PROTOCOL

Precision medicine in liver transplantation: a personalised approach to immunosuppression

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Sponsor/s:
Austin Health

CONFIDENTIAL

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

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STUDY SYNOPSIS

Title:	Precision medicine in liver transplantation: a personalised approach to immunosuppression
Short Title:	Personalising immunosuppression in liver transplantation
Design:	Single-centre prospective observational cohort study
Study Centres:	Austin Health
Hospital:	Austin Hospital
Study Question:	What is the utility of the combination of the novel QuantiFERON-Monitor (QFM) and donor-specific cell free DNA (dscfDNA) blood tests in monitoring and managing immunosuppression post liver transplantation (LT)?
Study Objectives:	To determine the utility of the combined QFM and DNA (QFM-dscfDNA) tests in monitoring and managing immunosuppression post LT.
Primary Objectives:	To examine if the QFM-DNA tests can be used to accurately diagnose acute rejection and infective complications after LT.
Secondary Objectives	To examine if the QFM-DNA tests can be used to predict acute rejection or infective complications, monitor treatment responses, and improve healthcare resource utilisation.
Inclusion Criteria:	• Age 18 years and above • Undergoing LT at Austin Health • Can provide written informed consent
Exclusion Criteria:	• Aged under 18 years • Undergoing multi-organ transplantation • Unable to provide written informed consent at any stage
Number of Planned Subjects:	210
Investigational product:	Nil
Safety considerations:	Participants will be required to have serial additional blood sampling, performed when blood tests for the standard of care for LT occur wherever possible. They may be subjected to mild physical or psychological distress during venepuncture. There will be no deviation from standard of care treatment.
Statistical Methods:	• Receiver-operator characteristics analysis. • Logistic regression analysis.
Subgroups:	Nil

1. GLOSSARY OF ABBREVIATIONS & TERMS

Abbreviation	Description (using lay language)
QFM	QuantiFERON-Monitor
dscfDNA	Donor specific cell free DNA
QFM-cfdsDNA	Combined QuantiFERON-Monitor and donor specific cell free DNA
LT	Liver transplantation
LFTs	Liver function tests
tBPAR	Treatment responsive biopsy proven acute rejection
FBE	Full blood count
UEC	Urea and electrolytes
AUC	Area under the receiver operator curve
PCR	Poylmerase chain reaction
MBS	Medicare Benefits Schedule
PBS	Pharmaceutical Benefit Scheme
ONJCRI	Olivia Newtown John Cancer Research Institute
LTU	Liver Transplant Unit

2. STUDY SITES

a. STUDY LOCATION

Site	Address	Contact Person	Phone	Email
Austin Health	145 Studley Rd, Heidelberg 3084	Dr Tess McClure	0400151816 0394965000	tess.mcclure@austin.org.au

3. INTRODUCTION/BACKGROUND INFORMATION

a. LAY SUMMARY

Liver transplantation is the only effective treatment for many patients with liver disease. Due to advances in medical care, liver transplantation now has acceptable mortality and morbidity. Both locally and globally, the number of liver transplants performed each year continues to increase.

Long-term, the success of a liver transplant depends on a fine balance: suppressing the immune system to avoid organ rejection, whilst maintaining it to prevent infection. Despite careful monitoring with standard blood tests, most patients will experience episodes of rejection and/or infections. Diagnosing these complications often requires expensive medical

imaging and an invasive liver biopsy, prior to treatment with immunosuppression adjustment. There is a clear need for innovative tools to 'personalise' immunosuppression, and improve patient outcomes, whilst reducing healthcare resource utilisation.

Researchers at Austin Health have pioneered the study of two novel blood tests - QuantiFERON-Monitor to assess immune function, and donor-specific cell free DNA to measure organ injury. This prospective observational cohort study aims to advance current knowledge, by determining the utility of combining these two tests in monitoring and managing immunosuppression post liver transplantation.

We aim to recruit 210 adult liver transplant recipients who will be followed up for 12 months. Participants will be required to have serial additional blood sampling, performed when blood tests for the standard of care for LT occur wherever possible.

b. INTRODUCTION

The long-term success of LT is finely balanced between adequately suppressing the recipient immune system to minimise organ rejection, while simultaneously maintaining it at a level that prevents infective complications. Immunosuppression is dosed empirically and adjusted according to the temporal changes to LFTs, drug levels or the onset of rejection or infective complications. Despite the judicious use of immunosuppression, nearly half the recipients develop an episode of rejection and up to 70% experience infective complications[1-4]. These complications impact on patient quality of life[5] and are costly to manage (up to USD $83,000 per episode)[6].

LFTs are extremely sensitive tests for organ injury but have poor specificity for LT complications[7]. As a screening tool, they can lead to a series of radiological and endoscopic investigations that often culminate in an invasive liver biopsy to confirm the clinical event[8]. Acute rejection is treated with increased doses of immunosuppressants while infection necessitates dosage reduction. This relatively 'reactive' approach precludes preventive intervention by predictive adjustments.

Researchers at Austin Health have shown that the use of an immune monitoring blood test, QuantiFERON-Monitor (QFM), identifies recipients at risk of early rejection or infective complications. Austin Health researchers have also developed a novel blood test to rapidly measure organ injury using donor-specific cell-free DNA (dscfDNA).

We propose a prospective observational cohort study of 210 patients who are LT recipients followed up for 12-months to determine the utility of combining the two blood tests (QFM-dscfDNA) in monitoring and managing immunosuppression post LT. Our primary hypothesis is that the QFM-dscfDNA tests can be used to accurately diagnose the occurrence of rejection or infective complications after liver transplantation. The secondary hypotheses are that QFM-dscfDNA tests can be used to predict acute rejection or infective complications, monitor treatment responses and improve healthcare resource utilisation.

There is a clear and urgent need for accurate clinical tools to personalise immunosuppression. Confirmation of our hypotheses facilitates a "predictive" rather than the current "reactive" approach to immunosuppression. Our approach can potentially minimise the need for complex diagnostic tests including ultrasound, computed tomography, magnetic resonance imaging, endoscopy and liver biopsies - with significant clinical and economic benefits.

If positive, the findings of this study will lead to a prospective randomised multi-centre trial comparing the standard and precision approaches in LT. If validated in LT, we foresee that the predictive approach to immunosuppression is readily translatable for evaluation in other solid organ transplantations. With over 125,000 solid organ transplantations being performed

worldwide annually, the clinical and economic implications of this study could be substantial.

c. BACKGROUND INFORMATION

i. SCIENTIFIC BACKGROUND

The long-term success of LT is finely balanced between adequately suppressing the recipient immune system to minimise organ rejection, while simultaneously maintaining it at a level that prevents infective complications. Immunosuppression is dosed empirically and adjusted according to the temporal changes to LFTs, drug levels or the onset of rejection or infective complications. Despite the judicious use of immunosuppression, nearly half the recipients develop an episode of rejection and up to 70% experience infective complications[1-4]. These complications impact on patient quality of life[5] and are costly to manage (up to USD $83,000 per episode)[6].

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ii. PRELIMINARY RESEARCH

Researchers at Austin Health have pioneered the study of two rapid and low-cost blood tests in LT: one that measures the immune function of the recipient, and one that quantifies organ injury.

The first of these is QFM. Based on the widely available QuantiFERON-Gold test, QFM involves stimulating whole blood collected from the patient with innate and adaptive immune ligands, and quantifying interferon gamma release. Sood et al. have evaluated QFM, and gained experience in its application in LT[9-11]. They found that QFM accurately identifies recipients at risk of developing biopsy-proven acute rejection requiring treatment (tBPAR) or infective complications within one month after LT.

The second test is a novel DNA-based laboratory assay based on the following concepts. Firstly, that during normal cellular turnover, the donor organ continuously sheds dscfDNA into the recipient circulation. Secondly, that when the donor organ is injured, dscfDNA increases. This can be quantified using next-generation sequencing techniques, however these are complex, labour-intensive and expensive[12-14]. Goh et al. therefore developed a polymerase chain reaction technique to quantify dscfDNA that is accurate, rapid and economic[15,16]. They subsequently performed a pilot study in patients post LT comparing dscfDNA to clinical events, and found that dscfDNA levels were low in well patients, but elevated in those with tBPAR[17].

4. STUDY OBJECTIVES

a. HYPOTHESIS

The primary hypothesis is that the QFM-dscfDNA tests can be used to accurately diagnose the occurrence of acute rejection and infective complications after LT.

The secondary hypotheses are that the QFM-dscfDNA tests can be used to:

- Predict acute rejection and infective complications.
- Monitor treatment responses.
- Improve healthcare resource utilisation.

b. STUDY AIMS

- To examine if the QFM-dscfDNA tests can be used to accurately diagnose acute rejection and infective complications after LT.
- To examine if the QFM-dscfDNA tests can be used to predict acute rejection or infective complications, monitor treatment responses, and improve healthcare resource utilisation.

c. OUTCOME MEASURES

i. DEFINITION OF CLINICAL EVENTS:

- Rejection: The gold standard for diagnosing acute rejection is based on histopathologic grading on liver biopsy. It is often difficult to differentiate mild rejection from ischemia-related cholestasis early after LT and clinical evaluation may lead to observation rather than active treatment if temporal improvement is demonstrated. We thus employed the strict definition requiring consistent histology and treatment of rejection (tBPAR), an acceptable standard for clinical trials[18,19].
- Infective complications: Infection endpoints will be defined according to the criteria of 'no', 'probable' or 'definite' infection derived from the international sepsis forum consensus conference on '*Definitions of Infection in the Intensive Care Unit*'[20].

i. VARIABLES TO BE MEASURED:

- Standard of care clinical variables: Donor and recipient factors such as donor risk index, model for end-stage liver disease score, waiting list time, primary indication for transplantation, operative factors and immunosuppression regimens can influence post LT outcomes. These characteristics will be prospectively recorded for analysis.
- Standard of care blood tests: Routine blood tests including full blood count (FBE), urea and electrolytes (UEC), LFTs and serum drug levels are used for clinical decision making as a standard of care. The results of each test will be prospectively recorded.
- QFM: Recipient blood samples will be processed within eight hours of venesection. The blood samples will be stimulated with the QFM immune ligands anti-CD3 and R848[14], incubated overnight at 37 °C and fractionated. The plasma component will then be stored at -80 °C. A batched analysis of the stored plasma will be performed at the end of the 12-month follow-up using ELISA to quantify IFNγ as IU/m, so results will not influence the decision-making of the treating clinicians.
- dscfDNA: Recipient blood samples will be processed within three hours of venesection. The blood samples will be fractionated into the leukocyte-rich 'buffy-coat' and plasma components. Donor blood samples will be obtained during organ procurement, in accordance with the Human Research Ethics Committee for Donate Life and the Australian Red Cross Blood Services (application in progress). All samples will be stored at -80 □C. Genomic DNA will first be extracted from the buffy coats of the organ donors and the pre-transplant recipient. High resolution melting analyses will be used to genotype each donor-recipient pair using a panel of small bi-allelic deletion/insertion polymorphisms[16]. This step will be performed once for each donor–recipient pair to determine a set of allelic sequences that are present in the donor and absent in the recipient for the detection of dscfDNA. Subsequently, we will utilise our rapid and readily performed methodology to quantify dscfDNA[17]. Our novel assay design enables the amplification of dscfDNA using allelic breakpoints that are only present in donor-specific alleles. Combined with the Bio-Rad droplet digital polymerase chain reaction (PCR) platform, dscfDNA can be measured with unprecedented accuracy in the recipient's blood, in copies/mL of recipient plasma. As with QFM, the samples of each recipient will be batched for analysis at the end of the 12-month follow-up, so results will not influence the decision-making of the treating clinicians.
- Health expenditure: The costs associated with inpatient and outpatient healthcare resource usage, investigations medications and the QFM-dscfDNA tests will be

retrieved from the Austin Health Department of Finance, Austin Health as well as the Medicare Benefits Schedule (MBS) and Pharmaceutical Benefit Scheme (PBS).

ii. PRIMARY ENDPOINTS:

The accuracy of QFM and dscfDNA, alone and in combination, compared to LFTs to diagnose:

- The first episode of tBPAR, as measured by the area under the receiver operator curve (AUC).
- The first episode of infective complication, as measured by the AUC.

iii. SECONDARY ENDPOINTS:

- The accuracy of QFM and dscfDNA, alone and in combination, in predicting the occurrence of imminent tBPAR and infective complications before clinical manifestation of the event.
- The performance of QFM and dscfDNA, alone and in combination, in monitoring treatment responses of tBPAR and infective complications, as compared to routine blood tests.
- Healthcare expenditures and cost-effectiveness of QFM-dscfDNA, as measured using hospital, PBS and MBS data.

5. STUDY DESIGN

a. STUDY TYPE & DESIGN & SCHEDULE

ii. STUDY DESIGN:

This is a prospective observational single-centre cohort study of 210 patients who are LT recipients followed up for 12-months, to determine the utility of the QFM-dscfDNA in monitoring and managing immunosuppression post LT.

Consenting adults (aged 18 years and over) undergoing LT at Austin Health will be invited for enrolment in the study in the pre-transplant clinic, during outpatient assessment or on admission to hospital prior to LT. Written informed consent will be obtained prior to enrolment. Recipients under 18 years of age, those who do not consent their participation and those who are undergoing multi-organ transplantation will be excluded from the study.

The design of this study will enable the researchers to examine if the QFM-dscfDNA tests can be used in LT recipients to accurately diagnose and predict acute rejection and infective complications, monitor treatment responses, and improve healthcare resource utilisation.

iii. INTERVENTION:

Participants will receive the standard of care after LT, with serial additional blood sampling required for QFM-dscfDNA testing:

- Standard of care after LT: Clinical care of LT recipients is directed by a team of experienced clinicians. Potential recipients are selected based on clinical urgency and donor–recipient matching. LT is performed as per protocol. Routine immunosuppression comprising steroids, a calcineurin inhibitor and an anti-metabolite will be prescribed. Routine protocol liver biopsies are not performed to monitor organ health. Routine blood tests (FBE, UEC, LFTs and serum drug levels) are performed regularly to monitor the clinical course. Recipients are closely followed up as outpatients after discharge. Complications after LT may result in the deterioration of clinical status and abnormalities on routine blood tests. Imaging,

endoscopy and/or liver biopsies are performed for diagnosis and managed accordingly.

- Additional blood sampling: The intervention requires serial additional blood sampling of recipients, which will be performed when routine blood sampling for standard of care is undertaken wherever possible, to reduce the need for additional venesection.

  As per **Figure 1**, 15-30ml will be collected to perform the QFM-dscfDNA testing and to be stored for future research purposes as per the extended consent obtained. Blood sampling will occur in the inpatient and outpatient setting at Austin Health, to ensure that investigators have direct and timely access to the blood samples. Blood samples will be processed in the Olivia Newtown John Cancer Research Institute (ONJCRI) and stored in a specially allocated -80°C freezer in the Liver Transplant Unit (LTU), both located at the same campus.

  As per **Table 1**, blood will be collected pre-transplant (baseline) and post-transplant on days 1, 3 and 5; week 1 and 2; and months 1, 2, 4, 6 and 12 from enrolled participants (11 time points x 210 recipients = 2310 tests). We estimate that approximately 150 clinical events of acute rejection and infective complications will occur throughout the course of this study. As a standard of care, these clinical events require close surveillance with routine blood tests. In conjunction with the routine blood tests, additional blood will be sampled to capture the dynamics of QFM-dscfDNA during these clinical events (3 time points taken 1-3 days apart, 3 x 150 events = 450 tests). A total of 2760 QFM-dscfDNA measurements will be performed in this study (2310 + 450 tests).

Figure 1: Process for additional blood sampling

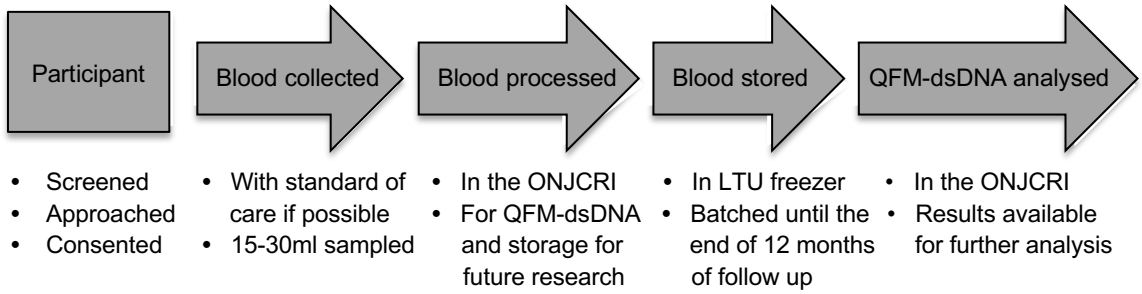

Table 1: Schedule of serial additional blood sampling		
Time		**Additional blood sampling**
Before LT		x 1
Post LT		
Day	1	x 1
	3	x 1
	5	x 1
Week	1	x 1
	2	x 1

Month	1	x 1
	2	x 1
	4	x 1
	6	x 1
	12	x 1
If a clinical event occurs		x 3 (taken 1-3 days apart)

The donor blood samples required for dscfDNA processing will be obtained during organ procurement, in accordance with the Human Research Ethics Committee for Donate Life and the Australian Red Cross Blood Services (application in progress).

iv. DATA COLLECTION, USE AND DISSEMINATION:

The following data (as defined in '**4.c OUTCOME MEASURES**' and '**5.a.iii INTERVENTION**') will be prospectively collected:

- Standard of care clinical variables
- Standard of care blood tests
- Clinical events
- Blood sampling
- Health expenditure

All data will be collected in identifiable form correlated to the patient's Austin Health UR, but will be re-identified or coded for use.

Results from this study will be published and presented at conferences in de-indentified or cohort form. Furthermore, Dr Tess McClure and Dr Daniel Cox will use the results from this study towards their PhDs in Medicine, Dentistry and Health Sciences at the University of Melbourne.

v. TIMELINE:

The aims of this study can be accomplished within a four-year period. Austin Health performs approximately 75 adult LTs per year, therefore recruitment of 210 participants is achievable within three years. The QFM-dscfDNA levels laboratory analyses will commence after each recipient has completed a 12-month follow-up, thus the completion of this will correlates with year four, when post trial analyses and preparation of results for publication will occur.

6. STUDY POPULATION

a. RECRUITMENT PROCEDURE

All patients who are being assessed for or awaiting a LT will be screened, approached, provided with recruitment documentation and consented prior to undergoing LT.

Austin Health performs approximately 75 adult LTs per year for the treatment of end-stage liver disease, fulminant liver failure and hepatocellular carcinoma. All patients referred for consideration of LT are managed by the LTU. They are therefore seen in one outpatient clinic, undergo assessment in one outpatient setting and treated by one inpatient team. This will assist in identification of appropriate patients.

Treating clinicians will be made aware of this cohort study and the identification of participants will require referral from the treating clinician. Patients will be recruited from outpatient clinics, during outpatient assessment and on the inpatient ward prior to LT.

Research coordinators will approach patients in person, provide them with recruitment documentation, and be available to explain the nature of the research. Individual written informed consent will be obtained prior to the patient undergoing LT.

b. Inclusion Criteria

The inclusion criteria are:

- Patients aged 18 years or above.
- Undergoing LT.
- Able to provide written informed consent.

c. Exclusion Criteria

The exclusion criteria are:

- Patients aged under 18 years.
- Unable to provide written informed consent at any stage.
- Undergoing multi-organ transplantation

Patients undergoing multi-organ transplantation will be excluded as dscfDNA will not specifically reflect donor liver injury in this setting.

d. Consent

Individual consent will be obtained from all participants without a waiver of consent.

In the event that a patient is too unwell and unable to consent, but is expected to recover and be able to provide consent (which may happen for example in fulminant hepatic failure), consent for initial enrolment will be obtained from the next of kin or person responsible/medical treatment decision maker. Following recovery from LT, patient consent for ongoing follow up and for use of data collected at enrolment will be obtained.

Consent will be extended in scope. This means that participants are consenting to the use of their blood samples or data in future research projects that are extensions of or closely related to this study, or in the same general area of research.

7. Participant Safety and Withdrawal

a. Risk Management and Safety

In this prospective observational cohort study, there will be no deviation from standard of care treatment.

Participants will be required to have serial additional blood sampling, performed when blood tests for the standard of care for LT occur wherever possible. This is the reduce the need for additional venepuncture, and is anticipated to be highly feasible due to the frequency of blood tests required for the standard of care post LT.

Participants may be subjected to mild physical (pain, bruising, dizziness) or psychological distress (anxiety) during venepuncture. They will be informed this low risk prior to enrolment, and provided with access to medical or counselling services if required.

b. Handling of Withdrawals

Participants who withdraw from this study will have the opportunity to have a withdrawal interview with the research coordinators, in order to voice their concerns and to receive

answers for any questions. Unless specified by the participant, already collected data will be kept for analysis and future research purposes. However, in cases where initial consent has been obtained from their person responsible and the patient then wishes to withdraw this consent, all of their data collected for research purposes will be securely destroyed.

c. REPLACEMENTS

Withdrawn participants will be replaced in this study

8. STATISTICAL METHODS

a. SAMPLE SIZE ESTIMATION & JUSTIFICATION

We propose enrolling a total of 210 LT recipients during a three-year recruitment period. This timeframe is anticipated to be easily feasible as the LTU performs an average of 75 adult LTs each year. Most recipients will fulfil the trial's inclusion and exclusion criteria; hence non-eligibility (including refusal of informed consent) is anticipated to be low (about 5%). In addition, lost-to-follow-up (including death) is anticipated to be very low (about 1-2%) during the 12-month follow-up[2].

In preliminary research from Austin Health[16], 31% of the recipients experienced tBPAR as the first event after LT while 41% experienced a 'probable'/'definite' infective complication as the first event after LT, both within a 12-month follow-up period. To be conservative, we assume 30% of our recruited recipients will experience at least one tBPAR and 40% at least one infective complication. The sample size of 210 LT recipients will allow us to estimate a fair AUC of 0.7 using a two-sided 95% confidence interval with precision ± 0.081 for tBPAR and ± 0.075 for infection and a good AUC of 0.8 with a precision of ± 0.075 for tBPAR and ± 0.069 for infection[21,22].

b. POWER CALCULATIONS

This sample size will provide a power of 99.7% to show that the combination has any ability to discriminate between those with and without tBPAR and 99.9% power between those with and without infection, (null hypothesis AUC of 0.5), assuming that we observe a fair AUC of 0.7 with 5% alpha[21].

c. STATISTICAL METHODS TO BE UNDERTAKEN

All participants will be included in the final analyses. Diagnostic performance of QFM and dscfDNA combined for the first tBPAR and the first 'probable'/'definite' infective complication will be assessed by evaluation of sensitivity, specificity, the positive and negative predictive value and diagnostic accuracy. Diagnostic accuracy will be evaluated by determining the AUC and two-sided 95% confidence interval. Given that QFM and dscfDNA outcomes are measured repeatedly over 12 months, we will initially summarise these outcomes across time-points using the average, nadir, standard deviation and slope and fit a logistic regression model.

Next, we will look at moving average, moving standard deviation, and change of slope and fit a mixed logistic regression model. Finally, a joint model for longitudinal tBPAR, respectively longitudinal infective complications, and QFM, dsfDNA and LFT outcomes will be explored. This model will allow investigation of the temporal dynamics of rejection and infection, and evaluate the usefulness of QFM and dscfDNA for monitoring purposes[23].

We will compare the AUC of using QFM alone or dscfDNA alone versus the combination. An optimal threshold of QFM and dscfDNA in identifying recipients at risk of tBPAR or infection will be established. A detailed statistical analysis plan will be prepared prior to locking of the

database.

9. Storage of Blood and Tissue Samples

a. Details of where samples will be stored, and the type of consent for future use of samples

Blood samples will be processed at the ONJCRI, Austin Health and stored in a specially allocated -80°C freezer in the LTU, Austin Health. The blood samples will be stored for seven years after study closure. After seven years, the blood samples will be disposed of in designated hazardous waste bins as per hospital/laboratory protocol.

The consent obtained will be extended in scope. This means that participants are consenting to the use of their blood samples or data in future research projects that are extensions of or closely related to this study, or in the same general area of research.

10. Data Security & Handling

a. Details of where records will be kept & How long will they be stored

The database will be stored through the electronic data management program REDCAP provided through the University of Melbourne. This will be password protected and the password will only be available to the main study investigators. 10 years following study completion the electronic database will be deleted

b. Confidentiality and Security

Blood samples will be processed in the ONJCRI, Austin Health and stored in a specially allocated -80°C freezer in the LTU, Austin Health. The blood samples will be stored for seven years after study closure. After seven years, the blood samples will be disposed of in designated hazardous waste bins as per hospital/laboratory protocol.

Records will be kept on a secure password-locked computer in LTU, Austin Health. The database will be stored stored through the electronic data management program REDCAP provided through the University of Melbourne, on a single computer with a password that is only available to the main study investigators. The UR numbers will be recorded against patient study numbers in a separate file with a separate password on the same computer. 10 years following study completion, the records and electronic database will be deleted.

No identifiable individual information will be presented in any publication. Any papers arising from this project will provide de-identified or cohort information only.

11. References

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