An experimental medicine study of seasonal influenza vaccination responses in Lymph nodE single-cell Genomics in AnCestrY (LEGACY01)

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Human Cell Atlas Method Development Community

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ABSTRACT

This is a collection of protocols of an experimental medicine study of seasonal influenza vaccination responses in Lymph nodE single-cell Genomics in AnCestrY (LEGACY01)

ATTACHMENTS

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GUIDELINES

GENERAL INFORMATION

This protocol was constructed using the Imperial College Research Governance and Integrity Team template (Template Ref: RGIT_TEMP_027, Template V6.0 04Nov2021). The authors wish to credit the MRC CTU at UCL for use of their Protocol Template version 8.0 in drafting of this protocol, which describes the LEGACY01 study and provides information about procedures for entering participants.Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

COMPLIANCE

The study will be conducted in compliance with the approved protocol, the Declaration of Helsinki 1996, the principles of Good Clinical Practice (GCP) ICH topic E6 (R2), and revision E6 (R3) EWG, General Data Protection Regulation and the UK Data Protection Act 2018, and the UK Policy Framework for Health and Social Care Research.

PROTOCOL DEVELOPMENT TEAM

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Patient and public involvement and engagement (PPIE) representatives:

Two PPIE representatives have reviewed the study protocol prior to regulatory submission:

Dolapo Ogunleye, Patient Representative for NHS DigiTrials and PPIE representative for the LEGACY Network

Saira Tamboo PPIE representative for the LEGACY Network

Statisticians:

Calliope Dendrou, Sir Henry Dale Fellow, Nuffield Department of Medicine, University of Oxford

Hashem Koohy, Associate Professor of Systems Immunology, University of Oxford

STUDY SITE AND COORDINATION CENTRE

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For general queries, supply of study documentation, and collection of data, please contact:
Study Coordinator: Aime Palomeras

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CLINICAL QUERIES

Clinical queries should be directed to Katrina Pollock who will direct the query to the appropriate person.

SPONSOR

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

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STUDY REGISTRATION

The LEGACY study is registered ISRCTN13657999
https://doi.org/10.1186/ISRCTN13657999

STUDY SUMMARY

	A	B
	TITLE	An experimental medicine study of seasonal influenza vaccination responses in Lymph nodE single-cell Genomics in AnCestrY
	ACRONYM	LEGACY01
	IRAS ID	314444
	SPONSOR	Imperial College London
	DESIGN	Experimental medicine study; single arm, non-randomised, open label
	SETTING	Secondary care (NHS) and academic research facilities
	AIM	To investigate human immune responses in lymph node cells before and after immunisation with a seasonal influenza vaccine
		Primary objective: To generate a single cell atlas of lymph node cells before and after immunisation with seasonal influenza vaccine.
		Secondary objective: To compare serum antibody responses before and after immunisation.

	A	B
	OBJECTIVES	Exploratory: To compare cellular immune responses in various immune compartments e.g. blood and lymph nodes, against antigens including influenza before and after immunisation with seasonal influenza vaccine.
		Exploratory: To compare immune responses in various immune compartments (e.g., blood and lymph nodes) against antigens including influenza before and after immunisation to help inform vaccine development and testing across different ethnicities.
		Capacity building and training: To build capacity with respect to staff expertise and resource between the three partner institutions to support this project and future similar research.
	OUTCOME MEASURES	Outcome measures may include but are not limited to the following assays
		1. Single cell RNA sequencing analysis of LNC and matched paired PBMC
		2. Binding ELISA specific for influenza/A antigens e.g. haemagglutinin
		3. Intracellular cytokine secretion or activation induced marker assay of PBMC and LNC
		4. Genotypic assays of areas of the genome of immunological relevance may include tests such as HLA-testing.
	POPULATION	Healthy adults aged 18 – 55 years n=30
		Cohort 1 in influenza season 2022 to 2023
		Cohort 2 in influenza season 2023 to 2024
	ELIGIBILITY	Individuals with African or Asian ancestry
	DURATION	Three years

FLOW DIAGRAM AND STUDY SCHEDULE

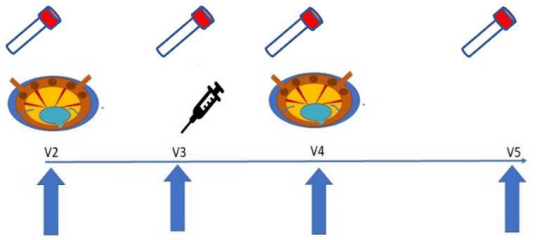

Figure 3. Timeline of study sampling and vaccination events for enrolled participants including lymph node FNA and influenza vaccination. Participants enrol at Visit 2 and undergo paired peripheral blood and lymph node sampling, vaccination at Visit 3, repeat paired lymph node sampling at Visit 4 and then phlebotomy at Visit 5.

Blood draw

Vaccine

FNA

Table 1. Schedule of investigations, treatments, and assessments

	A	B	C	D	E	F	G
	Study visit	**V1**	**V2**	**V2a**	**V3**	**V4**	**V5**
	Visit location	Site	Site	Remote (by phone)	Site	Site	Site
	Visit type	Screening	Enrolment: FNA1	Follow up	Vaccination	Follow-up: FNA2	Follow up
	Study week	minus 24 to minus 1	0	0	1	2	5
	Study day[4]	minus 168 to minus 1	0	5	7	12	35
	Window (days)	NA	NA	minus 1 to plus 1	0 to plus 161	minus 2 to plus 2*	minus 2 to plus 14
	Informed consent	x					
	Demographics	x					
	Medical history	x					
	Weight and height, calculate BMI	x					

	A	B	C	D	E	F	G
	Blood borne virus screen (approx. 6 mL)[1]	x					
	Laboratory safety tests (approx. 10 mL)[2]	x					
	Concomitant medication[3]	x	x	x	x	x	x
	COVID-19 symptoms and trigger COVID-19 test[3]	x	x	-	x	x	x
	Urinary pregnancy test[3]	x			x		
	Symptom directed physical examination[3]	x	x		x	x	x
	Inspection of the FNA site[3]	x	x		x	x	x
	Vital signs[3]	x	x		x	x	x
	Ultrasound scan[3]		x			x	
	Lymph node fine needle aspiration		x			x	
	Vaccination				x		
	Blood for serum immunoassays (6mL)[3]		x		x	x	x
	Blood for cellular and plasma immunoassays (42mL)[3]		x		x	x	x
	Blood for RNA PAXgene tube (2.5mL)[3]				x	x	
	Blood for HLA testing (3-4 mL)[3]		x				
	Adverse events check[3]		x	x	x	x	x
	Blood volume (approx.) (mL)	16	52	-	50.5	50.5	48

1. Detection of antibodies and/or antigen for HIV, hepatitis B and hepatitis C
2. Full blood count, liver function, renal function, non-fasting glucose
3. At visits which include FNA or vaccination, there will be an AE check and vital signs pre-FNA/pre-vaccination, and again at least 30 min after. At visits which include FNA, there will be an inspection of the FNA site pre-FNA, and again at least 30 min after. All other procedures/assessments at these visits will be pre-FNA/pre-vaccination only.
4. The timings of V2a and V3 are set according to that of V2; the timings of V4 and V5 are set according to that of V3.
* This is the preferred window. However, if the FNA2 visit cannot be scheduled within the preferred window, it can take place up to 21 days after the vaccination without being a protocol deviation. Every effort should be made to schedule the

FNA2 visit as close to 5 days post-vaccination as possible.

INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study. Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Resolution for NHS Trusts in England, which apply to this study.

SPONSOR

Imperial College London will act as the main Sponsor for this study.Delegated responsibilities will be assigned to Imperial College Healthcare NHS Trust.

FUNDING

The Chan Zuckerberg Initiative is funding this study. Participants will be paid for each visit they complete, for their inconvenience and travel, at the end of their participation in the study, as follows:

- Screening (V1): £10
- Visits which include an FNA (V2 and V4): £120
- Vaccination visit (V3): £80
- Follow-up visit V5: £60

PATIENT AND PUBLIC INVOLVEMENT

Patient and Public Involvement and Engagement (PPIE) in research is research being carried out 'with' or 'by' members of the public rather than 'to', 'about' or 'for' them. The term "patient and public" includes patients, participants, carers and people who use health and social care services as well as people from specific communities and from organisations that represent people who use services.
The protocol has been reviewed and approved by the LEGACY01 PPIE committee.

PUBLICATION POLICY

The preparation of a manuscript for publication in a peer-reviewed professional journal or an abstract for presentation, oral or written, to a learned society or symposium will be discussed on the study calls and with the PPIE Advisory Group. Details of dissemination can be found in the study specific communication plan.

Authorship will reflect work done by the investigators and other personnel involved in the analysis and interpretation of the data, in accordance with generally recognised principles of scientific collaboration.

PROTOCOL AMENDMENTS

The protocol v2.0 will be the first version approved for use.

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APPENDIX 1. INFLUENZA VACCINE USE IN THE UK

Table S1 All influenza vaccines marketed in the UK for the 2022 to 2023 season (as of 14 Apr 2022)

	A	B	C	D	E	F
	Supplier	**Name of product**	**Vaccine type**	**Age indications**	**Ovalbumin content micrograms per dose**	**Contact details**
	AstraZeneca UK Ltd	Fluenz® Tetra	Quadrivalent LAIV (live attenuated influenza vaccine) supplied as nasal spray suspension	From 24 months to less than 18 years of age	Less than 0.024 micrograms per 0.2 ml dose	0845 139 0000
	MASTA	Quadrivalent influenza vaccine	QIVe (standard egg-grown quadrivalent influenza vaccine), split virion, inactivated	From 6 months	Equal to or less than 0.05 micrograms per 0.5 ml dose	0113 238 7552

	A	B	C	D	E	F
	MASTA	Quadrivalent Influvac® sub-unit Tetra▾	QIVe (standard egg-grown quadrivalent influenza vaccine), surface antigen, inactivated	From 6 months	Equal to or less than 0.1 micrograms per 0.5 ml dose	0113 238 7552
	Sanofi Pasteur	Quadrivalent influenza vaccine	QIVe (standard egg-grown quadrivalent influenza vaccine), split virion, inactivated	From 6 months	Equal to or less than 0.05 micrograms per 0.5 ml dose	0800 854 430
	Viatris (formerly Mylan)	Quadrivalent Influvac® sub-unit Tetra▾	QIVe (standard egg-grown quadrivalent influenza vaccine), surface antigen, inactivated	From 6 months	Equal to or less than 0.1 micrograms per 0.5 ml dose	0800 358 7468
	Seqirus UK Ltd	Cell-based quadrivalent influenza vaccine Seqirus▾	QIVc (cell-grown quadrivalent influenza vaccine), surface antigen, inactivated	From 2 years	Egg-free	08457 451 500
	Sanofi Pasteur	Supemtek▾	QIVr (quadrivalent influenza vaccine (recombinant, prepared in cell culture))	From 18 years	Egg-free	0800 854 430
	Seqirus UK Ltd	**Adjuvanted Quadrivalent Influenza Vaccine Seqirus▾**	**aQIV (adjuvanted egg-grown quadrivalent influenza vaccine) surface antigen, inactivated, adjuvanted with MF59C.1**	**From 65 years**	**Equal to or less than 1 micrograms per 0.5 ml dose**	**08457 451 500**

The vaccine to be used in LEGACY01 study is in bold text.
Source: https://www.gov.uk/government/publications/influenza-vaccines-marketed-in-the-uk/all-influenza-vaccines-marketed-in-the-uk-for-the-2022-to-2023-season

Table S2 All influenza vaccines marketed in the UK for the 2021 to 2022 season (as of 23 Jun 2021)

	A	B	C	D	E	F
	Supplier	**Name of product**	**Vaccine type**	**Age indications**	**Ovalbumin content** micrograms/dose	**Contact details**
	AstraZeneca UK Ltd	Fluenz® Tetra	Quadrivalent LAIV (live attenuated influenza vaccine) supplied as nasal spray suspension	From 24 months to less than 18 years of age	Less than 0.024 micrograms per 0.2 ml dose	0845 139 0000
	MASTA	Quadrivalent Influenza vaccine	QIVe (standard egg-grown quadrivalent influenza vaccine), split virion, inactivated	From 6 months	Equal to or less than 0.05 micrograms per 0.5 ml dose	0113 238 7552
	Sanofi Pasteur Vaccines	Quadrivalent Influenza vaccine	QIVe (standard egg-grown quadrivalent Influenza vaccine), split virion, inactivated	From 6 months	Equal to or less than 0.05 micrograms per 0.5 ml dose	0800 854 430
	Viatris (formerly Mylan)	Quadrivalent Influvac® sub-unit Tetra▼	QIVe (standard egg-grown quadrivalent Influenza vaccine), surface antigen, inactivated	From 6 months	Equal to or less than 0.1 micrograms per 0.5 ml dose	0800 358 7468

	A	B	C	D	E	F
	Seqirus UK Ltd	Flucelvax® Tetra▼	QIVc (cell-grown quadrivalent Influenza vaccine), surface antigen, inactivated	From 2 years	Egg-free	08457 451 500
	Sanofi Pasteur Vaccines	Supemtek▼	QIVr (quadrivalent Influenza vaccine (recombinant, prepared in cell culture))	From 18 years	Egg-free	0800 854 430
	Seqirus UK Ltd	Fluad Tetra▼	aQIV (egg-grown quadrivalent Influenza vaccine), surface antigen, inactivated, adjuvanted with MF59C.1	From 65 years	Equal to or less than 1 micrograms per 0.5 ml dose	08457 451 500

Source: https://www.gov.uk/government/publications/influenza-vaccine-ovalbumin-content/influenza-vaccines-2020-to-2021-flu-season

COMPOSITION

One 0.5 mL dose of aQIV contains 15 µg of haemagglutinin from two A and two B strains of influenza propagated in hens' eggs and adjuvanted with MF59C.1 which contains per 0.5 mL dose, squalene (9.75 mg), polysorbate 80 (1.175 mg), sorbitan trioleate (1.175 mg), sodium citrate (0.66 mg) and citric acid (0.04 mg).

By comparison, one 0.5 mL dose of Supemtek contains 45 µg of influenza virus haemagglutinin from two A strains and two B strains produced by recombinant DNA technology using a baculovirus expression system in an insect cell line derived from *Spodoptera frugiperda*.

Table S3 Comparison of Supemtek and aQIV: safety data

	A	B	C	D	E

	A	B	C	D	E
		Very common	**Common**	**Uncommon**	Rare
		(≥1/10)	(≥1/100 to <1/10)	(≥1/1,000 to <1/100)	
	aQIV: Adverse reactions reported following vaccination in elderly subjects 65 years and older in clinical trials	Headache, injection site pain, fatigue	Nausea, diarrhoea, myalgia, arthralgia, ecchymosis, chills, erythema, induration, ILI	Vomiting, fever ≥38C	
	Supemtek Adverse reactions reported following vaccination in adults 18 years and older during clinical trials and post-marketing surveillance	Headache, fatigue, myalgia, arthralgia, local tenderness, local pain	Nausea, firmness / swelling, redness, fever, shivering / chills,	Cough, oropharyngeal pain, diarrhoea, pruritus, dermatitis, rash flu-like symptoms, injection site pruritus,	Dizziness, urticaria

aQIV: no post marketing data are yet available. Fluad trivalent formulation has post marketing reports of thrombocytopaenia, lymphadenopathy, extensive limb swelling, allergy/anaphylaxis, angioedema, muscular weakness, Encephalomyelitis, Guillain-Barré syndrome, convulsions, neuritis, neuralgia, paraesthesia, generalised skin reactions including erythema multiforme, urticaria, pruritus or non-specific rash, and vasculitis with transient renal involvement.

Supemtek: Hypersensitivity including anaphylaxis has been reported with an unknown frequency. Guillain-Barre syndrome has been reported with an unknown frequency and a causal relationship has not been established.

Table S4 Comparison of Supemtek and aQIV: immunogenicity data in older adults*

	A	B	C	D	E
	Lineage	A	A	B	B
		A/H1N1	A/H3N2	B/Yamagata	B/Victoria
	aQIV: 65 years and older GMT	65	294.9	24.7	30.8
		(57.8; 73.1)	(261.9; 332.1)	(22.7; 26.8)	28.3;33.5
	aQIV: 65 years and older seroconversion rate	35.2	39.3	16.4	13.4
		(32.0; 38.5)	(36.1; 42.7)	(14.0; 19.0)	(11.2; 15.9)
	Supemtek	A/California/7/2009 (H1N1)	A/Texas/50/2012 (H3N2)	B/Massachusetts/02/2012 (Yamagata lineage)	B/Brisbane/60/2008 (Victoria lineage)

	A	B	C	D	E
	adults > 50 years	190 (164;221)	522 (462;589)	55 (48;64)	29 (26;33)
	GMT				
	Supemtek	44.9 (39.3; 50.6)	54.5 (48.8; 60.1)	38.9 (33.4; 44.5)	21.0 (16.6; 25.9)
	adults > 50 years				
	seroconversion rate				

*immunogenicity data for younger adults are not on the SmPC for aQIV

Supemtek solution for injection in pre-filled syringe - Summary of Product Characteristics (SmPC) - (emc) (medicines.org.uk)

Adjuvanted Quadrivalent Influenza Vaccine (Surface Antigen, Inactivated) Seqirus suspension for injection in pre-filled syringe Influenza vaccine, Adjuvanted with MF59C.1 - Summary of Product Characteristics (SmPC) - (emc) (medicines.org.uk)

APPENDIX 2 FINE NEEDLE ASPIRATION OF THE LYMPH NODE

A medical practitioner will carry out the FNA using clinical facilities at Imperial College Healthcare NHS Trust, London, 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 PI deems significant to warrant exclusion from the FNA

Participants will have a set of observations performed including temperature, blood pressure and pulse rate.

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 full lymphatic system, and then under US guidance. A sterile needle and syringe will be used to aspirate material from lymph nodes on each side using 3-5 passes. Where necessary local anaesthesia will be employed to numb the area prior to sampling, using a standard

local anaesthetic e.g., 1% lidocaine.

At each visit for FNA sampling a paired peripheral blood sample will be taken using standard non touch aseptic phlebotomy technique.

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

Participants will be observed for a minimum of 00:30:00 after the procedure. There will be an AE check and FNA site inspection at least 00:30:00 post-FNA.

EXPECTED ADVERSE EVENTS AND GRADING

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, and participants at increased risk due to blood-thinning medication will be excluded. Bruising may occur but is expected to fade after 2 weeks. Participants will be provided with information regarding expected adverse events in a participant information leaflet and adverse events will be monitored and reported as per standard AE reporting for the LEGACY01 study.

APPENDIX 3. POST MARKETING SURVEILLANCE FOR FLUAD

Adverse reactions reported in post marketing surveillance of the aTIV, FLUAD include thrombocytopenia including severe thrombocytopaenia in very rare cases, lymphadenopathy, asthenia, Influenza-Like Illness (ILI), swelling and redness of injected limb, allergic reactions including, rarely anaphylactic shock, anaphylaxis and angioedema, pain in the extremity, muscular weakness, encephalomyelitis, Guillain-Barré Syndrome, convulsions, neuritis, neuralgia, paraesthesia, syncope, presyncope, generalised skin reactions including erythema multiforme, urticaria, pruritus or non-specific rash, vasculitis which possibly associated with transient renal involvement. https://www.medicines.org.uk/emc/product/9223/smpc

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Protocol

NAME
LEGACY01: INTRODUCTION

VERSION 1

CREATED BY
Katrina M PollockUniversity of Oxford

OPEN →

Protocol

NAME
LEGACY01: STUDY DESIGN

VERSION 1

CREATED BY
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LEGACY01: PARTICIPANT ENTRY

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LEGACY01: ADVERSE EVENTS

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LEGACY01: ASSESSMENT AND FOLLOW-UP

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