Study Title: An experimental medicine study of early tissue response in vaccination with lipid encapsulated non-amplifying mRNA in Lymph nodE single-cell Genomics in AnCestrY and ageing

Short title: Mechanism of early tissue responses in vaccination with mRNA vaccines (MechRNA)

Study code: 2025/01 LEGACY04

Ethics Ref: 25/NW/0164

IRAS Project ID: 347668

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Funder:	UK Research and Innovation, Medical Research Council

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Declaration of interests: 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

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2. LAY SUMMARY

Messenger RNA (mRNA) vaccines are a new type of vaccine. While effective for many, some groups, including older adults and individuals on certain immunosuppressive treatments, respond less strongly or for less time, requiring regular booster shots. To overcome this, we need to understand precisely how mRNA vaccines trigger immune cells.

For older adults and people on specific immunosuppressive medications, such as those for inflammatory bowel disease (IBD) that block a protein called tumour necrosis factor (TNF), mRNA vaccines do not appear to work as well. We want to understand why by examining samples taken directly from the lymph nodes in the armpit after an mRNA vaccine and comparing them with samples from younger adults without immunosuppressive therapies. By doing so, we aim to improve future vaccine designs, especially for those who need new vaccines the most such as older people and people with immune conditions.

Lymph nodes are bean-shaped organs that play a central role in producing immune cells after a vaccine is given. Using ultrasound scanning, we can collect cells from these lymph nodes to see how the body's immune system reacts to an mRNA vaccine. This is called ultrasound guided fine needle aspiration (FNA) and is commonly used for patients in the clinic.

This study will look at how the lymph nodes respond when people from three groups— younger adults, older adults, and individuals on anti-tumour necrosis factor therapy (anti-TNF)—are given a licensed COVID-19 mRNA vaccine. Comparing these groups will help reveal what goes on in the immune cells in each case. We will study why some people have a less robust response and others have a stronger and more sustained response. Ultimately, this work will guide the development of improved vaccines that better protect everyone, especially those who are most at risk from future infection and pandemic threats.

3. SYNOPSIS

Short title	LEGACY04 Study	
Study code	OVG2025/01	
Study registration		
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 Older adults, aged 65 years or over Adults aged 18 - 50 years on anti-TNF immunosuppressive therapy	
Sample Size	Sample size: Up to 45 participants • Up to 15 participants aged 18-45 years • Up to 15 participants aged 65 years or over • Up to 15 participants aged 18-50 years on anti-TNF immunosuppressive therapy Aiming for a minimum 12 analysable datasets from participants per group	
Intervention(s)	• Study injection: COVID-19 mRNA vaccine given as intramuscular injection into **left arm at Day 0** • Fine needle aspiration of axillary lymph nodes at Day 14 and 112 (optional Day 42) • Non-diagnostic ultrasound • Seasonal Influenza vaccine into **right arm at Day 28**	
Planned Study Period	Total length of the project: 3 years Duration of an individual participant's involvement: 6 months	
Planned Recruitment period	Start date for recruitment: 01 July 2025 End date for recruitment: 31 March 2026	
	Objectives	Outcome Measures
Primary Objective	To measure the LN GC response to mRNA vaccination in people on anti-TNF therapy and compare that with healthy adults.	The frequency of GC B cells in the ipsilateral and contralateral LNs at day 14 post mRNA immunisation in younger adult volunteers compared with those on anti-TNF therapy.
Secondary Objective	To measure the LN response to mRNA vaccination in younger adults versus older people and to identify key cellular processes that are disrupted that lead to an impaired humoral response.	The frequency of GC B cells in the draining LN at day 14 post-mRNA immunisation in older people versus healthy controls or individuals on anti-TNF therapy, and the cell signalling pathways active in these cells.

Exploratory Objective 1	To measure waning of the LN response to mRNA vaccines	The frequency of GC T and B cells in the lymph node and serological responses in the blood
Exploratory Objective 2	To define reactive lymph nodes using bedside imaging at baseline and after intramuscular immunisation, in older compared with younger volunteers or individuals on anti-TNF therapy	Ultrasound measurements of secondary lymphoid tissue
Exploratory Objective 3	To collect participant reported measures of axillary pain, bruising, swelling and tenderness, after fine needle aspiration of axillary lymph nodes	Participant reported outcome collected by means of eDiary
Exploratory Objective 4	All three study 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 compare tissue-based responses at an early and late phase following vaccination (day 14 and 112) with the antibody and memory B cell profile at 6 months (day 182). c. To measure serological responses to SARS-CoV-2 and other pathogens in blood d. To measure the inflammatory response after immune challenge in blood and lymph node cells e. To measure lymph node cell yield after axillary lymphoid tissue sampling f. 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 g. To identify B cell clones with immunoglobulin genes encoding broadly neutralising antibodies against SARS-CoV-2 strains h. Other immunological objectives relevant to the study may be carried out	Including but not limited to: • Single cell ribonucleic acid sequencing 5-prime (5' scRNA-seq) to measure cell by cell transcriptomes in lymph node and blood cells • Cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) to measure cellular antigens on lymph node and blood cells • Single cell T cell receptor sequencing (scTCR-seq) to measure T cell receptor diversity in lymph node and blood cells • Immunoglobulin gene sequencing (Ig-seq) to measure B cell receptor and antibody diversity in lymph node and blood 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 • Genotyping / Human Leukocyte Antigen (HLA) typing • ELISA and other serological assays to measure antibody responses • Other immunological assays relevant to the study may be carried out

4. ABBREVIATIONS

AE	Adverse event
AIM	Activation induced marker
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Adverse reaction
AST	Aspartate aminotransferase
BMI	Body mass index
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CI	Chief Investigator
COVID-19	Coronavirus disease 2019
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
IBD	Inflammatory Bowel Disease
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
MoA	Mechanism of action
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
TNF	Tumour Necrosis Factor
TOPS	The Over-Volunteering Prevention System
US	Ultrasound
WHO	World Health Organization
µmol/L	Micromole per litre

5. BACKGROUND AND RATIONALE

5.1. Characteristics of the disease being studied

Lipid nanoparticle-encapsulated mRNA (mRNA) is a ground-breaking vaccine platform, having shown remarkable success during the COVID-19 pandemic. However, mRNA vaccines have proven subsequently less effective for long-term infection and symptomatic disease control, particularly in immunocompromised and older populations. Understanding the mechanism of action (MoA) of these vaccines at the cellular level is crucial for addressing these challenges and enhancing vaccine design.

Previous studies have indicated that TNF signalling is important in the MoA of mRNA vaccines in secondary lymphoid tissue. Patients with inflammatory bowel disease (IBD) taking anti-TNF immunosuppressive therapy have intentionally disrupted TNF signalling, and this is associated with impairment of the antibody response to COVID-19 mRNA vaccines[1,2]. Conversely patients with IBD taking a gut-immune specific immunomodulator, vedolizumab, have little or no impairment in their response to COVID-19 mRNA vaccines when compared with an adult control group[3]. Taken together, these data indicate that the responding secondary lymphoid tissue located in the axilla after an intramuscular injection with mRNA into the deltoid muscle is pivotal to generating the serological response, and that this mechanism is TNF dependent.

Similarly, ageing affects the inflammatory set-point, known as inflammaging[4]. Changes in TNF signalling may be involved in this process, reducing vaccine efficacy in older individuals[5]. Studying the effects of TNF signalling on vaccine responses will provide insights into the MoA of mRNA vaccines and guide the development of next-generation vaccines for vulnerable populations.

To further study the mechanism of mRNA vaccines, immune function can be tested by challenging the immune system through immunisation in different groups. Investigating this requires tissue-based research. Located in the axilla, reactive secondary lymphoid tissue is the major target for the MoA 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[6].

In this study we will examine the responses of secondary lymphoid tissue to an immune challenge in the form of a licensed COVID-19 mRNA vaccine given as intramuscular injections. We will compare responses across three groups: younger adults, older adults, and individuals on anti-TNF therapy.

5.2. Description of the population to be studied

5.2.1. Name, description and characteristics of the study intervention

Older people are at risk from severe disease from all pathogens with pandemic potential[7,8]. 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[9]. 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[10]

Similarly, individuals receiving anti-TNF therapy also experience diminished vaccine responses, especially to mRNA vaccines[1–3]. Whilst anti-TNF therapy is effective in treating various autoimmune conditions, it compromises immune responses, leaving many individuals vulnerable to severe disease. This also underscores the critical role of TNF signalling in the function of mRNA vaccines and may explain suboptimal responses observed in older adults, given the age-related pro-inflammatory changes that could disrupt TNF signalling pathways. Investigating the draining secondary lymphoid tissue in both healthy individuals and those with impaired immunity (older people and individuals on anti-TNF therapy) will be essential for understanding the MoA of mRNA vaccines in these populations.

5.2.2. Summary of findings from previous studies

Multiple studies have shown that individuals receiving anti-TNF therapy have reduced vaccine induced antibodies after mRNA vaccination. In the VIP study, patients with inflammatory bowel disease (IBD) receiving anti-TNF therapy infliximab had significantly reduced anti-SARS-CoV-2 spike protein antibody responses after two COVID-19 vaccine doses compared with healthy controls, whereas those on thiopurines, ustekinumab, or vedolizumab had responses comparable to controls[3]. Infliximab-treated patients had 10-fold lower antibody responses, and within 92 days of two vaccine doses, 55% of infliximab monotherapy patients and 48% of combination (thiopurine plus infliximab) patients had antibody concentrations more than two geometric standard deviations below the mean of controls. Similarly, in the Clarity-IBD study, patients with IBD treated with infliximab had significantly lower neutralising antibody titres (NT50) against wild-type SARS-CoV-2 and omicron subvariants (BA.1 and BA.4/5) after three vaccine doses[1]. Notably, 13.7% of infliximab-treated patients experienced breakthrough infections. These observations are further supported by the findings in the OCTAVE cohort study and OCTAVE-DUO randomised clinical trial, which similarly reported lower mRNA vaccine-induced antibodies and neutralising titres in individuals on anti-TNF therapy[2,11]. Overall, these data suggest a key role for TNF signalling in the cellular mechanisms underpinning mRNA vaccine responses. Taken together, these findings suggest a role of TNF signalling in the cellular function of mRNA vaccines.

Older adults have lower responses after mRNA vaccination, necessitating additional vaccine boosters to maintain adequate protection. Tut, Lancaster, and Krutikov et al. report a 2.5-fold reduction in SARS-CoV-2 neutralising antibody titres following a third mRNA vaccine dose (i.e. booster) in individuals over 65 years of age (418 IC50) compared with those under 65 (163 IC50)[12]. Similarly, the phase 1 trial evaluating the safety and immunogenicity of the Pfizer-BioNTech mRNA vaccines BNT162b1 and BNT162b2 found that older participants had 1–3-fold lower geometric mean concentrations of S1-binding IgG than younger participants[13]. This is likely linked to ageing-related chronic inflammation, characterised by raised pro-inflammatory cytokines such as TNF and interleukin-6, often termed “inflammaging”[4,5]. Human studies have shown that the diminished immune response to vaccination in older adults correlates with a decline in T and B cell function[14,15]. However, the extent of TNF disruption in ageing and its effect on lymph node cellular responses post-vaccination remains to be investigated.

5.3. Name, description and characteristics of the study intervention

5.3.1. Fine needle aspiration of lymph nodes

The Oxford Vaccine Group has an established experimental medicine programme using US guided FNA of lymph nodes established by the PI (Katrina Pollock), LEGACY01 (ISRCTN13657999), LEGACY02 study (ISRCTN11688703, LEGACY03 (ISRCTN12928349) and an established track record in the safety, feasibility and acceptability of these studies[6,16–18].

This study will use FNA of lymph nodes to investigate the tissue based immune responses to boosting with a vaccine encoding or containing recall antigens *i.e.,* the S glycoprotein from SARS-CoV-2 in the previously experienced host. This characterisation in younger and older adults and individuals on anti-TNF therapy will expose differences in the boosting response.

This approach will highlight how TNF signalling affected by therapy and ageing influence vaccine-critical immune pathways in the secondary lymphoid tissue, including germinal centre (GC) formation, induction of GC B cell and T follicular helper cell responses. The immunological insights gained from this study will prove critical in the design and delivery of novel age-appropriate vaccines and offer understanding of responses against respiratory pathogens.

5.3.2. COVID-19 and Seasonal influenza vaccine administration

The COVID-19 vaccine will be used in this study as an immune challenge to investigate tissue-based responses to mRNA vaccines. SARS-CoV-2, the causative agent of COVID-19, has driven the development of several vaccines. In January 2022, a new Omicron variant characterised by multiple mutations in the S protein became the dominant circulating variant in England. Omicron has since generated multiple subvariants, most recently including XBB 1.5, JN.1, and KP.3. XBB 1.5 remained dominant until June 2023, when JN.1 emerged and became the predominant strain by December 2023. As of October 2024, KP.3 is now the dominant strain. Vaccines targeting the XBB 1.5 and JN.1 variants were approved for the Spring 2024 and Autumn 2024 national vaccination programmes respectively.

Participants in this study will also receive the seasonal influenza vaccine as part of the usual vaccination programme for at-risk groups (as defined by the UK JCVI), and to provide additional benefit to those taking part in the study. Both COVID-19 and influenza vaccines used in this study are licensed and used routinely in the UK national vaccination programmes.

The COVID-19 vaccine and seasonal influenza vaccine will be administered at separate times. Further details regarding the study vaccines administration can be found in Section 7 (Study design) and 9.6 (Description of study interventions).

5.3.3. 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 COVID-19 and seasonal influenza. 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.

5.4. Rationale for the study

The rational design of mRNA vaccines based on mechanistic insight into individuals with immune modulation is needed. Here we propose to study lymphatic tissue that is responding to an immune stimulus in the study groups above to build this insight. The study focuses on the cellular pathway that induces antigen-specific neutralising antibody through the induction and maintenance of T follicular helper cells which provide help to B cells in specialised structure called germinal centres (GC). These GC T and B cells may be vulnerable to the effects of TNF blockade in the lymph node. The relationship between TNF signalling where it is optimal (young adults), deliberately blocked (adults on anti-TNF medication) or suboptimal due to the ageing process (older adults), and the immune response to an mRNA vaccine is therefore of interest to study as a potential target for future vaccine design. The waning of this response is important to measure as it may underpin the need for booster vaccination in vulnerable populations such as the elderly and immunocompromised.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome Measures*	Timepoint(s)
Primary Objective	To measure the LN GC response to mRNA vaccination in people on anti-TNF therapy and compare that with healthy adults.	The frequency of GC B cells in the ipsilateral and contralateral LNs at day 14 post mRNA immunisation in younger adult volunteers compared with those on anti-TNF therapy.	Day 14
Secondary Objective	To measure the LN response to mRNA vaccination in younger adults versus older people and to identify key cellular processes that are disrupted that lead to an impaired humoral response.	The frequency of GC B cells in the draining LN at day 14 post-mRNA immunisation in older people versus healthy controls or individuals on anti-TNF therapy, and the cell signalling pathways active in these cells.	Day 14
Exploratory Objectives 1	To measure waning of the LN response to mRNA vaccines	The frequency of GC T and B cells in the lymph node and serological responses in the blood	Day 112
Exploratory Objectives 2	To define reactive lymph nodes using bedside imaging at baseline and after intramuscular immunisation, in older compared with younger volunteers or individuals on anti-TNF therapy	Ultrasound measurements of secondary lymphoid tissue	All study timepoints (Day 0, 14, 28, 112, 182) after study injection

Exploratory Objective 3	To collect participant reported measures of axillary pain, bruising, swelling and tenderness, after fine needle aspiration of axillary lymph nodes	Participant reported outcome collected by means of eDiary	7 days after each FNA procedure
Exploratory Objective 4	All three study 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 compare tissue-based responses at an early and late phase following vaccination (day 14 and 112) with the antibody and memory B cell profile at 6 months (day 182). c. To measure serological responses to SARS-CoV-2 and other pathogens in blood d. To measure the inflammatory response after immune challenge in blood and lymph node cells e. To measure lymph node cell yield after axillary lymphoid tissue sampling f. 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 g. To identify B cell clones with immunoglobulin genes encoding broadly neutralising antibodies against SARS-CoV-2 strains h. Other Immunological objectives relevant to the study may be carried out	Including but not limited to*: • Single cell ribonucleic acid sequencing 5-prime (5' scRNA-seq) to measure cell by cell transcriptomes in lymph node and blood cells • Cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) to measure cellular antigens on lymph node and blood cells • Single cell T cell receptor sequencing (scTCR-seq) to measure T cell receptor diversity in lymph node and blood cells • Immunoglobulin gene sequencing (Ig-seq) to measure B cell receptor and antibody diversity in lymph node and blood 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 • Genotyping / Human Leukocyte Antigen (HLA) typing • ELISA and other serological assays to measure antibody responses • Other immunological assays relevant to the study may be carried out	All study timepoints where blood is collected may be relevant

*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 as an intramuscular injection (the study injection) (Figure 1). Participants will then receive a seasonal influenza vaccine on Day 28, administered in the arm contralateral to the one used for the COVID vaccine.

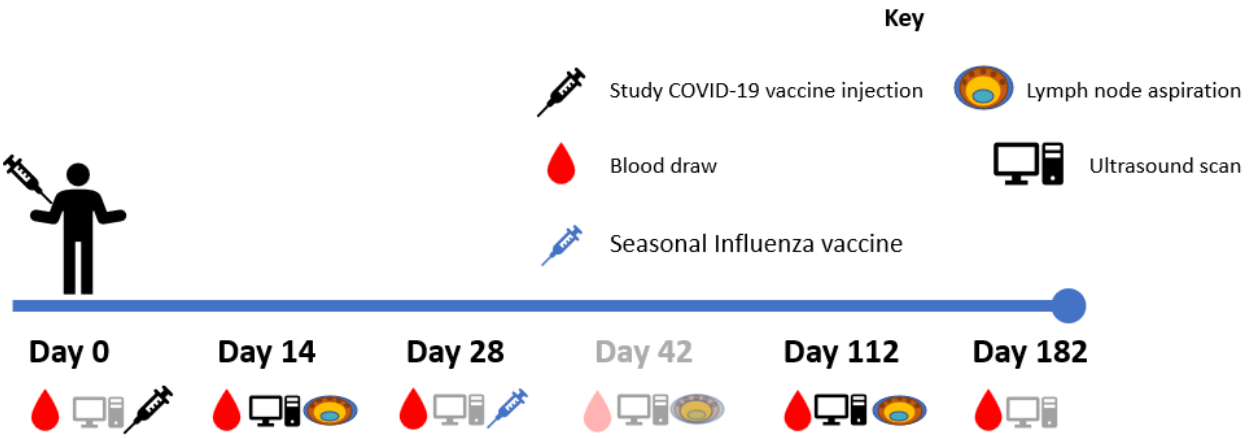
Participants will be adults in three groups: 18-45 years, ≥65 years and individuals aged 18-50 years on anti-TNF therapy. All participants will receive one dose of COVID-19 booster vaccination at Day 0 (study enrolment). Participants will then have a fine needle aspiration (FNA) biopsy of axillary lymph nodes on both sides at two timepoints at Day 14 or 112 after the study injections.

On Day 42 following the COVID-19 study vaccine injection, an additional, optional FNA with blood draw will be offered to up to 15 participants, with no more than five from each study group. This FNA procedure will only be carried out on the arm that received the seasonal influenza vaccine. The visit and procedures are subject to staff capacity and resources, and will be performed on a first-come, first-served basis.

Further study schedule of events is detailed in tables in Appendix A and B.

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 and all procedures listed at Day 42 represent optional interventions.



7.1. Study groups

36 – 45 participants will be enrolled in this study across one winter season. Participants will be assigned depending on age and whether they are receiving anti-TNF therapy (Sample size determination is further listed in Section 11.2).

Group	Number of participants	Age
A	12 -15	18 – 45 years
B	12 -15	≥65 years
C	12 -15	18 – 50 years individuals on anti-TNF therapy

7.2. Study duration

The total duration of the study will be 26 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 participants who meet the inclusion and exclusion criteria described below. Group A & B and Group C will have separate exclusion criteria.

8.2. Inclusion criteria for all groups

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 OR aged 18 to 50 years on anti-TNF immunosuppressive therapy
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
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.

For Group C participants (participants taking anti-TNF therapy), they would also have to satisfy the following criteria in addition to above to be eligible for the study:

11. Have a diagnosis of inflammatory bowel disease (Crohn's disease, ulcerative colitis, or inflammatory bowel disease unclassified)

12. On stable anti-TNF immunosuppressive therapy for the preceding 12 months prior to enrolment.

8.3. Exclusion criteria for all groups

1. Receipt of an investigational product within 12 weeks prior to enrolment or planned within the trial period.
2. Participation in another research study, in which procedures performed could compromise the integrity of this study (such as significant volumes of blood taken) or are planning to do so within the trial period.
3. Body mass index ≥35
4. Administration of immunoglobulins and/or any blood products within the three months of study enrolment.
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 vaccine including hypersensitivity to the active substance or to any of the excipients of the vaccine or to local anaesthetic such as lidocaine.
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. 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)

14. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units per week.
15. Suspected or known injecting drug use within the 5 years preceding enrolment.
16. Detectable circulating hepatitis B surface antigen (HBsAg).
17. Seropositive for hepatitis C virus (antibodies to HCV).
18. Seropositive for HIV.
19. A history of pericarditis, myocarditis or other cardiac inflammation deemed significant by the investigator.
20. 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.
21. 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. Specific additional exclusion criteria for Group C (participants taking anti-TNF therapy)

Participants for Group C may not enter the study if any exclusion criteria from section 8.3 apply or the following apply:

1. Any confirmed or suspected immunodeficient state unrelated to anti-TNF therapy, including HIV infection; asplenia; 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).
2. Participants taking any other systemic immunomodulator apart from / concurrently with anti-TNF immunosuppressive therapy
3. Any evidence of known inflammatory disease episode while on anti-TNF immunosuppressive therapy within a 12-month period

8.5. Temporary exclusion criteria for all Groups at vaccination visits

The following apply when determining if a participant will receive the study vaccines. 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.

8.6. Temporary exclusion criteria for FNA procedure for all groups

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.
2. Signs of infection at the site of biopsy in the axillae.

8.7. 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.

Although established contraception it is not a requirement of study entry, participants should not be planning a pregnancy during study participation and may wish to use effective contraception. 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 e.g., condoms.

Male participants are not required to use barrier methods for the purposes of contraception.

9. PROTOCOL PROCEDURES

9.1. Recruitment

Advertisements for recruitment will be distributed through methods including but not limited to posters, leaflets, websites, newspapers, radio, public engagement events, and/or social media, using advertising material containing wording from approved study documents to invite participation in the study. Potential participants may be contacted by methods including but not limited to email, telephone, and/or mail, using an approved invitation letter. Individuals who are taking anti-TNF therapy may also be identified and contacted (including but not limited to) by their doctor, nurse or clinical staff responsible for their care in the inpatient and outpatient setting at Oxford University Hospital and/or by the study team through research volunteer databases such as the Translational Gastroenterology and Liver Unit (TGLU) Biobank / Inflammatory Bowel Disease Cohort, if volunteers have consented to be contacted for research.

The details of other recruitment methods which may be used are outlined below:

- **Email campaign**: We may contact representatives of local tertiary education establishments and local employers and ask them to circulate approved posters and a link to the study website by email or hard copy.
- **Volunteers' databases**: Direct email and link may be sent to members of the public who have registered their interest in potentially volunteering for clinical trials. These are secure databases where members of the public registered here have given consent to have their details recorded and be contacted expressly for the purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to participating for any trial they are contacted about.
- **Media advertising**: Approved local media, newspaper and website advertisements may be 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 the information booklet may be placed on study websites and other appropriate platforms for vaccine trial advertising.
- **Social media**: Approved advertisements may be placed on trial 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 may exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.
- **SMS/text messages**: SMS/text message (or emails) may be sent to potential participants identified by GPs from their databases (which will require Participant Identification Centres [PIC] agreements to be set up with the GP surgeries), subject to ethics approval via an amendment to add PIC sites.
- **Royal Mail Leaflet**: Royal Mail door-to-door service with delivery of leaflets or invitation letters enclosed in envelopes may be sent to every household within certain postcode areas.

9.2. Pre-screening Questionnaire

Information about the study will direct volunteers to the study website, where a full participant information sheet will be available. Volunteers will also have access to the study team contact details to

communicate with the team directly. Volunteers who are willing to proceed will be asked to complete an initial online 3-part questionnaire.

Part 1: Will include major inclusion and exclusion criteria. If a volunteer is deemed ineligible based on any of the replies, they will be informed, the questionnaires will stop, and no demographic information will be recorded. Those eligible will be directed to e-consent in part 2.

Part 2: e-consent for access to and storage of medical history and vaccination records (via the volunteer GP or NHS databases) and recording and storage of personal information. Completion of the e-consent directs the volunteer to part 3.

Part 3: Records demographic information, NHS number, medical history, and medication use.

Volunteers that remain eligible will be invited for a full screening and consent visit, where their full eligibility will be assessed by a member of the clinical research team (see Section 9.9.1 below). Clarification of history provided may be discussed with the volunteer by telephone, prior to the face-to-face screening visit.

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.

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.
- The risks of participation in the study / study procedures will be discussed
- 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.
- The samples may be used for the commercial development of therapeutics, drugs and/or vaccines
- FNA at Day 42 of study will be on consent forms as an additional optional choice, and the visit and procedure will be carried out dependent on staff capacity and resources.

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. Blinding and Unblinding

This is an open-label study, and as such no blinding procedures will be performed.

9.5. Description of study interventions

9.5.1. Immune challenge with COVID-19 vaccine

The COVID-19 vaccine for the study is an mRNA COVID-19 vaccine, such as Spikevax (Moderna) or Comirnaty (Pfizer).

The vaccine targeting the specific COVID-19 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 2024-2025 season, Spikevax Omicron XBB1.5 (50 micrograms/50micrograms)/mL dispersion for injection or Spikevax JN.1 0.1 mg/mL dispersion for injection are recommended[17,18].

One 0.5 mL dose of Spikevax contains one type of COVID-19 mRNA vaccine embedded in SM-102 lipid nanoparticles. One 0.3 mL dose of Comirnaty contains one type of COVID-19 mRNA vaccine embedded in ALC-0315 lipid nanoparticles.

The Spikevax 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. The Comirnaty vaccine is presented in a 2 mL clear vial (type I glass) with a stopper (synthetic bromobutyl rubber) and a grey flip-off plastic cap with aluminium seal.

Storage and handling

For the Spikevax vaccine, 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. For the Comirnaty vaccine, unopened vials can be stored for 18 months when stored at -90 °C to -60 °C. Within the 18-month shelf life the thawed (previously frozen) vials may be stored at 2 °C to 8 °C for up to 10 weeks. the unopened vial can be stored for up to 12 hours at temperatures up to 30 °C. Once punctured, the vaccine must be used within 12 hours. Both vaccines cannot be refrozen once thawed.

9.5.2. Seasonal influenza vaccine administration

Seasonal influenza vaccine will also be given to participants within this study as part of the usual seasonal vaccination programme for at risk groups (as defined by the UK JCVI), and to provide potential benefit to participants who are joining the study.

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

The vaccine strains are updated every year in line with recommendations from the WHO. Vaccines currently used in the UK are now trivalent (contain three subtypes); two A subtypes and one B subtype, as opposed to the traditional quadrivalent vaccine. 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]. The WHO has since recommended the use of trivalent vaccines[22].

Based on UK JCVI guidance, the preferred seasonal influenza vaccine for the study is the inactivated recombinant influenza vaccine (IIVr) Supemtek suspension for injection in pre-filled syringe (Sanofi Pasteur). If this is unavailable, inactivated influenza cell-culture vaccine (IIVc) (Surface Antigen, Inactivated), Seqirus suspension for injection in pre-filled syringe will be used[23].

Table 2: Summary of influenza vaccines for 2025 to 2026 season from UK JCVI (adapted from [23])

Age or risk group	Vaccine preference	If the preferred vaccine is unavailable
Over 65 years of age	aIIV, IIV-HD, IIVr	IIVc
18 to 64 years in a risk group	IIVc, IIVr or aIIV (in those aged 50 to 64 years) or IIV-HD (in those aged 60 to 64 years)	IIVe
2 to under 18 years	LAIV	IIVc
2 to under 18 years but unable to have LAIV	IIVc	IIVe
6 months to under 2 years in a risk group	IIVc	IIVe

Note: LAIV is the vaccine of choice for children aged 2 to 17 years.

One 0.5 mL dose of IIVr contains 45 micrograms HA from four strains of influenza produced by recombinant DNA technology using a baculovirus expression system in a continuous insect cell line that is derived from Sf9 cells of the fall armyworm, *Spodoptera frugiperda*. One 0.5mL dose of IIVc contains 15 micrograms HA from four strains of influenza in Madin Darby Canine Kidney (MDCK) cells. Both vaccines are licensed for prophylaxis of influenza in people aged 65 years and over and at-risk adults aged 18 to 64 years of age. In this study, the IIVr / IIVc vaccine will be given to participants aged 18 – 45 years in an off license-indication and in people aged 65 years and over and individuals aged 18 – 50 on anti-TNF therapy as a licensed indication.

The IIVr may contain traces of octylphenol ethoxylate, and excipients include Polysorbate 20 (E432), Sodium chloride, Sodium phosphate monobasic, Sodium phosphate dibasic and Water for injections. It is presented as a pre-filled syringe containing 1 dose of 0.5 mL as a clear and colourless solution. It is given as an intramuscular injection and the preferred site is the deltoid muscle of the upper arm.

The IIVc may contain traces of beta-propiolactone, cetyltrimethylammonium bromide, and polysorbate 80. Excipients include Sodium chloride, Potassium chloride, Magnesium chloride hexahydrate, Disodium phosphate dihydrate, Potassium dihydrogen phosphate and Water for injections. It is presented as a pre-filled syringe containing 1 dose of 0.5 mL as a clear to slightly opalescent liquid. It is given as an intramuscular injection and the preferred site is the deltoid muscle of the upper arm.

Storage and handling

Both the IIVr and IIVc vaccine is stored in a refrigerator (2 °C – 8 °C) protected from light and must not be frozen

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.

9.5.3. 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.

9.5.4. 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.

9.5.5. 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. Description of study procedures

9.6.1. Fine needle aspiration of lymph nodes

Fine needle aspiration (FNA) will be carried out by an appropriately trained medical practitioner at the clinical facilities or other appropriate clinical areas within the 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 up to 6 passes judged to be successful by

the investigator. 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 (except the optional Day 42 visit) 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. The Day 42 visit will involve sampling from the right axilla, ipsilateral to the influenza vaccine.

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. 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 which are CE marked and equipped with appropriate probes 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.

9.7. Potential risks to participants

Study related risks are summarised below.

9.7.1. 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 two studies lead by the chief investigator, adverse events attributable to the FNA were mild (grade 1) in nature and resolved within 5 days[18]. It is also possible for the needle used in FNA to damage underlying structures; however, such occurrences are extremely rare. Among these, a rare but potential complication of FNA is a pneumothorax. This occurs when air leaks into the space between the lung and chest wall, which can cause pain and, in some cases, difficulty

breathing. A small pneumothorax can heal by itself with rest. FNA procedures are conducted under ultrasound guidance to prevent this from happening. Participants will be provided with information regarding these adverse events in the participant information sheet and adverse events will be monitored and reported.

9.7.2. Risks related to COVID-19 and seasonal influenza vaccine injections

9.7.2.1. COVID-19 Vaccines

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. Potential participants with a history of these conditions will be excluded.

9.7.2.2. Seasonal Influenza Vaccines

The safety of IIVr has been collected from 998 adults 18-49 years of age (Study 1) and 4328 adults 50 years of age and older (Study 2).

The most common reactions occurring after vaccine administration were injection-site reactions (tenderness and pain) reported overall by 48% and 37% of study participants 18-49 years of age receiving Supemtek respectively. In study participants 50 years of age and older, injection site tenderness was reported by 34% and injection site pain reported by 19%. The severity of the reactions was mild to moderate. Onset usually occurred within the first 3 days after vaccination. All resolved without sequelae.

The safety of IIVc in adults 18 years and older was evaluated in a randomised, controlled study (V130_01), in which 1334 subjects received Cell-based Quadrivalent Influenza Vaccine (Surface Antigen, Inactivated)

Seqirus suspension for injection in pre-filled syringe. Similar rates of solicited local and systemic adverse reactions were reported in subjects who received Cell-based Quadrivalent Influenza Vaccine (Surface Antigen, Inactivated) Seqirus suspension for injection in pre-filled syringe and cell-based trivalent influenza vaccine comparator in this clinical trial.

The most commonly reported (≥ 10%) reactions in subjects who received Cell-based Quadrivalent Influenza Vaccine (Surface Antigen, Inactivated) Seqirus suspension for injection in pre-filled syringe were pain at the injection site (34%), headache (14%), fatigue (14%), myalgia (12%), injection site erythema (13%) and injection site induration (10%).

Both influenza vaccines are licensed vaccines used routinely in the UK Influenza Vaccination Programme. Further adverse reactions are listed in the Summary of Product Characteristics.

9.7.3. Pregnancy and lactation

Both IIvr , IIVc and COVID-19 mRNA vaccines are licensed for use in pregnancy and lactation. JCVI recommend either IIvr or IIVc in pregnancy[23]. Where applicable, a pregnancy test will be carried out during the study; if a participant is pregnant, they will not be included in the study as this may affect study outcome measures.

9.7.4. 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. These findings are not diagnostic however, participants will be informed if further investigation is required, and, with their consent, their general practitioner (GP) or other appropriate medical professional will be contacted.

9.8. Baseline Visits

9.8.1. 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) and any previous COVID-19 infection within the last three years prior to the screening visit. For Group C participants, history of inflammatory bowel disease will also be collected (i.e date of diagnosis, current and previous symptoms, duration of anti-TNF therapy and previous medication used to treat inflammatory bowel disease)
- History of COVID-19 and influenza vaccines received within the last five years prior to the screening visit and which arm they received them in.
- 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. Permission to access the volunteer medical records either via the electronic patient record (EPR) or GP will also be sought (if possible) prior to the screening visit for study purposes. Patient medical summary, vaccination and prescribed medication history may then be reviewed 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.

9.8.2. 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 study injections at Day 0. For each of these participants, a minimal set of screening failure data will be recorded, including demographic details and the reason for screening failure.

9.9. 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. Participants will also be asked about COVID-19 symptoms at every subsequent visit. Presence of highly likely COVID-19 symptoms in the opinion of the investigator will trigger a PCR or lateral flow test (which can be self-taken).

9.9.1. 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 8.7), and perform a targeted physical examination (if required to reassess eligibility)
- Review concomitant medications (if applicable)
- Record temperature, pulse, and blood pressure
- Targeted physical examination (if indicated)
- Perform urinary pregnancy test for participants of child-bearing potential
- Perform ultrasound scans of both axillae (if feasible)
- Take blood sample
- Administer COVID-19 mRNA vaccine by IM injection into deltoid muscle of the **left arm**
- Schedule next visit

9.9.2. FNA visits

The following procedures will be performed at FNA visits:

- Review of SAEs related to study procedures, as appropriate, since the last visit
- Review concomitant medications (if applicable)
- Targeted physical examination (if indicated)
- Record oral temperature, pulse, and blood pressure
- Perform ultrasound (see Section 9.7.2)
- Inspect FNA site
- Perform FNA (see Section 9.7.1)
- Monitor post-FNA for 30 mins
- Take blood sample

On the FNA visits, the participant will be provided with access and training to use the eDiary (on REDCap, with link sent via email). If required, a paper version will also be available for participants in case they are unable to access the eDiary online.

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

9.9.3. Seasonal influenza vaccination visit (at D28)

The procedure for the study injection visit will be as follows:

- Ensure that participant consent remains valid and confirm continued consent
- Check temporary exclusion to vaccination (see Section 8.5), and perform a targeted physical examination (if required)
- Review eDiary entries (if applicable)
- Review concomitant medications (if applicable)
- Record temperature, pulse, and blood pressure
- Targeted physical examination (if indicated)
- Perform urinary pregnancy test for participants of child-bearing potential
- Perform ultrasound scans of both axillae (if feasible)
- Take blood sample
- Administer seasonal influenza vaccine by IM injection into deltoid muscle of the **right arm**
- Schedule next visit

9.9.4. Follow up visits

Follow-up visits require the following procedures:

- Review of SAEs that are related to study procedures, as appropriate, since the last visit (further definitions of AEs and SAEs related to study procedures in section 10)
- Review eDiary entries (if applicable)
- Targeted physical examination and vital signs (if indicated)
- Take blood sample
- Perform ultrasound scans of both axillae (if feasible)
- In addition, the next visit will be scheduled/confirmed.

9.9.5. 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).

9.9.6. 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.

9.9.7. Electronic diary (eDiary)

Following each FNA, e-diaries to collect information on axillary symptoms will be used as part of safety monitoring for 7 days (8 days in total including the day of FNA). Participants will be asked if they have experienced pain, bruising, swelling or tenderness in the left and right axillae after the FNA.

9.10. Sample handling

9.10.1. 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)
- Diagnostic serology
    - 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) (screening visit only)
    - Serology test for recent COVID-19 infection, including anti-nucleocapsid and anti-spike antibody tests
- Immunology (first vaccination visit only):
    - Human Leukocyte Antigen (HLA) typing
- Blood glucose point of care test
- Anti-TNF medication monitoring +/- test (Group C only)

Additional safety blood tests may be performed if clinically relevant at the discretion of the medically qualified investigator(s).

9.10.2. 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 pseudonymised. Informed consent for this will be gained from the volunteers at screening. Immunological assays will be conducted according to local SOPs.

9.10.3. 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[24-26].

Flow cytometry data will be analysed using software packages such as 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.

9.10.4. 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 pseudonymised 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 according to relevant OVG SOPs.

9.10.5. 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.

9.10.6. Retention of samples

Participants will be asked if they consent to remaining samples being used in future research. If they do not consent to this, the samples will be destroyed at the end of the study.

9.11. 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.

9.12. 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 adverse events for 7 days after the FNA procedure, and serious adverse events (SAE) that are related to the study procedure as assessed by Investigators from the point of enrolment after study procedures have been performed. This is to ensure the 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. Adverse events

Adverse events will be solicited by means of an eDiary on the day of the FNA procedure visit and for 7 days afterwards. Unsolicited adverse events will not be collected unless they meet the criteria for a serious adverse event.

10.2. Definition of serious adverse events

A SAE 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

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.

10.3. Reporting procedures for Serious Adverse Events

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

SAEs related to the study procedures 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 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. 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

The primary study endpoint is to compare the frequency of GC B cells in the ipsilateral and contralateral LNs at day 14 post immunisation in younger adult volunteers compared to those on anti-TNF therapy. To assess this endpoint, we will utilise the MASC (mixed-effects association of single-cells) algorithm, which is designed for case-control abundance analysis of scRNA-seq data while controlling for fixed and random effects. Odds ratios of enrichment of a given cell type by group will be determined. Secondary and exploratory endpoints will be conducted according to standard laboratory protocols for immunophenotyping and multi-omic analysis.

11.2. Sample size determination

Given that lymph node (LN) germinal centre (GC) B cell frequencies in humans on anti-TNF therapy or in older adults remain unpublished, previous studies investigating vaccine immunogenicity are used as surrogate data. Studies suggest a 3–10-fold reductions in immunogenicity in people on anti-TNF therapy compared to healthy controls. For instance, following COVID-19 mRNA vaccination, individuals on anti-TNF showed up to a 10-fold decrease in geometric mean anti-spike antibody titres, while those over 65 years displayed a 2.5–3-fold reduction. Conversely, lymph node assessments in young, healthy volunteers indicate ~13% GC B cells (SD ~9%), and sample size modelling suggests that enrolling 12 participants per group (n=12) yields 81–93% power for detecting potential 3–10-fold reductions in LN GC B cell frequencies.

Finally, to account for ~20% of participants with unanalysable samples during the study, up to n=15 per group (45 participants) is proposed. To meet the minimum sample size requirements, additional enrolments may occur if participants have unanalysable study samples or an ultrasound guided FNA with insufficient yields during the course of the study.

11.3. Analysis populations

All participants with any available data will be included in the analyses. Participants will be analysed according to their assigned study group in an intention to treat analysis population, including participants whose FNA visits were out of window. If deemed appropriate, participants will also 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, with details fully described in the Data Management Plan.

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 the screening visit. Names or identifying details are not included in any electronic file, containing study data. The exception to this is the electronic diaries, for which consent will be obtained to store the participant email address, which is necessary for the system to function. 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 where data are first recorded, and from which participants' CRF data are obtained. 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 vaccination/pharmacy records, diaries, ultrasound images, 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

All study data will be recorded directly into eCRFs within 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.

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. An independent Data and Safety Monitoring Committee (DSMC) will also be appointed for this study. 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.

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 Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the Sponsor, Ethics and funder, where required.

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 visits; £90 for follow-up visits; £30 for the diary card and £150 for each FNA procedure visit. 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 £960 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 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 may be stored electronically on a secure server, 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[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 (e.g., smoking and alcohol history)[2]	X
Vital signs (heart rate, temperature, blood pressure)	X
Measure height and weight, calculate BMI	X
Screening physical examination[2]	X
TOPS registration *www.tops.org.uk*	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
Review anti-TNF medication monitoring +/- test (Group C only)	x

[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, initial consent will be obtained prior to this. Information obtained in this way will be reviewed at the face-to-face screening visit. Physical examination will be performed as needed

[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	D14	D28	Optional visit D42§	D112	D182
Study day	0	14	28	42	112	182
Window (days)	NA	-2 to +2	-2 to +2	-2 to +2	-7 to +7	-7 to +7
Purpose	Vaccine 1	FNA 1	Vaccine 2	FNA optional	FNA 2	Follow up
Visit procedures						
Review contraindications, inclusion and exclusion criteria	x					
Concomitant medications	x	x	x	x	x	x
COVID-19 vaccine injection	x					
Recording of vital signs	x	x	x	x	x	(x)
Symptom directed physical examination	(x)	(x)	(x)	(x)	(x)	(x)
Review SAE related to study procedures	x	x	x	x	x	x
COVID-19 symptoms and trigger COVID-19 test	x	x	x	x	x	x
Seasonal influenza vaccination			x			
Urinary pregnancy test	(x)		(x)			
Ultrasound and lymph node fine needle aspiration procedures#						
Inspection of the FNA site		x		x	x	
Ultrasound examination of axillary lymph nodes†	(x)	x	(x)	x	x	(x)
Lymph node fine needle aspiration		x		x	x	
Post FNA check		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)	
FNA ediary set-up		x		x	x	
FNA ediary review			x		x	x
Blood samples						
Blood for serum immunoassays (approx. 10mL)	x	x	x	x	x	x
Blood for cellular and plasma immunoassays (approx. 40 – 60 mL)	x	x	x	x	x	x
Blood for RNA PAXgene tube (approx. 2.5mL)	x	x				
Blood for HLA testing (approx. 3-4mL)	x					
Review anti-TNF medication monitoring +/- test (Group C only)			x			x

() if applicable
FNA can be offered irrespective of study visit window if the visit cannot be scheduled within window
† Ultrasound visits at day 0, 28, 182 are dependent on-site staff capacity and resources
§ Day 42 study visit and study procedures are additional and optional, and will be carried out depending on participant interest to take part and site staff capacity and resources

24. APPENDIX C: Grading the severity of symptoms recorded on eDiary after FNA procedure

Adverse event	Grade	Definition
Any symptom	0	Absence or resolution of symptom
	1	Does not interfere with daily activity.
	2	Interferes with daily activity, no treatment except paracetamol or other simple painkiller e.g., ibuprofen
	3	Prevents daily activity or requires treatment

25. APPENDIX C: Amendment history

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