M.O.T. AMR

Mechanistic Evaluation of Treatments for Acute Antibody-Mediated Rejection of the Kidney Transplant

Version 1.4 27/05/2020

MAIN SPONSOR: Imperial College London
FUNDERS:
Imperial College BRC, Immunology theme will fund transplant biopsy analysis
Cambridge University BRC will fund blood collection and storage for peripheral blood transcriptomics
NIHR EME 129029 will fund transplant biopsy analysis, protocol biopsy procedure cost, and extra DSA testing
Funding for peripheral blood transcriptomics and peripheral blood B cell assays: pending

STUDY COORDINATION CENTRE: Imperial College JRCO number: 19HH5204
NRES reference: 19/SW/0240
IRAS Project ID: 264179

Protocol authorised by:

Name & Role	**Date**	**Signature**

Study Management Group

Chief Investigator: Dr Candice Roufosse

Co-investigators: Dr Michelle Willicombe, Dr Olivia Shaw, Dr Thomas Hiemstra, James Hutchinson (lay representative)

Statistician: Dr Simon Bond

Study Management: Dr Candice Roufosse

Study Coordination Centre

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: Dr Candice Roufosse
Address: Centre for Inflammatory Diseases, Imperial College, Hammersmith Campus, Commonwealth Building 9th floor, DuCane Road, London W12 0NN
Tel: 020 3313 8252
E-mail: c.roufosse@imperial.ac.uk

Clinical Queries

Clinical queries should be directed to Dr Candice Roufosse 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:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 215, Level 2, Medical School Building
Norfolk Place
London, W2 1PG
Tel: 0207 594 1862

Funder

Imperial College BRC, Immunology theme will fund transplant biopsy analysis.
Cambridge University BRC will fund blood collection and storage for peripheral blood transcriptomics.
NIHR EME 129029 will fund transplant biopsy analysis, protocol biopsy procedure cost, and extra DSA testing

Funding for peripheral blood transcriptomics and peripheral blood B cell assays: pending

Samples will be analysed in 4 centres:

- Imperial College will co-ordinate sample collection; and store and carry out analyses on biopsy samples and serum samples
- Cambridge University will store and carry out analyses on peripheral blood transcriptomic and genomic samples
- Guys' and St Thomas' NHS Trust will store and carry out analyses on serum samples (DSA testing)
- Birmingham University will store and carry out analyses on serum samples (BAFF testing) and peripheral blood B cell analyses

This protocol describes the MOT-AMR 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.

Table of Contents

GLOSSARY OF ABBREVIATIONS

(A)(C)AMR	(Acute)(Chronic) Antibody-Mediated Rejection
BAFF	B-cell activating factor
C1q	Complement factor 1q
C3	Complement factor 3
C4d	Complement factor 4d
Cg	Banff lesion score chronic glomerular
Ci	Banff lesion score chronic interstitial
Ct	Banff lesion score chronic tubular
Cv	Banff lesion score chronic vascular
CTU	Clinical Trials Unit
DMEC	Data Monitoring and Ethics Committee
(dn)DSA	(de novo) Donor specific antibodies
(e)GFR	(Estimated) Glomerular Filtration Rate
EM	Electron microscopy
ENDAT	Endothelial cell associated transcripts
ESKD	End stage kidney disease
FPFV	First patient first visit
g score	Banff Lesion score Glomerulitis
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HLA	Human Leucocyte Antigen
ICF	Informed Consent Form
IFN	interferon
IFTA	Interstitial fibrosis/tubular atrophy
iIFTA	Banff lesion score interstitial inflammation in areas of Interstitial fibrosis/tubular atrophy
IMP	Investigational Medicinal Product
IVIg	Intravenous immunoglobulins
KRUK	Kidney Research UK
MFI	Mean Fluorescence Index
MVI	Microcirculation inflammation
MHRA	Medicines and Healthcare products Regulatory Agency
NK	Natural killer
NHSBT	NHS Blood and Transplant registry

PEX	Plasma exchange
PIS	Participant Information Sheet
ptc score	Banff Lesion score Peritubular capillaritis
R&D	Research and Development
REC	Research Ethics Committee
RCT	Randomised controlled trial
RRT	Renal Replacement Therapy
SOC	Standard of Care
SOCR	Standard of Care plus Rituximab
t	Banff lesion score interstitial inflammation in non-scarred areas
TAR:GET-1	Transplant Antibody Mediated Rejection: Guiding Effective Treatments clinical trial
TCMR	T-cell mediated rejection
ti	Banff lesion score interstitial inflammation in total cortex
TMG	Trial Management Group
TSC	Trial Steering Committee
UPCR	Urinary protein:creatinine ratio
v score	Banff lesion score Arterial inflammation

KEYWORDS

Kidney transplant, antibody-mediated rejection, transplant biopsy, gene expression analysis, donor-specific antibody, B cells

STUDY SUMMARY

Title of Study: Mechanistic Evaluation of Treatments for Acute Antibody-Mediated Rejection of the Kidney Transplant
Design: Prospective observational study of patients enrolled in TAR:GET-1
Objectives: TAR-GET-1 (starting in 2019) is a multi-centre UK clinical trial that will compare standard of care (SOC) treatment of Acute Antibody-Mediated Rejection of the Kidney Transplant (AAMR) to standard of care plus rituximab (SOCR). This trial presents a rare opportunity to investigate the mechanisms of action of treatments (including rituximab) in AAMR. The rationale for using rituximab is the depletion of CD20-positive precursors to antibody-producing cells. However, rituximab has effects on other B cell subtypes and not all studies have shown antibody level reductions. Furthermore, AAMR is a heterogeneous disease, as defined by variability in mechanistic markers of AAMR in blood (B cell and antibody characteristics) and in transplant biopsies (histological features and gene expression). The objectives of this study are: • To investigate how treatment with rituximab affects these mechanistic markers, by comparing SOC to SOCR • To predict treatment benefit early, by analysing the effect of treatment on the mechanistic markers
Methodology: In patients enrolled in TAR:GET-1 (n=170), we will analyse mechanistic markers with established proof of concept (B cell and antibody characteristics, histological and transcriptional markers in the biopsy) at baseline and on follow-up, making use of clinical data, surplus diagnostic material from TAR:GET-1, and material from optional extra blood samples for research taken as part of TAR:GET-1, at 3,6 and 12 months. We will also offer an optional protocol biopsy at 6 months post treatment. Differences pre- and post-treatment will be analysed comparing SOC and SOCR. We will use mediation analysis models to decompose total effect of treatment effect on graft loss at 4 years (primary end point) into an indirect effect, which measures how much of the effect acts through the intermediate variable(s), and a residual direct effect.
Number of Subjects and Study populations: 170 subjects enrolled in TAR:GET-1
Outcome measures: same as primary outcome of TAR:GET-1 (graft loss at 4 years after initiation of treatment)
Inclusion Criteria: All patients enrolled in TAR:GET-1 Signed informed consent prior to any study specific procedures.

Exclusion Criteria:
None

Investigational Product: N/A

Duration of study: 8 years (duration is dictated by the need for primary outcome of TAR-GET-1 to be reached)

Statistical Methods:

Data from this research project will be merged and linked with data from the TAR:GET-1 clinical trial by the statistician team.

Descriptive data

This will include description of all the biological characteristics analysed in 2 populations (rituximab + SOC and SOC) at baseline and at all follow-up time points. Summary statistics (mean, median, SD, range for continuous variables, and frequency tables for categorical variables) will be reported broken down by treatment, with exploratory figures (box-and-whisker plots for continuous, and stacked bar-charts for categorical).

Outcome analysis

Appropriate measures of differences between treatment groups will be estimated with point estimates, 95% confidence intervals, and p-values. Depending on the type of variable (continuous, categorical, time-to-event), regression techniques (linear, logistic, cox) and transformation of the endpoints will be considered.

Differences in characteristics comparing pre- to post-treatment samples at sequential time points will be analysed comparing the 2 groups of patients (SOCR versus SOC) using r mixed effect models repeat measurement techniques.

Mediation analysis, a special case of regression analysis, will be used to document that the treatment effects a change in the biomarker. We will use mediation analysis models to decompose total effect of treatment effect on graft loss into an indirect effect, which measures how much of the effect acts through the intermediate variable(s), and a residual direct effect. All the mediation analyses will be based on the Intention-to-Treat principle. We will fit the same form of outcome models as for the efficacy analyses, including the mediator as a covariate. We will use the mediation package within R which was developed specifically to make valid causal inference in explanatory analyses of the mechanisms of treatment-induced change in clinical outcomes in randomised clinical trials. All estimates of treatment differences will be summarised using effect size estimates and 95% confidence intervals (Dunn 2013). Collaborator for mediation analysis: Prof Ian White, Professor of Statistical Methods for Medicine, UCL.

1. INTRODUCTION

1.1 BACKGROUND

Impact of Antibody-mediated rejection (AMR) on kidney allograft survival

Kidney transplantation is the treatment of choice for patients with end stage kidney disease resulting in improved health, patient survival and quality of life (Gibbons 2016), as well as health economic benefit in comparison to remaining on dialysis (Baboolal 2008). The UK adult prevalence of renal replacement therapy (RRT) is 962 per million population (pmp)(Renal Registry Report 2017). The UK prevalent transplant population in 2016 was 34,286 (Pyart 2018).

The use of current immunosuppression regimens, along with careful immunological selection of transplant recipients, has improved short term renal allograft survival. However long term allograft survival remains unchanged, with the average lifespan of a kidney transplant up to 15 years at most (Meier-Kriesche 2004). Rejection is an important cause of transplant failure, mostly due to chronic AMR (Loupy 2018).

TAR:GET-1 : a clinical trial for the treatment of AAMR

There is currently no high quality evidence from randomised trials to guide treatment of acute AMR (Velidedeoglu 2018). Clinical trials of existing and new drugs for AMR are a recognised priority for patients and researchers in the transplant community, as noted in the NIHR James Lind Alliance priority setting partnership 2016 (www.transplantpsp.org). The recently published UK Renal Research Strategy document (drafted following extensive patient and public consultation) similarly highlights the complete lack of evidence in treating AAMR and identifies this as a key research priority (www.kidneyresearchuk.org/research/ukkrc). This need for trials in acute AMR is recognised globally; the US FDA convened workshops aimed at assessing AMR treatments during 2010 and 2017 (Velidedeoglu 2018).

The TAR:GET-1 trial is due to start in early 2019. This trial provides a time-limited opportunity to carry out research into the mechanisms of action of rituximab in acute AMR (AAMR), which are poorly understood, using samples accrued in a randomised controlled trial, with uniform treatment and follow-up.

MOT-AMR : a time-limited opportunity to advance our understanding of the mechanisms under-pinning AMR and how treatments affect them

Kidney transplantation occurs most frequently across a degree of HLA incompatibility. Despite immunosuppression, this HLA disparity frequently leads to B cell activation and production of donor-specific antibodies (DSA), some of which cause antibody-mediated injury to the graft. In addition, depending on local protocols, some recipients already have antibodies against the HLA epitopes of the graft before transplantation (sensitised patients with pre-formed antibodies and positive cross match transplantation) (Lefaucheur 2008).

A diagnosis of AAMR rests on the identification of serum DSA plus the presence in the graft biopsy of specific histological and/or transcriptional features related to the effect of antibody on the graft endothelium (Haas 2018a). The biopsy is necessary to prove that graft dysfunction is being caused by the antibody, as not all DSA cause injury. These diagnostic features are predictive of outcome and are used to enrol patients in clinical trials of AAMR. However, AAMR is a heterogeneous disease, as defined by variability in mechanistic markers of AAMR in blood (B cell and antibody characteristics) and in transplant biopsies (histological features and gene expression). In addition, individual variations in genes

involved in co-ordinating the patient's immune response (e.g. Fc gamma receptors) may influence B cell activation (Steri 2017; Andres Floto 2005).

Rituximab is a chimeric murine-human monoclonal antibody directed against the B-cell surface molecule CD20, which depletes B cells with CD20 on their surface. Use of Rituximab in AMR has been developed on the basis that, following a primary challenge, in the presence of rituximab, B cells would be prevented from undergoing proliferation and would undergo apoptosis via complement-mediated and complement independent mechanisms. This may lead to reduction in antibody titre, and reduced antibody-mediated graft injury (Fehr 2009; Vo 2010). However, on the one hand, the effects of rituximab on the immune system are wider ranging, and on the other hand, the limited studies that have investigated response to rituximab have not consistently demonstrated the expected effect (Gudbrandsdottir 2018, Cornec 2016, Ehrenstein 2016, Ikemiyagi 2017, Pallier 2010). Investigations of the transcriptional profile of peripheral blood B cells and B cell regulatory potential show promise in elucidating the link between B cell phenotype and AMR (Clatworthy 2009 and 2014, Banham 2018).
This research will investigate the effect of rituximab along the pathophysiological pathway of development of AMR: changes in B cell characteristics; changes in anti-donor antibody titres and characteristics; and changes in histological and transcriptional evidence of AMR in the graft tissue. A better understanding of the mechanisms of rituximab in comparison to standard of care will provide evidence for its use in AAMR, and may provide information on the characteristics of patients with AAMR that are likely to respond to the drug, and information on how to measure response to the drug. These are vital to improving an effective and efficient use of drugs in treating AAMR.

1.2 RATIONALE FOR CURRENT STUDY

This research proposal is a bolt-on mechanistic study, affiliated to an NIHR HTA/Kidney Research UK-funded UK multicentre randomised controlled trial to assess the safety and efficacy of rituximab + standard of care (SOCR) compared with SOC in treating acute antibody-mediated rejection (AAMR) in kidney transplantation (TAR:GET-1)(Transplant Antibody Mediated Rejection: Guiding Effective Treatments; ISRCTN pending, EudraCT ref 2018-002882-20). This phase 3, 2-arm, open-label randomised controlled trial will enrol patients with biopsy-proven AAMR and randomise them to SOCR versus SOC (85 patients in each arm). The trial is scheduled to start in Spring 2019, and patients will be recruited over 40 months, then followed up for 4 years post treatment (follow up complete late 2026). The primary objective is efficacy, with graft survival at 4 years the primary end point.

Analysis of B cell, antibody and biopsy characteristics of patients enrolled in TAR:GET-1 will address several questions:

1) Which patients with a diagnosis of AMR require additional immunosuppression?

It is recognised that AAMR is a heterogeneous disease, and there is evidence that severity of the individual diagnostic features at presentation (HLA antibody strength, biopsy Banff scores) is associated with outcome. Novel parameters not yet used in routine diagnosis also hold promise in increasing diagnostic accuracy and directing treatment; these include complement-activating ability of DSA (Viglietti 2017, Sicard 2015, Bouquegneau 2018), the AAMR-related gene expression signature level (Loupy 2014, Halloran 2016), and B cell characteristics (genomic, transcriptional and functional). The data from past investigations is contradictory, most often derived from retrospective observational studies without uniform

follow-up and treatment, and often lacks robust multivariate analyses. There is insufficiently robust evidence to promote risk stratification and selection of patients for additional treatment.

2) How can we tell if treatment is working?

Serial measurements of the strength of DSA and severity of biopsy features have been used to describe the natural history of AAMR and to determine response to treatments, including rituximab. There is evidence in the literature that treatment of AAMR may reduce antibody strength and/or affect complement-activating ability (Cioni 2018, Viglietti 2018b, Bailly 2018, Lefaucheur 2018) and may have a positive effect on biopsy scores (Viglietti 2018a, Sautenet 2016, Eskandary 2018, Choi 2017) or transcript levels (Eskandary 2018, Lefaucheur 2018). Immunosuppression can induce changes in B cell gene expression and functional profile (Banham 2018). However, the evidence is inconclusive and in places contradictory, and has not been validated in the context of randomised controlled trials. Therefore, serial measurements are not yet used to guide treatment decisions.

3) The need for surrogate endpoints for clinical trials in AMR

Given that the end point for trials in AAMR (graft loss) is remote, development of surrogate end points for AAMR trials is a priority (Velidedeoglu 2018). Longitudinal assessment of biomarkers in patients with AAMR has been shown to assist in outcome prediction, but here again data is limited (Viglietti 2018a). This project provides an opportunity to investigate potential surrogate markers of outcome.

<u>This MOT-AMR research proposal has 2 hypotheses:</u>

Hypothesis 1: AMR is a heterogeneous disease, with outcome and response to treatment accurately predicted by mechanistic biomarkers (B cell, DSA and biopsy features) at baseline, including more recently characterised complement-activating ability of DSA and AAMR gene expression biopsy signature.

Hypothesis 2: Although the clinical endpoint of TAR:GET-1 is graft loss, effective AAMR therapy will be reflected in changes in mechanistic features in serum and in biopsies. In the case of treatment with rituximab, this is achieved through favourable changes in B cell characteristics.

2. STUDY OBJECTIVES

The primary objective is to investigate the mechanisms of treatments including rituximab in AAMR, by measuring the effect of SOCR versus SOC on mechanistic markers of AMR (B cell characteristics, antibody characteristics, end organ damage assessed by histology and transcript analysis)

The secondary objective is to predict treatment benefit early, by decomposing the effect of treatment on graft loss at 4 years (primary end point) into an indirect effect, which measures how much of the effect acts through the changes in mechanistic markers, and a residual direct effect.

3. STUDY DESIGN

Patient Cohort
This is a prospective cohort study using samples from all patients enrolled in the TAR:GET-1 (170 patients, 85 in each arm). The TAR:GET-1 trial will recruit patients starting in June 2019, over a period of 4 years, and patient will be followed up with further biological samples taken over a further period of 4 years. This will need to be analysed for which we estimate a further 2 years. The total duration of the study is therefore 10 years starting June 2019.

We aim to collect all samples from each patient to maximise the information obtained, whilst incurring negligible extra risk to the study participants.
Some of the samples are already obtained as part of the TAR:GET-1 protocol and we will use left over material to carry out analyses.
Some of the samples are not routinely obtained for TAR:GET-1 enrolment, but the TAR:GET-1 protocol and consent process covers their (optional) collection.
The only extra samples that requires a new consent process as part of M.O.T.-AMR is an optional post-treatment protocol biopsy.

The study aim is to be a comprehensive assessment of antibody-mediated rejection biomarkers pre- and post-treatment, but has below been artificially divided into Parts 1 and 2, Part 1 being funded by Imperial BRC, Cambridge BRC and NIHR EME (funding secured) whereas applications for funding for Part 2 are in process. A funding approval letter will be provided to the Sponsor and REC committee prior to commencing any element of part 2 of the study, and will specify which further specific elements of the study have received funding approval.

Samples taken as part of TAR:GET-1:
Patients enrolled in TAR:GET-1 will have a number of samples taken, and consent for enrolment in TAR:GET-1 includes agreement to the use of extra material left after diagnosis for research. We will therefore be able to access in 100% of patients enrolled in TAR:GET-1:

- Enrolment biopsy material
- Serum samples taken for DSA measurements
- Post-treatment indication biopsies taken as part of routine clinical care.

TAR:GET-1 is already assessing DSA MFI, DSA target and B lymphocyte numbers at baseline and 3 months post-treatment. In addition to these,M.O.T.-AMR will analyse in left over material from these routine samples:

1) Serum DSA characteristics at an extra timepoint (costs not covered in TAR:GET-1; 1 year), plus more detailed DSA analyses at all 3 timepoints including not only anti-HLA antibodies (IgG subtype, complement-activating ability) but also non-HLA antibodies;

2) Biopsy Banff histological scores (Roufosse 2018); The following data will be obtained for each biopsy:

- Total number of glomeruli
- Number of glomeruli with global sclerosis
- Number of glomeruli with segmental sclerosis
- Number of scorable glomeruli (excluding those with global/segmental sclerosis and ischaemic changes)
- Percentage of cortical scarring [tubular atrophy, interstitial fibrosis]

- Full Banff lesion scores (t,i,ti,v,g,ptc,ct,ci,cg,cv,ah, C4d) and the microcirculation inflammation score (MI=g+ptc)
- Extent of peritubular capillary inflammation (% of cortex)
- Number of glomeruli with glomerulitis
- Number of glomeruli with capillary wall double contours
- Result of SV40 immunostain (positive/negative)
- Banff Classification for Allograft Pathology diagnostic category(ies) and subcategory(ies)
- Ultrastructural features: presence and distribution of electron dense deposits; extent of foot process effacement; extent of glomerular and peritubular capillary basement membrane mulitlayering; extent of ultrastructural endothelial activation; presence of tubuloreticular inclusions
- If performed, immunofluorescence staining pattern for IgG, IgA, IgM, C3, C1q, kappa light chain and lambda light chain;

3) Biopsy gene expression with be assessed using Nanostring nCounter technology (Adam 2016; Dominy 2019); this will include the AMR-gene expression signature (Haas 2018), and a B cell signature. As literature is evolving and it is possible to test for hundreds of genes on the same sample, the list of genes to be studied may evolve.

4) Serum BAFF (B cell activating factor) levels

Funding:
Part 1 (covered by Imperial BRC, Cambridge BRC and NIHR EME 129029): Shipping of the samples, 1), 2) and 3).
Part 2: 1) and 4); we will be applying to other funding sources, including Kidney Research UK and local NHS Trust research charity funds.

<u>Optional samples covered by TAR:GET-1 consent process</u>

The additional samples are optional and M.O.T.-AMR is not dependent on them being obtained. As part of TAR:GET-1, patients will be offered the option of contributing extra tubes of blood at baseline, 3 months, 6 months and 1 year, and may decline any of them or all of them.
The extra samples will be: 1) lithium heparin tubes for PBMC isolation in order to carry out B cell immunophenotyping (flow cytometry); peripheral blood B cell stimulation assay (stimulation assay utilising intracellular staining for IL10 and IL6 and deriving a ratio to study regulatory potential of peripheral blood B cells)(Banham 2018)(extra tube of blood at 3 time points, fresh sample needed); and DNA analysis; and 2) PAXgene tubes for peripheral blood transcriptomics (whole blood transcriptomic analysis)(extra tube of blood at 3 time points).

Samples for 1) need to be shipped rapidly and we do not expect to take these samples until funding is secured, so estimate 50% of patients will contribute these, in the later stages of the trial. Samples for 2) can be frozen and batch-shipped so we expect them to be obtained in about 90% of patients.

Funding:
Part 1: Funding for taking and shipping of the PAXgene tubes will be covered by Cambridge BRC.
Part 2: We are applying for funding for the lithium heparin tube samples, and their collection will not be started until funding is secured. We are also applying for funding for the transcript analysis and DNA analysis.

Optional post-treatment renal transplant protocol biopsy

Specifically for M.O.T-AMR, patients enrolled in TAR:GET-1 will be offered an optional protocol biopsy at 6 months post-treatment. This additional sample is optional and M.O.T.-AMR is not dependent on it being obtained. This protocol biopsy will be carried out in the local centre, by the local clinical team. A post-treatment protocol biopsy is a biopsy taken in a patient with stable transplant function and no indication for a biopsy to be taken. The literature suggests that this a useful tool for monitoring response to treatment (Viglietti 2018a), but strong evidence from randomised controlled trials such as TAR:GET-1 is lacking for its routine introduction into clinical care and therefore, in most centres a post-treatment protocol biopsy is not offered to patients. The protocol biopsy will be collected for research and costed as research. Biopsy results on these protocol biopsies will be made available to the clinicians if any unexpected findings that need to be acted upon for patient benefit are noted. We will carry out the same histological and transcriptional investigations on these biopsies as for the enrolment and indication biopsies. A conservative estimate of protocol biopsy acceptance is 50%, based on our local experience (85 samples), and this is the number of samples we aim to achieve.

Funding:
Part 2: Funding for taking these biopsies is covered by NIHR EME 129029. Shipping and analysis will be partly covered by Imperial BRC.

This table illustrates how visits and samples in TAR:GET-1 will be used for investigations in M.O.T.-AMR. The additional samples needed for M.O.T.-AMR are in italic.

TAR:GET-1 trial visits	**Baseline**	**Unscheduled**	**Visit 3 (month 3)**	**Visit 4 (month 6)**	**Visit 5 (month 12)**
Biopsy histology and gene expression analysis	Use leftover sample	Indication biopsy for graft dysfunction: Use leftover samples		*Optional protocol biopsy*	
Serum DSA characteristics and BAFF	Use leftover sample		Use leftover sample		Use leftover sample
B cell characteristics and Peripheral blood transcriptomics	*2 extra blood tubes*		*2 extra blood tubes*	*2 extra blood tubes*	*2 extra blood tubes*

Sample storage:
Central storage will be at Imperial College. Samples will be dispatched to other centres for processing. Samples will be stored at Imperial College and Cambridge University, using -80C freezers for frozen samples and room temperature storage for slides and paraffin blocks. For B cell immunophenotyping, we will have samples sent fresh to Birmingham University for PBMC preparation, immediate flow cytometry, and storage of left over frozen PBMC. Samples may also be stored at Viapath.

3.1 STUDY OUTCOME MEASURES

Primary outcome is graft survival 4 years post-treatment.

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

None

4.2 INCLUSION CRITERIA

All patients enrolled in TAR:GET-1 are eligible.

4.3 EXCLUSION CRITERIA

None. If some patients decline extra samples to be taken for research, we can still analyse left over tissue from routine clinical samples for some aspects of the study.

4.4 WITHDRAWAL CRITERIA

- Subject decision. A subject is free to withdraw consent and discontinue participation in the study at any time
- Patients who withdraw from TAR:GET-1 will also be withdrawn from MOT-AMR and withdrawal criteria is the same as TAR:GET-1.

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 and unexpected 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.

5.3 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.

5.3.2 Serious AEs

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

All SAEs should be reported to the South West -Frenchay Research Ethics Committee 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 SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
jrco@imperial.ac.uk

6. ASSESSMENT AND FOLLOW-UP

Assessment and follow-up of subjects is part of the TAR:GET-1 trial.

7. STATISTICS AND DATA ANALYSIS

The patient cohort size is dictated by TAR:GET-1: 170 patients, 85 in each arm. Under plausible modelling assumptions, for any biomarker that is on the mechanistic pathway between treatment and primary outcome, the power of the main study (90%) will be matched or exceeded when comparing the mean biomarker values between treatment groups. The mediation analysis will estimate for each biomarker the influence on allograft survival rate per unit change in biomarker, above and beyond, the direct effect of treatment. This will decompose the overall, marginal, treatment effect into a proportion attributable to a mechanism via the biomarker, and the compliment attributable to direct effects, or other mechanisms. A similar multivariable analysis will proceed to estimate the mediation effects of multiple biomarkers acting in parallel, as an exploratory analysis using variable selection methods to control for over-fitting.

Data from this research project will be merged and linked with data from the TAR:GET-1 clinical trial by the statistician team.

Descriptive data

This will include description of all the biological characteristics analysed in 2 populations (rituximab + SOC and SOC) at baseline and at all follow-up time points. Summary statistics (mean, median, SD, range for continuous variables, and frequency tables for categorical variables) will be reported broken down by treatment, with exploratory figures (box-and-whisker plots for continuous, and stacked bar-charts for categorical).

Outcome analysis

Appropriate measures of differences between treatment groups will be estimated with point estimates, 95% confidence intervals, and p-values. Depending on the type of variable (continuous, categorical, time-to-event), regression techniques (linear, logistic, cox) and transformation of the endpoints will be considered.

Differences in characteristics comparing pre- to post-treatment samples at sequential time points will be analysed comparing the 2 groups of patients (SOCR versus SOC) using r mixed effect models repeat measurement techniques.

Mediation analysis, a special case of regression analysis, will be used to document that the treatment effects a change in the biomarker. We will use mediation analysis models to decompose total effect of treatment effect on graft loss into an indirect effect, which measures how much of the effect acts through the intermediate variable(s), and a residual direct effect. All the mediation analyses will be based on the Intention-to-Treat principle. We will fit the same form of outcome models as for the efficacy analyses, including the mediator as a covariate. We will use the mediation package within R which was developed specifically to make valid causal inference in explanatory analyses of the mechanisms of treatment-induced change in clinical outcomes in randomised clinical trials. All estimates of treatment differences will be summarised using effect size estimates and 95% confidence intervals (Dunn 2013). Collaborator for mediation analysis: Prof Ian White, Professor of Statistical Methods for Medicine, UCL

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Chief Investigator has obtained approval from the Health Research Authority (HRA) and Research Ethics Committee. The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT

As part of the consent procedure for enrolment in TAR:GET-1 patients will:

- Consent for use of left over material from samples taken as part of TAR:GET-1 to be used for research
- Consent to be offered optional extra tubes of blood for research during the scheduled blood drawing events of TAR:GET-1

We will separately obtain consent for use of TAR:GET-1 samples for this specific project (M.O.T.-AMR), and for an optional post-treatment protocol biopsy at 6 months after initiation of treatment.

The ICF and process for obtaining informed consent must comply with the applicable national and international laws, rules, and regulations. The ICF will document the study-specific information the Investigator or his/her designee provides to the subject and the subject's agreement to participate in the study and to comply with the instructions of the Investigator and study staff. The Investigator/designee will fully explain, in terms understandable to the subject, the nature of the study, along with the aims, methods, anticipated benefits, potential risks, and any discomfort that participation in the study may entail. The ICF must be signed and dated by the subject before the subject participates in any study-related activities. The original and any amended signed and dated ICFs must be retained in the subject's file at the study site, and a copy must be given to the subject at the time that it is signed by the subject. The Investigator must also maintain a log of all informed consents obtained.

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 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 (delete as applicable)

8.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

Funding for some aspects of the research will be provided by the Imperial Biomedical Research Centre (Immunology theme), the Cambridge BRC, and NIHR EME.
Applications for funding of some aspects of the study are still on-going.

8.7 AUDITS

The study is subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Candice Roufosse at Imperial College, Centre for Inflammatory Diseases.

10. PUBLICATION POLICY

After conclusion of the study, Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media, the results of the study.

10. REFERENCES

Adam B, Afzali B, Dominy K, Chapman E, Gill R, Hidalgo LG et al. **2016** Multiplexed Color-Coded Probe-Based Gene Expression Assessment for Clinical Molecular Diagnostics in Formalin-Fixed Paraffin-Embedded Human Renal Allograft Tissue. Clin Transplant. 30:295-305

Andres Floto R, Clatworthy M, Heilbronn K, Rosner D, MacAry P, Rankin A et al. **2005** Loss of function of a lupus-associated FcgRIIb polymorphism through exclusion from lipid rafts. Nat Medicine; 11(10):1056-1058

Baboolal K, McEwan P, Sondhi S, Spiewanowski P, Wechowski J, Wilson K. **2008** The cost of renal dialysis in a UK setting - a multicentre study. Nephrol Dial Transplant; 23(6):1982-9.

Bailly E, Anglicheau D, Blancho G, Gatault P, Vuiblet V, Chatelet V, et al. Prognostic Value of the Persistence of C1q-Binding Anti-HLA Antibodies in Acute Antibody-Mediated Rejection in Kidney Transplantation. 2018 Apr;102(4):688-698.

Banham G, Flint SM, Torpey N, Lyons PA, Shanahan DN, Gibson A et al. **2018** Belimumab in kidney transplantation: an experimental medicine, randomised, placebo-controlled phase 2 trial. Lancet 30;391(10140):2619-2630

Bouquegneau A, Loheac C, Aubert O, Bouatou Y, Viglietti D, Empana JP et al. 2018 Complement-activating donor-specific antiHLA antibodies and solid organ transplant survival: A systematic review and metaanalysis. PLoS Med 15(5): e1002572.

Choi J, Aubert O, Vo A, Loupy A, Haas M, Puliyanda D et al. **2017** Assessment of Tocilizumab (Anti-Interleukin-6 Receptor Monoclonal) as a Potential Treatment for Chronic Antibody-Mediated Rejection and Transplant Glomerulopathy in HLA-Sensitized Renal Allograft Recipients. Am J Transplant;17(9):2381-2389

Cioni M, Nocera A, Tagliamacco A, Basso S, Innocente A, Fontana I, et al. **2018** Failure to remove de novo donor-specific HLA antibodies is influenced by antibody properties and identifies kidney recipients with late antibody-mediated rejection destined to graft loss - a retrospective study. Transpl Int; doi: 10.1111/tri.13325. [Epub ahead of print]

Clatworthy M, Watson CJ, Plotnek G, Bardsley V, Chaudhry AN, Bradley JA, et al. **2009** B-cell-depleting induction therapy and acute cellular rejection. N Engl J Med;360(25):2683-5.

Clatworthy M. **2014** B-cell regulation and its application to transplantation. Transpl Int;27(2):117-28

Cornec D, Costa S, Devauchelle-Pensec V, Jousse-Joulin S, Marcorelles P, Berthelot JM, et al. 2016 Blood and salivary-gland BAFF-driven B-cell hyperactivity is associated to rituximab inefficacy in primary Sjogren's syndrome. J Autoimmun;67:102-10.

de Kort H, Willicombe M, Brookes P, Dominy K, Santos-Nunez E, Galliford J et al. 2013 Microcirculation inflammation predicts outcome in renal transplant patients with de novo donor-specific antibodies. Am J Transplant;13:485-92

Dominy KM, Willicombe M, Al Johani T, Beckwith H, Goodall D, Brookes P et al. **2018** Molecular Assessment of C4d Positive Renal Transplant Biopsies Without Evidence of Rejection. Kidney Int Rep. Sep 18;4(1):148-158

Ehrenstein MR, Wing C. 2016 The BAFFling effects of rituximab in lupus: danger ahead? Nat Rev Rheumatol;12(6):367-72.
Eskandary F, Regele H, Baumann L, Bond G, Kozakowski N, Wahrmann M et al. 2018 A Randomized Trial of Bortezomib in Late Antibody-Mediated Kidney Transplant Rejection. J Am Soc Nephrol;29:591-605
Fehr T, Rusi B, Fischer A, Hopfer H, Wuthrich RP, Gaspert A. **2009** Rituximab and intravenous immunoglobulin treatment of chronic antibody-mediated kidney allograft rejection. Transplantation 87: 1837
Gibbons A BC, ATTOM investigators. **2016** The effects of kidney alone or simultaneous pancreas and kidney transplant on quality of life and health status: Findings from ATTOM-programme cohorts with baseline data. Nephrology, Dialysis, Transplantation.;31(Suppl 1):i439--i53.
Gudbrandsdottir S, Brimnes M, Kollgaard T, Hasselbalch HC, Nielsen CH. 2018 Effects of rituximab and dexamethasone on regulatory and proinflammatory B-cell subsets in patients with primary immune thrombocytopenia. Eur J Haematol;100(1):45-52.
Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D et al. **2018** The Banff 2017 Kidney Meeting Report: Revised Diagnostic Criteria for Chronic Active T Cell-Mediated Rejection, Antibody-Mediated Rejection, and Prospects for Integrative Endpoints for Next-Generation Clinical Trials. Am J Transplant 2018 Feb;18(2):293-307
Halloran P, Famulski K.Reeve J. **2016** Molecular assessment of disease states in kidney transplant biopsy samples. Nat Rev Nephrol. Sep;12(9):534-48
Ikemiyagi M, Hirai T, Ishii R, Miyairi S, Okumi M, Tanabe K. **2017** Transitional B Cells Predominantly Reconstituted After a Desensitization Therapy Using Rituximab Before Kidney Transplantation. Ther Apher Dial;21(2):139-49.
Lefaucheur C, Suberbuelle-Boissel C, Hill GS, Nochy D, Andrade J, Antoine C et al. **2008** Clinical relevance of pre-formed HLA donor-specific antibodies in kidney transplantation. Am J Transplant; 8: 324 – 331
Lefaucheur C, Viglietti D, Hidalgo L, Ratner L, Bagnasco S, Batal I et al. **2018** Complement-Activating Anti-HLA Antibodies in Kidney Transplantation: Allograft Gene Expression Profiling and Response to Treatment. J Am Soc Nephrol. Feb;29(2):620-635
Loupy A, Lefaucheur C, Vernerey D, Chang J, Hidalgo L, Beuscart T et al. **2014** Molecular microscope strategy to improve risk stratification in early antibody-mediated kidney allograft rejection. J Am Soc Nephrol 25(10):2267-77
Loupy A, Lefaucheur C. **2018** Antibody-Mediated Rejection of Solid-Organ Allografts. N Engl J Med. 20;379(12):1150-1160
Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. **2004** Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant;4(3):378-83
Pallier A, Hillion S, Danger R, Giral M, Racape M, Degauque N, et al. 2010 Patients with drug-free long-term graft function display increased numbers of peripheral B cells with a memory and inhibitory phenotype. Kidney Int;78(5):503-13
Pyart R, Wong E, Sharples E, Casula A, Byrne C. **2018** Chapter 3 Demographic and Biochemistry Profile of Kidney Transplant Recipients in the UK in 2016. Nephron.;139 Suppl 1:75-104.
Roufosse C, Simmonds N, Groningen MC, Haas M, Henriksen K, Horsfield C et al. **2018** A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. Transplantation; doi: 10.1097/TP.0000000000002366. [Epub ahead of print]
Sautenet B, Blancho G, Buchler M, Morelon E, Toupance O, Barrou B, et al. **2016** One-year Results of the Effects of Rituximab on Acute Antibody-Mediated Rejection in Renal Transplantation: RITUX ERAH, a Multicenter Double-blind Randomized Placebo-controlled Trial. Transplantation.;100(2):391-9

Sicard A, Ducreux S, Rabeyrin M, Couzi L, McGregor B, Badet L et.al. 2015 Detection of C3d-binding donor specific anti HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. J Am Soc Nephrol ; 26: 457 – 467
Steri M, Orrù V, Idda ML, Pitzalis M, Pala M, Zara I et al. 2017. Overexpression of the Cytokine BAFF and Autoimmunity Risk. N Engl J Med;376:1615-26
Velidedeoglu E et al. **2018** Summary of 2017 FDA Public Workshop: Antibody-mediated Rejection in Kidney Transplantation. Transplantation.
Viglietti D, Loupy A, Vernerey D, Bentlejewski C, Gosset C, Aubert O et.al. **2017** Value of donor-specific anti-HLA antibody monitoring and characterisation for risk stratification of kidney allograft loss. J Am Soc Nephrol; 28: 702 – 715
Viglietti D, Loupy A, Aubert O, Bestard O, Van Huyen JD, Taupin JL et al. **2018a** Dynamic Prognostic Score to Predict Kidney Allograft Survival in Patients with Antibody-Mediated Rejection. J Am Soc Nephrol
Vo AA, Peng A, Toyoda M, Kahwaji J, Cao K, Lai CH et al. 2010 Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. Transplantation; 89:1095