Study Title: An experimental medicine study of Crimean–Congo haemorrhagic fever (CCHF) vaccine immune challenge responses in Lymph nodE single-cell Genomics in AnCestrY and ageing

Short title: Examining lymph node cells to assess how age affects immune responses

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Chief Investigator:

Dr Katrina Pollock
Oxford Vaccine Group
Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital
Oxford
OX3 7LE

Investigators:

Name	Address	Email and telephone
Teresa Lambe (TL)	Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ	teresa.lambe@paediatrics.ox.ac.uk

Sponsor:

University of Oxford
Research Governance, Ethics & Assurance Team (RGEA)
Boundary Brook House, Churchill Drive, Headington,
Oxford, OX3 7GB
Tel: 01865 616480
Email: RGEA.sponsor@admin.ox.ac.uk

Funder: UK Research and Innovation, Medical Research Council (MRC)

Chief Investigator Signature: Katrina Pollock, Oxford Vaccine Group, University of Oxford

Statistician Signature: Yiyuan Zhang, Oxford Vaccine Group, University of Oxford

Declaration of interests: Dr K Pollock is a member of a data safety monitoring board (DSMB) for a commercially (ModernaTX, Inc) sponsored clinical trial NCT05575492 and has been a DSMB member for another commercially sponsored trial, NCT05249829.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so. The document is part of the LEGACY programme of research and is developed in line with other LEGACY clinical study protocols.

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1. KEY CONTACTS

Chief Investigator	Dr Katrina Pollock Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital Oxford OX3 7LE Tel: 01865 611400 Email: katrina.pollock@paediatrics.ox.ac.uk
Sponsor	University of Oxford Research Governance, Ethics & Assurance Team (RGEA) Boundary Brook House, Churchill Drive, Headington, Oxford, OX3 7GB Tel: 01865 616480 Email: RGEA.sponsor@admin.ox.ac.uk
Principal Investigators / Trial Sites	**Site 1:** PI Katrina Pollock Oxford Vaccine Group, University of Oxford Centre for Clinical Vaccinology & Tropical Medicine (CCVTM), Churchill Hospital Oxford OX3 7LE **Site 2:** PI Katrina Pollock Oxford University Hospitals Foundation Trust (non-recruiting site) Headley Way, Headington, Oxford OX3 9DU
Funder(s)	UK Research and Innovation, MRC
Clinical Trials Unit	**Primary Care and Vaccine Collaborative Clinical Trials Unit,** Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital Old Road, Headington Oxford United Kingdom OX3 7LE
Statistician	Yiyuan Zhang Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital Oxford OX3 7LE Tel: 01865 611400 Email: yiyuan.zhang@paediatrics.ox.ac.uk
Committees	**Data safety monitoring committee** Chair: Martha Nason mnason@niaid.nih.gov

	Lymph node single cell genomics ancestry and ageing (LEGACY) Network Chair: Dr Katrina Pollock Email: Katrina.pollock@paediatrics.ox.ac.uk
Clinical Trial Monitor	Oxford Vaccine Group (OVG) internal quality assurance team Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital Oxford OX3 7LE

2. LAY SUMMARY

This study aims to understand how immune cells in lymph nodes respond to a new immunisation and how this response changes with ageing. This information will help design future vaccines (for example, for future pandemics), to tailor vaccination strategies in different patient populations including in older people.

Older people respond less well to vaccines than younger adults, and they are more severely affected by infectious diseases, so it is important to understand how age influences lymph node responses.

Immune responses are typically tested by taking blood samples and measuring antibodies and immune cells (lymphocytes). These responses occur in lymph nodes (in the case of injection in the arm, in armpit lymph nodes). Cells from lymph nodes can be sampled using ultrasound-guided fine needle aspiration (FNA) and core needle biopsy (CNB), well-established techniques, which enable direct testing of the responses of immune cells.

Participants in the study will receive ChAdOx2 CCHF, a new immunisation against Crimean-Congo haemorrhagic fever (CCHF), a potentially fatal viral illness spread by ticks. The World Health Organisation estimates 3 billion people are at risk. The vaccine is being developed by the University of Oxford, using similar technology to the Oxford/AstraZeneca COVID-19 vaccine, and its first clinical trial is underway.

In this study the immunisation, or study injection is used as a challenge to the immune system (called an immunogen) to stimulate the lymph nodes. We are testing how lymph nodes respond to an immunogen that people have not previously encountered. This is why we are using the CCHF immunogen; because most people will not have had this infection in the past and will not have been vaccinated. This means we can really understand how ageing affects the ability of lymph nodes to respond.

The study will recruit 16 healthy adults (8 aged 18-45 years; 8 aged 65 years or above). All will receive two doses of the immunogen injection, 12 weeks apart. All will have FNA from both armpits, before receiving the study injection and at 7 days after each injection. Participants will be assessed for eligibility at a screening visit; those eligible to take part will attend a further 8 visits, scheduled over 24 weeks. Blood samples will be taken at each visit. Safety will be closely monitored.

In a separate arm of the study, we will recruit up to 4 healthy adults aged 18-45 years or 65 years and over to receive two doses of the immunogen injection and have a core needle biopsy (CNB) 7 days after receiving the study injection.

3. SYNOPSIS

Study Title	An experimental medicine study of Crimean–Congo haemorrhagic fever (CCHF) vaccine immune challenge responses in Lymph nodE single-cell Genomics in AnCestrY and ageing		
Short title	Examining lymph node cells to assess how age affects immune responses		
Study code	OVG2023/03 LEGACY02		
Study registration	ISRCTN11688703		
Sponsor	University of Oxford		
Funder	UK Research and Innovation Medical Research Council (MRC) Polaris House, North Star Avenue, Swindon, SN2 1FL		
Study Design	Experimental medicine study, open label		
Study Participants	Healthy adults, aged 18-45 years, and aged 65 years or over		
Sample Size	Total maximum sample size: 24 • 8 participants aged 18-45 years • 8 participants aged 65 years or over • Up to 4 participants aged 18-45 years or 65 years and over into the core needle biopsy study		
Planned Study Period	Total length of the project: 2 years Duration of an individual participant's involvement: Main cohort: 24 weeks, Core Needle Biopsy cohort: 16 weeks		
Planned Recruitment period	Start date for recruitment: July 2024 End date for recruitment: April 2025		
	Objectives	Outcome Measures	Timepoint(s)
Primary	To determine the frequency, phenotype, and function of immune cells, in axillary secondary lymphoid tissue and compare with the blood after intramuscular immunogen challenge with	Multi-parameter analysis of lymph node cells using single cell ribonucleic acid sequencing 5-prime (5' scRNA-seq), and/or multiparameter flow cytometry	Baseline/Day 0 and Day 7 after first and second study injections

	ChAdOx2 CCHF, in older and younger age volunteers.		
Secondary	To measure immunogen-reactive lymph nodes using ultrasound imaging	Ultrasound measurements of secondary lymphoid tissue	Baseline/Day 0, Day 7, and Day 28 after first and second study injections
Exploratory	Comparing younger with older age groups, before and after injection 1. To observe self-reported measures of axillary swelling and tenderness 2. To determine the T and B cell responses in axillary secondary lymphoid tissue after intramuscular immunisation 3. To measure serological responses to CCHF 4. To measure the inflammatory response in the lymph node after immunisation 5. To perform high-resolution tracking of T and B cell clones from LNC (Lymph Node Cells) and PBMC (Peripheral Blood Mononuclear Cells) as they develop after immune challenge 6. To observe self-reported measures of axillary swelling, tenderness and bruising after ultrasound guided core needle biopsy (core needle biopsy study cohort only) 7. To measure the intra-nodal spatial relationship between lymph node cells responding to the immunogen injection	Outcome measures may include but are not limited to the following: • Single cell ribonucleic acid sequencing 5-prime (5' scRNA-seq) to measure cell by cell transcriptomes in lymph node cells • Cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) to measure cellular antigens on lymph nodes cells • Single cell T cell receptor sequencing (scTCR-seq) to measure T cell receptor diversity in lymph node cells • Immunoglobulin gene sequencing (Ig-seq) to measure B cell receptor and antibody diversity in lymph node cells • Phenotypic and functional T cell assays to measure T cell subsets and function, particularly T follicular helper cells using for example an activation induced marker (AIM) assay, multi-dimensional flow cytometry and ELISpot • ELISA to measure binding antibody responses against CCHF • Imaging-based techniques to measure spatial architecture of lymph node core needle biopsy such as single cell spatial transcriptomics or Cell DIVE™	Measured at any or all pre and post injection timepoints
Intervention(s)	Fine needle aspiration of axillary lymph nodes Non-diagnostic ultrasound Immune challenge agent: ChAdOx2 CCHF candidate vaccine		

4. ABBREVIATIONS

AE	Adverse event
AESI	Adverse Events of Special Interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Adverse reaction
AST	Aspartate aminotransferase
BMI	Body mass index
CBF	Clinical Biomanufacturing Facility
CCHF	Crimean-Congo haemorrhagic fever
CCHFV	Crimean-Congo haemorrhagic fever virus
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
ChAd63	Chimpanzee Adenovirus serotype 63
ChAdOx1	Chimpanzee Adenovirus Ox1
ChAdOx2	Chimpanzee Adenovirus Ox2
CI	Chief Investigator
CLS	Capillary leak syndrome
CRF	Case Report Form
CTIMP	Clinical Trial of an Investigational Medicinal Product
CVST	Cerebral venous sinus thrombosis
DNA	Deoxyribonucleic acid
DSMC	Data Safety Monitoring Committee
DSUR	Development Safety Update Report
EBV	Epstein Barr virus
EDC	Electronic Data Capture
ELISA	Enzyme linked immunosorbent assay
ELISpot	Enzyme linked immunospot assay
FDA	Food and Drug Administration
FNA	Fine needle aspiration
GBS	Guillain-Barre Syndrome
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
HBsAg	Hepatitis B surface antigen
HCG	Human Chorionic Gonadotrophin
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRA	Health Research Authority
IB	Investigators Brochure
IFN	Interferon
IM	Intramuscular/intramuscularly
IMP	Investigational medicinal product
ISF	Investigator Site File
ITP	Immune thrombocytopaenia purpura
IU	Infectious units

IUD	Intrauterine device
IUS	Intrauterine system
JCVI	Joint Committee on Vaccination and Immunisation
LVLV	Last volunteer last visit
MedRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	Messenger ribonucleic acid
NCT Number	National Clinical Trial number
OVC	Oxford Vaccine Centre
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
PIS	Participant information sheet
POCBP	Participant of childbearing potential
QP	Qualified Person
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance (formerly Clinical Trials and Research Governance)
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SmPC	Summary of Product Characteristics
SOC	System Organ Classes
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TTS	Thrombosis with thrombocytopenia syndrome
UKHSA	United Kingdom Health Security Agency
US	Ultrasound
VITT	Vaccine-induced immune thrombotic thrombocytopaenia
vp	Viral particles
WHO	World Health Organization

5. BACKGROUND AND RATIONALE

5.1. Tissue based immunogen challenge studies in older people

Older people are at risk from severe disease from pathogens with pandemic potential. The adult population aged 65 years and over was the largest vulnerable patient group during the COVID-19 pandemic. This population continues to grow, increasing the at-risk patient population facing any future pandemic. Age-related immune decline, comorbidity and risk of exposure to infection in health and social care facilities all contribute to this risk. Vaccines are the major tool to protect against this but suffer from a lack of efficacy in older people. This is because the immunogens are not designed with the ageing immune system in mind.

Tissue-based research to improve immunogen design iteratively (including dose selection, dose scheduling, platform design, adjuvant selection, formulation and delivery) is needed in older adults. Located in the axilla, reactive secondary lymphoid tissue is the major target for the mechanism of action of immunogens delivered by intramuscular (IM) injection. Our group has established an experimental medicine model that uses ultrasound (US) guided fine needle aspiration (FNA) of the axillary lymph nodes to investigate immune-responsive cells.[1] In addition, our group has expertise in performing core needle biopsies (CNB) of axillary lymph nodes, a procedure that can be utilised to study the spatial relationships of the immune-responsive cells under investigation, including stromal cells within the lymph nodes. In this study we will compare the lymph node responses of younger and older adults with the response in the blood, using a novel immunogen to stimulate the immune response (ChAdOx2 CCHF)

The World Health Organisation (WHO) has prioritised twelve diseases for pandemic research; second on the list after COVID-19 is Crimean-Congo haemorrhagic fever (CCHF).[2] This is a tick-borne viral haemorrhagic fever that can also be transmitted during animal slaughter and through human-to-human transmission. Most people have not been exposed to CCHF, meaning they are immune naïve. For this reason, glycoprotein-based immunogens from CCHF, expressed by the adenoviral-vectored candidate vaccine ChAdOx2 CCHF can be used to investigate *de novo* lymph node responses in younger and older people. Data from this study will have several important uses in combating ageing. Not only this will inform the design and delivery of immunogens used in vaccines against pandemic infections but provide significant insight into the critical immune response pathways that are negatively affected by age.

CCHF and the novel replication deficient adenoviral-vectored vaccine ChAdOx2 CCHF are described in more detail in the following section.

5.2. Crimean-Congo haemorrhagic fever (CCHF) and ChAdOx2 CCHF

5.2.1. Crimean-Congo haemorrhagic fever (CCHF)

In 1944, an outbreak of haemorrhagic fever occurred in Russian troops re-occupying the Crimean peninsula.[3] Cases of the disease were soon linked to tick exposure. The causative organism was identified in 1967, using suckling mice to cultivate the virus.[4,5] In 1969, it was realized that the virus was the same as a virus isolated in the Belgian Congo in 1956.[6] The disease has been referred to as Crimean-Congo haemorrhagic fever (CCHF) since the early 1970s.

The causative agent is an *Orthonairovirus* (genus *Nairoviridiae*, family *Bunyaviridales*), a negative-sense, single-stranded RNA virus. The virion is spherical, approximately 80-120nm in diameter. The outer membrane is studded with two glycoproteins, G_N and G_C. The virions contain three RNA genome segments:

small (S), medium (M) and large (L). A precursor for the surface glycoproteins is coded by the M segment. The S segment encodes nucleoprotein; the L segment encodes an RNA-dependent RNA polymerase.[4]

The virus causes asymptomatic infection in many wild and domesticated vertebrates, including cattle, sheep, goats, camels and ostriches. It is usually transmitted by ixodid ticks, particularly those of the genus *Hyalomma*.[7] The northern limit of distribution of these ticks is approximately 50°N.[8,9] Transmission can also occur by contact with infected body fluids and tissues.[4]

Human infections have been reported in numerous countries in southern Europe, the Middle East, Africa and south-west Asia.[10,11] A few cases acquired by travellers from endemic countries have been diagnosed in the UK.[12] Turkey, Iran, Russia and Uzbekistan all report more than 50 cases per year. Recently, cases have been reported in Spain.[13] The WHO estimates that 3 billion people live in areas at risk, and that CCHF infects 10,000 to 15,000 people per year, causing 500 deaths.[9]

Serological surveys in high-risk areas of Turkey suggest that about 88% of human infections are subclinical.[14] In humans who develop disease, the incubation period may be from 1 to 13 days; typically, it is 1 to 5 days following a tick bite, or 5 to 7 days following exposure to infected blood or tissues. The disease is characterised by sudden onset fever, headache and myalgia; vomiting, abdominal pain and diarrhoea may also occur.[15] A haemorrhagic phase typically begins around 3 to 5 days later. Petechial skin rashes may progress to extensive subcutaneous ecchymoses; subconjunctival and other mucosal haemorrhages may occur; there may be bleeding from the gastrointestinal and urinary tracts; cerebral haemorrhage may also occur. In fatal cases, death is usually in the second week of the illness, from haemorrhage, shock and organ failure. High viral load ($\geq 10^8$ copies/ml) correlates with fatal outcome.[16]

Supportive therapy, including replacement blood products, is the mainstay of treatment.[15] Ribavirin has been observed to reduce viral load and lethality in laboratory suckling mice, but its efficacy in humans has not been definitively demonstrated in randomised clinical trials.[17] Specific immunoglobulin therapy has been used for post-exposure prophylaxis and treatment, but efficacy has not been confirmed by case-control studies.[17,18]

5.2.2. Rationale for study injection

CCHF is one of twelve diseases currently listed by the WHO as a priority disease for research and development, based on its risk to public health, epidemic potential and inadequacy of countermeasures.[19] It is listed as a high consequence infectious disease by the UK Health Security Agency.[20] As the host tick is not distributed in the UK, younger and older adults entering the study are not expected to have any previous immunity. This provides a relevant model for studying immune priming and boosting in older adults who are vulnerable to severe infectious disease.

5.2.3. ChAdOx2 CCHF study injection

This study will investigate the tissue based immune responses to priming and boosting by intramuscular injection with a ChAdOx2 construct encoding novel antigens, the Gn and Gc glycoproteins from CCHF, in the previously naïve host. This immunogen has been selected because volunteers will have had no previous exposure to these antigens allowing full characterisation of a truly *de novo* response. This characterisation in younger and older adults will expose differences in the priming and boosting response. Through avoidance of immune-imprinting, a major confounder in studies using a recall antigen such as haemagglutinin (the antigen in seasonal influenza vaccine), this approach will highlight how age affects critical immune pathways in the secondary lymphoid tissue, including germinal centre (GC) formation, induction of T follicular helper cell- and GC B cell responses. Given that any future pandemic will, by

definition, involve widespread exposure to novel antigens, and this will occur in a rapidly ageing global population, the immunological insights gained from this study will prove critical in designing new tools to protect the ageing population from pandemic diseases.

ChAdOx2 is a recombinant simian adenovirus viral vector developed by the University of Oxford. It was derived from wild-type replication-competent isolate AdC68 (species adenovirus E, also known as SAdV-25 and Pan 9), which has been rendered replication-deficient through deletion of the E1/E3 gene region (which is essential for viral replication), with further modification to the E4 region. To produce ChAdOx2 CCHF, a human codon optimised gene encoding CCHFV glycoprotein under the control of the short cytomegalovirus promoter was inserted into the vector. The viral vector was then rescued and grown in the HEK293 derived TRex cell line, prior to CsCl purification and sterile filtration.

The CCHFV M segment gene codes for a glycoprotein precursor (GPC). After expression, this is processed by a range of proteases into five glycoproteins: MCL, GP38, Gn, NSm, and Gc. Of these glycoproteins, the Gn and Gc subunits show greatest conservation across CCHFV strains; Gn and Gc antigens have been shown to induce protective responses in some mouse challenge studies, and antibodies targeting Gc have been shown to neutralise CCHFV tec-VLPs.[21] [22]. The Gn and Gc glycoproteins are the antigens found on the surface of CCHFV virus and are thought to be responsible for viral tropism and cell entry, though the cell surface target is unknown.

The immunogenicity of ChAdOx2 CCHF has been compared with a similar ChAdOx1-vectored vaccine (ChAdOx1- CCHFV GPC) in BALB/C mice.[22] Vaccination with ChAdOx2 CCHF induced significantly greater anti-CCHFV Gc antibody titres in mice than vaccination with ChAdOx1- CCHFV GPC, although no further significant differences were measured between vectors with regards to cellular or neutralisation responses induced by vaccination.

A phase 1 clinical trial to assess the safety and immunogenicity of ChAdOx2 CCHF in healthy adults aged 18 to 55 years is currently being undertaken in Oxford (CCHF01, REC ***[***23/LO/0420***]*** EudraCT Number 2022-003889-20).

Participants will be enrolled into LEGACY02 when the first cohort of six participants in CCHF01 [23/LO/0420], the phase 1 study, have received their second dose of vaccine and after the first formal DSMC safety review has been completed. This safety review occurred on 2 October 2023 once all six participants had completed 7 days post first vaccination. The date for completion of second vaccine for cohort 1 in the phase 1 study was 3rd January 2024.

5.2.4. Other ChAdOx2-vectored vaccines in clinical trials

The safety and T-cell immunogenicity of another vaccine based on the ChAdOx2 platform, expressing four genes from the *Mycobacterium avium* subspecies *paratuberculosis* has been assessed in humans, using a 'three-plus-three' dose escalation study design.[23] The vaccine was found to be safe and well tolerated in doses up to $5x10^{10}$vp. A rabies vaccine, ChAdOx2 RabG, has also been assessed in a phase I clinical trial, in which participants were sequentially allocated to receive low ($5x10^{9}$vp), middle ($2.5x10^{10}$vp) or high ($5x10^{10}$vp) doses.[24] Participants reported predominantly mild-to-moderate reactogenicity and there were no serious adverse events. The middle and high dose groups developed an encouraging immunogenicity profile, and at follow up one year after vaccination, 6/7 maintained a protective level of neutralising antibody.

5.2.5. Rationale for selected dose

The regimen and dose level of ChAdOx2 CCHF ($5x10^{10}$ viral particles per dose, as a 2-dose administration) was selected on the basis of clinical experience with other ChAdOx1- and ChAdOx2-viral vectors, as well as other adenovirus vaccines (such as ChAd63).

ChAdOx1 nCoV-19 has been approved as a 2-dose schedule, administered 4 to 12 weeks apart, at a dose of $5x10^{10}$ viral particles per dose (or equivalent). This regimen is well tolerated and immunogenic. Although approved as a 2-dose schedule, immunogenicity occurs following the first dose. The second dose boosts binding and neutralising antibody responses to SARS-CoV-2. A 12-week interval between doses results in superior immunogenicity to a 4-week interval. The second dose produces lower rates of adverse reactions compared to the initial dose.[25-27] Studies have demonstrated its efficacy following both a single and two-dose regimen.

Many clinical trials of ChAdOx1-vectored vaccines have included doses up to $5x10^{10}$ viral particles. In every case, the safety and immunogenicity profiles were acceptable.

Two vaccines based on the ChAdOx2 platform have been assessed in phase 1 trials and found to be safe and well tolerated in doses up to $5x10^{10}$ vp.[23,24]

Based on these data, LEGACY02 study will use a 2-dose regimen of ChAdOx2 CCHF, each containing $5x10^{10}$ vp, given at a 12-week interval. This is the same dose regimen as is being tested for safety and immunogenicity in the Phase 1 clinical trial.

5.3. Potential risks to participants

Study related risks are summarised below.

5.3.1. Risks related to FNA of lymph nodes

Expected adverse events following lymph node aspiration include sample site pain or tenderness. Haematoma is a rare risk, and minimal bleeding may occur after the aspiration but should resolve spontaneously. Participants at increased risk due to blood-thinning medication will be excluded. Bruising may occur but is expected to fade after 2 weeks. In a study lead by the chief investigator, common adverse events attributable to the FNA (tenderness/pain, bruising, swelling) were mild in nature and resolved within 5 days[1]. The chief investigator is conducting studies that included >390 US guided axillary lymph node FNA procedures (NCT03816137, ISRCTN13657999, ISRCTN12928349, ISRCTN11688703) with no serious adverse events related to the procedure.

Damage to the underlying/adjacent structures is an extremely rare risk effectively minimized by direct visualization under ultrasound guidance. In a different study conducted at the University of Oxford (VAC096), a pneumothorax occurred after an FNA procedure and was considered by the investigators of that study to be related to study procedures.

Participants will be provided with information regarding both these expected and rare adverse events in the participant information sheet and adverse events will be monitored and reported.

5.3.1. Risks related to CNB of lymph nodes

The adverse events following CNB of axillary lymph nodes are similar to those for axillary lymph node FNA described above. As a larger bore needle is used than for FNA (typically 14-18 gauge for CNB compared with 21 gauge for FNA), there may be higher frequency of bruising and haematoma, however this is expected to fade after 2 weeks. The procedure is performed under direct US guidance to minimise this risk and risk of penetrating underlying structures. Similar to FNA, participants will be provided with information

regarding expected adverse events, and adverse events post CNB procedure will be prospectively monitored and reported.

5.3.2. Risks related to ChAdOx2 CCHF

The most likely side effects that recipients of ChAdOx2 CCHF may experience are short-lived local (primarily injection site pain) and systemic vaccine reactions (fatigue, headache, myalgia,) that resolve completely within days. There has been a wider clinical experience with ChAdOx1- than with ChAdOx2-viral vectors, particularly with ChAdOx1 nCoV-19 (Oxford/AstraZeneca COVID-19 vaccine). Given the close similarity between the two platforms, it may be anticipated that ChAdOx1- and ChAdOx2-viral vectors are likely to share similar safety profiles.

Subjects will have the details of a 24-hour contact study doctor and can be seen for unscheduled visits, if required.

The frequency of the commonest adverse reactions to ChAdOx1 nCoV-19 are shown in Table 1 below.

Table 1: Frequency of commonest adverse reactions to ChAdOx1 nCoV-19[28]

Adverse Reaction	Frequency (%)
Injection site tenderness	68%
Injection site pain	58%
Fatigue	53%
Headache	53%
Malaise	44%
Myalgia	44%
Feverishness	34%
Arthralgia	27%
Nausea	22%
Fever over ≥38°C	8%

Table 2: Frequency of solicited adverse reactions to ChAdOx2 CCHF vaccine for dose 1 and dose 2 reported in the CCHF01 Phase 1 clinical trial.

Adverse Reaction	Frequency (%)	
	Dose 1*	Dose 2**
Injection site Pain	63%	61%
Fatigue	57%	47%
Muscle pain (Myalgia)	57%	35%
Headache	48%	42%
Malaise	35%	26%

Joint pain (Arthralgia)	24%	7%
Feverishness	15%	23%
Nausea	15%	7%
Chills	11%	14%
Warmth	11%	19%
Fever (over ≥38°C)	7%	2%
Itch	2%	12%

* Please note that the denominator for dose 1 is 46
** Please note that the denominator for dose 2 is 43

Data for Table 2 were extracted from the ongoing Phase 1 clinical trial on 15 April 2024 (CCHF01, REC ***[***23/LO/0420***]*** EudraCT Number 2022-003889-20). Reactions to the ChAdOx2 CCHF vaccine in the Phase 1 clinical trial were self-limiting and the majority (99.3%) were mild or moderate in nature. There was one report of fatigue and one report of malaise which were both reported as grade 3 after the second dose. Two participants did not receive a second dose of ChAdOx2 CCHF in the trial; one participant developed mild allergy after the first dose, deemed related to the study injection, and one participant had a persistent neutropaenia deemed unrelated by the trial investigators. A third participant developed mild self-limiting thrombocytopaenia which fully resolved after both injections were given. No SAEs have been reported in the Phase 1 trial. The LEGACY02 protocol has taken account of these findings and mitigated by the criteria for inclusion and exclusion (allergy history) and continuous monitoring of safety blood test results.

Rarer adverse reactions associated with ChAdOx1 nCoV-19 are shown in Table 3, Section 10.3.

Post-marketing experience has revealed a very rare but serious side effect following vaccination with ChAdOx1 nCoV-19 (which has also been associated with the adenovirus- vectored Johnson & Johnson COVID-19 vaccine). This is known as thrombosis with thrombocytopenia syndrome (TTS) or vaccine-induced immune thrombotic thrombocytopaenia (VITT). It can present with venous thrombosis, often at unusual sites, such as cerebral venous sinus thrombosis (CVST) and splanchnic vein thrombosis.[29] The condition can also present with arterial thrombosis.[30] It can lead to death or serious long-term disability.

There has been marked geographical variation in the reporting rates of TTS/VITT. The WHO Strategic Advisory Group of Experts (SAGE) noted reporting rates have been far lower outside of the UK and EU, despite the widespread use of the vaccine in many countries outside of Europe.[31] An analysis of the AstraZeneca global safety database has shown reporting rates (in the 21 days after vaccination) vary, with 17.6 cases per million doses in Nordic countries, 10.0 cases per million doses in the UK and 0.2 cases per million doses in Brazil, South Korea and the Philippines.[32]

A review of 170 definite and 50 probable cases of VITT in the UK found a case mortality rate of 22%.[30] Most cases occurred between 5 and 30 days after vaccination (median 14 days, maximum 48 days). The age range was 18 to 79 years (median, 48), with no sex preponderance and no identifiable medical risk factors. Another UK study of over 3.7 million recipients of ChAdOx1 nCoV-19 found that after the first dose there was an increased risk of venous thromboembolism (standardized incidence ratio: 1.12 [95% CI: 1.05 to 1.20]), and of cerebral venous sinus thrombosis (standardized incidence ratio: 4.14 [95% CI: 2.54 to 6.76]).[33] Up to 23 November 2022, the MHRA had received Yellow Card reports of 445 cases of major thromboembolic events with concurrent thrombocytopenia in the UK following vaccination with ChAdOx1 nCoV-19 (221 in females, and 219 in males; age range from 18 to 93 years).[34] Fifty-one of the 445 reports

have been reported after a second dose. The overall case fatality rate was 18% with 81 deaths, six of which occurred after the second dose. Cerebral venous sinus thrombosis was reported in 161 cases (average age 46 years) and 284 had other major thromboembolic events (average age 54 years) with concurrent thrombocytopenia. The estimated number of first doses of COVID-19 Vaccine AstraZeneca administered in the UK by 23 November 2022 was 24.9 million and the estimated number of second doses was 24.1 million. The reported incidence of major thromboembolic events with concurrent thrombocytopenia following the first dose was higher in the younger adult age groups compared to the older groups (21.8 per million doses in those aged 18-49 years compared to 11.3 per million doses in those aged 50 years and over). This contrasts with incidence of these events after the second dose, which were more common in the older age group (1.0 per million doses in those aged 18-49 years compared to 2.1 per million doses in those aged 50 years and over).

It is widely recognised that VITT is more likely to occur after a first dose of ChAdOx1 nCoV-19 than after a second dose. An international consortium has set up a registry which provides data on cases of cerebral venous sinus thrombosis occurring within 28 days of ChAdOx1 nCoV-19 vaccination. Of the 124 cases, 120 were after a first dose (61 definite, 20 probable, 10 possible, and 29 unlikely VITT), and 4 were after a second dose (1 definite, 1 probable, 1 possible, and 1 unlikely VITT).[35] The interval between receiving the second vaccine dose and symptom onset varied between 1 and 6 days.

Cerebral venous sinus thrombosis (CVST) has been the most closely monitored manifestation of VITT. Using data from the European Medicines Agency's EudraVigilance database (until June 13, 2021), the absolute risk of cerebral venous sinus thrombosis (CVST) within 28 days of first-dose vaccination with ChAdOx1 nCoV-19 was estimated to be 7.5 per million (95% CI: 6.9-8.3).[36] In an age-stratified analysis, the absolute risk was the highest in the 18- to 24-year-old group (total CVST 11.0 per million [95% CI 5.0–23.9]). In the age groups 60 to 69 years and ≥70 years, the risk of CVST was the lowest (2.2 per million (95% CI 1.4–3.3) and 1.3 per million (95% CI 0.6–2.9), respectively).

The pathophysiological mechanism behind VITT appears to be activation of platelets by antibody to platelet factor 4 (PF4).[37,38] This closely resembles the pathophysiology of heparin-induced thrombocytopenia, which is also caused by anti-PF4 antibodies.

Other very rare serious reactions have been identified as part of post-marketing surveillance of ChAdOx1 nCoV-19 (Oxford/AstraZeneca COVID-19 vaccine). These include anaphylaxis, Guillain-Barré syndrome (GBS), transverse myelitis, capillary leak syndrome (CLS), and immune thrombocytopenic purpura (ITP). By 1st December 2022, the Yellow Card reporting system had received 888 reports of anaphylaxis or anaphylactoid reactions, 514 reports of suspected Guillain-Barré syndrome, 129 reports of transverse myelitis and 18 reports of capillary leak syndrome, after about 50 million doses of ChAdOx1 nCoV-19. The risk of ITP is about 5 cases per million doses of the vaccine.[34]

It is currently unknown whether these very rare reactions will occur with other ChAdOx1- or ChAdOx2-vectored vaccines. As ChAdOx2 CCHF has similarities to ChAdOx1 nCoV-19, participants will be informed about these conditions as part of the informed consent process for the study. Investigators will be aware of potential signs of these conditions.

Given existing safety data which supports the use of ChAdOx1 nCoV-2 use in pregnant women, there is no reason to believe ChAdOx2 CCHF would be harmful to women or the foetus during pregnancy. However, there are no data on the use of ChAdOx2 CCHF in pregnancy. Therefore, pregnant women will be excluded from the study and participants of childbearing potential will be required to use effective contraception (see Section 8.5).

5.3.3. Other study related risks

Blood sampling during the study may cause slight pain, bruising, light-headedness or fainting. The total volume of blood taken in the study is approximately 506ml, so should not compromise healthy participants (for comparison, a *single* donation to the NHS blood bank would be approximately 470ml). Intramuscular injections carry a risk of bleeding in patients with very low platelet counts or coagulopathies. A baseline full blood count (with a platelet count) taken prior to study injection reduces this risk.

The medical tests carried out during the study screening and follow up have the potential to find incidental medical problems that may require referral of volunteers for further investigation. Participants will be informed of these, and, with their consent, their general practitioner (GP) will be contacted.

5.4. Potential benefits to participants

The recruitment population will not directly benefit from participation in the study. This is because the individual's risk of becoming infected with CCHF is currently low. Furthermore, ChAdOx2 CCHF clinical efficacy against CCHF infection has not been established and will not be established by this study. Participants will be informed that they should not anticipate any protection from potential future CCHF infection following participation in this study. No specific additional medical care will be provided through participation, and medical procedures are performed with the aim of determining eligibility and safety during the study.

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	**Outcome Measures***	**Timepoint(s) of evaluation of this outcome measure (if applicable) compared with baseline**
Primary Objective To determine the frequency, phenotype, and function of immune cells, in axillary secondary lymphoid tissue and compare with the blood after intramuscular immunogen challenge with ChAdOx2 CCHF, in older and younger age volunteers.	Multi-parameter analysis of lymph node cells using single cell ribonucleic acid sequencing 5-prime (5' scRNA-seq), and/or multiparameter flow cytometry	Baseline/Day 0 and Day 7 after first and second study injections
Secondary Objective To measure immunogen-reactive lymph nodes using ultrasound imaging	Ultrasound measurements of secondary lymphoid tissue	Baseline/Day 0, Day 7, and Day 28 after first and second study injections
Exploratory Objectives		Measured at any or all pre and post injection timepoints

Comparing younger with older age groups, before and after injection 1. To observe self-reported measures of axillary swelling and tenderness	Outcome measures may include but are not limited to the following: • Single cell ribonucleic acid sequencing 5-prime (5' scRNA-seq) to measure cell by cell transcriptomes in lymph node cells • Cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) to measure cellular antigens on lymph nodes cells • Single cell T cell receptor sequencing (scTCR-seq) to measure T cell receptor diversity in lymph node cells • Immunoglobulin gene sequencing (Ig-seq) to measure B cell receptor and antibody diversity in lymph node cells • Phenotypic and functional T cell assays to measure T cell subsets and function, particularly T follicular helper cells using for example an activation induced marker (AIM) assay, multi-dimensional flow cytometry and ELISpot • ELISA to measure binding antibody responses against CCHF • Imaging-based techniques to measure spatial architecture of lymph node core needle biopsy such as single cell spatial transcriptomics or Cell DIVE™	
2. To determine the T and B cell responses in axillary secondary lymphoid tissue after intramuscular immunisation		
3. To measure serological responses to CCHF 4. To measure the inflammatory response in the lymph node after immunisation 5. To perform high-resolution tracking of T and B cell clones from LNC (Lymph Node Cells) and PBMC (Peripheral Blood Mononuclear Cells) as they develop after immune challenge 6. To observe self-reported measures of axillary swelling, tenderness and bruising after ultrasound guided core needle biopsy (core biopsy study cohort only) 7. To measure the intra-nodal spatial relationship between lymph node cells responding to the immunogen injection		

* *Outcome measures are not limited to these example assays

7. STUDY DESIGN

This is an open label, interventional experimental medicine study to investigate human immune responses in lymph node cells and compare with the blood in younger and older adults. A novel Crimean–Congo haemorrhagic fever immunogen presented as a non-replicating adenoviral vector, (ChAdOx2 CCHF) and given by intramuscular injection, will be used to stimulate the lymph nodes. Participants will be healthy adults in two age groups: 18-45 years and ≥65 years. All participants will receive two doses of $5x10^{10}$ vp ChAdOx2 CCHF, 12 weeks apart. Participants in the FNA cohort will have a lymph node FNA prior to and at 7 days after each study injection (three in total). Participants in the CNB cohort will have a lymph node CNB at 7 days after first study injection (one CNB in total). The study will be conducted at a single site, the experimental medicine clinical research facility at CCVTM, Oxford.

7.1. Study groups

Cohort	Group	Number of participants	Age	Timing of FNA/CNB (Days after each injection)	Follow up
FNA cohort	**A**	8	18-45 years	7 days*	24 weeks
	B	8	≥65 years	7 days*	24 weeks
CNB cohort	**C**	4	18-45 or ≥65 years	7 days*#	16 weeks

*Window: 7-10 days post injection. Where an FNA cannot be performed within this window, it can be rearranged at a later date, provided this is 7 days or more before or after the study injections.

#Participants in CNB cohort will only have one CNB 7 days after first study injection. Participants will also have a follow-up phone call 7 days after the CNB at D14.

7.2. Study duration

The total duration of the study will be approximately 6 months from the day of enrolment for each volunteer for the FNA cohort and approximately 4 months for each volunteer for the CNB cohort from the day of enrolment.

For the FNA cohort, Participants will be considered enrolled into the study at the point of the first FNA (FNA1). For the CNB cohort, participants will be considered enrolled in the study at the point of study injection administration.

7.3. Definition of start of study

The start of the study is defined as the date of the first FNA of the first volunteer.

8. PARTICIPANT IDENTIFICATION

8.1. Study participants

This study will be conducted in healthy adults, who meet the inclusion and exclusion criteria described below.

8.2. Inclusion criteria

Participants must satisfy all the following criteria to be eligible for the study:

1. Adults aged between 18 to 45 years (inclusive) OR aged 65 years and over at screening visit.

2. Medically stable (i.e., according to investigator judgement, it is not anticipated that the participant will require hospitalisation within the study period or that they will need to withdraw from the study for medical reasons before completion of protocol-specified follow-up). A stable medical condition is

defined as disease not requiring significant change in therapy or hospitalisation for worsening disease during the 90 days prior to enrolment.

3. Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of electronic diary cards.

4. Willing and able to give informed consent for participation in the study.

5. Agree to allow study staff to contact their GP or equivalent NHS databases to access the participant's vaccination records, medical history and have their opinion solicited as to the participant's appropriateness for inclusion.

6. Willing to allow their GP and/or consultant, if appropriate, to be notified of participation in the study.

7. Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS).

8. Agree to refrain from blood donation whilst in the study.

9. For participants of childbearing potential only (as defined by protocol Section 8.5): willing to use effective contraception established prior to receiving the first study injection and for the duration of enrolment in the study (and for a minimum of 18 weeks after final study injection) AND have a negative pregnancy test on the days of screening and study injection.

10. Has previously received any viral vectored vaccine, except for ChAdOx2 CCHF

8.3. Exclusion criteria

1. Participation in another research study involving an investigational product, or which includes procedures that could compromise the integrity of this study (such as significant volumes of blood already taken), within the 12 weeks prior to enrolment, or planned participation in such a study within the study period.

2. Body mass index >35

3. History of previous confirmed or suspected CCHF infection.

4. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the study injection.

5. Administration of regular anticoagulation medication likely to induce bruising or bleeding on fine needle aspiration.

6. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; severe infection(s); receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months, or long-term systemic corticosteroid therapy (including for more than 7 consecutive days within the previous 3 months).

7. History of anaphylaxis in relation to vaccination, or local anaesthetic such as lidocaine.

8. History of allergic disease or reactions likely to be exacerbated by any component of the study injection including hypersensitivity to the active substance or to any of the excipients of the study injection or to local anaesthetic such as lidocaine.

9. History of hereditary angioedema, acquired angioedema, or idiopathic angioedema.

10. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ) that is not fully resolved.

11. History of any serious psychiatric condition likely to affect participation in the study.

12. For participants of childbearing potential only: participants who are pregnant, breastfeeding or lactating, or are planning pregnancy during the study.

13. History of a bleeding disorder (e.g., factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture.

14. History of confirmed major thrombotic event (including cerebral venous sinus thrombosis, deep vein thrombosis, pulmonary embolism); history of antiphospholipid syndrome, or history of heparin induced thrombocytopenia or thrombosis with thrombocytopaenia syndrome.

15. History of episodes of capillary leak syndrome.

16. Severe and/or uncontrolled cardiovascular disease, respiratory disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder, or neurological illness, as judged by the Investigator (note, mild/moderate well-controlled co-morbidities are acceptable)

17. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 14 units per week.

18. Suspected or known injecting drug use within the 5 years preceding enrolment.

19. Detectable circulating hepatitis B surface antigen (HBsAg).

20. Seropositive for hepatitis C virus (antibodies to HCV).

21. Seropositive for HIV.

22. Any clinically significant finding on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing (at the discretion of an Investigator) within the recruitment timeline of the study.

23. Member of the study team. This is deliberately loosely defined, but at a minimum will include: anyone on the delegation log; anyone who might be anticipated to be placed onto the delegation log in the course of the study; anyone who has access to personal data on study participants (beyond name, contact details, DOB); and anyone who attends meetings where details of the study are discussed, for example safety updates.

8.4. Temporary exclusion criteria (for study injection visits)

The following apply to **study injection** visits. If the temporary exclusion resolves within the time constraints of the study, progression in the study can continue.

1. Receipt of any systemic corticosteroid (or equivalent) treatment within 14 days prior to study injection, or for more than 7 days consecutively within the previous 3 months.
2. Febrile illness (oral temperature ≥37.5°C) or systemically unwell on the day of study injection.
3. Receipt of systemic antibiotics will result in study injection being postponed until 7 days after the last antibiotic dose. This does not apply to topical antibiotic preparations.
4. Use of antipyretics in the 4 hours prior to study injection.
5. Occurrence of a laboratory adverse event, which in the opinion of the Investigator, requires further time and/or investigation to resolve or stabilize prior to study injection being administered.
6. Occurrence of any illness or adverse event, which in the opinion of the investigator, requires further time and/or investigation to resolve or stabilize prior to study injection being administered.

7. Receipt of any vaccines administered within 30 days of study injections (before or after) EXCEPT for influenza and COVID-19 vaccines, which preferably should not be given within 14 days of the study injection (before or after).
8. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer if included in the study, affect the ability of the volunteer to participate in the study, or impair interpretation of the study data.

8.5. Pregnancy and contraception

The viral vector component of ChAdOx2 CCHF lacks the E1 gene region necessary for replication *in vivo*. No safety signal related to pregnancy has been observed with ChAdOx1 nCov-19 vaccine. The risk of human teratogenicity/fetotoxicity with ChAdOx2 CCHF is therefore considered to be low.

However, the possible adverse effects of the ChAdOx2 CCHF injection on the outcome of pregnancy are unknown; therefore, pregnant and breastfeeding/lactating women will be excluded from the study. Should a participant become pregnant during the study, with their ongoing consent, they will be followed up for clinical safety assessment until the pregnancy outcome is determined. The baby will be followed up for up to 3 months post-delivery. Venepuncture and blood sampling will not be performed in a pregnant volunteer unless there is clinical need.

Participants of childbearing potential will be required to use an effective form of contraception. A participant is considered of childbearing potential (*i.e.,* fertile) from the point following menarche 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 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient, and effective contraception would need to be used.

Effective contraception should be established prior to receiving the first dose of immunogen, and for the duration of the study. Acceptable forms of effective contraception for participants of child-bearing potential include:

- Established use of oral, injected or implanted hormonal methods of contraception that inhibit ovulation
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomised male partner
- Sexual abstinence from heterosexual sex, when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (*e.g.*, calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal methods are NOT acceptable methods of contraception.

Barrier methods of contraception are **not** considered highly effective.

Male participants are not required to use barrier methods for the purposes of contraception, as the risks of immunogen excretion at mucosal surfaces and in semen are negligible.

9. PROTOCOL PROCEDURES

9.1. Recruitment

Several recruitment strategies may be employed, including but not limited to:

- Poster advertising: Display of posters advertising the study throughout local hospitals and doctor's surgeries, tertiary education institutions and other public places with the permission of the owner/ proprietor.
- Direct mail-out / SMS/text message/telephone / emails: Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases and other databases as described below. For the NHS databases, mailouts will be used only with CAG approval and should this be required, an amendment will be submitted. Initial contact to potential participants will not be made by the study team. Instead, study invitation material will be sent out on our behalf by an external company, CFH Docmail Ltd (or equivalent company), to preserve the confidentiality of potential participants. CFH Docmail Ltd (or equivalent company) is accredited as having exceeded standards under the NHS Digital Data Security and Protection Toolkit (ODS ID – 8HN70). For mail-outs via the electoral register, the study team will obtain access to the names and addresses of individuals who are on the open electoral register (which contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH Docmail system (or equivalent company), and the study invitation pack will be sent out by CFH Docmail (or equivalent company). Volunteers may also be recruited using direct SMS/text message, or emails to potential participants identified by GPs from their databases, subject to approval via an amendment (PIC agreements to be set up with the GP surgeries as required).
- Email campaign: We will contact representatives of local tertiary education establishments and local employers and ask them to circulate posters and link to study website by email or hard copy.
- Oxford Vaccine Centre (OVC) database for healthy volunteers/other databases: The study may be advertised on the electronic newsletter sent out to those potential participants signed up to the Oxford Vaccine Centre's Healthy Volunteers Database. Additionally, by email distribution to potential participants registered on the OVC Healthy Volunteers Database or similar databases (where members of the public have given their consent to be contacted when studies open for recruitment and understand that this is not a commitment to participate), or to a group or list only with the express agreement of the network administrator or with equivalent authorisation.
- Media advertising: Local media, newspaper and website advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- Website advertising: Description of the study and copy of information booklet on the OVG website.
- Social media: Advertisements placed on OVG or University of Oxford Social media accounts or targeted social media platform advertisements including, but not restricted to, Twitter, Facebook and Instagram
- Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.

Potential participants who are interested in study participation will be able to contact the site by telephone, email, online or a reply slip.

9.2. Screening and eligibility assessment

If an individual is interested in the study, an information sheet can be downloaded from the study website by the potential participant, and/or sent to them via mail or email. If potential participants are willing to proceed, they will be asked to complete an initial online questionnaire which will include eligibility screening, e-consent to access medical and vaccination records and store personal information, and obtaining relevant medical history and personal information, before they are invited for a full screening and consent visit, where their eligibility will be assessed by a member of the clinical research team. Where potential participants are not able or willing to complete the online screening and e-consent for storing and accessing medical records, they can be invited to attend a face-to-face screening.

Potential participants who appear eligible will be invited to the screening visit (Section 9.7.1).

9.3. Informed consent

No study specific procedures will be performed until the individual has given informed consent and indicated this by signing and dating the informed consent form. The participant information sheet will be made available to the volunteer at least 24 hours prior to the full screening visit. At the in-person screening visit, the individual will be fully informed of all aspects of the study, its potential risks, and their obligations. The following general principles will be emphasised:

- Participation in the study is entirely voluntary.
- Refusal to participate involves no penalty or loss of medical benefits.
- The volunteer may withdraw from the study at any time.
- The individual is free to ask questions at any time to allow them to understand the purpose of the study and the procedures involved.
- The study involves research into lymph nodes using an immune challenge agent, which is also being developed as a candidate vaccine.
- There is no direct benefit to individuals from participating.
- The volunteer's GP will be informed of their participation in the study.
- Confirmation of their medical history will be required, *e.g.,* through a medical history summary from their GP practice or equivalent.
- The volunteer's samples may be sent outside of the UK and Europe to laboratories in collaboration with the University of Oxford. These samples will be de-identified.
- That long term storage of samples after the study is over is optional and will be covered under the Oxford Vaccine Centre Biobank Study protocol (REC 21/SC/0161), which will be consented to separately.
- The samples may be used for the commercial development of therapeutics, drugs and/or vaccines.

The individual will have the opportunity to discuss the study with a medically qualified investigator. Written informed consent will be obtained by means of a dated signature of the participant and a signature of the appropriately trained and delegated clinician. A copy of the signed informed consent will be given to the participant and the original signed form will be retained at the study site.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP, or other independent parties to decide whether they will participate in the study.

9.4. Randomisation

There is no randomisation in this study.

9.5. Blinding and code-breaking

There is no blinding in this study.

9.6. Description of study intervention(s), comparators and study procedures (clinical)

9.6.1. Description of study intervention: ChAdOx2 CCHF

See Sections 5.2.3 and 5.3.2 above, for a description of the ChAdOx2 CCHF injection and the potential risks associated with it.

9.6.1.1. ChAdOx2 CCHF manufacture and presentation

ChAdOx2 CCHF has been formulated and vialed under Good Manufacturing Practice conditions at the Clinical Biomanufacturing Facility (CBF), University of Oxford. At the CBF the immunogen injection will be certified and labelled for the study by a Qualified Person (QP) before transfer to the clinical site. The immunogen is supplied as a liquid in single use closed plastic Aseptic Technologies vials (consisting of Cycle Olefin Copolymer vial body, Thermo Plastic Elastomer stopper and Acrylonitrile Butadiene Styrene top and bottom ring, with a nominal volume of 2 ml) for intramuscular administration and will be stored at -70°C to -85°C in a secure freezer at the clinical site.

Batch number CC22A23A (used for this study) is presented in A438 formulation buffer: 10mM Histidine, 7.5% sucrose (w/v), 35mM NaCl, 1mM MgCl2, 0.1% PS80 (w/v), 0.1mM Edetate Disodium, 0.5% ethanol (v/v), pH 6.6. The appearance of ChAdOx2 CCHF is frozen liquid. Once thawed out it presents as a slightly opaque liquid, essentially free from visible particulates.

The dose of ChAdOx2 CCHF to be used in study will be $5x10^{10}$ virus particles per administration.

9.6.1.2. Storage of ChAdOx2 CCHF

ChAdOx2 CCHF requires storage between -70°C to -85°C throughout and will be transported to the study site after authorised release for use in the study by the CBF Qualified Person (QP) and study approval by REC. Movements of study medication between CBF and study sites will be documented in accordance with relevant SOPs.

Throughout the study, the study immunogen will be stored in temperature monitored freezers with an auditable temperature record in accordance with the manufacturer's instructions and relevant SOPs. Study freezers are connected to a monitoring system with 24-hour access to staff who can move the product in the event of significant temperature deviation.

9.6.1.3. Compliance with study treatment

The study injection will be administered by trained study personnel and will be documented according to GCP guidelines and relevant SOPs. Issues related to compliance are therefore the responsibility of study personnel who have received appropriate training.

9.6.1.4. Accountability of the study treatment

The ChAdOx2 CCHF immunogen will be manufactured, packaged, labelled and supplied by CBF. All immunogens (vials and boxes) are labelled with no less than the following:

- The study identifier (by reference code)

- The content of each vial
- Batch and serial number
- Expiry date
- Chief Investigator's name

The immunogen will be delivered and stored at the study sites pending authorised release for use in the study.

9.6.1.5. Concomitant medication

The use of all concomitant medication (prescribed or "over the counter") will be recorded in the CRF. There is no restriction on the use of concomitant medication, but the use of some prescribed medicines, such as immune suppressive agents, may result in the withdrawal of the participant at the discretion of the Investigator, while others, such as antibiotics, may result in a temporary exclusion.

9.6.1.6. Emergency medication and procedures

All clinical staff are trained, and can provide evidence of competency, in the acute management of anaphylaxis reactions, including the use of intra-muscular adrenaline. This is detailed in relevant SOPs and adrenaline is available at all times of study injection administration and subsequent observation.

9.6.2. Description of study procedures

9.6.2.1. Fine needle aspiration of lymph nodes

Fine needle aspiration (FNA) will be carried out by an appropriately trained practitioner at the clinical facilities at CCVTM, Oxford, UK.

Eligibility to undergo the procedure will be confirmed, paying attention to:

- Blood thinning medication likely to induce bruising taken prior to aspiration
- Signs of local infection
- Pain or swelling at any sites of potential lymph node sampling
- Allergy to local anaesthetic
- Any other medical reason, to warrant exclusion from the FNA

Before the procedure, the participant's temperature, blood pressure and pulse rate will be recorded.

The FNA will be conducted using standard aseptic technique under ultrasound guidance. During the procedure, the ipsilateral and contralateral lymph nodes in the axilla will be located by physical examination and under US guidance. A sterile needle and syringe will be used to aspirate material from lymph nodes on each side using multiple passes. Where necessary local anaesthesia will be employed to numb the area prior to sampling, using a standard local anaesthetic e.g., 1% lidocaine.

Each visit for FNA will involve sampling lymph nodes from both axillae. Samples from right and left sides will be placed in separate specimen pots which have been clearly labelled to indicate the side from which the biopsy has been taken.

At each visit for FNA sampling a paired peripheral blood sample will be taken.

Lymph node samples will be placed into pre-prepared and labelled specimen pots and placed with the blood tubes in an appropriate transportation container. They will be transferred to the receiving laboratory where they will be processed upon receipt. The equipment necessary will all be made available on the day, including an US machine, and equipment for FNA (disinfectant, local anaesthetic, needles, 5mL syringes, air-tight specimen tubes prepared with R10 transport medium).

Participants will be observed for a minimum of 30 minutes after the procedure, and a final check of the FNA site at the end of this time, before participant leaves the visit.

9.6.2.1. Core needle biopsy of lymph nodes

Similar to FNA, core needle biopsy (CNB) will be carried out by a medical practitioner at the clinical facilities at CCVTM, Oxford, UK. The eligibility to undergo the procedure will be confirmed as described above in the FNA section.

Before the procedure, the participant's temperature, blood pressure and pulse rate will be recorded.

The CNB will be conducted using standard aseptic technique under ultrasound guidance. During the procedure, the ipsilateral lymph nodes in the axilla, on the same side as study immunogen injection, will be located by US guidance.

A small incision (~5 – 8mm) will be made on the skin, and a sterile CNB needle will be introduced to obtain material from lymph nodes. Local anaesthesia will be employed to numb the area prior to incision and sampling, using a standard local anaesthetic e.g., 1% lidocaine. Samples will be placed in separate specimen pots which have been clearly labelled to indicate the participant's side from which the biopsy has been taken.

A paired peripheral blood sample will be taken.

Lymph node samples from CNB will be placed into pre-prepared and labelled specimen pots and placed with the blood tubes in an appropriate transportation container. They will be transferred to the receiving laboratory where they will be processed upon receipt. The equipment necessary will all be made available on the day.

Participants will be observed for a minimum of 30 minutes after the procedure, and a final check of the CNB site at the end of this time, before participant leaves the visit.

9.6.2.2. Ultrasound imaging

No device is being tested for the purposes of the research and therefore the study does not include an investigational device. The device described herein is a tool to facilitate the research.

A clinical grade ultrasound machine purchased and maintained for the purposes of research will be used during the study. However, there will be no endpoints directly related to the performance of the machine.

(a) *Device description*; a Siemens LogiqE10, Toshiba Aplio i700 or similar US machine with appropriate probe for imaging soft tissues will be deployed for the study. A medicinal practitioner with training in its use will perform the ultrasound scan.

(b) *Device safety*: the US machine will be checked by the Experimental Medicine Clinical Research Facility electrician for use.

(c) *Maintenance and storage of device*: the US machine will be maintained, stored and cleaned according to the manufacturer's instruction. Storage of the machine will be at the study site in the Experimental Medicine Clinical Research Facility, University of Oxford.

9.7. Baseline assessments

9.7.1. Screening visit

The schedule of events for the screening visit are shown in Appendix A.

Once informed written consent has been obtained, the following baseline assessments will be performed and recorded/confirmed as part of the assessment of inclusion/exclusion criteria:

- Participant demographics: age, sex and ethnicity
- Medical history
- Lifestyle history including smoking and alcohol intake history
- Contraception: participants of childbearing potential are asked if they are willing to use effective contraceptive measures for the duration of their enrolment in the study; if they withdraw from the study, effective contraceptive measures must be continued until at least 18 weeks after receipt of the last study injection.
- Use of concomitant medication (including over the counter medications, vitamins, illicit drug use and herbal supplements)
- Recording of resting pulse, blood pressure, temperature
- Recording of weight and height and calculation of BMI
- Physical examination: cardiovascular, respiratory, abdominal and gross neurological examination
- Urine pregnancy test (participants of childbearing potential only)
- Blood samples for full blood count, urea and electrolytes/renal function and liver function tests and random blood glucose
- Core needle biopsy cohort only (Group C) An axillary examination, including ultrasound to assess eligibility for US guided CNB procedure.

The medical, vaccination, and prescribed medication history are initially based on participant recall. However, with prior participant consent, patient medical summary, vaccination and prescribed medication history will be formally requested from the GP or accessed via the electronic patient record (if available) at the screening visit, if not already requested or accessed in advance. In addition, all participant GPs will be notified of an individual's participation in the study.

Consent will be taken to register the participant on The Over-volunteering Prevention System (TOPS) database to guard against the potential for harm that can result from excessive volunteering in clinical studies. This will be done using the participant's National Insurance number or passport number. The TOPS database will be checked for any conflicts at full screening, however formal registration will be done at enrolment.

9.7.2. Screening failures

Participants who have signed the informed consent form but are not subsequently enrolled in the study will be regarded as screening failures. Enrolment occurs following administration of first study injection. For each of these participants, a minimal set of screening failure data will be recorded, including demographic details and the reason for screening failure.

9.8. Subsequent visits

The procedures to be included in each visit are shown in the schedule of events table in Appendix B. Each visit is assigned a time-point and a window period, within which the visit will be conducted. If a participant cannot attend a visit, where possible, this will be re-arranged to an in-person visit within the time window. As scheduling is more difficult for FNA visits, staff should make all reasonable effort to book visits in window, but out of window visits may occasionally be necessary.

9.8.1. Study injection visits

The procedure for both study injection visits will be as follows:

- Ensure that participant consent remains valid and confirm continued consent
- Obtain and document interim medical history since the screening visit and check eligibility criteria, specifically temporary exclusion to study injection (see Section 8.4), and perform a targeted physical examination (if required to reassess eligibility)
- Record temperature, pulse and blood pressure
- Perform urinary pregnancy test for participants of child-bearing potential
- Perform ultrasound scan
- Take blood sample
- Administer ChAdOx2 CCHF by IM injection into non-dominant deltoid muscle. **Record which arm is used for the prime dose and administer the second study injection into the same arm.**
- Observe participant for a minimum of 30 minutes following study injection.
- Schedule next visit and remind participants of what is required of them (*e.g.*, eDiary entries, notify the study team of any SAE's)
- Provide participant with training on completion of eDiary or refresher training if required

On the first study injection visit, the participant will be provided with access and training to use the eDiary (on REDCap, with link sent via email).

AESIs and SAEs will be reviewed at every visit. The FNA site will be inspected, and any adverse events associated with the FNA will be reviewed at subsequent follow-up visits.

9.8.2. FNA visits

The following procedures will be performed at FNA visits:

- Review of AESIs/SAEs, as appropriate, since the last visit
- Review eDiary entries and laboratory blood tests
- Targeted physical examination (if indicated)
- Record oral temperature, pulse and blood pressure
- Perform ultrasound (see Section 9.6.2.2)
- Inspect FNA site
- Perform FNA (see Section 9.6.2.1)
- Take blood sample
- Monitor for safety post-FNA for 30 minutes

In addition, the next visit will be scheduled/confirmed.

9.8.3. CNB visits

The following procedures will be performed at CNB visits (only for CNB cohort):

- Review of AESIs/SAEs, as appropriate, since the last visit
- Review eDiary entries and laboratory blood tests
- Targeted physical examination (if indicated)
- Record oral temperature, pulse and blood pressure
- Perform ultrasound (see Section 9.6.2.2)

- Inspect CNB site
- Perform CNB on the same side as the arm that received the study injection (see Section 9.6.2.1)
- Take blood sample
- Monitor for safety post-CNB for 30 minutes

9.8.4. Follow up visits

Follow-up visits require the following procedures:

- Review of AESIs/SAEs, as appropriate, since the last visit
- Review eDiary entries (if applicable) and laboratory blood tests
- Inspect FNA site and review safety associated with FNA
- Targeted physical examination (if indicated)
- Record oral temperature, pulse and blood pressure
- Take blood sample
- Perform ultrasound (if indicated and feasible)

In addition, the next visit will be scheduled/confirmed.

9.8.5. Unscheduled visits

Additional visits or procedures may be performed at the discretion of investigators (*e.g.*, further medical history and physical examination, additional blood tests or other investigations, if clinically relevant, including testing for COVID-19).

9.8.6. Missed visits

In exceptional circumstances, only where follow-up visits would otherwise be missed entirely, visits may alternatively be conducted remotely via phone or video calling.

9.8.7. Electronic diary (eDiary)

Following each study injection, e-diaries to collect information on axillary responses to immunisation will be used to fulfil the secondary objective for 7 days. Participants will be asked if they have experienced pain, swelling or tenderness in the left and right axillae after the study injections.

For the CNB cohort only, on top of the e-diaries after immunisation, participants will also have a separate e-diary to collect information on axillary symptoms and reaction after CNB. Participants will be asked if they have experienced pain, swelling, tenderness or bruising in the axilla after CNB sampling.

9.9. Sample handling

9.9.1. Clinical laboratory samples

Blood will be drawn (at different time points as shown in Appendices A and B) for the following laboratory tests. Samples will be labelled with the participant's study ID label, unless the sample is being transferred to the OUH NHS FT laboratory, in which case it will be labelled according to their policy. All samples will be handled as set out in the Laboratory Analysis Plan. The processing and analysis of the blood will be carried out at an accredited clinical laboratory.

- Haematology:

- Full blood count (including haemoglobin, platelet count, total white cell count, neutrophil count, lymphocyte count, eosinophil count)

- Biochemistry:
  - Urea and electrolytes (including sodium, potassium, urea and creatinine)
  - Liver function tests (including ALT, ALP, Bilirubin, Albumin)
  - Random blood glucose (Screening visit only)
- Diagnostic serology (screening only):
  - Screening tests for Hepatitis B, Hepatitis C and HIV infection (including: HBsAg, HCV antibodies, standard clinical HIV test in a laboratory, *e.g.,* 4th generation HIV antigen/antibody test HIV antibodies)
- Immunology (first study injection visit only):
  - Human Leukocyte Antigen (HLA) typing

Additional safety blood tests may be performed if clinically relevant at the discretion of the medically qualified investigator(s). Acute infectious hepatitis is notifiable to the UK Health Security Agency. This may involve a referral to the participant's GP or specialist for further testing if a screening test is reactive.

9.9.2. Immunology blood samples

University of Oxford Research Laboratories:

Immunogenicity will be assessed by a variety of immunological assays. This may include single cell RNA-seq, CITEseq, VDJ Seq, ELISpot assays, flow cytometry assays, functional antibody assays and B cell analyses. Other exploratory immunological assays including cytokine analysis and other antibody assays, production of monoclonal antibodies, DNA analysis of genetic polymorphisms potentially relevant to immunogenicity and gene expression studies, amongst others, may be performed.

Other Research Laboratories

Collaboration with other specialist laboratories in the UK (including laboratories at study sites), Europe and outside of Europe for further exploratory immunological tests may occur. This would involve the transfer of serum, plasma and peripheral blood mononuclear cells (PBMCs) or lymph node cells (LNCs) to these laboratories, but these samples would remain de-identified. Informed consent for this will be gained from the volunteers. Immunological assays will be conducted according to local SOPs.

9.9.3. Lymph node biopsy samples

These will be handled similarly to previous studies conducted by the CI[1]. Samples will be sent with de-identified data to the handling laboratory at the University of Oxford to include sufficient information to identify when and where the sample was taken such as date and sample ID but without including identifying personal identification such as full name, according to OVG standard operating procedures. Examples of handling are detailed below.

Single cell RNA-Seq experiments typically undertake filtering and quality control to yield ~ 3,000-5,000 lymph node cells. Samples will be handled according to established single-cell RNA-seq best practice (*e.g.,* https://github.com/DendrouLab; COMBAT Consortium 2022[39]). Dimensionality reduction can determine cell clusters and immune cell subpopulations. Analysis software such as edgeR packages can be used to determine differential cluster abundance and gene expression using pseudobulk counts and applying a Benjamini-Hochberg multiple testing correction. Single-cell repertoire and bulk sequencing analyses can be performed as previously described using standard pipelines.[39-41] Flow cytometry data can be analysed

using FlowJo or similar programme, with appropriate controls (e.g., non-specific isotype controls and beads).

Ultrasound images will be collected using the software provided with the US machine operating system. These may be securely shared for storage on a secure password protected computer as pseudonymised images in the appropriate format such as .jpeg or DICOM using the appropriate applications. Ultrasound images can be stored according to the relevant OVG SOP.

9.9.4. Urine samples

For participants of childbearing potential only, urine will be tested for human chorionic gonadotrophin (hCG) at screening and immediately prior to each injection visit. Alternatively, β-hCG blood sampling may be used to confirm a female participant is not pregnant.

9.9.5. Retention of samples

Participants will be informed that they may opt-in to the Oxford Vaccine Centre Biobank study (REC 21/SC/0161) to allow long-term storage of biological samples collected under this protocol for use in possible future research. The OVC Biobank study is covered by a separate study protocol and consent process. Participants will be informed that declining to take part in the OVC Biobank study will not affect their participation in this study. If a participant elects to decline to take part in the OVC Biobank, all their remaining samples will be destroyed after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

9.10. Early discontinuation/withdrawal of participants

Each participant can exercise their right to withdraw from the study at any time without giving a reason. In addition to consent being withdrawn by a participant, the investigator may discontinue a participant from the study at any time for the following, although not exhaustive, reasons:

- The investigator considers it necessary for participant safety
- Significant non-compliance with study requirements
- The participant is lost to follow up

In circumstances pertaining to the safety of the participant, the DSMC chair, DSMC committee or Investigator may choose to discontinue further study injections and/or study procedures for an individual participant; however, with ongoing consent, monitoring for safety will be continued, *via* either scheduled or unscheduled visits. For example, such circumstances may include the following:

- Pregnancy
- An adverse event which requires discontinuation of the study injections or results in an inability to continue to comply with study procedures
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening)

Participants of child-bearing potential who withdraw from the study must continue effective contraception until at least 18 weeks after receipt of their last study injection.

Withdrawal from the study will not result in exclusion from analysis of existing data generated by the participant. The reason for withdrawal, if given, will be recorded in the CRF.

9.11. Definition of end of study

The end of the study is when the last laboratory assay has been performed to determine the primary and secondary objectives of the study protocol.

10. SAFETY REPORTING

For this experimental medicine study using a novel immunogen, safety reporting of AESIs and SAEs will be done for two purposes,

1. To ensure the study's ethical conduct for participants, reporting of AESIs and SAEs during the study will occur as described below.

2. To ensure the ethical conduct of the related phase 1 clinical trial of ChAdOx2 CCHF01, CTIMP (OVG2022/05), any serious adverse events (SAEs) and adverse events of special interest (AESIs) will be reported to the Sponsor, and the DSMC who are overseeing both studies. These SAEs occurring in LEGACY02, considered to be related to the study injection, will also be reported in the IMPD and IB for the CTIMP (OVG2022/05) noting that the events have occurred in another study.

The window for reporting AESIs and SAEs for each participant is from the enrolment for the duration of the study.

10.1. Definition of serious adverse events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.

Adverse Event (AE)	Any untoward medical occurrence in a participant including occurrences which are not necessarily caused by or related to the intervention or study procedures.
Adverse Reaction (AR)	An untoward and unintended response in a participant which is related to the intervention administered to that participant. The phrase "the intervention" means that a causal relationship between the intervention and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

	All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect*. Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial immunogen or procedure, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the immunogen in question set out: • In the case of a product with a marketing authorization, in the approved summary of product characteristics (SmPC) for that product, or • In the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question.

10.2. Reporting procedures for Serious Adverse Events

SAEs will be collected throughout the entire study period (from first enrolment to the final study visit or withdrawal). All SAEs must be recorded on a SAE form (on REDCap or paper backup).

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected non-fatal SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (see HRA website). Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. Any additional relevant information should be sent within 8 days of the report. All SUSARs will be reported to the Principal Investigator, Sponsor, DSMC, and relevant Research Ethics Committee. SUSARS will be reported for this candidate via the EudraCT registered CCHF01 Phase 1 trial. The CI or Co-Investigator will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

10.3. Adverse events of special interest (AESI)

AESIs will be monitored and recorded throughout the entire study period (from first enrolment to the final study visit or withdrawal). These will include the list below (Table 2). Additionally, other adverse events (*i.e.*, not listed below) may also be categorised by investigators as AESIs if scientifically warranted.

There is no previous clinical experience with ChAdOx2 CCHF, apart from the current CCHF01 trial. There is extensive clinical experience of ChAdOx1 nCoV-19 vaccine (AZD1222), marketed as Vaxzevria in the UK, a vaccine based on a similar vaccine platform technology. Adverse reactions based on five clinical trials and post authorisation experience are tabulated in Table 3 below.

Given that it is not known whether there will be any similarity in clinical safety profile between ChAdOx2 CCHF and ChAdOx1 nCoV-19 vaccine (AZD1222), monitoring of AESIs will include adverse reactions listed in the summary of product characteristics for Vaxzevria that are considered clinically significant.

Investigators managing a case of suspected vaccine-induced immune thrombocytopenia and thrombosis (VITT), also known as Thrombosis with Thrombocytopenia Syndrome (TTS), should refer to the NICE guidance on diagnosis and treatment[42].

Table 2 List of Adverse Events of Special Interest (AESIs)

Respiratory	Acute Respiratory Distress Syndrome (ARDS)
	Pneumonitis
Neurological	Transverse Myelitis
	Generalised convulsion
	Guillain-Barre Syndrome (GBS)
	Acute Disseminated Encephalomyelitis (AE)
	Encephalopathy
	Encephalitis
	Stroke
	Facial paralysis
Haematological / Vascular	Thrombocytopenia
	Thrombosis with Thrombocytopenia Syndrome (TTS)
	Major thrombosis (without thrombocytopenia)
	Heparin-Induced Thrombocytopenia
	Immune thrombocytopenic purpura
	Disseminated intravascular coagulation (DIC)
Immunological	Anaphylaxis
	Angioedema
	Urticaria
	Vasculitis
	Capillary Leak Syndrome (CLS)
	Other immune-mediated conditions
Other	Acute renal failure
	Muscle spasms

AESIs should be collected and recorded in the AE reporting form in REDCap throughout the duration of this study. These should also be reported as SAEs if they fulfil the definition criteria for SAEs. All AESIs not already reported as SAEs should be included in the reports to the DSMC.

Table 3 Adverse reactions to ChAdOx1 *nCoV-19* vaccine (AZD1222)

MedDRA SOC	Term	Frequency*	Monitored AESI**
Blood and lymphatic system disorders	Lymphadenopathy	Uncommon	No
	Thrombocytopenia, Immune thrombocytopenia	Uncommon	Yes
Immune system disorders	Anaphylaxis, hypersensitivity	Uncommon	Yes
Metabolism and nutrition disorders	Decreased appetite	Uncommon	No
Nervous system disorders	Headache	Very common	No
	Dizziness, somnolence, lethargy	Uncommon	No
	Facial paralysis	Rare	Yes
	Guillain-Barré syndrome	Very rare	Yes
	Transverse myelitis	Not known	Yes
Vascular disorders	Thrombosis with thrombocytopenia syndrome	Very rare	Yes
	Cerebrovascular venous and sinus thrombosis	Not known	Yes
	Capillary leak syndrome	Not known	Yes
Gastrointestinal disorders	Nausea	Very common	No
	Vomiting, diarrhoea	Common	No
	Abdominal pain	Uncommon	No
Skin and subcutaneous tissue disorders	Hyperhidrosis, pruritus, rash, urticaria	Uncommon	Yes (urticaria)
	Angioedema	Not known	Yes
Musculoskeletal and connective tissue disorders	Myalgia, arthralgia	Very common	No
	Pain in extremity	Common	No
	Muscle spasms	Uncommon	Yes
General disorders and administration site conditions	Injection site tenderness, pain, warmth, pruritus, bruising, fatigue, malaise, feverishness, chills	Very common	No
	Injection site swelling, erythema, induration, pyrexia, influenza-like illness, asthenia	Common	No

* very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1000); very rare (<1/10,000) and not known (cannot be estimated)

10.4. Causality assessment for AESIs and SAEs

The relationship of each adverse event to the study injection or study procedures must be determined by a PI-delegated clinician / Investigator. The relationship of the adverse event with the study procedures will be categorized as not related, possibly related, probably related or definitely related. The delegated clinician will use clinical judgement to determine the relationship using the following definitions (Table 4):

Table 4 Guidelines for assessing the relationship of immunisation to an Adverse Event

No Relationship		No temporal relationship to study product; ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product.
Related	Possible	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines.
Probable		Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***or*** Known pattern of response seen with other vaccines.
Definite		Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines.

10.5. Expectedness assessment

All serious adverse reactions (serious adverse events assessed as possible, probable or definitely related to ChAdOx2CCHF administration) will be assessed for expectedness by the investigator. Expectedness will be determined according to the information set out in the reference safety section of the ChAdOx2 CCHF IB. As no expected SARs are recorded in the reference safety section of the ChAdOx2 CCHF IB, and no SAR has been reported so far (20th April 2024, while submitting the LEGACY02; Protocol, Version 1.0, 01 February 2024) in the Phase 1 ChdOx2 CCHF vaccine trial, any SARs associated with ChAdOx2 CCHF will be classified as unexpected and reported as SUSARs in this study.

10.6. Severity assessment

The severity of clinical and laboratory adverse events will be assessed according to scales based on FDA toxicity grading scales for healthy volunteers enrolled in preventive vaccine clinical trials. Severity scales for clinical and laboratory adverse events are shown in Appendix C.

10.7. Physical Observations

Physical observations of the patient (*e.g.,* temperature, blood pressure) will be taken at each visit for the purposes of the safe conduct of the study. These will be recorded in the eCRF.

10.8. Solicited reactions

Following study injection axillary swelling and tenderness will be collected in an electronic diary for 7 days following administration of the study injection (from D0 to D7) to meet the secondary objective of the study.

10.9. Unsolicited AEs

Unsolicited adverse events, *i.e.* those collected through open questioning (*e.g.* "did you experience any new illnesses?"), that constitute SAEs or AESIs, will be collected.

10.10. Laboratory AEs

Severity grading for laboratory AEs is described in Appendix C (Section 24.3). All changes in laboratory values that potentially meet an AESI or SAE will be documented.

If a test is deemed clinically significant, it may be repeated to ensure it is not a single occurrence or spurious result. If a test remains clinically significant, the volunteer will be informed and advised about appropriate medical care. If abnormal laboratory values are the result of pathology for which there is an overall diagnosis, then this diagnosis will be reported as one AE only.

Laboratory results can be out of normal range for a number of reasons other than physiological disturbance (e.g. hot weather, delayed transit to processing laboratory).

10.11. Notes on recording AEs

Pre-existing medical conditions (present prior to enrolment into the study) are considered "concurrent medical conditions" and should not be recorded as AEs. However, if the participant experiences a worsening or complication of the condition, the worsening or complication should be recorded as an AE if it meets the criteria for SAE or AESI. Study staff will ensure that the AE term recorded captures the change in the condition (*e.g.*, "worsening of").

Each AE will be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) will not be recorded as additional AEs.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, in which case it would fall within the definition of "serious" and the congenital abnormality of birth defect would be reported as an SAE. Pregnancy notification and follow-up reports on pregnancy outcome will be provided to the DSMC with the ongoing consent of the participant.

10.12. Follow up of SAEs and AESIs

AESIs and SAEs considered related to the study injection will be followed until resolution, the event is considered stable or until non-study causality is assigned. At the end of the study all other ongoing/open AESIs and SAEs will be assessed by a PI-delegated clinician/Investigator, to ensure, if not already done so, that adequate medical follow-up (if required) has been arranged, *e.g.,* referral to the participant's GP.

All AESIs and SAEs that result in a participant's withdrawal from the study will be, subject to participant consent, followed up where possible until a satisfactory resolution occurs, or until a non-study related causality is assigned. This will involve an end of study assessment at which the requirement for further appropriate care under medical supervision will be determined. If required, the participant will be referred to their GP for ongoing medical supervision, until symptoms cease, or the condition is deemed resolved or stable.

10.13. Study Management Group

The CI and study site investigators will form the study management group (SMG) and will provide on-going management of the study.

10.14. Data Safety Monitoring Committee (DSMC)

For this study, an independent DSMC will be appointed. There will be a minimum of three appropriately qualified committee members of whom one will be the designated Chair. The DSMC will operate in accordance with the study specific DSMC charter, which will be established before recruitment starts. The Chair of the DSMC may also be contacted for advice where the Chief Investigator feels independent advice or review is required. The DSMC will be the same as the DSMC for the Phase 1 ChAdOx2 CCHF clinical trial.

10.15. Procedures to be followed in the event of abnormal findings

Laboratory parameters for inclusion/exclusion in the study will be considered on an individual basis, with investigator discretion for interpretation of results and the need for repeated tests. Abnormal clinical findings from medical history, examination, including ultrasound examination or blood tests will be assessed for clinical significance throughout the study. If a test is deemed clinically significant, it may be repeated to ensure it is not a single occurrence or spurious result. If a test remains clinically significant, the participant will be informed, and medical care arranged as appropriate, with the permission of the participant. Decisions to exclude the participant from enrolling in the study or to withdraw a participant from the study will be at the discretion of the Investigator.

10.16. Criteria for termination of the study

The CI and DSMC will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the Investigator will promptly inform the participants and will ensure appropriate therapy and follow-up. If the study is halted, the Sponsor and the relevant Ethics Committee will be notified within 15 days.

In the event of the study being terminated early, follow-up of enrolled participants will continue as planned for safety reasons, but further study injections will not be given, and study procedures will be modified to monitor safety only.

11. STATISTICS AND ANALYSIS

11.1. Statistical analysis plan (SAP)

The statistical aspects of the study are summarised here.

11.2. Description of the statistical methods

11.2.1. Descriptive analyses

A flow diagram can describe the number of participants enrolled, randomised and in each analysis group. Descriptive tables can summarise participant demographics and clinical characteristics. The analyses for this study will be descriptive in purpose and will not include any hypothesis testing or presentation of p-values for group comparisons or power calculation

11.2.2. Immunology analyses

Where appropriate, non-normal distributed immunology data will be log-transformed to render a normal distribution and geometric mean concentrations (GMC) and corresponding 95% confidence intervals (CI) will be reported by computing the anti-log of the mean of the log-transformed data, or medians and interquartile ranges if appropriate. Standard approaches will be used, e.g., geometric mean ratios (GMR) and corresponding 95% CIs between groups will be calculated to understand the difference between age groups and timepoints. There will be no formal hypothesis testing between study groups.

11.2.3. Lymph node analyses

Analyses will be conducted according to standard laboratory protocols for immunophenotyping.

11.3. Sample size determination

16 participants will be recruited to the FNA cohort and up to 4 participants will be recruited to the CNB cohort. Participants will be allocated to study groups detailed in section 7. Participants will be replaced where possible, if they have not received a study injection or if there is a failed FNA or CNB procedure. There has been no formal power calculation to determine this number as the study is primarily descriptive. The number of participants has therefore been chosen to achieve a sufficient description of the study cohorts in both age groups.

11.4. Analysis populations

All participants with any available data will be included in the analyses.

11.5 Decision points

Interim analysis will be conducted as data become available but will not affect the conduct of the trial.

11.5. Stopping rules

There are no formal stopping rules for futility, efficacy or lack of power. The CI reserves the right to pause the study or terminate the study on ethical or safety grounds.

11.6. The Level of Statistical Significance

There will be no statistical significance testing. All confidence intervals for descriptive analyses will be set to 95%.

11.7. Procedure for Accounting for Missing, Unused, and Spurious Data.

The level of the missing data in the baseline variables and outcomes will be reported. All available data will be used in the analyses and there will be no imputation for missing data.

12. DATA MANAGEMENT

The data management aspects of the study are summarised here. The Investigators will populate the content of the participants' CRFs, which will be in a paper and/or electronic format using an EDC system (e.g., REDCap database, or an appropriate alternative). The database will be stored for at least five years (de-identified data will be stored indefinitely) on a secure server located in UK and will have restricted access (password-protection) and accountability records. All information transcribed to and from the database will be done by encrypted (https) transfer.

Personal identifiable data will be recorded electronically to plan and schedule visits, set reminders, track payments, and generate reports on participant management to enable the study teams to track recruitment and visit compliance. This information is only accessible through the University network including VPN and will be restricted, with only delegated study members able to gain access.

Each study participant will have a unique participant number or code which will be allocated at the time of screening. Names and/or identifiable details are not included in the clinical electronic database capture system. Storage of participant email addresses for electronic diaries and electronic medical records access will be required for the system to function, which consent will be obtained. Only site research staff and sponsor data managers have access to view the email address. With the exception of clinical safety blood samples, which are sent to local clinical laboratories and follow local sample labelling requirements, samples sent to laboratories for processing will be identified by study number and participant number only.

12.1. Source data

Source documents are original documents, data, and records from which participants' CRF data are populated. These include, but are not limited to, hospital or GP records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. In this study, CRF entries will be considered source data where it is the site of the original recording. All documents will be stored safely under strict confidentiality and with restricted access. On all study-specific documents, other than the signed consent and the participant contact sheet, the participant will be referred to by the study participant number/code only.

12.2. Access to data

Direct access will be granted to authorised representatives from (or appointed by) the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data recording and record keeping

The Investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. REDCap, or similar), or onto a paper source document for later entry into the EDC system if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of

features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personnel. The servers are in a physically secure location in Europe, and data are backed up on secure servers operated by the University of Oxford IT Services, physically located in Europe. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. Weekly backup tapes are stored offsite. The servers provide a stable, secure, well-maintained, and high-capacity data storage environment. REDCap is a widely used, powerful, reliable, well-supported system. Access to the study's database will be restricted to the members of the study team by username and password.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (*e.g.* requesting to be informed of other trials), participant's personal details will not be used to contact them other than in exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

13. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

13.1. Risk assessment

A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the study to reflect significant changes to the protocol or outcomes of monitoring activities. Approved and relevant SOPs will be used at all clinical and laboratory sites.

13.2. Study monitoring

Regular monitoring will be performed according to the study specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the study specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13.3. Study Committees

See sections 10.13 and 10.14

14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g., consent process or administration of study intervention) or from Good

Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

15. SERIOUS BREACHES

A "serious breach" is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the study subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet, and required material will be submitted to an appropriate Research Ethics Committee (REC) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Other ethical considerations

Ultrasound scanning will be performed by a medical practitioner trained in using this imaging modality to support fine needle aspiration of lymph nodes for research purposes only.

In the unlikely event of seeing any possible structural abnormalities on a scan, the scan will either be checked by a clinical specialist or the participant will be asked to follow up with their GP. If the specialist feels that the abnormality was medically important, they will discuss the implications with the participant and arrange for further investigations as necessary. Participants will not be informed unless the doctor considers the finding has clear implications for their current or future health. It is important to note that scans are not carried out for diagnostic purposes, and therefore the scans are not a substitute for a clinical appointment. Rather, the scans are intended for research purposes only.

16.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

16.6. Transparency in research

Prior to the recruitment of the first participant, the study will have been registered on a publicly accessible database. Where the study has been registered on multiple public platforms, the study information will be kept up to date during the study, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the study declaration.

16.7. Participant confidentiality

The study will comply with the United Kingdom General Data Protection Regulation (UK GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s), with the exception of the consent forms, where participant name and initials will be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

16.8. Expenses and benefits

Volunteers will be compensated £110 for attending the screening visit and each study injection visit; £90 for follow-up visits; £30 for full completion of each diary; and £150 for each FNA procedure and £175 for the CNB procedure. Additional reimbursement for unscheduled visits at £90 per visit will be provided. This will not be given unless an unscheduled visit occurs.

The total amount of compensation for an individual participant will depend on the actual number of visits attended and whether any repeat or additional visits were necessary. If a participant withdraws consent for continued participation in the study or is withdrawn for any other reason, they will still be compensated for any study visits they attended. Each participant in the FNA cohort and CNB cohort can therefore receive a maximum of £1110 and £775 respectively for the scheduled study visits plus an additional amount, based on whether unscheduled visits were required and how many occurred.

17. FINANCE AND INSURANCE

17.1. Funding

The study is funded by UK Research and Innovation, MRC.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Data from the study may also be used as part of a thesis for a PhD or MD. Authors will acknowledge that the study was funded by UK Research and Innovation, MRC. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the study.

20. ARCHIVING

Study data will be stored electronically on a secure server operated by the University IT team, and paper notes will be kept in a secure location at the study site(s) or as outlined in local SOP's. All essential documents (for example TMF) will be retained up to 25 years, or as per national regulatory requirements. Pseudonymised research data may be stored indefinitely due to regulatory requirements or for scientific benefit, but with 5 yearly reviews. (Data will become de-identified at the point at which ICFs, payment information and contact sheets are destroyed as per local SOPs).

Participants' bank details will be stored for 10 years from the project end date in line with the University of Oxford financial policy. Volunteers who complete online screening only (before informed consent) will not have data kept beyond the end of the trial. General archiving procedures will be conducted in compliance to SOP OVC020 Archiving.

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22. APPENDIX A: Schedule of events for screening visit

Visit Number	S
Visit type	Screening
Timeline[1]	1 to 90 days before V1
Visit Procedures	
Informed consent	X
Review inclusion and exclusion criteria	X
Record demographic data	X
Medical history[2]	X
Vital signs (heart rate, temperature, blood pressure)	X
Measure height and weight, calculate BMI	X
Screening physical examination	X
Urine Samples	
Urinary HCG (POCBP only)	X
Blood Samples[3]	
HbsAg, HCV Ab, HIV serology (mL)	~5
Biochemistry, haematology, random blood glucose (mL)	~5
Blood volume per visit (mL)	~10
Cumulative blood volume (mL)	~10

[1]Additional unscheduled screening visits may occur (for example: to repeat a blood test, for safety or where clinically indicated)

[2]Medical history may be initially assessed by a telephone call prior to screening; information obtained in this way will be reviewed at the screening visit

[3]Minor differences in blood volumes may occur depending on the collection tubes and equipment used (~ = approximately); additional repeat blood draws may be required (for example, if there is a problem with the sample or result abnormality)

23. APPENDIX B: Schedule of events for study visits, from the first study injection

23.1. Schedule of events for FNA cohort

Study visit number	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**
Visit type	FNA1	Study injection 1	FNA2	Follow-up	Study injection 2	FNA3	Follow-up	Follow-up
Study day	-7 to -90	0	7	28	84	91	112	168
Window (days)	NA	NA	0 to +3	-1 to +2	-7 to +7	0 to +3	-1 to +2	-14 to +14
Relationship to study injection 1 or 2	-7 to -90	I1	I1+7	I1+28	I2	I2+7	I2+28	I2+84
Visit procedures								
Concomitant medication and review inclusion criteria	x	x	x	x	x	x	x	x
Study Injection		x			x			
Vital signs	x	x	x	x	x	x	x	x
Symptom directed physical examination	x	x	x	x	x	x	x	x
Adverse events check								
SAEs and AESI	x	x	x	x	x	x	x	x
Urine samples								
Urinary pregnancy test (if applicable)		(x)			(x)			
Lymph node fine needle aspiration procedures								
Inspection of the FNA site	x	x	x	x	x	x	x	x
Ultrasound scan (if applicable)*	x	(x)	x	x	(x)	x	(x)	(x)
Lymph node fine needle aspiration	x		x			x		
Post FNA adverse events check	x		x	x		x		
Lymph node cells (approx. number per sample)	10^5 to 10^7		10^5 to 10^7			10^5 to 10^7		
Ultrasound images per side	x	(x)*	x	(x)	(x)	x	(x)	x
Blood samples								
Biochemistry, Haematology (LFTs, U + Es, FBC) (approx.10mL)	x	x	x	x	x	x	x	x
Blood for serum immunoassays (approx. 10mL)	x	x	x	x	x	x	x	x
Blood for cellular and plasma immunoassays (approx. 40mL)	x	x	x	x	x	x	x	x
Blood for RNA PAXgene tube (approx. 2.5mL)		x	x		x	x		
Blood for HLA testing (approx. 3-4mL)	x							
Visit blood volume (approx. mL)	64	62.5	62.5	60	62.5	62.5	60	**60**
Cumulative blood volume (mL)	64	126.5	189	249	311.5	374	434	**494**

*These bracketed ultrasound events are dependent on staff availability

23.1. Schedule of events for CNB cohort

Study visit number	**V1**	**V2**	**V2a**	**V3**	**V4**	**V5**
Visit type	Study injection 1	CNB	Phone call	Follow-up	Study injection 2	Follow-up
Study day	0	7	14	28	84	112
Window (days)	NA	0 to +3	-1 to +1	-1 to +2	-7 to +7	-1 to +2
Relationship to study injection 1 or 2	I1	I1+7	I1+14	I1+28	I2	I2+28
Visit procedures						
Concomitant medication and review inclusion criteria	x	x	x	x	x	x
Study Injection	x				x	
Vital signs	x	x		x	x	x
Symptom directed physical examination	x	x		x	x	x
Adverse events check						
SAEs and AESI	x	x	x	x	x	x
Diary review		x		x	x	
Urine samples						
Urinary pregnancy test (if applicable)	(x)				(x)	
Lymph node core needle biopsy procedures						x
Inspection of the CNB site	x	x		x	x	(x)
Ultrasound scan (if applicable)*	(x)	x		(x)	(x)	(x)
Lymph node core needle biopsy		x				
Post CNB adverse events check		x	x	x		
Ultrasound images per side	(x)*	x		(x)*	(x)	(x)
Blood samples						
Biochemistry, Haematology (LFTs, U + Es, FBC) (approx.10mL)	x	x		x	x	x
Blood for serum immunoassays (approx. 10mL)	x	x		x	x	x
Blood for cellular and plasma immunoassays (approx. 40mL)	x	x		x	x	x
Blood for RNA PAXgene tube (approx. 2.5mL)	x	x			x	
Blood for HLA testing (approx. 3-4mL)	x					
Visit blood volume (approx. mL)	66.5	62.5	0	60	62.5	60
Cumulative blood volume (mL)	66.5	129	129	189	251.5	311.5

*These bracketed ultrasound events are dependent on staff availability

24. APPENDIX C: Severity Grading Scales

24.1. Measurable Solicited and Unsolicited Adverse Events Scale

Adverse event	Grade	Definition
Any symptom		
	1 (Mild)	Awareness of symptom but tolerated; transient or mild discomfort; little or no medical intervention required
	2 (Moderate)	Discomfort enough to cause limitation of usual activity; some medical intervention or therapy required
	3 (Severe)	Significant interference with daily activity

Adverse Event	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Redness at injection site	2.5 – 5.0 cm	5.1 – 10 cm	>10 cm
Swelling or Hardness at injection site	2.5 – 5.0 cm	5.1 – 10 cm	>10 cm
Fever (°C)	38.0 – 38.4	38.5 – 38.9	≥ 39.0

24.2. Vital Sign Adverse Event Grading Scale

Adverse Event *	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Temperature (°C) **	38.0 – 38.4	38.5 – 38.9	≥ 39.0

Tachycardia (beats per minute)	101 – 115	116 – 130	>130
Bradycardia (beats per minute) ***	50 – 54	45 – 49	<45
Hypertension (systolic, mmHg)	141 – 150	151 – 155	>155
Hypertension (diastolic, mmHg)	91 – 95	96 – 100	>100
Hypotension (systolic, mmHg)	85 – 89	80 – 84	< 80

*Participant should be at rest for all vital sign measurements

**Oral temperature

24.3. Laboratory Adverse Events (Oxford)

Adverse Event *	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Sodium: hyponatraemia (mmol/L)	132 – 134	130 – 131	< 130
Sodium: hypernatraemia (mmol/L)	146	147	> 147
Potassium: hypokalaemia (mmol/L)	3.3 – 3.4	3.1 – 3.2	< 3.1
Potassium: hyperkalaemia (mmol/L)	5.4 – 5.5	5.6 – 5.7	> 5.7
Urea (mmol/L)	8.2 – 9.3	9.4 – 11.0	> 11.0
Creatinine (µmol/L)	132 – 150	151 - 177	> 177
ALT (IU/L)	50 – 112	113 – 229	> 229
AST (IU/L)	46 – 105	106 – 213	> 213
Bilirubin, with increase in LFTs (µmol/L)	23.1 – 25	26 – 31	> 31
Bilirubin, with normal LFTs (µmol/L)	23.1 – 33	34 – 41	> 41
ALP (IU/L)	143 – 272	273 – 402	> 402
Albumin (g/L)	28 – 31	25 – 27	<25
Haemoglobin: decrease from baseline value (g/L)	10 – 15	16 – 20	> 20
White cell count: Elevated (x 10^9/L)	11.10 – 15.00	15.01 – 20.00	> 20.00
White cell count: Depressed (x 10^9/L)	2.50 – 3.50	1.50 – 2.49	< 1.50
Neutrophil count (x 10^9/L)	1.50 – 1.69	1.00 – 1.49	< 1.00
Lymphocyte count (x 10^9/L)	0.75 – 0.89	0.50 – 0.74	< 0.50
Eosinophil count (x 10^9/L)	0.65 – 1.50	1.51 – 5.00	> 5.00
Platelet count (x 10^9/L)	125 – 149	100 – 124	< 100

25. APPENDIX D: Amendment history

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

MA02	1.1	18 Oct 24	N Owino R Mahmud T Javed	Appendix C: Revision of AE grading to remove grade 4 and 5 in tables adapted from FDA toxicity grading model. Removal of statistician name and replaced with free text
SA01	2.0	20 Feb 25	T Javed R Mahmud	Section 5.3 Risks related to FNA of lymph nodes updated to reflect FNA-related SAE in a different study conducted at the University of Oxford and provided the safety profile from ongoing studies conducted by the PI. Section 10.10 removal of Grade 4 AE to reflect changes previously made to the Appendix. Addition of the following text: "Laboratory results can be out of normal range for a number of reasons other than physiological disturbance (e.g. hot weather, delayed transit to processing laboratory"
SA02	3.0	18 July 2025	T Chan N Owino K Pollock	Added a new cohort (Core Needle Biopsy cohort) to the study; changes made reflect the new study cohort: Section 2 update to the lay summary Section 3 update to the study Synopsis Section 5 update to the Background and Rationale. Risks associated with core needle biopsy of lymph nodes Section 6 added new exploratory objectives related to CNB, namely

				"Imaging-based techniques to measure spatial architecture of lymph node core needle biopsy such as single cell spatial transcriptomics or Cell DIVE™" Section 7 added new Core Needle Biopsy (CNB) cohort to study design and defined study duration for CNB cohort participants Section 9 added description of CNB procedure, description of the CNB study visit, and Electronic diary changes to monitor axillary responses after CNB procedure Section 11 made minor changes to sample size determination to account for up to 4 additional participants for the CNB cohort Section 16 added details of participant reimbursement for the CNB cohort participants Section 23 added Schedule of Events table for CNB cohort