Study Title: An experimental medicine study of influenza and COVID-19 vaccine immune challenge responses in Lymph nodE single-cell Genomics in AnCestrY and ageing

Short title: Lymph node flu & COVID-19 vaccine responses in younger or older adults

Study code: OVG2023/06 LEGACY03

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Additional input will be from the protocol development team within the LEGACY Network and within the Oxford Vaccine Group.

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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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2. LAY SUMMARY

This study will test the immune responses of cells in lymph nodes after the immune system has been challenged by two licensed vaccines at the same time (seasonal flu and COVID-19) and compare differences between older and younger adults.

Immune responses to vaccination are typically tested by taking blood samples and measuring proteins called antibodies and immune cells (lymphocytes). The immune responses to vaccination predominately occur in the lymph nodes, which are small bean shaped organs found throughout the body. The lymph nodes that respond to a vaccine given as an injection in the arm are found in the armpit. Cells from lymph nodes can be sampled using ultrasound-guided fine needle aspiration (FNA). This is a well-established and safe technique in the clinic, and in research enables direct testing of the responses of the immune cells. This information will help design future vaccines, select the right dose to give, and tailor vaccination strategies in different patient populations, for example older people.

Older people typically respond less well to vaccines than younger adults, and they are also more severely affected by diseases such as flu and Covid. It is important to understand how age influences immune responses to vaccination to better improve health outcomes.

Participants in the study will receive seasonal influenza vaccine and COVID-19 vaccine boosters. This study aims to understand how we respond to vaccine and how this changes as we age. This information will be valuable for developing vaccines against commonly circulating viruses and future pandemics.

The study will recruit 48 healthy adults (24 aged 18-45 years; 24 aged 65 years or above). All will receive two doses of the different vaccines, one in each arm. All participants will have two FNA from both armpits, either 7, 14 or 28 days after each vaccination (determined by randomisation) and then again 12 weeks later. Participants will be screened for eligibility at the screening visit, and then if eligible to take part, will attend a further 6 in-person visits scheduled over 12 weeks. Depending on which group, there will be one or two remote follow-up visits. Blood samples will be taken at each visit.

3. SYNOPSIS

Short title	Lymph node flu & COVID-19 vaccine responses in younger or older adults		
Study code	OVG2023/06 LEGACY03		
Study registration	ISRCTN12928349		
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; randomised open label		
Study Participants	Healthy adults, aged 18-45 years, and aged 65 years or over		
Sample Size	Total sample size: 48 • 24 participants aged 18-45 years (randomised equally into 3 groups) • 24 participants aged 65 years or over (randomised equally into 3 groups)		
Planned Study Period	Total length of the project: 2 years Duration of an individual participant's involvement: 13 weeks		
Planned Recruitment period	Start date for recruitment: 01 September 2023 End date for recruitment: 31 March 2025 Participants will be recruited into season 1; 2023-2024 or season 2; 2024-2025		
	Objectives	Outcome Measures	Timepoint(s)
Primary Objective	To determine the frequency, phenotype, and function of immune cells in axillary secondary lymphoid tissue and blood after intramuscular immunisation, in older compared with younger volunteers.	Single cell ribonucleic acid sequencing (scRNA-seq) to measure cell by cell transcriptomes in lymph node cells and/or multiparameter flow cytometry	Day 0, 7, Day 14 and Day 28 and Day 84 after study injection
Secondary Objective	To define reactive lymph nodes using bedside imaging at baseline and after intramuscular immunisation, in older compared with younger volunteers.	Ultrasound measurements of secondary lymphoid tissue	Day 0, 7, Day 14, Day 28, and Day 84 after study injection

Exploratory Objective 1	To collect participant reported measures of axillary pain, swelling and tenderness, at baseline and after intramuscular immunisation, in older and younger volunteers.	Participant reported outcome collected by means of eDiary and ultrasound measurements	Day 0 to day 7.
Exploratory Objective 2	In younger and older age groups before and after immunisation to perform detailed immunological profiling including for example a. To determine the T and B cell signalling pathways in axillary secondary lymphoid tissue after intramuscular immunisation b. To measure serological responses to influenza A and B subtypes and SARS-CoV-2 c. To measure the early inflammatory response after immune challenge d. To perform high-resolution tracking of T and B cell clones from lymph node cells and peripheral blood mononuclear cells as they develop after immunisation e. To identify B cell clones with immunoglobulin genes encoding broadly neutralising antibodies against influenza A and B subtypes and against SARS-CoV-2 strains	• 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 and other serological assays to measure antibody responses	All study timepoints where blood is collected
Intervention(s)	• Fine needle aspiration of axillary lymph nodes • Non-diagnostic ultrasound • Study injection: seasonal influenza vaccine, COVID-19 vaccine given contemporaneously as intramuscular injections into the right and left arms. The		

	influenza vaccine is given into the right arm and the COVID-19 vaccine into the left arm.

4. ABBREVIATIONS

AE	Adverse event
AIM	Activation induced marker
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Adverse reaction
AST	Aspartate aminotransferase
aQIV	Adjuvanted quadrivalent influenza vaccine
BMI	Body mass index
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CI	Chief Investigator
CRF	Case Report Form
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
EDC	Electronic Data Capture
ELISA	Enzyme linked immunosorbent assay
ELISpot	Enzyme linked immunospot assay
FNA	Fine needle aspiration
GC	Germinal centre
GCP	Good Clinical Practice
UK GDPR	United Kingdom General Data Protection Regulation
g/L	Grams per litre
GMC	Geometric mean concentrations
GMR	Geometric mean ratios
GP	General Practitioner
HI	Haemagglutination inhibition
HBsAg	Hepatitis B surface antigen
HCG	Human Chorionic Gonadotrophin
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRA	Health Research Authority
https	Hypertext Transfer Protocol Secure
Ig-seq	Immunoglobulin sequencing
IM	Intramuscular/intramuscularly
IU	Infectious units
IUD	Intrauterine device
IUS	Intrauterine system
JCVI	Joint committee on vaccination and immunisation
mmHg	Millimetres of mercury
mL	Millilitre
mmol/L	Millimoles per litre
MRC	Medical Research Council
mRNA	Messenger ribonucleic acid

ODS-ID	NHS Digital Organisational Data Service unique identifier
OVC	Oxford Vaccine Centre
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
PEG2000-DMG	1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIC	Participant identification centre
POCBP	Participant of childbearing potential
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance (formerly Clinical Trials and Research Governance)
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SMG	Study management group
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TOPS	The Over-Volunteering Prevention System
US	Ultrasound
WHO	World Health Organization
µmol/L	Micromole per litre

5. BACKGROUND AND RATIONALE

5.1.1. Characteristics of the disease being studied

Ageing is associated with a decline in immune function, which manifests clinically in the seventh decade of life. Vaccine immunogenicity engenders immune memory to prevent against infectious disease, which declines significantly from 65 years requiring alteration in vaccine design and delivery.

To investigate this age-related immune decline, immune function can be tested by challenging the immune system through immunisation in different age groups. Investigating this requires tissue-based research. Located in the axilla, reactive secondary lymphoid tissue is the major target for the mechanism of action of vaccines delivered by intramuscular (IM) injection into the deltoid muscle. Our group has established an experimental medicine model that uses ultrasound (US) guided fine needle aspiration (FNA) of the axillary lymph nodes to investigate vaccine-responsive cells[1].

In this study we will examine the responses of secondary lymphoid tissue to an immune challenge in the form of two licensed vaccines given as intramuscular injections, seasonal influenza, and COVID-19, comparing responses from older with younger adults.

5.2. Description of the population to be studied

Older people are at risk from severe disease from all pathogens with pandemic potential[2,3]. This population is rapidly growing, with over 60s predicted to account for 22% of the global population in 2050, increasing the at-risk population facing any future pandemic[4]. Responses to vaccination are blunted with age, with a gradual decline in late middle age that becomes clinically significant at 65 years and over. Efficacy of some vaccine platforms are particularly vulnerable to this, such as the inactivated COVID-19 vaccines[5.]

Vaccine responses to influenza vaccines decline with age with associated lower efficacy [6-8]. Adjuvanted vaccines have better immunogenicity and effectiveness in older people in some but not all studies[9]. Like COVID-19, there is a public health imperative to understand the precise immune mechanisms impacted by ageing that limit the magnitude and durability of the protective response after seasonal influenza immunisation.

In the case of the protein based seasonal influenza vaccine, poor immunogenicity in older adults has been partially overcome, by either increasing the dose of haemagglutinin fourfold (high dose influenza vaccine), or the addition of an adjuvant[10].

5.3. Name, description and characteristics of the study intervention

5.3.1. Immune challenge with COVID-19 vaccine

SARS-CoV-2 is the causative agent of COVID-19 against which vaccines have been developed[11,12]. In January 2022, the new omicron variant with multiple mutations in the S protein became the dominant circulating variant in England. Omicron has several sub-variants including BA.1, BA.2 and BA.4/5. BA.1 which dominated in England until April 2022, when BA.2 emerged before giving way to a peak of BA.4/5 later in the year[13]. Bivalent vaccines boost responses to the Omicron variants more effectively than the wild- type vaccines[14]. Bivalent vaccines targeting BA.4/5 were approved in November 2022, based on animal studies[15].

The primary schedule for vaccination in the UK is two doses of an approved or licensed COVID-19 vaccine. Protection is not lifelong and decline in protection is particularly evident in older people over 65 years. After the primary schedule, protection against Omicron wanes. The autumn 2022 booster offered to those aged 50 years and over, achieved 57.8 (51.6-63.3) effectiveness for the Moderna (Spikevax® bivalent Original/Omicron vaccine) at 2 weeks against hospitalisations, waning to 34.1 (29.2-38.7) at 10 weeks with similar responses for Pfizer BioNTech (Original/Omicron BA.1 Comirnaty®); 47.2 (39.4 to 54.1) to 38.0 (31.0 to 44.3) respectively[16].

The COVID-19 vaccine for the study is an mRNA COVID-19 vaccine, such as Spikevax.

The variant will be according to the latest JCVI guidance, which may vary year on year according to the evolving COVID-19 pandemic. For example, for the 2023-2024 season, Spikevax bivalent Original/Omicron BA.4-5 (50 micrograms/50micrograms)/mL dispersion for injection or Spikevax XBB.1.5 0.1 mg/mL dispersion for injection are recommended[17,18].

One 0.5 mL dose contains two types of COVID-19 mRNA vaccine embedded in SM-102 lipid nanoparticles.

The vaccine contains 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000-DMG) which is a potential allergen. It is presented as a multidose vial containing 2.5 mL dispersion in type 1 glass with a chlorobutyl rubber stopper and blue flip off plastic cap with aluminium seal.

Storage and handling

The unopened vial can be stored for 9 months at -50°C to -15°C. Once thawed, the unopened vaccine may be stored refrigerated at 2°C to 8°C, protected from light, for a maximum of 30 days. The unopened vaccine can be kept at room temperature (8°C to 25°C) for up to 24 hours before use and once punctured, the vaccine must be used within 6 hours. Thawed vaccine cannot be refrozen[17].

5.3.2. Immune challenge with seasonal influenza vaccine

Influenza is a globally endemic virus first isolated in the 1930s, with two types that predominantly circulate in humans; A, and B[19]. Influenza causes seasonal respiratory infection and disease and occasional pandemics. Nomenclature of the influenza virus is based on the immunodominant surface proteins haemagglutinin and neuraminidase (Table 1).

Table 1. Examples of influenza A and B virus classification (adapted from[20])

Type	**Subtype**	**Clade (Group) example**	**Sub-Clades (Sub-Groups)**	**Example vaccine strain**
Influenza A	A(H1N1)	6B.1	6B.1A	A/Victoria/2570/2019 IVR-215
Influenza A	A(H3N2)	3C.2a	3C.2a1	A/Darwin/6/2021 IVR-227
Type	**Lineage**	**Clade (Group) example**	**Sub-Clades (Sub-Groups)**	**Example vaccine strain**
Influenza B	B(Victoria)	V1A	V1A.1	B/Austria/1359417/2021 BVR-26

Influenza B	B(Yamagata)	Y1	None	B/Phuket/3073/2013 BVR-1B

The vaccine strains are updated every year in line with recommendations from the WHO. Vaccines currently used in the UK are quadrivalent (contain four subtypes); two A subtypes and two B subtypes. Control measures used to curb the spread of the COVID-19 pandemic also had an impact on the spread of influenza virus, initially curbing its spread in 2020 and 2021 with no Influenza B/Yamagata HA segment sequences uploaded to GISAID in these years[21]. Despite this, the WHO continues to recommend the use of quadrivalent vaccines containing a B/Yamagata lineage-like virus[22].

The seasonal influenza vaccine for the study is Adjuvanted Quadrivalent Influenza Vaccine (aQIV), (surface antigen, inactivated), Seqirus suspension for injection in pre-filled syringe.

One 0.5 mL dose contains 15 micrograms HA from four strains of influenza propagated in fertilised hens' eggs with adjuvant MF59C.1. It is licensed for prophylaxis of influenza in people aged 65 years and over. In this study, the aQIV vaccine will be given to participants aged 18-45 years in an off license-indication and in people aged 65 years and over as a licensed indication.

The MF59C.1 adjuvant contains per 0.5 ml dose: squalene (9.75 mg), polysorbate 80 (1.175 mg), sorbitan trioleate (1.175 mg), sodium citrate (0.66 mg) and citric acid (0.04 mg). The vaccine may contain traces of eggs e.g., ovalbumin or chicken proteins, kanamycin and neomycin sulphate, formaldehyde, hydrocortisone, cetyltrimethylammonium bromide (CTAB). It is presented as a pre-filled syringe containing 1 dose of 0.5 mL as a milky-white suspension. It is given as an intramuscular injection and the preferred site is the deltoid muscle of the upper arm.

Storage and handling

The aQIV vaccine is stored in a refrigerator (2 °C – 8 °C) protected from light and must not be frozen [23].

5.3.3. Fine needle aspiration of lymph nodes

This study will investigate the tissue based immune responses to boosting with a vaccine encoding or containing recall antigens; the S glycoprotein from SARS-CoV-2, and haemagglutinin from influenza, in the previously experienced host. This characterisation in younger and older adults will expose differences in the boosting response.

This approach will highlight how age affects vaccine-critical immune pathways in the secondary lymphoid tissue, including germinal centre (GC) formation, induction of T follicular helper cell- and GC B cell responses. The immunological insights gained from this study will prove critical in the rapid design and delivery of novel age-appropriate vaccines and offer insights to responses against currently circulating and highly impactful respiratory pathogens.

5.4. Summary of findings from previous studies

The Green Book recommendations for co-administration of COVID-19 and influenza vaccines state that apart from the Novavax COVID-19 vaccine, co-administration of vaccination is acceptable and that participants should be warned of the likely systemic side effects.

In the ComFluCOV study, six different combinations of licensed influenza vaccine and COVID-19 vaccine were co-administered to adult volunteers[24]. Reactions were largely mild or moderate in nature. There were no safety concerns raised on the study and the authors did not find evidence of blunting of the immune response with co-administration. Immunogenicity measured as anti-spike IgG and haemagglutination inhibition against all four vaccine types (A/H1N1, A/H3N1, B/Yamagata and B/Victoria) was unaffected by sequencing of vaccination or combination of vaccines given, except for BNT162b2 given with recombinant QIV where responses were higher against A/H1N1 and both B types.

A second study enrolled 154 participants aged 60 years and over who had received their primary course of COVID-19 vaccination. Although there was weak evidence of immune blunting, given the choice of vaccine and scheduling, the data from this study are not generalisable to the UK [25].

A phase 2, randomised, open-label study examined the safety and immunogenicity of a high-dose QIV given concomitantly with the mRNA-1273 COVID-19 vaccine in adults aged 65 years and over who had previously received primary schedule of the mRNA-1273 COVID-19 vaccine[26]. The most frequent grade 3 reactions were injection site pain and erythema. Headache, malaise, myalgia, and fatigue occurred in 50-60% of those receiving both vaccines. There were no SAEs or deaths up to day 22. With regards to immunogenicity, there was no evidence of immune interference with the HI titres or the SARS-CoV-2 IgG responses to immunisation. These were equivalent across groups where either vaccine was given. Axillary swelling and tenderness were not different between the groups and were uncommonly reported (<=5% frequency). In the pivotal licensure study, these AEs were reported more commonly in the younger age groups (<65 years) than in the older age groups[27].

5.5. Potential risks to participants

Study related risks are summarised below.

5.5.1. 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, adverse events attributable to the FNA were mild in nature and resolved within 5 days[1]. Participants will be provided with information regarding these expected adverse events in the participant information sheet and adverse events will be monitored and reported.

5.5.2. Risks related to study vaccine injections

The most likely side effects that recipients of mRNA vaccines may experience are short-lived local (primarily injection site tenderness or pain) and systemic vaccine reactions (fatigue, headache, malaise, feverishness) that resolve completely within days (Table 3).

Table 3: Frequency of adverse reactions to mRNA COVID-19 vaccine Spikevax original

Adverse Reaction	Frequency (%)	Adverse Reaction	Frequency (%)
Injection site pain	92	Chills	45.4
Fatigue	70	Nausea/vomiting	23
Headache	64.7	Axillary swelling/tenderness	19.8
Myalgia	61.5	Fever	15.5
Arthralgia	46.4	Injection site swelling	14.7

Spikevax increases the risk of myocarditis and pericarditis following immunisation; the condition typically develops within 14 days, often after the second dose. Vaccinated individuals are recommended to seek medical attention if they experience chest pain, shortness of breath or palpitations particularly in the 2 weeks following receipt of this vaccine. Spikevax has been associated with flares in capillary leak syndrome.

The safety of the aQIV was investigated in two studies of individuals aged 65 years and over (V118_20 and V118_18) and findings are summarised in Table 4. The most common reactions were injection site pain (16 and 32%), fatigue (11 and 16%) and headache (11 and 12%).

Table 4: Frequency of adverse reactions to aQIV in adults aged 65 years and over in two clinical studies V118_20 and V118_18

Adverse Reaction	Very common (≥1/10)	Common (≥1/100 to <1/10)	Uncommon (≥1/1,000 to <1/100)
Injection site pain	*		
Fatigue	*		
Headache	*		
Nausea, diarrhoea		*	
Myalgia, arthralgia		*	
Ecchymosis, erythema, induration		*	
Chills, influenza-like illness		*	
Vomiting			*
Fever over ≥38°C			*

Post marketing surveillance data are available for the trivalent formulation (Fluad) and have indicated the following rare reactions, thrombocytopenia (very rarely severe); lymphadenopathy; extensive swelling of injected limb > one week; injection-site cellulitis-like reaction; allergic reactions including anaphylactic shock, anaphylaxis, and angioedema; muscular weakness; neurological conditions including encephalomyelitis, Guillain-Barré syndrome, convulsions, neuritis, neuralgia, paraesthesia; skin reactions

including erythema multiforme, urticaria, pruritus or non-specific rashes; vasculitis with transient renal involvement.

5.5.3. Pregnancy and lactation

The aQIV vaccine is not recommended in women who are pregnant or breast-feeding because there are no data on safety of its use in this group. Animal studies do not indicate that inadvertent administration in this group would be harmful[23]. There are no data on Spikevax variant vaccines such as bivalent Original/Omicron BA.4-5 during pregnancy or breastfeeding, however, observational and animal data with the Spikevax original indicate there is no risk to the foetus or newborn infant.

5.5.4. Other study-related risks

Blood sampling during the study may cause slight pain, bruising, light-headedness, or fainting. The volume of blood taken in the study is less than that taken by regular blood donors over the same period, 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 vaccination 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) or other appropriate medical professional will be contacted.

5.5.5. Potential benefits to participants

The recruitment population may directly benefit from participation in the study. This is because the individuals will be vaccinated with licensed vaccines against influenza and COVID-19. 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.

5.6. Rationale for the study

The rational design of vaccines for older or younger people based on mechanistic insight into the ageing immune system is needed. Here we propose to study lymphatic tissue that is responding to an immune stimulus in these age groups to build this insight. The study focuses on the cellular pathway that induces neutralising antibody through the induction and maintenance of T follicular helper cells which provide help to B cells. These cells may be vulnerable to the effects of ageing through immune decline (loss of thymic output, loss of T cell clonal diversity and T cell exhaustion) and are therefore of interest to study as potential targets for future vaccine design.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome Measures*	Timepoint(s)
Primary Objective	To determine the frequency, phenotype, and function of immune cells in axillary secondary lymphoid tissue and blood after intramuscular immunisation, in older compared with younger volunteers.	Single cell ribonucleic acid sequencing (scRNA-seq) to measure cell by cell transcriptomes in lymph node cells and/or multiparameter flow cytometry	Day 0, 7, Day 14 and Day 28 and Day 84 after study injection
Secondary Objective	To define reactive lymph nodes using bedside imaging at baseline and after intramuscular immunisation, in older compared with younger volunteers.	Ultrasound measurements of secondary lymphoid tissue	Day 0, 7, Day 14, Day 28, and Day 84 after study injection
Exploratory Objective 1	To collect participant reported measures of axillary pain, swelling and tenderness, at baseline and after intramuscular immunisation, in older and younger volunteers.	Participant reported outcome collected by means of eDiary and ultrasound measurements	Day 0 to day 7.
Exploratory Objective 2	In younger and older age groups and after immunisation to perform detailed immunological profiling including for example a. To determine the T and B cell signalling pathways in axillary secondary lymphoid tissue after intramuscular immunisation b. To measure serological responses to influenza A and B subtypes and SARS-CoV-2	• Single cell ribonucleic acid sequencing (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	All study timepoints where blood is collected

	c. To measure the early inflammatory response after immune challenge d. To perform high-resolution tracking of T and B cell clones from lymph node cells and peripheral blood mononuclear cells as they develop after immunisation e. To identify B cell clones with immunoglobulin genes encoding broadly neutralising antibodies against influenza A and B subtypes and against SARS-CoV-2 strains	• 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 and other serological assays to measure antibody responses	

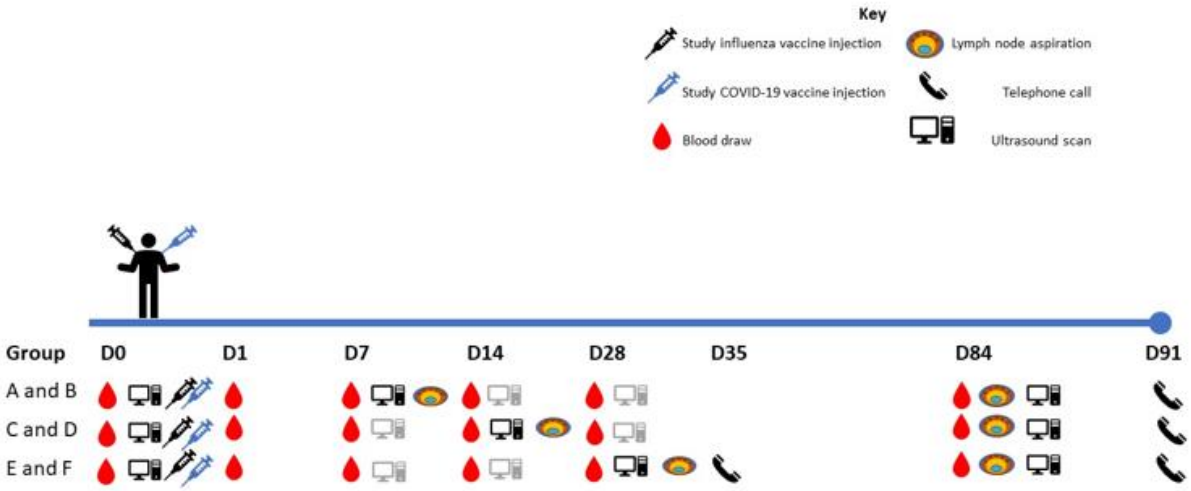
*Outcome measures are not limited to these example assays

7. STUDY DESIGN

This is an open label, observational, experimental medicine study to investigate human immune responses in lymph node cells after immune challenge with a COVID-19 vaccine and a seasonal influenza vaccine given contemporaneously as intramuscular injections (the study injections) (Figure 1). Participants will be healthy adults in two age groups: 18-45 years and ≥65 years. All participants will receive one dose of COVID-19 booster vaccination and one dose of seasonal influenza vaccine into opposite arm. Participants will be randomised to have a fine needle aspiration (FNA) biopsy of axillary lymph nodes on both sides at two timepoints; either 7 days, 14 days, or 28 days (determined by randomisation) and at 84 days after the study injections.

The study will be conducted at Oxford Vaccine Group, CCVTM, Oxford part of the University of Oxford, and will be supported by Oxford University NHS Foundation Trust at Experimental Medicine Clinical Research Facility as a non-recruiting site.

Figure 1 Study design. Images in grey represent optional interventions.



7.1. Study groups

48 participants will be enrolled over two seasons: 2023/24 and 2024/25. There is no restriction to the number of participants that can be recruited in a given season to make up the total number per group.

Group	Number of participants	Age	Timing of First FNA
A	8	18-45 years	D7 (-2)
B	8	≥65 years	D7 (-2)
C	8	18-45 years	D14 (+/- 1)
D	8	≥65 years	D14 (+/- 1)
E	8	18-45 years	D28 (+/-1)
F	8	≥65 years	D28 (+/-1)

All participants will have a second FNA at D84 followed by a phone call at D91.

7.2. Group randomisation

All participants will be block randomised (1:1:1) to determine whether their first FNA biopsy is performed at 7 days, 14 days, or 28 days after the study injections.

7.3. Study duration

The total duration of the study will be 13 weeks from the day of study injection for each volunteer. Participants will be considered enrolled into the study at the point of their injections.

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.
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 diaries.
4. Willing and able to give informed consent for participation in the study.
5. Agree to allow study staff to contact his or her GP or equivalent NHS databases to access the participant's vaccination records, medical history.
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 for the duration of enrolment in the study AND have a negative pregnancy test on the days of screening and study injections.
10. Have received at least a primary (two dose) schedule of any MHRA, UK authorised or licenced COVID-19 vaccine.

8.3. Exclusion criteria

Participants may not enter the study if any of the following apply:

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. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.
4. Administration of regular anticoagulation medication likely to induce bruising or bleeding on fine needle aspiration.
5. 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).

6. History of anaphylaxis in relation to vaccination, or local anaesthetic such as lidocaine.
7. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine including hypersensitivity to the active substance or to any of the excipients of the experimental vaccine or to local anaesthetic such as lidocaine.
8. History of hereditary angioedema, acquired angioedema, or idiopathic angioedema.
9. History of cancer that is not resolved (except basal cell carcinoma of the skin and cervical carcinoma in situ).
10. History of any serious psychiatric condition likely to affect participation in the study.
11. For participants of childbearing potential only: participants who are pregnant, breastfeeding or lactating, or are planning pregnancy during the study.
12. 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.
13. 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.
14. History of episodes of capillary leak syndrome.
15. 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)
16. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units per week.
17. Suspected or known injecting drug use within the 5 years preceding enrolment.
18. Detectable circulating hepatitis B surface antigen (HBsAg).
19. Seropositive for hepatitis C virus (antibodies to HCV).
20. Seropositive for HIV.
21. A history of pericarditis, myocarditis or other cardiac inflammation deemed significant by the investigator.
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 D0 Study Injection (vaccination) visit)

The following apply when determining if a participant will receive the study injections. 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 vaccination, 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 vaccination.

3. Receipt of systemic antibiotics will result in vaccination 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 vaccination.
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 a dose of vaccine 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 a dose of vaccine being administered.
7. 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. Temporary exclusion criteria for FNA procedure

The following apply when determining if a participant will receive the study injections. If the temporary exclusion resolves within the time constraints of the study, progression in the study can continue.

1. Receipt of any aspirin or medication that may increase risk of bleeding 7 days before each procedure.

8.6. Pregnancy and contraception

Participants of childbearing potential will be asked 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 for the duration of the study. Acceptable forms of effective contraception for participants of child-bearing potential include:

- 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 when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (*e.g.*, calendar, ovulation, symptothermal, post-ovulation methods), 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 vaccine 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, 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 (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 9.6.3).

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 full 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 the immune system.
- The study may involve genetic testing such as tissue typing.
- 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 may be required at investigator's discretion, *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 16/SC/014), 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 member of staff. 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.

9.4. Randomisation

Participants will be randomised 1:1:1, to have FNA either 7 days, or 14 days, or 28 days after the study injections. Randomisation will occur as part of screening after confirmation of eligibility when invited for and booking in the study injection visit. This will occur before the study injections visit to allow study activity management.

9.5. Blinding and code-breaking

There is no blind in this study.

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

9.6.1. Description of study interventions

See Sections 5.3 above, for a description of the mRNA COVID-19 and seasonal influenza vaccine and the potential risks associated with it.

9.6.1.1. Study vaccine presentation and storage

Throughout the study, the study vaccines will be stored in temperature monitored fridges or 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.

9.6.1.2. Compliance with study treatment

The study vaccines 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.

9.6.1.3. 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 due to potential effect on immune system compromising the objectives of the study.

9.6.1.4. 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 vaccine 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 medical 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, which the practitioner deems significant to warrant exclusion from the FNA

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

Given that vaccination may cause pain and swelling in the axilla, its' presence will not prevent sampling; if there are no signs of localised infection, sampling can proceed at the practitioner's discretion.

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 of the lymphatic system, and then under US guidance. A sterile needle and syringe will be used to aspirate material from lymph nodes on each side using 3-5 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 biopsy 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 (including disinfectant, local anaesthetic, needles, syringes, specimen tubes prepared with 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.

9.6.2.2. Ultrasound imaging

No device is being tested for the purposes of the research and 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. There will be no endpoints directly related to assessment of the performance of the machine.

(a) *Device description*; A GE LogiqE10s, Toschiba 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 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.

9.6.3. Screening visit

Screening visits will be conducted up to 120 days before D0. The schedule of events for the screening visit are shown in Appendix A.

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

- Participant demographics: age, sex, and ethnicity

- Medical history including lifestyle factors (i.e., smoking and alcohol history)
- Contraception: participants of childbearing potential are asked if they are willing to use effective contraceptive measures for the duration of their enrolment.
- 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 as required.
- 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 and blood borne viruses.

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 may be requested from the GP or accessed via the electronic patient record (if available) at the screening visit, if deemed required by the investigator. 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.

Group randomisation will be done once participant is deemed eligible for the study (see Section 9.4).

9.6.4. 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 study injections at Day 0. Both injections must be administered to be deemed enrolled. For each of these participants, a minimal set of screening failure data will be recorded, including demographic details and the reason for screening failure.

9.7. 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.

9.7.1. Study injection (vaccination) visit (D0)

The procedure for the study injection visit 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 vaccination (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 scans of both axillae
- Take blood sample
- Administer vaccines by IM injection into deltoid muscles of each arm. The aQIV will be given into the right arm and the COVID-19 vaccine into the left arm
- Schedule next visit

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

9.7.2. FNA visits

The following procedures will be performed at FNA visits:

- Review of SAEs, as appropriate, since the last visit
- Review eDiary entries (if applicable) 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)
- Monitor post-FNA for 30 mins
- Take blood sample

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

9.7.3. Follow up visits

Follow-up visits require the following procedures:

- Review of SAEs, as appropriate, since the last visit
- Review eDiary entries (if applicable) and laboratory blood tests
- Targeted physical examination (if indicated)
- Take blood sample
- Perform ultrasound (if indicated and feasible)

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

9.7.4. 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).

9.7.5. Missed visits

In exceptional circumstances, only where follow-up visits would otherwise be missed entirely, participants may be contacted remotely e.g., by phone or email to facilitate on-going study engagement.

9.8. E-diaries

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 vaccine injections.

9.9. Sample handling

9.9.1. Clinical laboratory samples

Blood will be drawn (as shown in Appendices A and B) for the following laboratory tests. 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
- 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 vaccination 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).

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, TCR and IgG-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 vaccine immunogenicity and gene expression studies, amongst others, may be performed.

Other Research Laboratories

Collaboration with other specialist laboratories in the UK study, 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) and/or lymph node cells to these laboratories, but these samples would remain de-identified. Informed consent for this will be gained from the volunteers at screening. Immunological assays will be conducted according to local SOPs.

9.9.3. Lymph node biopsy samples

These will be handled for processing similar to previous studies conducted by the CI[1].

Where appropriate, single cell RNA-Seq experiments will undertake filtering and quality control of lymph node cells. Samples will be handled according to established single-cell RNA-seq best practice. Data will typically be analysed using ANOVA, Wilcoxon rank, and Fisher exact tests. Dimensionality reduction will determine cell clusters and immune cell subpopulations. edgeR packages will 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 will be performed similar to previously described using standard pipelines[28-30].

Flow cytometry data will be analysed using FlowJo, with appropriate controls (non-specific isotype controls and beads). For serological and cell function analyses, assays will be performed in duplicate or triplicate. Experiments will be repeated where necessary to test reproducibility. Appropriate tests (e.g., t-tests, Wilcoxon rank-sum tests) for assessing inter-group differences will be performed using the appropriate statistical package e.g., R, python, or GraphPad Prism.

9.9.4. Ultrasound images

Ultrasound images will be collected using the software provided with the ultrasound machine operating system. These may be securely shared for storage on a secure password protected computer as anonymised images in the appropriate format such as .jpeg using the appropriate applications. Ultrasound images can be stored with the unique study identifier for each participant, the date of the scan and the initials of the person performing the examination.

9.9.5. Urine samples

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

9.9.6. Retention of samples

Participants will be informed that they may opt into the Oxford Vaccine Centre Biobank study (REC 16/SC/014) 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 declines 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, including unable to obtain sample from first FNA
- The participant is lost to follow up

In circumstances pertaining to the safety of the participant, the Investigator may choose to discontinue further study procedures for an individual participant. If a participant is withdrawn before first FNA they will be replaced. 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

There is no safety endpoint in the study; participants will be asked about serious adverse events from the point of enrolment, to ensure on-going ethical conduct of the study and to meet any regulatory guidance for post-marketing surveillance of licensed products. At the Chief Investigator's discretion, side effects of study vaccine injections that are suspected adverse reactions will be reported to the MHRA via its Yellow Card Scheme: www.mhra.gov/yellowcard

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.

10.2. Reporting procedures for Serious Adverse Events

SAEs will be collected throughout the entire study period (from study injections to the final study visit or withdrawal).

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 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).

11. STATISTICS AND ANALYSIS

11.1. Description of the statistical methods

The statistical aspects of the study are summarised here.

11.1.1. 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.

An interim analysis will be conducted on data collected for the primary, secondary and exploratory endpoints as appropriate, and as data become available, for example between seasonal cohorts. The purpose of interim analyses will be to understand assay capability and for initial data interpretation, to deliver on primary, secondary and exploratory endpoints. The interim analyses will not affect the treatment of participants or their future collected data. No formal sample size or power calculation was conducted, and the sample size or assumptions won't change.

11.1.2. Immunology analyses

These will be conducted where appropriate as previously described[28-30]. Where appropriate, non-normal distributed immunology data will be log-transformed to render a normal distribution and geometric mean concentrations (GMC). Corresponding 95% confidence intervals 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.

11.1.3. Lymph node analyses

11.2. Analyses will be conducted according to standard laboratory protocols for immunophenotyping. Sample size determination

48 participants will be randomised and enrolled to the study as detailed in section 7. Participants may be replaced if they do not have an ultrasound guided FNA with sufficient yield at D7, D14 or D28. 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 pragmatically reflect logistical and budgetary constraints.

11.3. Analysis populations

All participants with any available data will be included in the analyses. Participants who are randomised will be analysed according to their randomised study group in an intention to treat analysis population, including participants whose FNA visits were out of window. If deemed appropriate, participants who received their randomised study group will be analysed in a per-protocol analysis population, excluding participants whose FNA visits were out of window.

11.4. Stopping rules

There are no formal stopping rules. The CI reserves the right to pause the study or terminate the study on ethical or safety grounds.

11.5. The Level of Statistical Significance

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

11.6. 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 on a secure server located in Europe 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 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 informed consent forms 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. Participants will be identified by the unique study-specific participant number and/or code, allocated at the screening visit. 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 studies), 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.

Bank details will be stored for a minimum of 7 years in line with the site financial policy.

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

The OVG study team will form the study management group (SMG) and will provide on-going management of the study. As these are licensed vaccines with known safety profiles, a study specific Safety Committee will not be convened.

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 CI, 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), regulatory authorities, 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.

In the unlikely event of seeing any possible structural abnormalities on a scan, the scan will either be checked by a clinical specialist and 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 usually 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 vaccination visit; £90 for follow-up visits; £30 for the diary card and £150 for each FNA 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 can receive a maximum of £820 for the 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 the Medical Research Council and UK Research and Innovation.

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.

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 may be stored electronically on a secure server operated by the University IT team, and paper notes will be kept in a in a secure location at the study site. All essential documents, which includes research data and identifiable information, will be retained for a minimum of 5 years after the study has finished. Research data will be stored indefinitely, subject to adjustments in clinical trials regulations. Participants' bank details will be stored for a minimum of 7 years in line with the site financial policy. 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[1]
Timeline	120 days before D0
Visit Procedures	
Informed consent	X
Review inclusion and exclusion criteria[2]	X
Record demographic data[2]	X
Medical history including lifestyle factors (i.e. smoking and alcohol history)[2]	X
Vital signs (heart rate, temperature, blood pressure)	X
Measure height and weight, calculate BMI	X
Screening physical examination	X
TOPS registration *www.tops.org.uk*	X
Randomisation to study group	X
Urine Samples	
Urinary HCG (POCBP only)	X
Blood Samples[3]	
HBsAg, HCV Ab, HIV serology (mL)	~5
Biochemistry, haematology (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]Inclusion/exclusion criteria, demographic data and medical history may be initially assessed in part by a telephone call prior to screening; information obtained in this way will be reviewed at the face-to-face 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

Study visit number	D0	D1	D7	D14	D28	D35[$]	D84	D91
Study day	0	1	7	14	28	35	84	91[†]
Window (days)	NA	0	-1 to +2	-1 to +2	-1 to +2	-1 to +1	-7 to +7	-1 to +1
Visit procedures								
Follow up phone call						x		x
Review contraindications, inclusion and exclusion criteria	x							
Concomitant medications		x	x	x	x	x	x	x
Influenza / COVID-19 vaccine injections	x							
FNA vital signs			(x)*	(x)*	(x)*		x	
Symptom directed vital signs and physical examination	x	x	x	x	x		x	
SAEs		x	x	x	x	x	x	x
Urine samples								
Urinary pregnancy test (if applicable)	(x)							
Lymph node fine needle aspiration procedures (if applicable) *								
Inspection of the FNA site			(x)	(x)	(x)		x	
Ultrasound scan	x		(x)	(x)	(x)		x	
Lymph node fine needle aspiration			(x)	(x)	(x)		x	
Post FNA 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)		10^5 to 10^7	
Ultrasound examination of axillary lymph nodes	x		(x)	(x)	(x)		x	
Blood samples								

Blood for serum immunoassays (approx. 10mL)	x		x	x	x		x	
Blood for cellular and plasma immunoassays (approx. 40mL)	x	x	x	x	x		x	
Blood for RNA PAXgene tube (approx. 2.5mL)	x	x						
Blood for HLA testing (approx. 3-4mL)	x							
Visit blood volume (approx. mL)	56.5	42.5	50	50	50		50	
Cumulative blood volume (mL)	56.5	99	149	199	249		299	

*Depending on Group
[$] Group E and F only following D28 FNA
[†] 7 days after the participant has their D84 visit
() if applicable
Ultrasound visits at day 7, 14 and 28 are optional dependent on site staff capacity and may not be required for those not having an FNA.

If a participant misses a scheduled phone call visit, the study team can send a message or email to the participant requesting them to contact the study team if they have any concerns, without it being a protocol deviation.

24. APPENDIX C: Amendment history

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1.1	24 October 2023	KM Pollock H Robinson N Owino	5.3.1: Updated to allow use of JCVI recommended COVID-19 mRNA vaccine Synopsis and 6: Clarification of outcome measure Appendix B: Clarification of elements within schedule of events table Throughout: Corrected typos
2	1.2	15 December 2023	J Cotton W Smith	9.7 updated to allow out of window FNA visits. 23. Appendix B updated to remove the requirement for 6 ultrasound images per side. Minor typographical corrections throughout.
3	1.3	24 January 2024	J Cotton	23. Appendix B updated to clarify D91 phone call timepoint as 7 days after the participant has their D84 FNA.
4	1.4	15 April 2024	W Smith	9.6.3 Screening visits section updated: lifestyle factors (i.e., smoking and alcohol history) will be gathered at screening 22. Appendix A updated: lifestyle factors (i.e., smoking and alcohol history) will be gathered at screening
5	1.5	06 June 2024	M Greenland K Pollock T Madupuri N Owino	11.1. updated to clarify an interim analysis will be conducted on data collected for the primary, secondary and exploratory endpoints as appropriate.