Imperial College London

The MELODY Study

Mass evaluation of lateral flow immunoassays for the detection of SARS-CoV-2 antibody responses in immunosuppressed people

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FUNDERS: Medical Research Council [MR/W029200/1]
STUDY COORDINATION CENTRE: NHS Blood and Transplant Clinical Trials Unit

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REC reference: **21/HRA/4858**

Protocol authorised by:

Name & Role	Date	Signature
M.Willicombe (CI)	07.03.2022	

Imperial College London

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Clinical Queries

Clinical queries should be directed to the study co-ordinator, who will direct the query to the appropriate person

Sponsor

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

The study is funded by the Medical Research Council, UKRI infections and immunity research grant.

This protocol describes the MELODY 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.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

BAU	Binding Antibody Units
CPI	Confidential patient information
DABA	Double binding antigen ELISA
HES	Hospital Episode Statistics
JCVI	Joint Committee of Vaccination and Immunisation
LFIAs	Lateral flow immunoassays
NCARDRS	National Congenital Anomaly and Rare Disease Registration Service
NCRAS	National Cancer Registration and Analysis Service
NDRS	National Disease Registration Service
NHSBT	NHS Blood and Transplant
NIMS	National Immunisation Management Service
ONS	Office of National Statistics
PHE	Public Health England
REACT-2	REal-time Assessment of Community Transmission-2
RT-qPCR	Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction
SACT	Systemic anti-cancer therapy
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAP	Statistical analysis plan

KEYWORDS

Antibodies; Immunosuppressed; Lateral flow immunoassays; COVID-19 / SARS-CoV-2

STUDY SUMMARY

TITLE Mass evaluation of lateral flow immunoassays for the detection of SARS-CoV-2 antibody responses in immunosuppressed people (The MELODY Study)

DESIGN Observational epidemiological study

AIMS To determine:

1. The proportion of immunosuppressed people who have detectable SARS-CoV-2 antibodies following a primary vaccine course (at least 3 doses), and the demographic, disease, and treatment characteristics that influence antibody status.
2. If the detection of antibodies inversely correlates with subsequent risk of severe acute respiratory syndrome coronavirus-2 infection and/or severity of disease in immunosuppressed people.

OUTCOME MEASURES

1. The proportion of people with and without antibodies to SARS-CoV-2 at least 21 days post three vaccine doses will be presented.
2. The proportion of people with and without antibodies to SARS-CoV-2 after a 4th vaccine dose will be presented.
3. The incidence of participants having at least one RT-qPCR proven infection in the 6-month follow-up will be presented for those with and without antibodies to SARS-CoV-2 after 3rd or 4th vaccine.
4. The incidence of participants hospitalised due to COVID-19 and deaths due to COVID-19 by 6 months will be presented for those with and without antibodies to SARS-CoV-2 following 3rd or 4th vaccine, and compared as described above if there are sufficient events.
5. Rates of those with and without antibodies to SARS-CoV-2 after 3rd or 4th vaccine will be presented for different clinical characteristics and sociodemographic factors.

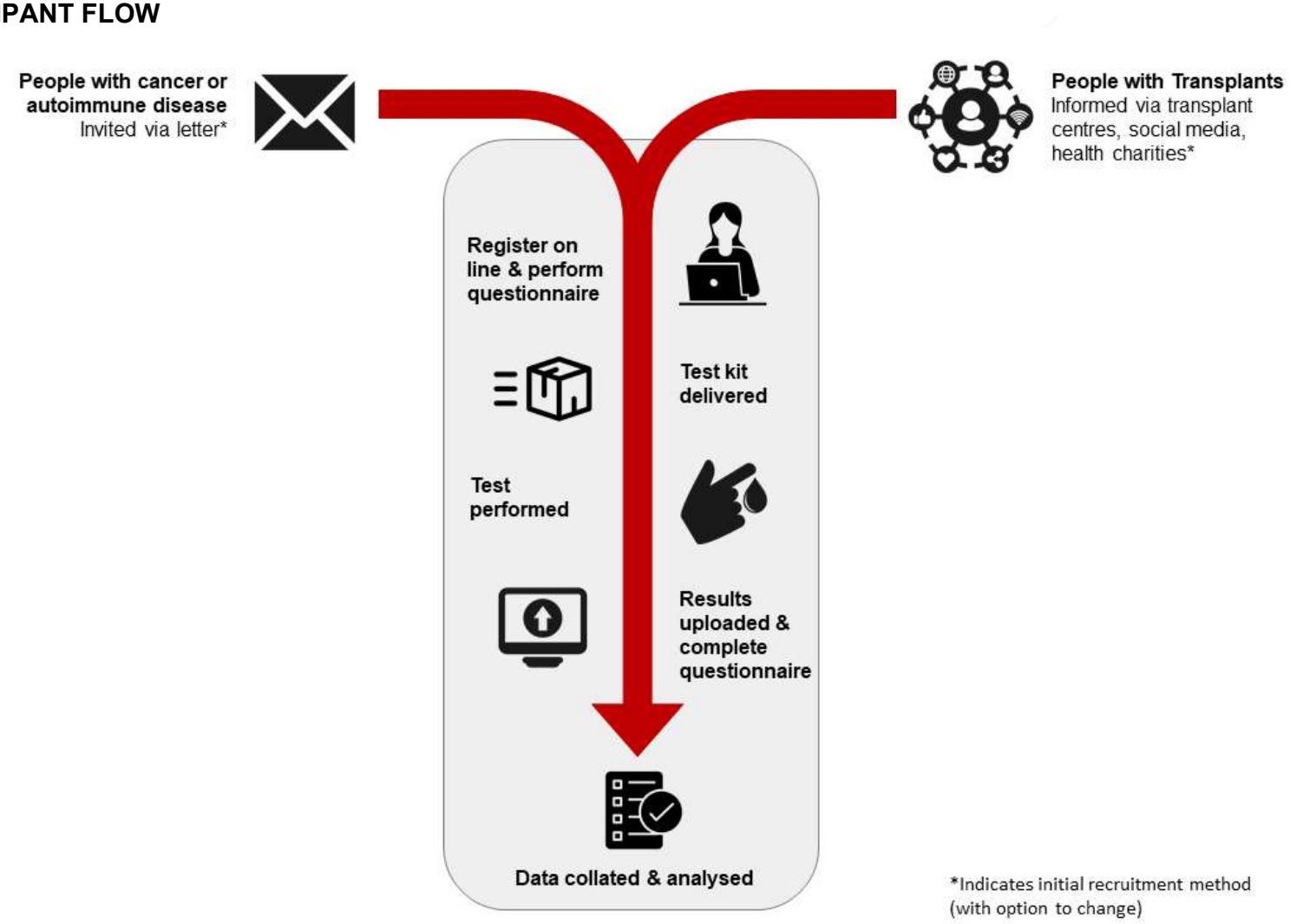
POPULATION Immunosuppressed people, with either:

1. A solid organ transplant
2. A rare autoimmune disease
3. A haematological malignancy

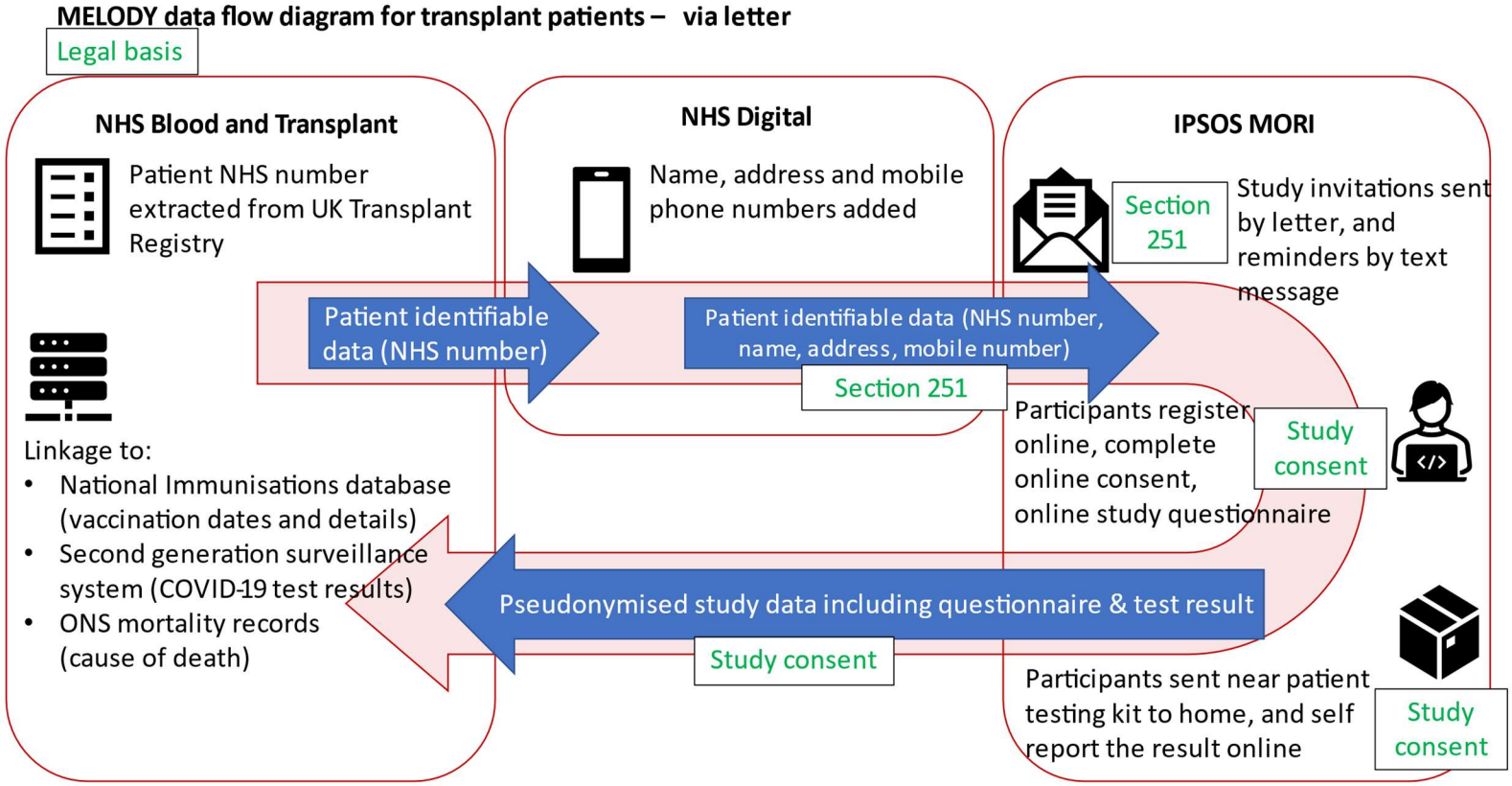
ELIGIBILITY	Immunosuppressed adults (≥18 years old) or young persons (aged 12 to 17 years) who have received at least 3 doses of a COVID-19 vaccine.
DURATION	6 months (from the point of registration)

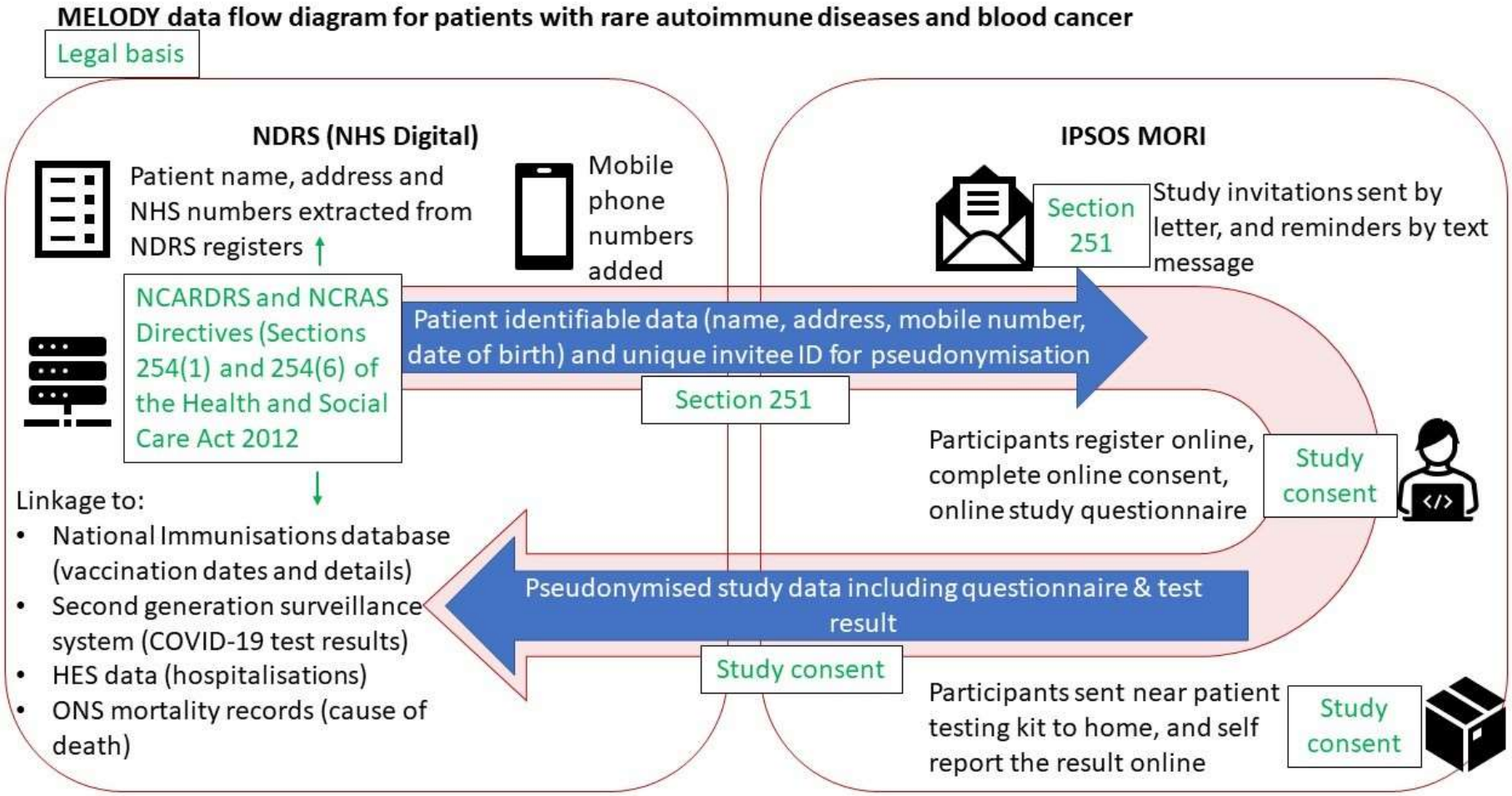
REFERENCE DIAGRAM

A. PARTICIPANT FLOW



B. PROCESS FLOW DIAGRAM




MELODY data flow diagram for patients with rare autoimmune diseases and blood cancer
Legal basis
NDRS (NHS Digital)
Patient name, address and NHS numbers extracted from NDRS registers
Mobile phone numbers added
NCARDRS and NCRAS Directives (Sections 254(1) and 254(6) of the Health and Social Care Act 2012
Linkage to:
• National Immunisations database (vaccination dates and details)
• Second generation surveillance system (COVID-19 test results)
• HES data (hospitalisations)
• ONS mortality records (cause of death)
IPSOS MORI
Section 251
Study invitations sent by letter, and reminders by text message
Patient identifiable data (name, address, mobile number, date of birth) and unique invitee ID for pseudonymisation
Section 251
Participants register online, complete online consent, online study questionnaire
Study consent
Pseudonymised study data including questionnaire & test result
Study consent
Participants sent near patient testing kit to home, and self report the result online
Study consent

1. INTRODUCTION

1.1. BACKGROUND

Immunocompromised individuals make up 500,000 of the UK population and comprise diverse population groups including those with autoimmune disease, solid organ transplants, and cancer. These individuals, especially those receiving concomitant immunosuppression, have a poor prognosis to infection with SARS-CoV-2. Whilst vaccination has conferred protection against severe infections for most individuals, the immunogenicity of SARS-CoV-2 vaccines have been shown to be suboptimal in immunocompromised individuals [1-4]. Although immunogenicity does not necessarily correlate with clinical efficacy, the following data supports the continued risk of COVID-19 to immunocompromised individuals: 256 COVID-19-related deaths were reported by The Office of National Statistics in doubly vaccinated individuals from January to July 2021, 13.1% of whom were immunocompromised[5]; 40% of 152 doubly vaccinated hospitalised COVID-19 patients were immunocompromised as a result of chronic corticosteroid use, solid organ transplantation or anti-cancer therapy[6]; early data from NHS Blood and Transplant (NHSBT) suggests the mortality rates from SARS-CoV-2 infected vaccinated transplant patients is as high as 8%. With these emerging data, the Joint Committee of Vaccination and Immunisation (JCVI) approved 3rd vaccine doses for immunosuppressed people, in September 2021. Existing data on serological responses to 3rd and 4th dose vaccines in immunocompromised individuals is limited, but reports suggest that up to 25% of immunosuppressed individuals will remain unprotected even after 3 doses of vaccine [7,8].

Serological responses to 2 vaccine doses are known to be influenced by clinical factors, such as disease type, type of immunosuppression, timing of immunosuppression in relation to vaccine and type of vaccine[1,9]. The reported real-world clinical efficacy of SARS-CoV-2 vaccines in immunocompromised individuals in the UK is likely biased by patients' behaviour, with many continuing to self-impose shielding because of anxiety over lack of vaccine efficacy. Therefore, there is little data on true infection risk in this patient population, clinical factors contributing to lack of immunogenicity and clinical efficacy. Such information could help provide stratified advice to specific patient groups, rather than classify all immunosuppressed patients at risk. This information could also help estimate and/or identify the number of patients who may benefit from additional or alternative methods of prophylaxis, such as monoclonal antibody therapy[10,11].

The REal-time Assessment of Community Transmission-2 (REACT-2) study (IRAS ID 210407), used lateral flow immunoassays (LFIAs) to track SARS-CoV-2 seroprevalence in the UK, coinciding with the vaccine roll out[12]. Evaluating the prevalence of SARS-CoV-2 antibodies, coupled with sociodemographic information, provided informative epidemiological data. The LFIAs used within the REACT-2 study

have been validated in a cohort of kidney transplant patients, with a test performance adequate to be used for population screening[13]. Using similar methodology to the REACT-2 study, we propose to investigate the proportion of immunosuppressed people with detectable SARS-CoV-2 antibodies following three and four vaccine doses, and correlate antibody status with subsequent infection risk and severity.

With the emergence of the Omicron variant, the UK government announced an expedited approach to the COVID-19 vaccination programme on 29th November 2021[1]. This involved people who are severely immunosuppressed being eligible for a 4th vaccine dose 3 months after their 3rd primary dose. Early data from France, suggest that up to 42% of transplant patients will develop de novo antibody responses following a 4th dose, if they have previously been non-responsive[2,3]. Antibody concentrations remain generally low in this population, however, and are unlikely to be associated with significant protection.

1.2. RATIONALE FOR CURRENT STUDY

Effective protection and management strategies of SARS-CoV-2 infection in immunocompromised adults have wide-reaching implications. For example, evidence has shown; a) they are more likely to have severe disease, which is associated with morbidity and mortality[14-17]; b) they are more likely to have prolonged infection, and viral shedding[18]; c) they are a risk for promoting viral evolution and escape mutations[19].

Immunocompromised young persons (aged 12-17 years old) are also considered to be at heightened risk from SARS-CoV-2 infection. Recent studies have suggested that this group mounts stronger responses following SARS-CoV-2 vaccination than are observed in vaccinated immunosuppressed adults, but numbers of participants were small, and the Joint Committee on Vaccination and Immunisation (JCVI) has recommended that the immunocompromised young also should receive a booster vaccine following three primary vaccines [4-6]. Clarification of how this group respond to SARS-CoV-2 vaccination will inform future immunisation strategies.

The aim of this proposal is to assess at a population level; 1) the proportion of immunosuppressed people who have detectable SARS-CoV-2 antibodies following at least 3 vaccine doses, and the sociodemographic, disease, and treatment characteristics that influence antibody status; 2) if the detection of antibodies inversely correlates with subsequent risk of SARS-CoV2 infection and/or severity of disease in immunosuppressed individuals.

We aim to target patient groups least likely to mount an immune response to vaccination[1,2,9]: a) solid organ transplant recipients; b) patients with a rare autoimmune disease; c) patients with haematological malignancies, specifically lymphoid malignancies. We will use comprehensive registries to identify and recruit patients

from these groups, and utilise the existing linkages these registries already have to obtain COVID-19 outcome information.

We hypothesise that a sizeable proportion of immunosuppressed people will have no detectable SARS-CoV-2 antibodies following either their third or fourth vaccine dose, and that this cohort is particularly susceptible to SARS-CoV-2 infection and death.

2. STUDY OBJECTIVES

AIM:
The aim of this proposal is to evaluate the detection of SARS-CoV-2 antibodies at a population level in immunosuppressed individuals, by determining:

1. The proportion of immunosuppressed people who have detectable SARS-CoV-2 antibodies following a primary vaccine course (at least 3 doses), and the demographic, disease, and treatment characteristics that influence antibody status.
2. If the detection of antibodies inversely correlates with subsequent risk of SARS-CoV-2 infection and/or severity of disease in immunosuppressed individuals.

OBJECTIVES:

1. To estimate the proportion of immunosuppressed people with detectable SARS-CoV-2 antibodies by LFIA testing following a primary vaccine course (3 doses).
2. To estimate the proportion of immunosuppressed people with detectable SARS-CoV-2 antibodies by LFIA testing following a 4th vaccine dose.
3. To correlate antibody status with risk of subsequent RT-qPCR proven infection and disease severity in immunosuppressed people.
4. To describe clinical characteristics, e.g. immunosuppression burden and sociodemographic factors, associated with lack of detectable SARS-CoV-2 antibodies.

3. STUDY DESIGN

This is an observational epidemiology study, combining self-reported near patient testing results, questionnaire data and routinely collected electronic health records.

Duration of study: 12 months

Number of participants: 36,000 immunosuppressed adults, and 750 immunosuppressed young adults (12-17 years old).

3.1. STUDY OUTCOME MEASURES

1. The proportion of participants with and without antibodies to SARS-CoV-2 at least 21 days post 3rd vaccine will be presented.
2. The proportion of people with and without antibodies to SARS-CoV-2 after a 4th vaccine dose will be presented.
3. The incidence of participants having at least one RT-qPCR proven infection in the 6-month follow-up will be presented for those with and without antibodies to SARS-CoV-2 after 3rd or 4th vaccine.
4. The incidence of participants hospitalised due to COVID-19 and deaths due to COVID-19 by 6 months will be presented for those with and without antibodies to SARS-CoV-2 following 3rd or 4th vaccine, and compared as described above if there are sufficient events.
5. Rates of those with and without antibodies to SARS-CoV-2 after 3rd or 4th vaccine will be presented for different clinical characteristics and sociodemographic factors.

4. RESEARCH PLAN

4.1. METHODS

We propose to undertake a survey of seroprevalence using self-administered lateral flow assays in 36,000 immunosuppressed adult participants and 750 young people, who are at least 21 days after completion of their primary COVID-19 vaccination course (3 vaccines of which may include a mix of vaccine types). People who have received a 4th booster dose, will also be included. Infection data will be recorded over a 6-month period (from the date of registration).

4.2. ELIGIBILITY

Adults and young people ≥12 years of age, and are classified as being part of one of the following patient groups:

1. A solid organ transplant recipient (n=12,000)
2. Patients with a rare autoimmune disease (n=12,000)
3. Patients with lymphoid malignancies (n=12,000)

The above patients will all be registered within the following national patient registries:

<u>NHS BLOOD AND TRANSPLANT (NHSBT)</u>

I. The UK Transplant Registry held by NHSBT records all people in the UK with a functioning solid organ transplant. It already has successful linkage with Public Health England (PHE), National Immunisation Management Service (NIMS) and NHS Digital Tracing Service, providing data on vaccines, RT-qPCR COVID-19 infections and COVID-19 mortality data[14,20]. Linkage for COVID-19 hospitalisations will be pursued for this project.
II. Any adult or young person ≥12 years of age with a functioning transplant who has received 3 vaccines, will be eligible to participate.

THE NATIONAL DISEASE REGISTRATION SERVICE (NDRS)

NDRS, part of NHS Digital, comprises the National Congenital Anomaly and Rare Disease Registration Service (NCARDRS) and the National Cancer Registration and Analysis Service (NCRAS).

NCARDRS Rare disease register (NCARDRS)

I. The REgistration of COmplex Rare Diseases – Exemplars in Rheumatology (RECORDER) project within NCARDRS has already identified a cohort of 170,000 people with rare autoimmune diseases, many of whom are on immunosuppressants. Early results from this project has shown this group have a COVID-19-related mortality 2.4 times higher than people of the same age and sex in the general population. This cohort have validated disease diagnoses, and the registry can further identify those treated with Rituximab who are most at risk of lack of seroconversion following COVID-19 vaccination.
II. The RECORDER team already have access to National Immunisation Management Service (NIMS) data, RT-qPCR COVID-19 infection data, hospitalisation data (Hospital Episode Statistics) and Office for National Statistics mortality data for this cohort.
III. Sampling will start with those people who have received treatment with Rituximab within the last 2 years, and a random sample of people with ANCA-associated vasculitis (n=9,854) or Lupus (n=41,261), or myositis or scleroderma or Giant Cell Arteritis ≥18 years of age who have received 3 vaccines will be eligible

National Cancer Registration and Analysis Service (NCRAS)

I. The National Cancer Registration and Analysis Service (NCRAS) is responsible for cancer registration in England to support cancer epidemiology, public health, service monitoring and research.
II. NCRAS actively records from health care data all patients with a diagnosis of cancer, and captures those patients receiving systemic anti-cancer therapy (SACT).
III. People ≥18 years of age with a haematological malignancy who have received 3 vaccines will be eligible.

4.3. RECRUITMENT

To ensure study start time is aligned with vaccine roll out, recruitment of transplant participants (via NHSBT) will differ from cancer and autoimmune participants. This is a pragmatic decision based on differences in patient identifiable data recorded by the transplant and NDRS registries. The recruitment plans for the different cohorts are outlined below. Those wishing to participate in the study will register via a web portal developed by Ipsos MORI, as per REACT-2. They will consent electronically for participation in the study, subsequent data linkage on outcomes and willingness to be contacted about any future interventional research.

Participate information sheets will be available for all potential recruits, both paper and electronic access, depending on method of recruitment.

People with solid organ transplants: Transplant recipients will be invited to join the study by self-registration via the study web portal. Eligibility for transplant recipients will be assessed by them providing their NHS/CHI number and year of most recent transplant at the time of opting-in to the study. This will be immediately verified against the data held by the UK Transplant Registry before the patient proceeds to register for the study.
Participants will be invited to join ('opt-in' method) via communications via the British Transplant Society, NHSBT, transplant centres, patient charities and social media. Linkage to the NHSBT and NHS Digital registries will provide confirmation that the applicant is a solid organ transplant recipient and details of their vaccine status. Young transplant recipients (aged 12-15) will be additionally asked for their parent / guardian details upon registering on the study web portal.
Simultaneous application for access to NHS Digital will be made. If the target number of 12,000 participants is not reached via the opt-in methods by the time approvals are obtained, invitations will be sent out to a random sample of patients on the transplant registry akin to the patients on the NDRS databases below. Baseline participant characteristics of all participants on the transplant registry are available and will enable a description of the study participants in relation to the whole registry.
As of 14th February 2022, 2,500 adult solid organ transplant recipients have self-registered with the trial web portal and returned the results of the LFIA they received following their 3rd vaccine dose. Depending on availability of LFIA kits, participants who had an initial negative test will be further invited to perform an additional LFIA test, after receipt of their fourth or subsequent vaccine dose. These additional tests will provide data on the proportion of transplant recipients who seroconvert after their fourth or subsequent vaccine dose, despite being negative for anti-SARS-Cov-2 IgG antibodies after their third dose.

People with cancer and autoimmune disease: A random selection of eligible patients with autoimmune disease or haematological malignancy registered on the NCARDRS and NCRAS databases respectively, will be invited to join the study via personalised letter. Up to 2 reminders will be sent to potential participants. Patients responding to the invitation will register via the Ipsos MORI web portal. Using the experience from REACT-2, we estimate a response rate of 70% at registration and 85% at lateral flow test completion.

Following registration on the web portal and completion of a short online questionnaire including information on immunosuppression and medical history, sociodemographic variables and COVID-19-related behaviours and COVID-19 infection history, participants will be sent a LFIA test, which is provided with a detailed instruction booklet and link to an instruction video. For consenting young persons aged 12-15, the LFIA kit will be addressed to the parent / guardian whose name they have provided. Participants will be asked to carry out the test and follow the instructions to read the result and to take a photograph of the completed test, if possible. They will then provide their results via the web portal. A telephone helpline will be established to deal with any queries that may arise. Usability of the test has been assessed in REACT-2 study.

4.4. WITHDRAWAL CRITERIA

All participants are free to withdraw at any time without giving reasons and without prejudice. Data provided after registration will remain in the web portal as they cannot be deleted. Participants who have received a test kit but wish to withdraw can simply discard the test kit.

5. ADVERSE EVENTS

5.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded- it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the trial end points.

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours. However, infections and death due COVID-19, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the London-Central REC where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Contact details for reporting SAEs
RGIT@imperial.ac.uk
CI email (and contact details below)
m.willicombe08@imperial.ac.uk

6. ASSESSMENT AND FOLLOW-UP

Direct participant involvement will finish after the results of the LFIA test are uploaded. Follow up thereafter will be via the respective registries for 6 months. The study will end at the time the last participant reaches 6 months from the point of their registration on the study web portal.

Participants will have access to an information booklet which describes the meaning of the test results. Participant's GPs will not be informed of recruitment.

7. STATISTICS AND DATA ANALYSIS

Data collection will be undertaken in collaboration with implementation partner Ipsos MORI. The data from the participants is self-entered into an online questionnaire hosted on a secure web portal developed and managed by Ipsos MORI.

Ipsos MORI will be responsible for setting up and managing the study web portal, receiving the questionnaire data, setting up and entering the questionnaire data into the study database and linking in the uploaded results of lateral flow assay. Once data is complete, a copy of the study database for each patient population will be sent securely from Ipsos MORI to NHSBT and NDRS where it will be linked to routinely collected healthcare data on COVID-19 infection, and severe outcome over the subsequent 6 months and analysed.

Analysis for the a) solid organ transplant groups and b) rare autoimmune disease and cancer groups will be conducted using a common protocol and statistical analysis plan, but separately by NHSBT and NDRS respectively, due to the complexities of data sharing between the two registries and existing restrictions on the data sharing they have with NIMS and NHS Digital. The high-level analysis plan is as follows:

1. The proportion of patients (and 95% CI) with and without antibodies to SARS-CoV-2 at least 21days post 3rd vaccine and prior to 4th vaccine dose will be presented.
2. The proportion of patients (and 95% CI) with and without antibodies to SARS-CoV-2 following a 4th vaccine dose will be presented
3. The cumulative incidence (and 95% CI) of participants having at least one RT-qPCR proven infection in the 6-month follow-up will be presented for those with and without antibodies to SARS-CoV-2 at the time the study LFIA was undertaken. Risk-adjusted cumulative incidence rates will be compared using a Fine and Gray model. These methods allow for participants who may die during the follow-up period.
4. The cumulative incidence (and 95% CI) of participants hospitalised due to COVID-19 and deaths due to COVID-19 by 6 months will be presented for those with and without antibodies to SARS-CoV-2 at the time the study LFIA was undertaken and compared as described above if there are sufficient events.
5. Rates of those with and without antibodies to SARS-CoV-2 after 3rd or 4th vaccine (and 95% CI) will be presented for different clinical characteristics and

sociodemographic factors. Logistic regression models will be used to identify which of these factors are independently associated with developing antibodies following completion of the primary vaccine course.

6. The proportion of re-sampled transplant recipients (and 95% CI) with and without antibodies to SARS-CoV-2 following 4th dose who did not have antibodies after 3rd dose.

All outcomes will be adjusted for other known risk factors, and receipt of a subsequent 4th vaccine dose, as and where appropriate. Multiple imputation based on full conditional specification will be used to impute values of potential risk adjustment factors. The set of variables that will feature in the multiple imputation models will be described in the SAP. Sensitivity analyses will be conducted with cases with missing primary outcome data excluded.
A descriptive analysis aligned with above will additionally be performed for the young adult population.

Sample size

Each participant population will recruit 12,000 participants, and of these, we expect 85% to return their LFIA result (n=10,200). Following a third vaccine, we estimate approximately a third of participants in each group will have no SARS-CoV-2 antibodies, so this sample size will give a 95% confidence interval for the proportion of participants with no antibodies of ±0.91% for each participant group [7,8]. Even if the proportion of participants with no antibodies is 50%, the confidence interval would be ±0.97%, so the large study size gives very good precision.
The study has been powered to detect a difference of 2% in the proportion of participants free from RT-qPCR proven infection in the 6-month follow-up period, with 90% power and a 5% two-sided significance level. We have assumed a 2:1 ratio between those with and without SARS-CoV-2 antibodies, as described above. In the group with SARS-CoV-2 antibodies it has been estimated that 92.4% will be free from infection at 6 months and to detect a decrease to 90.4% in the group without SARS-CoV-2 antibodies using survival analysis methods, we require 9,273 participants in total in each participant population (justification for the expected rate is provided in Appendix 1 (Reproducibility statistical and design annex). Our planned recruitment of 10,200 LFIA results per group therefore allows for up to a 9% withdrawal rate, which we think is very achievable as patients will have opted-in to the study initially. Higher LFIA return rates, a lower withdrawal rate, or combining data across the patient populations would enable smaller differences to be detected. We think that the timing of the study, and early evidence of strong engagement, would mean that only a relatively small number of patients would have received their 4th dose at the time of recruitment. With the emergence of Omicron, we expect infection event rates observed in the study to now be higher than those used in the original sample size calculation, even if the 4th dose reduce rates of RT-qPCR positive infection.

DIAGNOSTIC ACCURACY OF THE IMMUNOASSAYS

Using the threshold value for positivity on serological testing of ≥7.10 BAU/ml (n=183) on Abbott Architect Assay and confirmatory DABA results for available discordant samples (n=3) as the reference standard, the overall performance of the test in these combined cohorts produce an estimate of sensitivity of 92.0% (115/125; 95% CI 85.8% to 96.1%) and specificity of 95.1% (58/61; 95% CI 86.3% to 99.0%).

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

The Study has obtained approval from the London-Central Research Ethics Committee (REC) and Health Regulator Authority (HRA).

8.2. CONSENT

Participants interested in taking part will register their interest on the Ipsos MORI web portal and give separate consent to participate in the study, for subsequent data linkage on outcomes and willingness to be contacted about any future interventional research. At registration, young persons aged 12-15 years will be additionally asked to provide consent from their named parent / guardian, with the LFIA then posted to that parent / guardian.

8.3. CONFIDENTIALITY AND DATA PROTECTION

The Chief Investigator will hold the overall responsibility of preserving the confidentiality of participants taking part in the study from the Sponsor.

Section 251 of the NHS Act 2006 will be used as the basis for sharing patient identifiable data (PID) without prior consent. Approval for Section 251 support was granted by the Confidentiality Advisory Group at the Health Research Authority.

Ipsos MORI

All personal data will be held securely in line with ISO27001 Information Security Standard and in accordance with data protection policies.

NDRS

All data will be held securely in accordance with NHS Digital's data security standards and the National Congenital Anomaly and Rare Disease Registration Service's (NCARDRS') confidentiality policy. These are in line with ISO27001.

NHSBT

All data will be held securely in accordance with the NHSBT's Information Security and Confidentiality and Data Protection policies. These follow the principles of ISO27001. NHSBT's ICO Data protection registration Number is Z9210360.

At the end of the study, Ipsos MORI will delete all personal data relating specifically to this study from their systems (i.e. exclusively obtained via study procedures). NHSBT and NDRS will hold data for up to 10 years

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5. SPONSOR

Imperial College London will act as the Sponsor for this study.

8.6. FUNDING

The Medical Research Council are funding this study [MR/W029200/1], in collaboration with health charities (Kidney research UK, Blood Cancer UK, Vasculitis UK and the Cystic Fibrosis Trust).

8.7. AUDITS

The study may be subject to audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through NHSBT CTU. A study management group will be convened to provide ongoing oversight of operational activity.

10. PUBLICATION POLICY

The study management group will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Any publications arising will also be approved by funders. Authors will acknowledge that the study funding as detailed in Section 8.6 above. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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12. SUMMARY OF CHANGES

Version	Author	Date	Reason for revision
2.1	Michelle Willicombe Fiona Pearce	07.12.2021	To adjust endpoint to *at least* 21 days post 3rd vaccine and take into account Government notification of 4th doses for clinically vulnerable people.

			1. Outcome measures: Study Summary, page 6 and Eligibility p7 2. 4th dose in Background Section 1.1, p13 3. New Study Objective listed in section 2, p 14 4. New Outcome Measure re 4th dose added and outcome 1 updated to at least 90 days Section 3.1, p 15 5. Addition to Research Plan, Section 4.1, page 14 6. Additions to the Statistics and Data Analysis listed in Section 7, pages 20 and 21. 7. Data Flow diagram on page 10 updated to include Date of Birth 8. Data Flow diagram on page 9 to include NHSBT data flow process akin to NDRS 9. Trial coordinator details added to page 2
3.3	Gillian Powter Gavin Pettigrew Michelle Willicombe	15/02/2022	• Page 7- Eligibility - Addition of Immunosuppressed young adults aged 12 to 17 years • Page 14 – Amended to Adults and young people ≥12 years • Page 14 NHSBT Cohort – amended to Any adult or young person ≥12 • Page 15 NDRS cohort – amended to adults or young people ≥12 years • Page 16 - To re-test adult transplant recipients who have completed MELODY study participation following their third vaccine dose, after receipt of their fourth dose. • Page 21 - young persons aged 12-15 years will be additionally asked to provide consent from their named parent / guardian • Page 26 – 3 additional references Nos 4 - 6

13. APPENDIX 1. REPRODUCIBILITY AND STATISTICAL DESIGN ANNEX

This is an observational cohort study which will recruit 12,000 participants to each of 3 patient populations - 36,000 in total. We anticipate a LFIA result return rate of 85%, giving data on 10,200 cases in each participant population. Participants will either be invited at random to participate (autoimmune and cancer), or sign-up themselves (transplant), minimising potential recruitment bias, although self-selection bias could occur for either approach. This can be assessed by comparing those recruited against wider data on the registries participating in the study, and risk adjustment for any important confounders associated with outcome will be conducted.

LFIA diagnostic accuracy.

The lateral flow device to be used is the Fortress COVID-19 Total Antibody Device for the detection of IgM and IgG SARS-CoV-2 against the spike protein. The device has been evaluated for use in detecting anti-S in transplant patients and found to have a sensitivity of 92% and specificity of 95%[13]. The sample size calculation is described previously. The event rate of freedom from infection at 6 months in the group with antibodies was estimated from data in the PHE report "COVID-19 vaccine surveillance report - week 37". Table 2 of this report presents case rates per 100,000 for those who have received 2 doses of vaccine, which we have taken as our best estimate for event rates in a group with SARS-CoV-2 antibodies. The age-adjusted mean event rate from this data shows a 0.79% infection rate over a 4 week period in August 2021. Multiplying this up for our 6-month (180 day) follow-up period would suggest an event rate of 5.08%. However, this data relates to the UK population at large, not the higher risk immunosuppressed population that will be recruited to MELODY. Recent data on COVID-19 infection among those with rare autoimmune rheumatic disease showed that the age-standardised infection rate in this group was 1.54 times higher than in the general population[17]. We have therefore inflated our anticipated event rate by 50% to give the final event rate of 7.62% for this group (freedom from infection rate of 92.4%). Using the same process for the PHE data on unvaccinated individuals would suggest an infection rate 2.12% higher in the group with no SARS-CoV-2 antibodies. We have therefore powered the study to detect a 2% difference between those with and without antibodies. The statistical design and analysis was developed with co-applicants Helen Thomas, Fiona Pearce and Helen Ward.

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