is inserted in place of structural genes downstream of a sub-genomic promoter (SGP), which drives their transcription and surface expression on cells that take up the RNA vector. To ensure efficient uptake after intramuscular administration, the naked saRNA is formulated in lipid nanoparticles (LNPs). These particles are composed of a mixture of ionisable cationic lipid (C12-200), phosphatidylcholine, cholesterol, and polyethylene glycol (PEG)-lipid. The saRNA is encapsulated within the LNPs, which protects the RNA from degradation and delivers it to the cytoplasm of cells following the endocytosis of the LNP. This approach builds on the successful use of LNPs in mRNA and saRNA COVID-19 vaccines, including those licensed by Moderna and Pfizer [25, 26].

Following intramuscular injection, the formulated saRNA is absorbed into the cytoplasm of target cells. This results in 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, it does not produce viral particles; the amplification process is self-limiting (days), and there is no further transmission of the saRNA to other cells. The saRNA is strictly confined to the cytoplasm and does not enter the nucleus. It cannot affect chromosomal DNA and, as a result, causes no changes to the cells' genetic makeup.

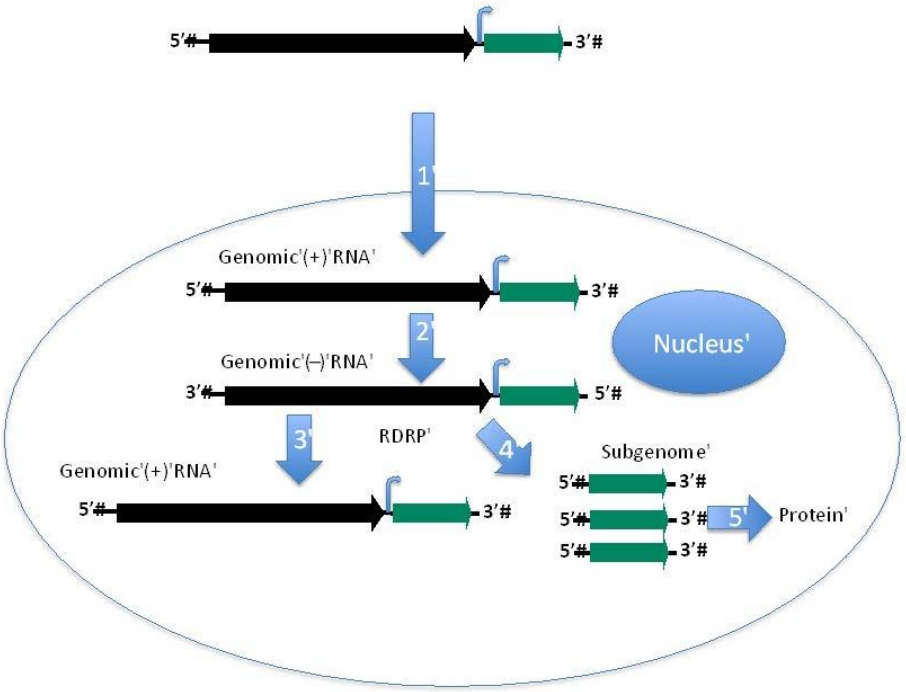


Figure 2. *On delivery into cells (1), the polymerase machinery of the VEEV backbone is preferentially expressed. This, in turn, leads to amplification of the RNA through production of negative (2) and positive copies of the saRNA vector (3). Within a few hours, sequential cleavage of the polymerase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

3.1.5 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 protects 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). Several 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].

The rabies saRNA vaccine has been refined following our earlier SARS-CoV-2 vaccine tested in the COVAC1 trial [14, 15]. The LNP-RABVsaRNA01 construct has been codon optimized to enhance translation while retaining the same amino acid sequence, the manufacturing process has been improved to generate saRNA with increased full-length integrity (from <50% in COVAC1 to >70%) and reduce dsRNA impurities (from >5% in COVAC1 to < 1%) and the LNP formulation has been optimized for saRNA delivery [36].

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, which protect the RNA from degradation and deliver the payload to the cytoplasm of cells following the 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 licensed 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 Rabies virus infection, that causes Rabies. Despite promising pre-clinical data, at this stage equipoise remains as to relative risks and benefits.

Based on the background risks and mitigations described above, it is considered safe and appropriate to enter a clinical early-phase immunogenicity and safety trial with a Rabies saRNA vaccine.

3.4 Rationale for the current trial

Despite the availability of effective vaccines, rabies still causes at least 55,000 human deaths annually. Therefore, ensuring a global supply of safe, effective, and affordable rabies vaccines remains a priority. A synthetic, low-dose, low-cost self-amplifying RNA rabies vaccine could greatly enhance vaccine manufacturing and distribution. Since a known correlate of protection exists—defined as a VNA titre of ≥0.5 IU/mL according to the World Health Organization—this study's results will benchmark this saRNA platform against competing technologies. It will also help evaluate its potential usefulness against rabies infection and, more broadly, as a vaccine platform. The pre-clinical data outlined above (and more detailed within the IB) suggests that LNP-RABsaRNA will be protective against rabies infection.

3.5 Rationale for dose selection

Preclinical studies in pigs showed a strong induction of neutralising antibodies to the LNP saRNA when administered at a 5.0 µg dose, achieving levels of 1000 IU per dose, significantly exceeding the WHO correlate of protection (0.5 IU per ml). This study did not investigate lower doses of saRNA; therefore, we aim to determine if similar responses can be elicited in humans at either 1.0 µg or 0.2 µg (a five-fold reduction). Previous results in our COVAC-1 clinical trials with saRNA demonstrated good tolerability for LNP-formulated saRNA at doses ≤10.0 µg. A recent phase I study (NCT06048770) of an alternative LNP-formulated rabies saRNA (RBI-4000) [16, 17] found no serious adverse events in healthy participants when administered at doses of 0.1, 1.0, or 10.0 µg in a prime-prime schedule (0 and 8 weeks). The pivotal toxicology study also supports the choice of dose. Three

intramuscular injections of the full human dose, 5.0 µg in 500 µl of RABV-saRNA, administered to Sprague Dawley rats at one-week intervals, caused no clinical observations or test-article-related oedema or erythema at the injection site. Muscle degeneration and regeneration with localised inflammatory infiltrates, associated with the large volume immunisation (250 µl per thigh), were confined to the administration site; however, all changes had resolved by four weeks after the final administration. The LNP-RABsaRNA-01 will be assessed at three different doses in this trial: 0.2 µg (very low), 1.0 µg (low) and 5.0 µg (moderate).

4. STUDY OBJECTIVES

RAB-Vac is a trial investigating a new RNA vaccine against rabies. The rabies virus spreads to humans through the saliva of an infected mammal, typically via a bite or scratch, or through contact with mucous membranes or open wounds. The highest risk areas are in Africa and Asia (95% of infections), where rabies is endemic in dog populations. Still, it also poses a risk in other regions where wild or domestic animals can be infected. The trial aims to assess the safety (reactogenicity and tolerability) of this vaccine, as this will be the first time it has been used in humans. As this is a first-in-human trial, accrual will be limited to healthy adults between 18 and 50 years old. 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**). Through this clinical study, we aim to determine the dose response profile (0.2 µg, 1.0 µg, and 5.0 µg) and evaluate the impact of a short (4 weeks) and longer (12 weeks) interval between the first and second priming doses (0-4 weeks and 0-12 weeks). Our previous clinical studies (COVAC-1 [15]) indicated that a 12-week interval was superior to a 4-week interval and we wish to determine if this remains the case with the LNP-RABVsaRNA-01 vaccine.. A third, final booster will be administered to all groups at 24 weeks to assess boosting potential. Provided the threshold for pausing vaccines in the trial is not crossed, the primary immunogenicity endpoint will be determined by the quantity of neutralising antibodies 4 weeks after the second injection. This will be measured using the standardised Fluorescent Antibody Virus Neutralisation Test (FAVN) together with WHO reference serum and reported as international units per ml (IU/ml). A titre of **≥ 0.5 IU/mL** indicates is the accepted correlate of protection according to WHO and OIE. Comparison against this known correlate of protection will enable this vaccine to be benchmarked against other vaccine candidates. As a placebo does not inform the analysis for either of these endpoints, there will be no allocation to a placebo group.

This trial is NOT examining whether the vaccine is 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' binding 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 priming immunisations. Peripheral blood mononuclear cells (PBMCs) from participants will be processed to isolate antigen-specific B cells, aiming to obtain neutralising monoclonal antibodies. This will enhance understanding of targeted epitopes, with priority given to those showing higher serum neutralising antibody titres.

Objectives	➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-

	01 at a 0.2 µg dose administered IM at 0, 4 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 1.0 µg dose administered IM at 0, 4 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 5.0 µg dose administered IM at 0, 4 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 0.2 µg dose administered IM at 0, 12 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 1.0 µg dose administered IM at 0, 12 and 24 weeks in 8 participants age 18-50 years. ➢ To evaluate the reactogenicity, tolerability, and immunogenicity of three immunisations with LNP-RABsaRNA-01 at a 5.0 µg dose administered IM at 0, 12 and 24 weeks in 8 participants age 18-50 years.

4.1 Study Design

This is the first-in-human trial of the LNP-RABsaRNA 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 have demonstrated that doses of up to 10.0 µg are well tolerated [14, 15]. Additionally, they indicated that a 12-week interval between doses is more effective than a four-week interval in terms of the magnitude of elicited responses [17]. We will assess whether a 4-week interval is similar to a 12-week interval.

4.1.1 LNP-RABsaRNA-01 EVALUATION

The initial dosing of the sentinel participant for each of groups 1-3 will proceed through administering either 0.2 µg, 1.0 µg, or 5.0 µg of LNP-RABsaRNA-01. Participants and laboratory staff will be blinded to the administered dose:

1. The first sentinel participant will receive 0.2 µg of LNP-RABsaRNA 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 review their diary. If the reactions are Grade 1-2, or transient Grade 3 that resolve within 24 hours, the second participant may receive 1.0 µg of LNP-RABsaRNA.
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 5.0 µg of LNP-RabsaRNA.
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 dose evaluation cohort of the remaining 45 participants may start to be enrolled and randomised into one of the six groups to receive their first immunisation.
8. 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 45 participants may receive their second dose.

The steps above describe the fastest plan to enrol into the 6 groups and is justified based on previous experience with this self-amplifying RNA platform. However, if persistent Grade 3 reactions are observed in any of the sentinels, the team will invite the affected participant to the trial site to evaluate these reactions 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 have 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 neutralising antibody responses to the Rabies virus surface glycoproteins 4 weeks after the second vaccinations
Exploratory Aims/Objectives	➢ To characterise the humoral and cellular immune responses to LNP-RABsaRNA-01 administered at 0.2 µg, 1.0 µg, or 5.0 µg dose administered IM at either 0, 4 and 24 weeks or 0, 12 and 24 weeks
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 ➢ Kinetics and durability of neutralising antibody responses ➢ Serum binding antibodies in an ELISA 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 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, 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 Over-volunteering 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-RABsaRNA is 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 anaphylaxis or angioedema
4. History of severe or multiple allergies to drugs or pharmaceutical agents
5. 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
1. Ever received an experimental or authorised vaccine against Rabies virus
6. Receipt of any immunosuppressive agents within 18 weeks of screening by any route other than topical
7. Detection of antibodies to hepatitis C
8. Detection of antibodies to HIV
9. ALT/AST exceeding the upper limits of normal ≤1.0
10. Abnormal urine analysis result (3 x repeats >24 hours apart are allowed)
11. 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

12. Participating in another clinical trial with an investigational drug or device or treated with an investigational drug within 28 days of screening.
13. 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 their 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, they 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 RAB-Vac trial.

6.2 Unblinding

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

In the event of an emergency, the Investigator is solely responsible for determining whether 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 justified, the Investigator may contact the Sponsor to discuss the situation before 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. If 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-enrollment in other clinical trials involving an investigational or non-investigational drug or device is not permitted. Individuals who have received 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 three IM doses of LNP-RABsaRNA at 0.2 µg (Group 1), 1.0 µg (Group 2) or 5.0 µg (Group 3) administered at 0, 8 and 24 weeks, or 0.2 µg (Group 4), 1.0 µg (Group 5) or 5.0 µg) (Group 6) administered at 0, 12 and 24 weeks, into the deltoid muscle (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 RAB-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-RABsaRNA dispensing schedule

Study component	Description	Route	Dose	Visit 2 (day/week) Prime	Visit 5 (day/week) Prime	Visit 8 (day/week) Boost
Group 1	LNP-RABsaRNA-01	IM	0.2 µg (0.5 mL)	D0/W0	D28/W4	D168/W24
Group 2	LNP-RABsaRNA-01	IM	1.0 µg (0.5 mL)	D0/W0	D28/W4	D168/W24
Group 3	LNP-RABsaRNA-01	IM	5.0 µg (0.5 mL)	D0/W0	D28/W4	D168/W24
Group 4	LNP-RABsaRNA-01	IM	0.2 µg (0.5 mL)	D0/W0	D84/W12	D168/W24
Group 5	LNP-RABsaRNA-01	IM	1.0 µg (0.5 mL)	D0/W0	D84/W12	D168/W24
Group 6	LNP-RABsaRNA-01	IM	5.0 µg (0.5 mL)	D0/W0	D84/W12	D168/W24

7.1.1 LNP-RABsaRNA-01 VACCINE

LNP-RABsaRNA-01 is an aqueous formulation of RNA encapsulated in Lipid Nano Particles (LNP), which is provided at a target concentration of 10 µg /mL for IM injection.

LNP-RABsaRNA-01 vaccine is manufactured by Centre for Process Innovation (CPI) in accordance with GMP standards on behalf of the sponsor, who is also responsible for the product development.

LNP-RABsaRNA-01 is fill finished by Nova Laboratories and shipped directly to the trial site. Nova Laboratories will supply the IMP with RAB-Vac-specific labels, according to GMP.

The drug product is supplied as a white to off-white suspension in Type I glass vials, sealed with a butyl rubber stopper and aluminium crimp cap. A 0.65 mL fill is provided, which allows for 0.5 mL to be used for administration, in accordance with the instructions in the RAB-Vac pharmacy manual.

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

7.1.2 Placebo Injections

There are no placebo injections or groups planned for this trial. A placebo group has been omitted because the main aim of this phase I trial is to assess reactogenicity, tolerability, and immunogenicity, not efficacy. A placebo or active control is usually added in phase II trials. The primary comparison in this phase I trial is between different dose levels, not between treatment and placebo. Our goal is to see whether the new product causes any toxicity or adverse reactions. Including a placebo group in this small study would decrease the number of participants receiving the active product and limit the ability to collect sufficient safety data on the investigational agent. Ethically, exposing healthy volunteers to a placebo injection would offer no medical benefit, only risk, discomfort, and inconvenience.

7.1.3 Administration

Vaccine 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.

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

7.1.4 Storage

The vaccine will be stored in a secure, limited-access storage area under the specified storage requirements (-80^{0}C +/-10). The vaccine 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 the scheduled injection, leading the investigator to consider deferring the injection. An oral 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 Trial Steering Committee (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 (greater than 72h) 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-RABsaRNA 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 recommend whether immediate reporting to the TSC is necessary. 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 to the authorities (see **section 8.3**) by the Chief Investigator. The TSC may recommend to the Sponsor that injections be 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-RABsaRNA 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.

7.3 Dispensing and Accountability

7.3.1 Dispensing

The pharmacist, or another person delegated by the PI, will ensure that the vaccines are dispensed in accordance with the protocol, 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 is 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 5.0 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.

Although not reported for saRNA, for mRNA vaccines, scarce instances of heart inflammation (myocarditis or pericarditis), particularly in adolescent males, have been reported, but this remains uncommon (1 in 37,000). A history of either of these conditions or an abnormal ECG on screening is an exclusion criterion. ECG will be used to assess any unexplained chest pain, palpitations (tachycardia and/or arrhythmias), dyspnoea, fainting, fever, signs of heart failure (e.g. peripheral oedema) that occur during the trial. If abnormal and the symptoms suggest an acute cardiac event, the participant will be taken to the CWHFT emergency department for treatment and follow-up (the GP will be informed for long-term FU).

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 5.0 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. It should be followed to outcome and monitored 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 their consent.

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 the 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 assessment

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-RABsaRNA Investigator's Brochure. A SAR that is more frequent or more severe than stated in the reference safety information would also be considered 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 emailed 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.

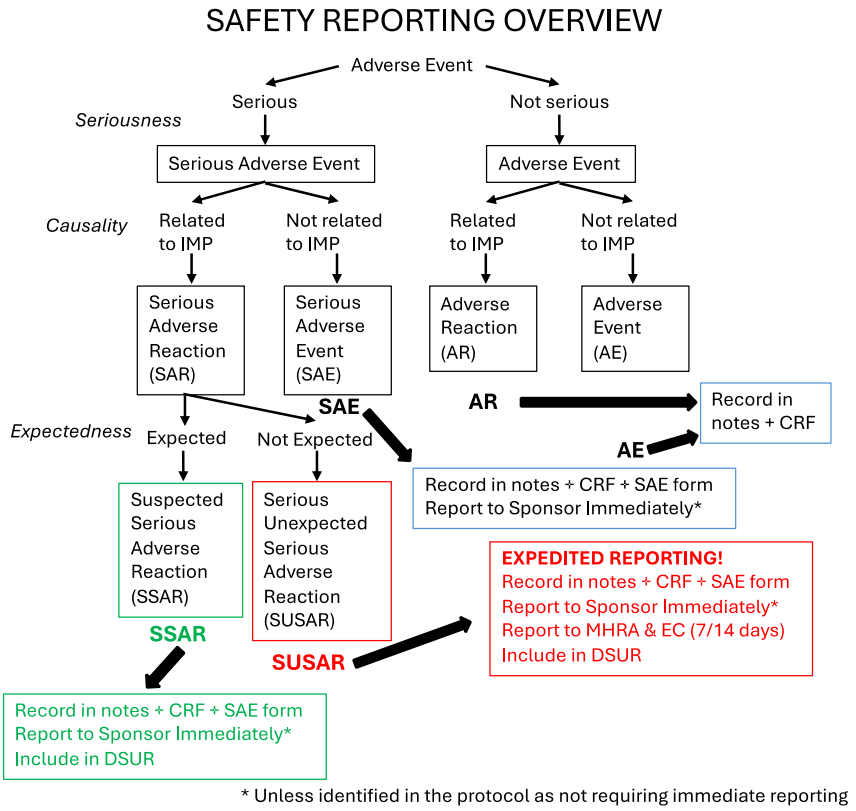
The trial coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the trial according to the following timelines: fatal and life-threatening within 7 days of notification and non-life-threatening within 15 days. All investigators will be informed of all SUSARs occurring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

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

The SAE details should be entered into the EDC system by an investigator (a clinician named on the Signature List and Delegation of Responsibilities Log, responsible for the participant's care), with due care 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 and 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 RAB-Vac trial, who completes 52 weeks of follow-up, is approximately 512 mL. These volumes do not include the blood volume required for additional diagnostic tests or for repeated safety tests. The required volumes per visit, along with sample collection and processing guidelines, are detailed in the Laboratory Manual.

The trial assessment schedule includes the following visits: a screening visit (Visit 1) and an enrolment visit, which must take place within 8 weeks of the screening visit and is defined as Week 0 (Visit 2). For groups 1-3 injection visits are at Weeks 0 (visit 2), 4 (visit 5) and 24 (visit 8) and safety visits at Weeks 1, 2, 4, 8, 10, 12, 24, 26, 28 and 36 (Visits 2a, 3, 4, 5a, 6, 7, 9, 10, 11) and a final visit at Week 52 (Visit 12). For groups 4-6 injection visits are at Weeks 0 (visit 2), 12 (visit 5) and 24 (visit 8) and safety visits at Weeks 1, 2, 4, 12, 14, 16, 24, 26, 28 and 36 (Visits 2a, 3, 4, 5a, 6, 7, 9, 10, 11) and a final visit at Week 52 (Visit 12). The visit windows are provided in **Table 2.1 and 2.2**.

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 Rabies virus infection

- That the vaccine does not contain any infectious component and cannot cause symptoms associated with Rabies 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 provide their informed consent in writing before 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 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 medications 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 (in kilograms), height (in centimetres), temperature, blood pressure, pulse, oxygen saturation, inspection of the skin to exclude severe eczema, and examinations of the respiratory, cardiovascular, abdominal, and neurological systems. An assessment of cervical, axillary and inguinal lymph nodes will also be undertaken.

Clinical Investigators should carefully assess participants with pre-existing conditions, considering the maximum severity of the condition in the past, the extent of treatment needed to control it at the time of screening, and how long the participant has been stable 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
- Rabies 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 (up to three times >24 hours apart). They 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) 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 the injection, participants should remain in the clinic for at least 60 minutes to assess and record solicited adverse events within 25-60 minutes following the injection. Study staff will review the details to be added to the online vaccine diary cards with participants and explain how and when to complete them over the next 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, both before 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 in the 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.

An upper arm motor function assessment to evaluate if vaccination leads to muscle degeneration or damage will be carried out on participants on the day of injection and day 1 and 14 for the 2 priming doses and on the day of injection and 14 days later for the boost.

During these visits, any upper arm symptoms will be assessed (skin colour change and skin temperature change) and the motor function of the deltoid muscles (deltoid contracture and shoulder abduction) will be graded using the MRC grading scale. Any signs of infection will be treated with antibiotics.

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 the FAVN Rabies 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 and is discovered unexpectedly while 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 participant may need to be withdrawn or vaccinations discontinued, as per **Section 5.4**, and their GP informed, provided consent 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 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-RABsaRNA vaccine at one of three doses (0.2 µg, 1.0 µg or 5.0 µg) administered at either 0, 4 and 24 weeks or 0, 12 and 24 weeks, 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 neutralising antibody responses 4 weeks after the second vaccination

The exploratory outcome measures are:

- The kinetics and durability of neutralising antibodies following each vaccination
- The titre of serum binding antibodies by ELISA
- 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 enough 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 dose and administration schedule 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 48 participants will be included in the randomised evaluation of immunogenicity, 8 participants per group. The primary analysis will compare neutralising antibody responses to the Rabies virus (FAVN assay), 4 weeks after the second immunisation (primary endpoint). 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 1-6 is to assess the immune responses to the three different doses (0.2 µg, 1.0 µg and 5.0 µg) administered at either 0, 4 and 24 weeks or 0, 12 and 24 weeks. No formal hypothesis testing will be performed for immunogenicity endpoints; all comparisons will be descriptive. Ideally, neutralising antibodies should be observed in 100% of participants. With a sample size of 8 participants per group, the lower 95% 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 second 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 serum. More information on the assay and definition of positive results will be supplied in the SAP. Titres of neutralising antibodies will be described by timepoint and group and compared using rank tests, where appropriate.

10.4 Analysis Plan

A complete 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 5.0 µg, and a report of 7-day reactogenicity will be provided when the first four 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 an 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 will be 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 guide the development of procedures regarding informed consent, confidentiality, trial monitoring, and audit, and will lead to the creation of a Data Management Plan (DMP), Safety Reporting Plan, and Monitoring Plan. This risk assessment will be updated as required during the trial.

11.1.1 Safety and Rights of Participants

The pre-clinical data available for LNP-RABsaRNA in three species (mice, rats, and pigs) suggest that this product will be similar to licensed RNA vaccines, causing mild to moderate reactions that are transient. However, this is a first-in-human trial, and therefore, a sentinel cohort will be established with one individual allocated to each dose 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 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 consistently 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 Chelsea and Westminster hospital on different trials including vaccines in 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 throughout 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. The 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 2013, 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.

*The trial will be conducted in accordance with the Clinical Trials Directive as implemented in the UK statutory instrument 2004/1031. As the new UK Clinical Trials Regulations (The Medicines for Human Use (Clinical Trials) (Amendment) Regulations 2025) come into force before trial completion (anticipated from April 2026), the sponsor and investigators will ensure continued compliance by aligning trial conduct with the updated requirements and MHRA guidance.

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 retained for a minimum of 10 years after the trial concludes. 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 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 the 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 at least 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 has been offered, and time has been allowed for consideration. Signed participant consent should be obtained. The participant's right to refuse to participate without giving reasons must be respected. After the participant has entered the trial, the clinician remains free to provide alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant’s best interest. Still, the reasons for doing so should be recorded. In these cases, the participants remain within the trial for 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 The Coalition for Epidemic Preparedness and Innovation (CEPI).

12.8 Audits and Inspections

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

13. TRIAL MANAGEMENT

There are several 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 the 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, particularly advising 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 who are identified to reflect the broader 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 necessary to address the problem. If the TMT or TMG identify issues 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 trial will be registered and reported on ISRCTN registry. 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, 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 RAB-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 v1.0, dated 10th November 2025. Any subsequent amendments will be recorded below.

Version	Date	Reason for change
1.0	10th Nov 2025	New protocol

16. REFERENCES

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