Clinical Trial Protocol

Trial Title: A multicentre randomised controlled trial to assess the efficacy of adding rituximab to standard of care in treating acute antibody-mediated rejection in kidney transplantation (TAR:GET-1)

Short Title: Transplant **A**ntibody-Mediated **R**ejection: **G**uiding **E**ffective **T**reatments (**TAR:GET-1**)

TAR:GET-1

Protocol Version: Version 3.0 (18 May 2020)

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CCTU Cambridge Clinical Trials Unit

1 PROTOCOL SIGNATURES

I give my approval for the attached protocol entitled “A multicentre randomised controlled trial to assess the efficacy of adding rituximab to standard of care in treating acute antibody-mediated rejection in kidney transplantation (TAR:GET-1)” version 3.0 dated 18 May 2020.

Chief Investigator

Name: _____

Signature: _____ Date: _____

Site Signatures:

I have read the attached protocol entitled “A multicentre randomised controlled trial to assess the efficacy of adding rituximab to standard of care in treating acute antibody-mediated rejection in kidney transplantation (TAR:GET-1)” version 3.0 dated 18 May 2020 and agree to abide by all provisions set forth therein.

I agree to comply with the conditions and principles of Good Clinical Practice as outlined in the European Clinical Trials Directives 2001/20/EC and 2005/28/EC, the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) and any subsequent amendments of the clinical trial regulations, the Sponsor’s SOPs, and other regulatory requirements as amended.

Principal Investigator

Name: _____

Signature: _____ Date: _____

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This protocol describes the TAR:GET-1 trial and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the trial, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version. Problems relating to this trial should be referred, in the first instance, to the trial coordination centre. This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the General Data Protection Regulation (EU GDPR) 2016/679, the Data Protection Act 2018 and other regulatory requirements as appropriate.

Contents

3 GLOSSARY OF ABBREVIATIONS

ABO	Blood group antigens
AE/AR	Adverse Event/Adverse Reaction
AMR	Antibody-Mediated Rejection
ATG	Anti-Thymocyte Globulin
BK	BK polyomavirus
BTS	British Transplant Society
CI	Chief Investigator
CD	Cluster of Differentiation
CCTU	Cambridge Clinical Trials Unit
cg	Chronic Glomerulopathy
CKD-EPI	Chronic kidney disease – Epidemiology Collaboration equation
CMV	Cytomegalovirus
CRF	Case Report Form
CRP	C-reactive protein
CTIMP	Clinical Trial of Investigational Medicinal Product
DMEC	Data Monitoring and Ethics Committee
DP	HLA Variant
DQA	HLA Variant
DQB1	HLA Variant
DRB1	HLA Variant
DSA	Donor Specific Antibody(ies)
DSUR	Development Safety Update Report
EBV	Epstein-Barr Virus
eGFR	Estimated Glomerular Filtration Rate
EQ-5D-5L	Health-related Quality of Life Questionnaire
ESKD	End stage kidney disease
EU	European Union
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FBC	Full Blood Count
FFP	Fresh Frozen Plasma

FPFV	First Patient First Visit
g score	Glomerulitis score
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GFR	Glomerular Filtration Rate
GP	General Practitioner
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRA	Health Research Authority
IA	Immunoadsorption
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomised Controlled Trials Number
ITT	Intent-To-Treat
IUD	Intrauterine Device
IV	Intravenous
IVIg	Intravenous immunoglobulins
KDIGO	Kidney Disease Improving Global Outcomes
KRUK	Kidney Research UK
LPLV	Last Patient Last Visit
MFI	Mean Fluorescence Index
MHRA	Medicines and Healthcare products Regulatory Agency
MMF	Mycophenolate mofetil
MP	Methylprednisolone
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NIHR	National Institute for Health Research
PEX	Plasma exchange
PCP	Pneumocystis Pneumonia
PI	Principal Investigator

PIS	Participant Information Sheet
PPI	Proton Pump Inhibitor
ptc score	Peritubular capillaritis score
QoL	Quality of Life
R&D	Research and Development
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RNA	Ribonucleic Acid
RSI	Reference Safety Information
SAE/SAR	Serious Adverse Event/Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SOC	Standard of Care
SOCR	Standard of Care plus Rituximab
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAR:GET	Transplant Antibody-mediated Rejection: Guiding Effective Treatments
TMG	Trial Management Group
TRALI	Transfusion-Related Acute Lung Injury
TSC	Trial Steering Committee
UPCR	Urinary Protein:Creatinine Ratio
v score	Arterial inflammation score
VOI	Value of Information

4 TRIAL SUMMARY

Trial Title	A multicentre randomised controlled trial to assess the efficacy of adding rituximab to standard of care in treating acute antibody-mediated rejection in kidney transplantation
Clinical Phase	3
Trial Design	An open label, 2-arm randomised controlled trial
Trial Population	Renal transplant recipients with acute antibody-mediated rejection
Trial Setting	UK renal transplant units
Primary Objective	Efficacy: Allograft survival
Secondary Objectives	Efficacy: Allograft function, in terms of serum creatinine, estimated GFR (CKD-EPI) and proteinuria (urinary protein:creatinine ratio (UPCR)). Assessment of donor specific antibody (DSA) levels at 3 and 12 months will also be performed. Safety: Adverse event rate Patient reported outcomes (Quality of Life (QoL)) Economic evaluation of cost effectiveness and a value of information analysis
Sample size	Approximately 170 patients, randomised 1:1
Interventional arm	Standard of care plus rituximab (SOCR)
Control Arm	Standard of care (SOC, consisting of plasma exchange, intravenous immunoglobulins and corticosteroids)
Summary of Eligibility Criteria **Inclusion Criteria:**	• Provision of informed consent by patient or by a parent or legal guardian for patients <16 years • Aged 5 years old or older • A diagnosis of acute AMR as defined by: o The presence of ≥1 DSA o An adequate renal transplant biopsy with histological features consistent with active AMR with no evidence of chronicity as defined by the Banff histological classification of allograft pathology(1):

	▪ If C4d positive (2 or 3): • v score ≥1 and/or • thrombotic microangiopathy and/or • g score ≥1 and/or • ptc score ≥1 • or if co-existing cellular rejection, a g score ≥1 Or ▪ If C4d negative (0 or 1): • microcirculation inflammatory score (g + ptc) ≥2 • or if co-existing cellular rejection, a g score ≥1 and (g + ptc) ≥2 AND ▪ Chronic glomerulopathy (cg) score 0 or 1a ▪ Tubulo-interstitial fibrosis <50% and glomerular obsolescence <50% (see APPENDIX 1)
Exclusion Criteria	• Patients who have received an ABO incompatible transplant • Patients who have received rituximab as part of induction or post-transplant for any other indications within the preceding 12 months (e.g. recurrent focal and segmental glomerular sclerosis) • Patients who have completed PEX treatment prior to the index biopsy on the suspicion of acute AMR in the absence of histology • Have active infection including bacterial, viral (including CMV and EBV), fungal or tuberculosis, which in the investigator's opinion could affect the conduct of the trial • Co-existing BK nephropathy • Patients with active hepatitis B (patients with prior exposure to hepatitis B may be enrolled at the discretion of the PI) • Have active hepatitis C (patients may be included if a negative hepatitis C recombinant immunoblot assay is confirmed or have a negative hepatitis C virus RNA [qualitative] test)

	• Have human immunodeficiency virus (HIV) • Active malignancy which would pose a contraindication to any of the trial interventions • Patients with known allergy, intolerance or contraindication to the treatments in the standard of care arm or rituximab as outlined in the Summary of Product Characteristics (SmPCs) • Clinically significant comorbidity • Females must be either post-menopausal for at least 1 year, surgically sterile or, if of child-bearing potential, must not be pregnant or lactating. If sexually active, female participants must agree to use an acceptable method of birth control for 12 months post treatment with rituximab. Female participants must also agree not to breastfeed for 12 months post treatment with rituximab.
Feasibility analyses	Assessed 12 and 21 months after first randomised patient, first visit (FPFV): • Research site activation (12 month analysis only) • Recruitment rate • Efficacy: o Transplant function (serum creatinine and proteinuria) o Change in DSA levels (3 months post-randomisation) • Safety – adverse event rate
Duration	Recruitment: 40 months Per patient follow-up: a minimum of 48 months

5 REFERENCE DIAGRAM

A detailed flow diagram of the patient pathway is shown in Figure 1.

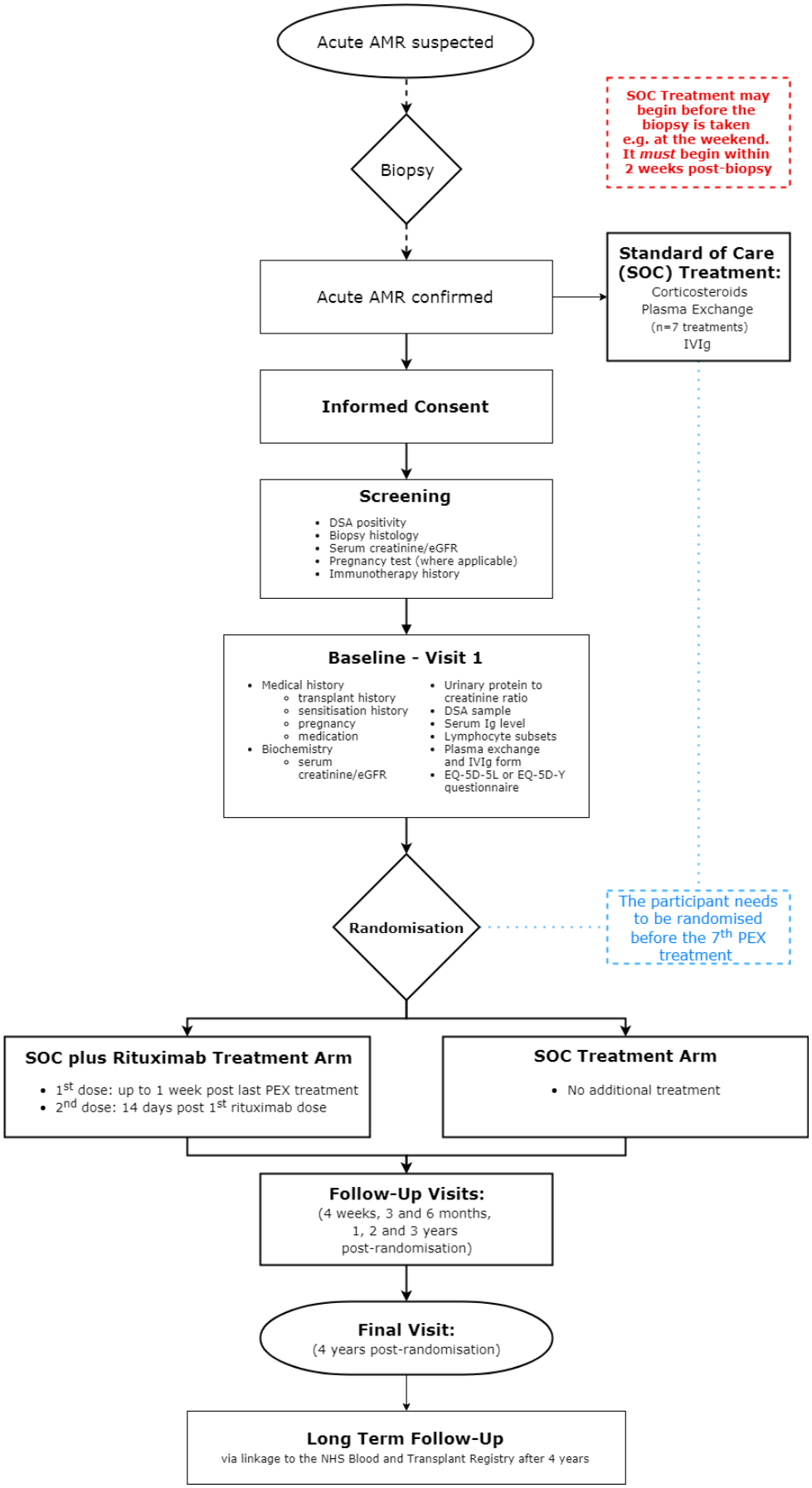

Figure 1 – Trial Flow Diagram of Participant Pathway

6 INTRODUCTION

6.1 BACKGROUND

Kidney transplantation is the treatment of choice for most patients with end stage kidney disease (ESKD), resulting in improved health and patient survival (2-6), quality of life (7-11) and health economic benefit (12-14) in comparison to remaining on dialysis.

The use of current immunosuppression regimens, along with careful immunological selection of transplant recipients, has improved short term renal allograft survival over recent decades (15). However, long term allograft survival has remained unchanged, with the average lifespan of a kidney transplant being up to 15 years (15). Chronic antibody-mediated rejection (chronic AMR) remains the leading cause of allograft failure and one of the commonest causes of 'ESKD' as patients with failed allografts have to return to dialysis and the transplant wait list (15, 16). Of the 7% of patients who will experience acute AMR in the first-year post-transplant, half (3.5%) will develop histological evidence of chronic AMR within one year (17). Despite its impact on the development of chronic AMR and graft failure, the current evidence comes from one under-powered randomised controlled trial and several cohort studies to guide treatment of acute AMR (18-21). Existing treatments are expensive and their use by UK and international transplant centres is, at best, driven by anecdotal evidence, clinical experience and resources, and at worst, determined by chance (18, 22). There is an urgent need to provide high quality data on the safety and effectiveness of treatments currently used in the UK to treat acute AMR.

6.1.1.1 BACKGROUND IN PAEDIATRIC PATIENTS

Kidney transplantations can be performed in paediatric patients from the age of less than 1 year old with comparable success to older children (23). The incidence of very young children requiring a kidney transplant is much lower than the incidence for older children. The Annual Report on Kidney Transplantation 2016/2017 from NHSBT shows that only 24% of paediatric patients on the transplant waiting list are aged 0 – 5 years old. A long-term follow-up of 300 children showed that there is no significant difference in the mortality rate of paediatric renal transplant recipients greater than and less than 5 years old at the time of transplantation (24). However, the survival time after transplantation is greater for patients >5 years old. Very young children are more susceptible to infectious complications and as such have been determined ineligible by the protocol committee. Therefore, paediatric patients 5 years old and older will be eligible for this trial.

6.1.2 Existing Trials in AMR

A systematic review of treatment of acute AMR published in 2012 identified the lack of robust evidence available (18). The 5 randomised controlled trials of acute AMR treatments available at that time had small sample sizes and were not adequately powered to assess efficacy or safety. Furthermore, 4 of these studies were historic and predated the introduction of the modern Banff histological classification of acute AMR in 2001 (25-28). These trials therefore have extremely limited value in informing current clinical practice. In

2016 the first RCT for AMR treatments aimed to determine the efficacy of rituximab in treating acute AMR. 38 patients with acute AMR were randomly assigned to rituximab or placebo in addition to SOC (29). The primary endpoint was graft loss or failure to improve function after 12 days, and participants were followed for only 12 months. More than a third of the patients in the control arm also received rituximab as 'rescue therapy', and this, together with the relatively short follow-up and small sample size, rendered the trial uninformative.

6.1.2.1 Existing Trials in Paediatric Patients

Chronic AMR is also a leading cause of kidney transplantation failure in paediatric patients and is associated with a poor outcome (30). The prevalence of chronic AMR may be even higher in paediatric patients than it is in adults (31). There is a lack of consensus in how to treat AMR and treatment options vary between centres (32, 33).

6.2 CLINICAL DATA OF RITUXIMAB

Rituximab is a genetically engineered chimeric mouse/human monoclonal antibody representing a glycosylated immunoglobulin with human IgG1 constant regions and murine light-chain and heavy-chain variable region sequences. It is designed to recognise and bind specifically to the protein CD20, a non-glycosylated phosphoprotein, present on the surface of pre-B and mature B-lymphocytes. The antigen is expressed on both normal and malignant B cells, but not on haematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissue. In the presence of rituximab, B cells are prevented from undergoing proliferation and undergo apoptosis via complement and complement-independent mechanisms, resulting in depletion of B-lymphocyte numbers.

6.2.1 Efficacy

The efficacy of rituximab in non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, rheumatoid arthritis and granulomatosis with polyangiitis and microscopic polyangiitis has been studied extensively, as described in the MabThera® SmPC. Although there have been several cohort and case-controlled studies investigating rituximab in acute AMR, efficacy has not been tested in sufficiently-powered randomised trials.

6.2.1.1 Efficacy of Rituximab in Paediatric Participants

There is limited data on the efficacy of rituximab in paediatric renal transplant recipients with one prospective controlled study reported (34). Rituximab is often used as combination therapy to treat steroid-resistant nephrotic syndrome and post-transplant lymphoproliferative disease (PTLD) (35).

6.2.2 Safety and Tolerability

The Summary of Product Characteristics (SmPC) reports that the safety of rituximab has been examined in several studies. The main adverse reactions are infusion related reactions, infections and cardiovascular events (see section 10.3.2.6).

6.2.2.1 Paediatric Participants

Safety data for rituximab use in paediatric patients has not been established (section 4.8 MabThera® SmPC). However, data can be extrapolated from its use to treat different conditions in children and adolescents with underlying renal disease. In the systematic review by Jellouli *et al*., rituximab was reported to be well tolerated in paediatric patients with similar adverse reactions as reported in adults (36).

6.2.3 Pharmacodynamics & Pharmacokinetics

6.2.3.1 Pharmacodynamics

CD20 does not internalise upon rituximab binding and is not shed from the cell surface. CD20 does not circulate in the plasma as a free antigen and, thus, does not compete for antibody binding. The Fab domain of rituximab binds to the CD20 antigen on B lymphocytes and the Fc domain can recruit immune effector functions to mediate B cell lysis.

Peripheral B cell counts declined below normal following completion of the first dose of rituximab.

6.2.3.2 Pharmacokinetics

The pharmacokinetics of rituximab administered four times once weekly at a dose of 375 mg/m^2 in patients with granulomatosis with polyangiitis and microscopic polyangiitis are an estimated median terminal elimination half-life of 23 days. Mean clearance and volume of distribution were 0.313 L/day and 4.5 L, respectively. The pharmacokinetic parameters of rituximab in these patients appear similar to what has been observed in rheumatoid arthritis patients. Rituximab was detectable in the serum of patients 3 – 6 months after completion of the last treatment.

7 RATIONALE FOR CURRENT TRIAL

Contemporary treatment for acute AMR is heterogeneous, and includes plasma exchange (PEX), intravenous immunoglobulin (IVIg), glucocorticoids, anti-thymocyte globulin (ATG), rituximab, bortezomib and eculizumab. These treatments are used in differing combinations depending upon local experience, physician preference and resources (22). Clinical practice guidelines published by the British Transplant Society (BTS) and Kidney Disease Improving Global Outcomes (KDIGO) initiative have concluded that firm recommendations of optimal

treatments could not be provided given a lack of evidence (19-21). Treatment for patients presenting with AMR in the UK has been entirely dependent upon the hospital they present to and the clinician they see. This situation is particularly undesirable given the high human and economic cost of treatment failure, and the high side-effect burden and cost most of these treatments confer.

Given the complete lack of evidence to guide treatment of acute AMR and the absence of existing trials of the most promising interventions already in use in UK transplant centres, there is an urgent need to conduct a high quality randomised trial to evaluate efficacy and safety of candidate treatments in the context of the NHS.

We propose a 2-arm open-label randomised controlled trial of SOC (consisting of PEX, glucocorticoids and IVIg) versus SOCR for the treatment of acute AMR across the UK transplant centres.

7.1.1 Definition of and Rationale for Standard of Care (SOC)

A combination of PEX and IVIg is the accepted standard of care for the treatment of acute AMR in the UK (19, 37). Our preliminary work found that 21/23 (91.3%) adult transplant centres use PEX, IVIg and glucocorticoids as standard of care (17). However the exact combination and specifics for each treatment varies considerably across transplant centres. Despite the overall paucity of evidence for AMR management, both plasma exchange and IVIg have been individually shown to be beneficial in treating acute AMR (38, 39).

IVIg showed equivalent efficacy but a better safety profile in a randomised trial of 30 patients with ‘steroid resistant rejection’ (38). IVIg is also effective at reducing HLA antibodies in highly sensitised patients awaiting transplantation, as demonstrated by Jordan *et al.* in a randomised trial of 101 patients (40). Other uncontrolled cohort studies have supported the use of IVIg (18, 19, 37, 41, 42). IVIg has therefore been approved for use in AMR in the UK (42, 43).

PEX (or immuno-adsorption) was assessed in 5 randomised trials, 4 of which predated the Banff classification (25-28). These 4 trials showed conflicting results. The most recent trial by Bohmig and colleagues employed immunoadsorption (IA) in acute AMR, and was terminated early after treatment of only 10 patients due to efficacy in all 5 IA treated patients, and treatment failure in 4/5 control patients (39). On the basis of this evidence, PEX (or IA) is used by all UK transplant centres.

The use of corticosteroids, PEX and IVIg is defined as standard of care for this trial (see section 10.3.1 for more details).

7.1.2 Rationale for Rituximab Plus Standard of Care (SOCR)

Rituximab is currently indicated for the treatment of Non-Hodgkin’s lymphoma, chronic lymphocytic leukaemia, rheumatoid arthritis and granulomatosis with polyangiitis and microscopic polyangiitis (44). Rituximab depletes the number of B cells and provides a rationale for its use in antibody-mediated rejection.

Successful treatment of acute AMR using rituximab in addition to PEX and IVIg has been shown in several cohort and case-controlled studies.

In the first of two notable case-controlled studies, Lefaucheur *et al.* compared 12 patients who received rituximab, PEX and IVIg with a control group of 12 patients who received high dose IVIg alone (45). The 3-year allograft survival in the rituximab group was 91.7%, compared with 50% in the IVIg arm. The authors also noted that the DSA levels were lower in the rituximab arm at 3 months, and that persistent DSA were associated with inferior graft survival (45). In the second study, Kaposztas *et al.* compared 26 patients who received rituximab plus PEX for acute AMR, against 28 patients who received PEX without rituximab (46). Both groups received IVIg depending on level of serum immunoglobulins. The 2-year allograft survival was reported as 90% in the rituximab group, and only 60% in the PEX alone group (46). In an uncontrolled cohort study, Becker *et al.* reported successful use of rituximab to treat refractory AMR in 27 patients (47). Only 3 of the grafts were lost subsequently, and allograft survival at 2 years was 85%. At 1-year follow-up, one graft was lost in each arm, however, function was superior and there was less evidence of ongoing antibody injury in the histological samples in the rituximab arm. Further benefits of rituximab use in terms of survival, histology or function have been shown in other small studies (48-50).

More recently, rituximab has been utilised in a multicentre, double-blind, placebo controlled RCT which compared the use of rituximab in addition to PEX and IVIg in 40 patients (29). This trial had serious limitations. Only 19 patients in each arm were included in the primary endpoint analysis; the primary outcome was graft loss or absence of improvement in function at day 12, and total follow-up was only 12 months. The primary endpoint was no different between trial arms, but no grafts in either group had failed after 12 days. There was no difference in graft function and survival after 12 months, but this was confounded by a high drop-in rate: 8/19 control arm patients received rituximab.

Despite the fact that the safety and efficacy of rituximab remains untested in adequately powered randomised trials, it has been used by 61% of UK renal transplant units for the treatment of acute AMR.

7.1.2.1 Rationale for Rituximab Use in Paediatric Participants

In a paediatric cohort, Zarkhin *et al.* reported the use of rituximab in addition to ATG and corticosteroids in 10 children, and compared the outcomes with another 10 cases treated with ATG and corticosteroids alone (34). Paediatric renal transplant recipients receive the equivalent treatment for antibody-mediated rejection as adults, with a dose adjustment made for body surface area.

8 TRIAL DESIGN

8.1 STATEMENT OF DESIGN

This is a phase 3, open-label, two-arm, randomised, controlled trial designed to evaluate the safety and efficacy of adding rituximab to standard of care (consisting of plasma exchange, intravenous immunoglobulins and corticosteroids) in treating acute antibody-mediated rejection (AMR) in kidney transplantation patients.

8.2 NUMBER OF CENTRES

This is a multi-centre trial and will take place at approximately 24 UK transplant centres.

8.3 NUMBER OF PARTICIPANTS

A total of approximately 170 participants with a new diagnosis of acute AMR following their renal transplant will be randomised in a 1:1 ratio to either SOC or SOCR.

8.3.1 Paediatric Participants

Paediatric patients 5 years old and older will be eligible to participate.

8.4 PARTICIPANTS' TRIAL DURATION

Trial duration will consist of up to 2 weeks for screening, up to a total of 5 weeks of treatment (including rituximab treatment, up to 3 weeks for SOC treatment) and a minimum of 48 months follow-up period. Eligible patients will be permitted to enter the trial up to 2 weeks post-biopsy to allow for diagnosis of AMR confirmation and as long as PEX treatment has not been completed.

8.5 TRIAL OBJECTIVES

8.5.1 Primary Objective

To assess the effectiveness of rituximab in addition to SOC, compared to SOC alone, in improving 4-year transplant allograft survival.

8.5.2 Secondary Objectives

1. To assess the efficacy of rituximab in addition to SOC, compared with SOC alone on allograft function in terms of serum creatinine, estimated glomerular filtration rate (eGFR) (CKD-EPI) and proteinuria (urinary protein:creatinine ratio (UPCR)), at 1, 3, 6, 12 months post-randomisation and then annually until 4 years
2. To assess the effect of rituximab in addition to SOC, compared with SOC alone on donor specific antibody (DSA) levels at 3 and 12 months post-randomisation
3. To assess the safety of rituximab in addition to SOC in the treatment of acute AMR. This will be determined by adverse event reporting
4. To assess the impact of SOCR versus SOC alone on quality of life (QoL)

5. To perform an economic evaluation and value of information analysis of rituximab in addition to SOC in the treatment of acute AMR. The economic evaluation will report both within-trial and decision model based incremental cost per quality-adjusted life year gained from the perspective of the NHS.

8.6 TRIAL OUTCOME MEASURES

8.6.1 Primary Outcome Measure

1. The primary outcome measure is allograft survival, where allograft failure is defined as the duration from the date of randomisation to the date of:
    a. eGFR measurement ≤15 mL/min/1.73 m^2 where:
        i. the eGFR measurement is not due to an acute reversible cause, as determined by the PI
        ii. a follow-up consecutive eGFR measurement ≤15 mL/min/1.73 m^2 is recorded (where the first date is recorded as the date of failure)
    b. renal replacement therapy (date of starting maintenance dialysis dependency, retransplantation, etc.)

    whichever occurs first.

8.6.2 Secondary Outcome Measure

1. Allograft function measured by serum creatinine, eGFR (CKD-EPI) and proteinuria (UPCR) at 1, 3, 6, and 12 months post-randomisation and then annually until 4 years
2. DSA positivity, number of DSA and mean fluorescence index (immunodominant)
3. Safety, determined by adverse event reporting
4. Health-related quality of life, assessed by EQ-5D-5L/EQ-5D-Y questionnaire
5. Economic analysis of cost per quality-adjusted life year gained from the perspective of the NHS

9 SELECTION AND WITHDRAWAL OF PARTICIPANTS

9.1 INCLUSION CRITERIA

- Willing and able to give written informed consent by patient aged 16 years and over; or by a parent or legal guardian for patients who are under 16 years old
- 5 years old or older

- A diagnosis of acute AMR as defined by:
    - The presence of ≥1 donor specific antibodies (DSA)
    - An adequate renal transplant biopsy with histological features consistent with active AMR with no evidence of chronicity as defined by the Banff histological classification of allograft pathology (1):
        - If C4d positive (2 or 3):
            - v score ≥1 and/or
            - g score ≥1 and/or
            - thrombotic microangiopathy and/or
            - ptc score ≥1
            - or if co-existent cellular rejection, a g score of ≥1

        OR

        - If C4d negative (0 or 1):
            - microcirculation inflammatory score (g + ptc) ≥2
            - or if co-existing cellular rejection, a g score ≥1 and (g + ptc) ≥2

        AND

            - Chronic glomerulopathy (cg) score 0 or 1a
            - Tubulo-interstitial fibrosis <50% and glomerular obsolescence <50%

9.2 EXCLUSION CRITERIA

- Patients who have received an ABO incompatible transplant
- Patients who have received rituximab as part of induction or post-transplant for any other indications (e.g. recurrent focal and segmental glomerular sclerosis)
- Patients who have completed PEX treatment prior to the index biopsy on the suspicion of acute AMR in the absence of histology
- Have active infection including bacterial, viral (including CMV and EBV), fungal or tuberculosis, which in the investigator's opinion could affect the conduct of the trial
- Co-existing BK nephropathy
- Patients with hepatitis B (patients with prior exposure to hepatitis B may be enrolled at the discretion of the PI)
- Have active hepatitis C (patients may be included if a negative hepatitis C recombinant immunoblot assay is confirmed or have a negative hepatitis C virus RNA [qualitative] test)

- Have human immunodeficiency virus (HIV)
- Active malignancy which would pose a contraindication to any of the trial interventions
- Patients with known allergy, intolerance or contraindication to treatments in the standard of care arm or rituximab as outlined in the Summaries of Product Characteristics (SmPCs)
- Clinically significant comorbidity
- Females must be either post-menopausal for at least 1 year, surgically sterile or, if of child-bearing potential, must not be pregnant or lactating. If sexually active, female participants must agree to use an acceptable method of birth control for 12 months post treatment with rituximab. Female participants must also agree not to breastfeed for 12 months post treatment with rituximab.

9.3 HISTOLOGICAL AND DSA CRITERIA

9.3.1 DSA

A detailed report on the HLA types of the donor and recipient, together with the degree of HLA sensitisation pre-transplant will be required at the screening/baseline visit and recorded in the 'Histocompatibility & Immunogenetics' Form. A clinical history of sensitising events will be recorded at the screening/baseline visit.

9.3.1.1 Definition of DSA positivity

DSA against HLA -A, -B, -Cw, -DRB1, -DRB3/4/5, -DQA, -DQB1, DPA or -DPB antigens are to be included.

Method of detection should be determined by local protocols.

Antibody levels determined to be positive should be determined by local policies.

5 mL of the index serum sample should be sent to the tissue biobank at Imperial College Healthcare NHS Trust, for central processing.

9.3.1.2 Post treatment, 3- and 12- month samples

Research serum samples taken post treatment, at 3 and at 12 months post-randomisation should be sent to the tissue biobank at Imperial College Healthcare NHS Trust, for central processing. Local processing of these samples can be performed at the discretion of the local units, depending on clinical need.

Samples should be sent for central processing at a time which is convenient with the local research site.

9.3.2 Histology

9.3.2.1 Inclusion biopsy

All patients should have acute AMR diagnosed by biopsy as an inclusion criterion to the trial. The histological inclusion and exclusion criteria are described in section 9.1 INCLUSION CRITERIA and APPENDIX 1.

9.3.2.2 Biopsies performed for clinical indications

Data on all biopsies performed subsequently to the inclusion biopsy should be collected, with appropriate filling of the histology case report forms.

9.3.3 Central Review of Biopsies

All biopsies will have central review for detailed Banff scoring to assess the severity of antibody-mediated rejection and biopsy material will be scanned to form part of the trial documentation. The process for this is as follows:

- Initial biopsy analysis that will inform eligibility is part of routine NHS care at each local centre
- Light microscopic examination and a C4d immunostain (by either immunoperoxidase or immunofluorescence, focal and diffuse positivity counts as positive) are necessary to determine eligibility
- The local pathologist will confirm eligibility by filling out the Enrolment Biopsy Form, and will sign out the biopsy report as usual
- Once sign out is complete, the biopsy material will be sent for central review by the Lead Pathologist for the trial. The central pathologist will perform full Banff scoring and the slides used to determine eligibility may be scanned with the images stored for trial monitoring. All material will then be returned to the referring site.
- If pathology scanning facilities are available, biopsy slides may be scanned rather than sent.

If for any reason, any additional renal biopsy is performed on patients enrolled in the trial (e.g. for a clinical indication), then every effort will be made to send these biopsy samples and results to the central pathology lab for scoring and scanning.

All material should be LINK-ANONYMISED prior to sending and identified by the participant's trial ID and partial date of birth.

9.4 TREATMENT ASSIGNMENT AND RANDOMISATION NUMBER

Randomisation will be carried out centrally using a central randomisation system coordinated by the Cambridge Clinical Trials Unit.

Randomisation will be stratified by age and baseline eGFR. Following the confirmation of eligibility, eligible participants will be randomised to either the standard of care arm or the standard care plus rituximab arm in a 1:1 ratio using a random block method. Randomisation will take place following receipt of the screening results.

At the site initiation, the trial coordinator will arrange for the members of the trial team delegated to randomise participants to be provided with a unique system username and password. This will allow them to access the central randomisation system. The trial coordinator will also train site staff in how to access and use the randomisation system.

In order to enrol a participant, data will be entered onto the central randomisation system via an internet-based interface. The following data will be required in order to randomise a participant:

- Confirmation that the participant satisfies all of the eligibility criteria
- Participant's date of birth
- eGFR measurement

A treatment arm will be allocated and a unique trial ID number will be assigned by the randomisation system for the participant.

If the central randomisation system is unavailable for any reason, a paper-based randomisation system is in place as a back-up. See Section 9.4.1 for further details.

9.4.1 Paper-based Randomisation System

If the central randomisation system is unavailable for use, participating site staff should contact the trial coordination team who will perform randomisation using the back-up paper-based system; details are given in the trial's Paper Randomisation Manual. Following randomisation, the participating site will be informed of the participant's trial treatment arm and trial ID by a TAR:GET-1 Randomisation Notification Email issued by the coordination team; this email will be sent to all relevant members of the participating site team, the trial CI, the lead pathologist and the CCTU team.

9.5 PARTICIPANT WITHDRAWAL CRITERIA

In accordance with GCP, a participant has the right to withdraw from the trial at any time and for any reason, without prejudice to his or her future medical care, and is not obliged to give his or her reasons for doing so. Any information already provided or results from tests already performed will continue to be used in this trial. Unless the participant requests destruction of the stored trial samples, research samples collected will be used according to initial consent. On participant withdrawal from the trial, permission to use future data collected as part of their routine care by their usual doctor will be sought.

9.5.1 Withdrawal from Trial Treatment

The Research Doctor or Principal Investigator may choose to withdraw a participant from the trial treatment. This may be because of any of the following reasons:

- The participant experiences a serious side effect, which may require different treatment or observation
- The Research Doctor/Principal Investigator feels it is in the best interest of the participant's health and welfare to discontinue
- Participant failure to comply with the trial requirements
- Participant becomes pregnant*
- The Sponsor or regulatory authorities stop the trial

If a participant experiences any serious side effects during the course of the trial which has led to their withdrawal from the trial, the local research team will follow up the progress of the side effect until it has stabilised or resolved.

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*Only participants who are diagnosed as pregnant prior to completion of the trial intervention i.e. rituximab treatment, and for 12 months post completion of rituximab treatment will be required to withdraw from the trial. Pregnancies occurring after 12 months post-rituximab treatment will not require withdrawal from the trial.

10 TRIAL TREATMENTS

10.1 TREATMENT PERMISSIBLE PRE-RANDOMISATION

Recruitment will be permitted in patients who meet the inclusion and exclusion criteria at any point until 2 weeks after the biopsy required for AMR diagnosis. It is foreseen that treatment as part of routine care may often be required to start prior to full information being available on inclusion criteria. Treatment with methylprednisolone and PEX for the indication of treating suspected acute AMR prior to biopsy is permissible, as long as PEX treatment has not completed. Participants will need to be randomised prior to the last PEX treatment. A summary of permissible treatments is described in APPENDIX 3 and APPENDIX 4.

10.2 TREATMENT SCHEDULES

Treatment schedules and doses are flexible to allow for logistical practicalities and patient preference, according to local practice, but it must be specified in advance prior to randomisation. A summary of treatment doses is described in APPENDIX 3 and examples of the permissible schedules are shown in APPENDIX 4.

10.3 TREATMENT ARMS

10.3.1 Standard of Care

10.3.1.1 Plasma exchange

All participants will receive exactly seven plasma exchange (PEX) treatments. PEX schedules are flexible, but they should be completed within a maximum period of 3 weeks. Some example schedules as guides can be seen in APPENDIX 4. Replacement solution will be based on weight at 60 mL/kg or a maximum volume exchange of 4 litres. Replacement solution should be given as per local practice. The use of fresh frozen plasma (FFP) is recommended for the first exchange and is encouraged thereafter over crystalloid or albumin to reduce the risk of bleeding post biopsy. Anticoagulation may be provided by citrate or by heparin citrate to reduce the risk of bleeding post biopsy.

The following parameters may be determined according to local practice:

- PEX may be performed by centrifugation or filter separation technique
- PEX may be performed via a temporary or semi-permanent central venous catheter or an arteriovenous fistula if patient already has established venous access
- Monitoring of coagulation parameters, FBC, bone profile (for calcium levels as citrate is being utilised) and serum immunoglobulin levels
- PEX dose may be reduced for PEX-related complications according to local best medical practice and indication and dose alteration noted for future analysis

Any PEX treatments considered outside of the treatment protocol should be discussed with the Chief Investigator and the details must be recorded in the participant's trial case report form.

10.3.1.1.1 Plasma exchange for paediatric participants

Paediatric participants will require dose adjustments based on size. PEX will consist of exchange volumes the equivalent of 1 – 1.5 plasma volumes.

10.3.1.2 Intravenous Immunoglobulin

Intravenous immunoglobulin should be used in accordance with local practice and availability. Infusion rates should be given as specified in the product's Summary of Product Characteristics (SmPC). The dose of IVIg is expected to be either 100 mg/kg (700 mg/kg in total) or a total of 2 g/kg (which can be administered in divided doses), according to local procedures. The schedule of IVIg administration should be timed appropriately with PEX sessions. Example schedules can be seen in APPENDIX 4.

10.3.1.2.1 Intravenous Immunoglobulin for paediatric participants

No dose adjustment is required for paediatric participants.

10.3.1.3 Corticosteroids

Participants who have not received methylprednisolone prior to recruitment should receive 3 doses of 500 mg methylprednisolone on days 1, 2 and 3. Following this, participants receiving maintenance oral corticosteroids should receive 30 mg once daily. Steroid naïve patients should have corticosteroids introduced at 30 mg once daily. Thereafter, the dose of oral prednisolone should be tapered to 10 mg per day, or according to local guidelines, by 3 months (please see Table 1 for an example of dose adjustments).

Table 1 – Schedule of prednisolone dose adjustment

Time	Dose of prednisolone per day (mg)
Day 1 to 14	30
Day 15 to 28	25
Week 5 to Week 8	20
Week 9 to Week 11	15
≥ Week 12	10 (may be reduced further to a minimum of 5mg a day)

10.3.1.3.1 Corticosteroids for paediatric participants

Paediatric participants will require dose adjustments of corticosteroids based on size. Doses are not to exceed adult doses. Methylprednisolone will be given at 600 mg/m^2 for 3 days, followed by oral prednisolone at a dose of 60 mg/m^2 per day, weaning to 10 mg/m^2 per day, or according to local guidelines, by 3 months.

10.3.2 Rituximab

10.3.2.1 Legal status

Rituximab (MabThera®) was approved by the FDA in 1997 and the EMA in 1998 for the treatment of adults with relapsed or refractory low-grade or follicular, B-cell non-Hodgkin's lymphoma. It has since received approval for the treatment of chronic lymphocytic leukaemia, rheumatoid arthritis and granulomatosis with polyangiitis and microscopic polyangiitis. It has been used off-label for the treatment of autoimmune diseases, including Systemic Lupus Erythematosus and vasculitis.

Any of the approved biosimilars of rituximab are permitted.

10.3.2.2 Supply

Rituximab is a licensed drug and will be dispensed and labelled through the local hospital pharmacies.

10.3.2.3 Packaging, labelling and storage conditions

Rituximab must be stored as per labelled direction. The container must be kept in the outer carton, in order to protect from light and stored in a refrigerator (2 – 8 °C).

10.3.2.4 Dosage and administration

Preparation and administration should be performed as described in the SmPC and adhere to local guidelines. Rituximab treatment will consist of two intravenous infusions, given at a dose of 375 mg/m^2, to a maximum dose of 1 g (see APPENDIX 3). The first dose of rituximab should be administered within 7 days of the last plasma exchange, followed by a second 375 mg/m^2, to a maximum of 1 g, intravenous infusion 14 days (± 2 days) following the first dose (see APPENDIX 2 and APPENDIX 3).

375 mg/m^2 and 1 g are the two standard regimens of rituximab dosing. 375 mg/m^2 is usually given as 4 weekly doses, based on its use in haematology patients, whilst 1 g rituximab is given as a fixed dose 2 weeks apart in rheumatoid arthritis patients (51). As discussed in section 7.1.2, 375 mg/m^2 was chosen based on the trial by Lefaucheur *et al*.(45). The amount of rituximab received by each participant is the same, depending on body size. The dose is limited to a maximum of 1 g to ensure that no participants will receive more than the standard accepted dose. It is extremely unlikely that any participant would require more than 1 g based on body surface area, as 1 g given at 375 mg/m^2 would require a BSA of 2.66 m^2, which is extreme.

The preparation and administration of rituximab is described in the SmPC, Pharmacy Manual and Trial Manual.

No dose modifications are recommended.

Rituximab administration requires premedication of the following recommended medications:

1. Methylprednisolone/hydrocortisone, according to local guidelines
2. Anti-histamine, according to local guidelines
3. Paracetamol, according to local guidelines

10.3.2.5 Dosage and administration for paediatric participants

Paediatric participants will receive two intravenous infusions of rituximab at 375 mg/m^2, to a maximum dose of 1 g. As the dose is calculated per body surface area, paediatric participants will receive a reduced total of dose of rituximab compared to adult participants. 375 mg/m^2 rituximab has been used in the majority of paediatric patients (36).

10.3.2.6 Known drug reactions

Rituximab's main adverse effects are described in the MabThera® SmPC (section 4.4) and in section 10.5.5 of the protocol.

Very Common (more than 1 in 10 people are affected)

- Infusion-related reactions (occurs in the majority of patients during the first infusion). The incidence of infusion-related symptoms decreases substantially with subsequent infusions. Infectious events (predominantly bacterial and viral) occur in approximately 30 – 55% of patients, severe bronchitis
- Angioedema
- Neutropenia, leucopenia, severe febrile neutropenia, severe thrombocytopenia
- Nausea
- Pruritus, rash
- Severe alopecia
- Headache, asthenia, fever, chills
- Decreased IgG levels

Common (between 1 in 10 and 1 in 100 people are affected)

- Sepsis (pneumonia, febrile infection, herpes zoster, respiratory tract infection, fungal infections, infections of unknown aetiology)
- Acute bronchitis, sinusitis
- Hepatitis B (including reactivation)
- Anaemia
- Severe pancytopenia, severe granulocytopenia
- Hypersensitivity
- Hyperglycaemia
- Weight decrease
- Peripheral oedema, face oedema
- Increased LDH (lactate dehydrogenase)
- Hypocalcaemia
- Paraesthesia, hypoesthesia
- Agitation, anxiety
- Insomnia
- Vasodilatation, dizziness
- Lacrimation disorder, conjunctivitis

- Tinnitus, ear pain
- Cardiac disorders (severe myocardial infarction, arrhythmia, severe atrial fibrillation, tachycardia, severe cardiac disorder)
- Hypertension, orthostatic hypotension, hypotension
- Bronchospasm, respiratory disease, chest pain, dyspnoea, increased cough, rhinitis
- Gastrointestinal disorders (vomiting, dysphagia, stomatitis, constipation, dyspepsia, diarrhoea, anorexia, throat irritation, abdominal pain)
- Urticaria, severe skin disorder
- Musculoskeletal disorders (hypertonia, myalgia, arthralgia, back pain, neck pain, pain)
- Tumour pain
- Flushing, malaise, cold syndrome, severe fatigue, severe shivering, sweating, night sweats
- Multi-organ failure

Uncommon (between 1 in 100 and 1 in 1000 people are affected)

- Coagulation disorders
- Aplastic anaemia, haemolytic anaemia
- Lymphadenopathy
- Depression
- Nervousness
- Dysgeusia
- Severe cardiac disorders (left ventricular failure, supraventricular tachycardia, ventricular tachycardia, angina, myocardial ischaemia, bradycardia)
- Asthma, bronchiolitis obliterans, lung disorder, hypoxia
- Abdominal enlargement
- Infusion site pain

Rare (between 1 in 1000 and 1 in 10000 people are affected)

- Serious viral infection, Pneumocystis jirovecii
- Severe cardiac disorders
- Anaphylaxis
- Interstitial lung disease

Very Rare (fewer than 1 in 10000 people are affected)

- Progressive multifocal leukoencephalopathy (PML)
- Transient increase in serum IgM levels
- Tumour lysis syndrome
- Cytokine release syndrome
- Serum sickness
- Peripheral neuropathy
- Facial nerve palsy
- Severe vision loss
- Heart failure
- Vasculitis, leukocytoclastic vasculitis
- Respiratory failure
- Gastro-intestinal perforation
- Severe bullous skin reactions, Stevens-Johnson syndrome, toxic epidermal necrolysis
- Renal failure

Not Known (frequency could not be estimated, identified only during post-marketing surveillance)

- Late neutropenia
- Infusion-related acute reversible thrombocytopenia
- Cranial neuropathy
- Hearing loss, loss of other senses
- Lung infiltration

10.3.2.7 Procedures for monitoring treatment compliance

Participants will be closely monitored during and after infusion for any reactions to the infusion, as per local hospital procedures.

10.4 OPTIMISATION OF BASELINE IMMUNOSUPPRESSION

All participants should have their baseline immunosuppression changed to tacrolimus and mycophenolate mofetil (MMF) (or mycophenolate sodium). Alternative anti-proliferative agents e.g. azathioprine are accepted for participants intolerant of MMF, based on local guidelines. It is expected that the majority of participants will already be taking these agents, in which case their dose will need to be optimised in order to achieve optimal drug levels in accordance with local practice. Any preparation of these agents, branded or generic, immediate release or extended release is acceptable.

For patients not already taking MMF or tacrolimus, starting doses should be line with local protocols. Protocol dosing of MMF for managing neutropenia or other adverse events should also follow local guidelines.

10.5 KNOWN POTENTIAL RISKS OF INTERVENTIONS

10.5.1 Plasma Exchange

Recognised complications of plasma exchange are:

- Bleeding, due to removal of coagulation factors. This can be minimised by using FFP.
- Respiratory complications, including acute respiratory distress syndrome and non-cardiogenic pulmonary oedema
- cardiac complications, including arrhythmias and hypotension

Both respiratory and cardiac complications are more common with plasma replacement than albumin replacement fluids. Hypocalcaemia may occur with the use of citrate anti-coagulation and calcium replacement should be administered in accordance with local protocol.

10.5.2 Intravenous Immunoglobulin

Patients must be closely monitored and carefully observed for any symptoms throughout the infusion period. Adverse reactions may occur more frequently if the infusion rate is high or when a patient is receiving human immunoglobulin for the first time. Preparation and infusion should follow the instructions as set out in the SmPC.

There is an association between IVIg administration and thromboembolic events which may be related to a relative increase in blood viscosity. Other reported adverse events include:

- aseptic meningitis syndrome
- haemolytic anaemia
- transfusion related acute lung injury (TRALI)
- transmission of infections
- circulatory overload

10.5.3 Corticosteroids

Recognised adverse effects of corticosteroids include:

- behavioural
    - irritability
    - sleep disturbance

    - anxiety
- weight gain
- dyslipidaemia
- glucose intolerance
- increased susceptibility to infections

10.5.4 Baseline Immunosuppression

Patients with receiving immunosuppressive medications will be at an increased risk of infection.

10.5.4.1 Mycophenolate mofetil and pregnancy

Mycophenolate mofetil (MMF) use during pregnancy is associated with an increased risk of malformations and first-trimester pregnancy loss. There is a theoretical risk to the babies of male patients taking MMF at the time of conception; however male participants do not need to use contraception for this purpose. Female participants who are planning pregnancy should arrange with their local clinician for alternative anti-rejection immunosuppression.

10.5.5 Rituximab

Infusion related reactions have been reported to occur in 50% of patients receiving rituximab but severe reactions only occurred in <1%. Premedication with corticosteroids reduces the incidence of infusion reactions, which is also dependent upon rate of infusion. Infusion reactions will not be included in safety comparisons unless they are serious adverse reactions.

Patients who develop evidence of severe reactions, especially hypotension, angioedema, stridor, or fall in oxygen saturations should have the infusion interrupted immediately and where necessary appropriate immediate support given (e.g. IV chlorpheniramine, hydrocortisone and where required adrenaline as per protocol for severe adverse reactions). Patients should then be evaluated by the trial physician or delegate for evidence of an acute allergic reaction. In all patients, the infusion should not be restarted until complete resolution of all symptoms. At this time, the infusion can be initially resumed at not more than one-half the previous rate. If the same severe adverse reactions occur for a second time, the decision to stop the treatment should be made. Mild or moderate infusion-related reactions, usually respond to a reduction in the rate of infusion. The infusion rate may be increased upon improvement of symptoms. All dose changes (including dose reductions or temporary stopping of the drug) should be recorded on the relevant forms.

The most notable adverse effect of rituximab is infections, some of which may be serious (including reactivation of hepatitis and extremely rare cases of progressive multifocal leukoencephalopathy caused by JC virus (44)). Hepatitis B virus (HBV) screening should be performed in all patients before recruitment, if status not already known. Patients with active hepatitis B disease are not eligible for the trial. Patients who have prior exposure to hepatitis

B may be enrolled at the discretion of the PI. Abnormalities in blood counts including neutropenia and thrombocytopenia have been reported in clinical trials in patients treated with rituximab. Other adverse effects reported include gastrointestinal, peripheral oedema, hypertension, cardiac and dermatological (44) (see 10.3.2.6).

10.5.5.1 Rituximab and paediatric participants

There have been case reports of hypogammaglobulinaemia in paediatric patients following treatment with MabThera® (51) (section 4.8 of the SmPC). Long-term substitution therapy with immunoglobulin has been required. The consequences of long term B cell depletion in paediatric patients are unknown.

10.5.5.2 Rituximab and pregnancy

Rituximab has a long retention time in B cell depleted patients, women of childbearing potential should use effective contraceptive methods during and for 12 months following treatment with rituximab.

IgG is known to cross the placental barrier. Transient B-cell depletion and lymphocytopenia have been reported in some infants born to mothers exposed to rituximab during pregnancy. Similar effects have been observed in animals (section 5.8 of the SmPC). Rituximab should not be administered to pregnant women unless the possible benefit outweighs the potential risk.

It is unknown whether rituximab is excreted in human milk. However, IgG is known to be excreted so women should not breastfeed whilst undergoing rituximab treatment and for the following 12 months.

Animal studies did not reveal deleterious effects of rituximab on reproductive organs.

There is no risk to the children of male participants conceived whilst undergoing rituximab treatment.

10.6 PREMEDICATION

10.6.1 Plasma Exchange

It is suggested that participants should be pre-medicated according to local protocols prior to PEX treatment.

10.7 CONCOMITANT THERAPIES

10.7.1 Prophylactic Medications

- All participants should receive a proton pump inhibitor (PPI) to cover at least the first 3 months of steroid administration

- All participants should receive prophylaxis against pneumocystis pneumoniae (PCP), which should include either co-trimoxazole or pentamidine nebulisers, as per local protocol
- All participants should either receive prophylaxis against CMV infection or have active monitoring for the development of CMV viraemia, as per local guidelines
- All participants should receive prophylaxis against oral fungal infection, as per local protocol
- All participants should receive prophylaxis against tuberculosis, as per local protocols
- Patients should receive erythropoietin therapy, as necessary, in order to minimise the risk of blood transfusion requirement during the early treatment period.

10.8 RESTRICTED MEDICATION AND INTERACTION WITH OTHER DRUGS

Management of all patients remains the responsibility of the doctors responsible for patient care. Patients should be managed as necessary for any complications, with no restriction imposed by the trial protocol.

If the responsible doctor feels any patient would benefit from an alternative anti-rejection therapy not included in the protocol (e.g. ATG), then this should be administered and treatment failure will be recorded for the purposes of the trial.

The CI should be informed as soon as possible of any deviations from the trial protocol.

There is no prohibited use of medications associated with the investigational medicinal product, rituximab.

11 TRIAL PROCEDURES, ASSESSMENTS AND SCHEDULE

A summary of the trial visits and assessments to be performed is shown in APPENDIX 2.

11.1 PARTICIPANT IDENTIFICATION

Participants will be identified by their local transplant team, following suspected diagnosis of acute AMR and biopsy. Potential participants will be asked if they are interested in TAR:GET and be given the participant information sheet (PIS). All potential participants will have the opportunity to ask questions of a trial physician or nurse and will be given adequate time to consider consenting to participate. Should a participant require a verbal translation of the trial documentation by a locally approved interpreter/translator, it is the responsibility of the individual investigator to use locally approved translators.

11.2 INFORMED CONSENT

The Informed Consent form used must be approved by the REC and must be in compliance with GCP, local regulatory requirements and legal requirements. The investigator or designee must ensure that each trial participant, or his/her legally acceptable representative, is fully informed about the nature and objectives of the trial and possible risks associated with their participation.

Written informed consent will be sought from all potential eligible participants or the parents/legal guardians of participants <16 years old before any trial-specific activity is performed. Written informed assent will also be sought from participants aged 5 – 15 years old. The informed consent and assent forms used for this trial and any changes made during the course of this trial must be prospectively approved by the REC. The principal investigator will retain the original of each participant's signed informed consent form (and assent form, if applicable).

Patients who do not consent to participating will receive acute AMR treatment as per local guidelines.

Any new information which becomes available, which might affect the participant's willingness to continue participating in the trial will be communicated to the participant as soon as possible.

Documentation of why patients do not agree to participate will be recorded and subsequently analysed.

TAR:GET-1 participants will be asked to give their consent to provide optional blood samples for lymphocyte subset and B-cell marker analysis at the baseline and 3-, 6- and 12-months post-randomisation visits (see sections 11.5 and 11.6 and APPENDIX 2). Participants who choose not to provide the optional blood samples are still eligible for the main trial.

11.3 DEFINITION OF TRIAL ASSESSMENTS

11.3.1 Medical History

Demographic data and list of co-morbidities will be documented during the baseline visit (visit 1). Any new relevant medical conditions should be documented as adverse events in subsequent visits, as scheduled in Table 3 (APPENDIX 2).

11.3.2 Physical Examination

A physical examination should take place at the screening visit and at visits scheduled in the trial visits and procedures table (Table 3), as clinically indicated (i.e. abnormal laboratory results or relevant symptomatology). A physical examination may include evaluation of the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. The participant's height and weight will also be recorded.

11.3.3 Laboratory Results

The following laboratory variables will be reported:

- serum creatinine
- eGFR
- FBC

- haemoglobulin
- platelet count
- total white cell count, including:
  - neutrophil count
  - leucocyte count

Other laboratory results will be collected as relevant and documented in the participant's case report form.

11.4 SCREENING

Trial specific assessments will only be conducted after participants have given written informed consent. Potential participants will undergo a screening evaluation within 2 weeks of the suspected AMR diagnosis, by a trial physician or qualified trial nurse. Participants must meet all of the inclusion criteria and none of the exclusion criteria.

The screening visit requires documentation of:

- DSA positivity
- Histology
- Serum creatinine
- eGFR
- Pregnancy test (where applicable)
- Screening tests for hepatitis B, hepatitis C and HIV. Status may already be known from testing at time of transplantation. If the participant's risk of exposure has changed since transplantation, these tests should be repeated.

11.5 BASELINE, VISIT 1

The baseline visit can occur at the same time as the screening visit, but must be before the 7th PEX treatment, in order for the baseline visit to be completed before randomisation. All participants will have a full medical history taken, a clinical examination, laboratory data recorded and will complete a self-reported questionnaire. This will include:

- Medical history, which will include:
  - Transplant history (allograft number, date of transplant, type of transplant (deceased donor, living donor), induction treatment)
  - Sensitisation history (sensitised pre-transplant, history of blood transfusions)
  - Pregnancy history (where applicable)
  - Medications
- Biochemistry, which will include serum creatinine and eGFR*
- Urinary protein:creatinine ratio (UPCR)
- DSA data (HLA class, MFI for each DSA present)

- Serum immunoglobulin level (optional for patients randomised to SOC)
- Lymphocyte subsets (optional for patients randomised to SOC)
- B-cell markers (optional)
- Histocompatibility & Immunogenetics form
- Plasma exchange & IVIg form
- Patient self-reported EQ-5D-5L questionnaire
    - Paediatric participants will complete the age-adjusted EQ-5D-Y questionnaire and their parent/legal guardian will complete the EQ-5D-Y proxy questionnaire

*Biochemistry tests include FBC, liver function, bone profile and C-reactive protein (CRP)

11.5.1 Randomisation

Participants will be randomised after the screening results are received and eligibility confirmed. Participants must be randomised before receiving the 7th PEX treatment.

11.6 TRIAL TREATMENT AND FOLLOW-UP

APPENDIX 2 describes the trial assessments that occur at each research visit.

11.6.1 Timing of Assessments

Please see Figure 1 and APPENDIX 2 for the schedule of assessments.

11.6.2 Rituximab Treatment

Participants randomised to the SOCR arm of the trial will receive two intravenous infusions of rituximab, as described in section 10.3.2.4)

11.6.3 Visit 2 – 4 Weeks Post-Randomisation (±1 week)

- Plasma exchange & IVIg Form
- Adverse event reporting
- Basic laboratory data (as described in section 11.3.3)
- Serum Immunoglobulin (optional for patients randomised to SOC)
- UPCR

11.6.4 Visit 3 – 3 Months Post-Randomisation (±2 weeks)

- Adverse event reporting
- Concomitant medications
- Basic laboratory data (as described in section 11.3.3)
- DSA
- Lymphocyte subsets (optional for patients randomised to SOC)
- B-cell markers (optional)
- UPCR
- Participant self-reported EQ-5D-5L questionnaire
    - Paediatric participants will complete the age-adjusted EQ-5D-Y questionnaire and their parent/legal guardian will complete the EQ-5D-Y proxy questionnaire

11.6.5 Visit 4 – 6 Months Post-Randomisation (±3 weeks)

- Adverse event reporting
- Concomitant medications
- Basic laboratory data (as described in section 11.3.3)
- B-cell markers (optional)
- UPCR

11.6.6 Visit 5 – 1 Year Post-Randomisation (±3 weeks)

- Adverse event reporting
- Concomitant medications
- Basic laboratory data (as described in section 11.3.3)
- DSA level
- Lymphocyte subsets (optional for patients randomised to SOC)
- B-cell markers (optional)
- UPCR
- Participant self-reported EQ-5D-5L questionnaire
    - Paediatric participants will complete the age-adjusted EQ-5D-Y questionnaire and their parent/legal guardian will complete the EQ-5D-Y proxy questionnaire

11.6.7 Visit 6 – 2 Years Post-Randomisation (±6 weeks)

- Adverse event reporting
- Concomitant medications
- Basic laboratory data (as described in section 11.3.3)
- UPCR
- Participant self-reported EQ-5D-5L questionnaire
    - Paediatric participants will complete the age-adjusted EQ-5D-Y questionnaire and their parent/legal guardian will complete the EQ-5D-Y proxy questionnaire

11.6.8 Visit 7 – 3 Years Post-Randomisation (±8 weeks)

- Adverse event reporting
- Concomitant medications
- Basic laboratory data (as described in section 11.3.3)
- UPCR
- Participant self-reported EQ-5D-5L questionnaire
    - Paediatric participants will complete the age-adjusted EQ-5D-Y questionnaire and their parent/legal guardian will complete the EQ-5D-Y proxy questionnaire

11.6.9 Visit 8 – 4 Years Post-Randomisation (±8 weeks)

- Adverse event reporting
- Concomitant medications
- Basic laboratory data (as described in section 11.3.3)
- UPCR
- Participant self-reported EQ-5D-5L questionnaire
    - Paediatric participants will complete the age-adjusted EQ-5D-Y questionnaire and their parent/legal guardian will complete the EQ-5D-Y proxy questionnaire

11.7 LONG-TERM FOLLOW-UP ASSESSMENTS

All participants will be asked to consent to linkage with the UK NHS Blood and Transplant Registry (NHSBT) at enrolment in the trial. Participants will be followed up via the NHSBT registry for the primary outcome measure until the trial is completed.

Participants withdrawing from the trial may do so with or without continued record linkage. Relevant data (transplant failure, death and graft function) required for analysis of the primary outcome measure can be obtained from the NHSBT Registry for those participants who withdraw from the trial but consent to continued record linkage.

Complete loss to follow-up is likely to be uncommon. Data capture on all transplant patients is achieved via the NHSBT Registry on an annual basis. Whilst a graft is functioning, patients will be known to a transplant team. So, the primary outcome measure of allograft survival will be captured as long as the patient remains a UK resident.

Participant identifiable data (NHS number (or equivalent), name, gender and date of birth) required for accurate linkage with the NHSBT Registry will be securely stored centrally at CCTU on encrypted servers with restricted use and access.

11.8 END OF TRIAL PARTICIPATION

Participation in the trial ends following completion of visit 8 at 48 months (±8 weeks) post randomisation (or earlier in the case of participants withdrawing from the trial). Participants will return to routine NHS care and any unresolved adverse events will be followed up clinically until resolution.

11.9 TRIAL RESTRICTIONS

Women of childbearing potential are required to use adequate contraception for the duration of the trial intervention (rituximab) and for 12 months after completion of rituximab treatment. This includes:

- Intrauterine Device (IUD, coil or intrauterine system)
- Hormonal based contraception (combined or progestogen only pill, contraceptive injection, implant, patch, etc.)
- Barrier contraception (condom and occlusive cap e.g. diaphragm or cervical cap with spermicide)
- Vaginal ring
- True abstinence (where this is in accordance with the participant's preferred and usual lifestyle)

In addition, the anti-rejection medication mycophenolate mofetil (MMF) used for all participants' baseline immunosuppression is not recommended for use during pregnancy. There is a theoretical risk alone, with no evidence of harm to babies of fathers taking MMF at the time of conception.

As kidney transplant patients are advised to delay conception for a minimum of one year post-transplant, it is highly unlikely that participants will become pregnant.

12 ASSESSMENT OF SAFETY

The Sponsor expects that adverse events are recorded from the point of Informed Consent, regardless of whether a participant has yet received a medicinal product. Individual adverse events should be evaluated by the Investigator. This includes the evaluation of its seriousness, causality and any relationship between drug therapy and/or concomitant therapy and the adverse event.

12.1 DEFINITIONS

12.1.1 Adverse Event (AE)

Any untoward medical occurrence in a participant or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.

12.1.2 Adverse Reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered. All AEs judged by either the reporting investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

12.1.3 Unexpected Adverse Reaction

An AR, the nature or severity of which is not consistent with the applicable reference safety information (RSI) in the Summary of Product Characteristics (SmPC). When the outcome of the adverse reaction is not consistent with the applicable RSI this adverse reaction should be considered as unexpected. The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious” which is based on participant/event outcome or action criteria.

Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.

12.1.4 Serious Adverse Event (SAE) or Serious Adverse Reaction

Any untoward medical occurrence or effect that at any dose:

- 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
- Is an important medical event – some medical events may jeopardise the participant or may require an intervention to prevent one of the above characteristics/consequences. Such events (hereinafter referred to as "important medical events") should also be considered as "serious"

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs 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.

12.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information set out in the RSI.

12.1.6 Reference Safety Information (RSI)

For adult participants: the Reference Safety Information (RSI) is Table 1 in section 4.8 of the latest approved version of the Summary of Product Characteristics (SmPC) for MabThera®.

For paediatric participants: the RSI is the "IgG Levels" section of "Description of Selected Adverse Reactions" section below Table 1 in section 4.8 of the latest approved version of the SmPC for MabThera®. The other listed adverse events are not relevant for paediatric participants.

12.2 EXPECTED ADVERSE REACTIONS/SERIOUS ADVERSE REACTIONS (AR/SARs)

Expected ARs are listed in the latest MHRA approved version of the RSI as specified in section 12.1.6. This must be used when making a determination as to the expectedness of the AR. If the AR meets the criteria for seriousness, this must be reported as per section 12.5.2 (see APPENDIX 5 for clarification).

12.3 EXPECTED ADVERSE EVENTS/SERIOUS ADVERSE EVENTS (AE/SAEs)

All adverse events, whether expected or not, will be recorded in this trial.

12.4 CAUSALITY

Most adverse events and adverse drug reactions that occur in this trial, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this trial. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in Table 2 below.

If any doubt about the causality exists the local investigator should inform the trial coordination centre who will notify the Chief Investigator (CI). Other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Table 2 – Assignment of causality (52)

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship and another documented cause of the AE is most plausible. **This is therefore an Adverse Event.**
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment). **This is therefore an Adverse Event.**
POSSIBLE	There is some clinically/biologically plausible evidence to suggest a causal relationship (e.g. there is a plausible time sequence between onset of the AE and administration of the IMP). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments). **This is therefore an Adverse Reaction.**
PROBABLE	There is highly plausible clinical/biological evidence suggesting a causal relationship and there is a plausible time sequence between onset of the AE and administration of the IMP and there is a reasonable response on withdrawal. **This is therefore an Adverse Reaction.**
DEFINITELY	A causal relationship is clinically/biologically certain and other possible contributing factors can be ruled out. **This is therefore an Adverse**

	Reaction.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

Unlikely and Unrelated causalities are considered NOT to be trial drug related. Definitely, Probably and Possible causalities are considered to be trial drug related.

A pre-existing condition must not be recorded as an AE or reported as an SAE unless the condition worsens during the trial and meets the criteria for reporting or recording in the appropriate section of the CRF.

12.4.1 Clinical Assessment of Severity

Mild – The participant is aware of the event or symptom, but the event or symptom is easily tolerated (52)

Moderate – The participant experiences sufficient discomfort to interfere with or reduce his or her usual level of activity (52)

Severe – Significant impairment of functioning; the subject is unable to carry out usual activities and/or the participant's life is at risk from the event (52)

12.4.2 Recording of Adverse Events

AEs and ARs should be recorded in the medical notes and the appropriate section of the CRF and/or AE/AR log. SAEs and SARs should be reported to the Sponsor as detailed in section 12.5.2.

12.5 REPORTING PROCEDURES

All adverse events should be recorded. 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 trial coordination centre in the first instance. The flowchart in APPENDIX 5 describes the classification and reporting process.

12.5.1 Non-Serious AE/ARs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant CRF and sent to the trial coordination centre within one month of the form being due.

12.5.2 Serious AE/ARs

All SAEs and SUSARs should be reported to CCTU within 24 hours of the local site becoming aware of the event. CCTU will be responsible for onward reporting to the CI and the Sponsor. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

SAEs/SARs

An SAE form should be completed and emailed to the trial coordination email address add-tr.target1trial@nhs.net within 24 hours. The trial coordination centre will notify the Sponsor (JRCO.CTIMP.TEAM@imperial.ac.uk) within 24 hours of receipt. However hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

12.5.2.1 Serious AE/ARs in paediatric participants

As rituximab is not licensed for use in paediatric patients and its safety in this population has not been determined, all SARs (SAEs that are possibly, probably or definitely related to the IMP) will be reported as SUSARs and subject to expedited reporting (please see section 12.6 for details). The only exception is hypogammaglobulinaemia, which is described in section 4.8 of the MabThera® SmPC.

12.6 REPORTING OF SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTIONS (SUSARs)

All suspected adverse reactions related to an IMP (the tested IMP and comparators) which occur in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. Please see section 12.1.6 for the respective RSI for adult and paediatric participants to be used in this trial.

Staff at the participating sites should:

- Complete the SAE case report form & send it immediately (within 24 hours), signed and dated to the trial coordination centre (add-tr.target1trial@nhs.net) together with relevant treatment forms and link-anonymised (labelled with the participants' trial ID number and partial date of birth) copies of all relevant investigations.

Or

- Contact the trial coordination centre by phone and then send the completed SAE form to the trial coordination centre within the following 24 hours as above.

12.6.1 Who Should Report and Whom to Report to

The Sponsor delegates the responsibility of notification of SUSARs to the Chief Investigator (CI) and trial coordination team. The CI must report all the relevant safety information previously described, to the:

- Sponsor
- Competent authorities in the concerned member states (e.g. MHRA)
- Ethics Committee in the concerned member states

The CI shall inform all investigators concerned or relevant information about SUSARs that could adversely affect the safety of participants.

Local investigators should report any SUSARs and/or SAEs as required by their Local Research & Development Office.

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

- Complete the SAE case report form & send it immediately (within 24 hours), signed and dated to the trial coordination centre (add-tr.target1trial@nhs.net) together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

- Contact the trial coordination centre by phone and then send the completed SAE form to the trial coordination centre within the following 24 hours as above.

The trial coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the trial according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the trial.

Local investigators should report any SUSARs and /or SAEs as required by their Local Research & Development Office.

12.6.2 When to Report

12.6.2.1 Fatal or life-threatening SUSARs

All parties listed in 12.6.1 must be notified as soon as possible but no later than **7 calendar days** after the trial team and Sponsor has first knowledge of the minimum criteria for expedited reporting.

In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to all parties within an additional **8 calendar days**.

12.6.2.2 Non-fatal and non-life-threatening SUSARs

All other SUSARs and safety issues must be reported to all parties listed in 12.6.1 as soon as possible but no later than **15 calendar days** after first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

12.6.3 How to Report

12.6.3.1 Minimum criteria for initial expedited reporting of SUSARs

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted within the time limits as soon as the minimum following criteria are met:

- A suspected investigational medicinal product
- An identifiable participant (e.g. trial participant code number)
- An adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship
- An identifiable reporting source

And, when available and applicable:

- A unique clinical trial identification (EudraCT number)
- A unique case identification (i.e. Sponsor's case identification number)

12.6.3.2 Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. Further available relevant information should be reported as follow-up reports.

In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

12.6.3.3 Format of the SUSAR reports

Electronic reporting is the expected method for expedited reporting of SUSARs to the competent authority. The format and content as defined by the competent authority should be adhered to. The trial will be registered by the Sponsor and reporting completed by the trial coordination team.

12.7 PREGNANCY REPORTING

Pregnancies within the trial (female trial participants in both arms of the trial) should be reported to CCTU (add-tr.target1trial@nhs.net) using the relevant Pregnancy Reporting Form within 24 hours of notification. CCTU will be responsible for onward reporting to the CI and Sponsor.

13 TOXICITY

Rituximab has been shown to be highly specific to the CD20 antigen on B-lymphocytes. Toxicity studies in cynomolgus monkeys have shown no other effect than the expected pharmacological depletion of B-lymphocytes in peripheral blood and in lymphoid tissue.

Developmental toxicity studies have been performed in cynomolgus monkeys and showed no evidence of toxicity to the foetus due to rituximab. However, dose-dependent pharmacological depletion of B-lymphocytes in the lymphoid organs of the foetuses was observed, which persisted postnatally and was accompanied by a decrease in IgG level in the newborn animals affected. B-lymphocyte counts returned to normal in these animals within 6 months of birth and did not compromise the reaction to immunisation.

There are limited data for overdosing with rituximab. The highest intravenous dose of rituximab tested in humans in clinical trials is 5000 mg (2250 mg/m^2). No additional safety signals were identified. Patients who experience overdose should have immediate interruption of their infusion and be closely monitored.

Five cases of rituximab overdose in the post-marketing setting have been reported. Three cases had no reported adverse events, two cases reported flu-like symptoms with 1.8 g rituximab and fatal respiratory failure with 2 g rituximab (see section 4.9 MabThera® SmPC).

14 EVALUATION OF RESULTS

14.1 RESPONSE CRITERIA

The primary outcome measure is allograft survival. Secondary outcome measures are detailed in the trial outcome measures section (section 8.6).

15 STORAGE AND ANALYSIS OF SAMPLES

Renal transplant biopsy samples will be collected at each centre, analysed as per standard practice at the local pathology laboratory and sent to the Imperial Biobank for further analysis, as described in the trial manual. If pathology scanning facilities are available, biopsy slides may be scanned rather than sent.

Blood and urine samples will be collected at each centre and analysed as per standard practice at the local Biochemistry and Haematology laboratories.

Blood samples for DSA analysis and the optional samples for B-cell marker analysis will be sent for central processing. Samples will be labelled with the participants' trial ID and partial date of birth.

All samples will be stored securely in a -80 °C freezer prior to analysis by a central laboratory, as described in the trial manual. Research samples may have genetic analysis performed on them. The results of genetic analysis will not be communicated to the participants. Explicit consent will be sought from the participants for genetic analysis.

16 STATISTICS AND DATA ANALYSIS

16.1 STATISTICAL METHODS

16.1.1 Analysis Populations

The following populations will be defined for efficacy and safety analyses:

- Intent-to-treat Population (ITT)

The ITT population is defined as all participants randomised in the trial, regardless of whether they actually received treatment. The treatment group will be analysed as randomised.

- Safety Population

The safety population comprises all participants randomised and having received at least one dose of trial treatment. The treatment group will be analysed as treated.

16.1.2 Efficacy Analyses

The primary efficacy outcome measure of the trial is allograft survival, where allograft failure is defined as the duration from the date of randomisation to the date of:

- an estimated GFR measurement of ≤15 mL/min/1.73 m^2,where:
    - the eGFR measurement is not due to an acute reversible cause, as determined by the PI
    - a follow-up consecutive eGFR measurement of ≤15 mL/min/1.73 m^2 is recorded (where the first date is recorded as the date of failure)
- renal replacement therapy (date of starting maintenance dialysis dependency, retransplantation etc.)

which ever occurs first.

Participants who have an eGFR measurement of ≤15 mL/min/1.73 m^2 will be adjudicated by an independent committee to confirm that the eGFR measurement is a true indication of

failure and not a temporary fluctuation in renal function. The primary analyses will be based on the results from the independent review committee.

The primary analysis will be a stratified log-rank test adjusted for the stratification factors (age, baseline eGFR function) for the difference in the distribution of allograft survival between the SOCR arm and the SOC arm (two-sided at an α-level of 5%). Assuming a proportional hazard holds, the hazard ratio together with the 95% confidence interval will be estimated using a Cox regression model adjusted for the stratification factors. Unstratified log-rank test and Cox regression model will also be performed to assess the robustness of the results. Kaplan-Meier estimates for allograft survival at 4-years and median allograft survival with the corresponding 95% confidence intervals by the treatment allocation will be presented.

Given that existing data show a low mortality in the target population (3% in a UK observational cohort study of 160 patients with AMR and a median follow-up of 2.64 years), the primary analysis for the primary outcome measure will not include death as a competing risk. We will however perform a competing risk sensitivity analysis accounting for death. In addition, we expect that the dropout rate will be low as all patients on renal replacement therapy have important data captured (return to dialysis, graft loss, death, death with a functioning allograft) by the NHS Blood and Transplant Registry. Dropout for the primary outcome measure analysis will only occur in the event a participant withdraws their consent for clinical follow-up and data capture.

Allograft function, in terms of serum creatinine, eGFR (CKD-EPI) and proteinuria (UPCR) at a time point specified in the secondary outcome measure will be compared using a standard t-test or a Wilcoxon rank test. Donor specific antibody (DSA) positivity, number of DSA and mean florescence index (immune dominant) at a time point specified in the secondary outcome measure will be compared using a chi-squared test or a t-test test when it is appropriate. Mixed models with repeated measures will also be applied.

Efficacy analyses will be performed according to ITT.

16.1.2.1 Censoring

Participants with no follow-up information following randomisation will be censored at the time of randomisation.

For allograft survival, participants who have neither started renal replacement therapy (returned to maintenance dialysis, had re-transplantation, etc) nor had an eGFR of ≤15 mL/min/1.73 m^2 or who are lost to follow-up will be censored at the date that they were last known not to have the allograft fail events.

16.1.3 Safety Evaluation

The safety analyses will be based on the safety population. All safety parameters will be summarised. Summary tables will be presented for incidence rates (number of patients with at least one incidence) of AEs, SAEs, AEs that led to premature withdrawal of trial treatment and interruptions/dose modifications, as well as summaries of severity.

A statistical analysis plan to include the detailed efficacy and safety analyses will be drafted prior to activation of the trial.

16.2 FEASIBILITY ASSESSMENTS

It is planned to conduct an internal feasibility assessment at 12 and 21 months after randomisation of the first patient (APPENDIX 6). These assessments will consider site activation, recruitment rate and acceptability of the protocol. The 12 month assessment will review research site activation, with an aim to have 24 sites active. Recruitment rate will also be assessed at this time point, with a required average recruitment of 1 patient per site every 20 weeks. A further assessment will be made at 21 months, and the trial will be considered feasible if 75 patients have been enrolled, or the average recruitment rate required of one participant per site every 20 weeks is equalled or exceeded at this point. All interim analysis reports will be reviewed by the Data Monitoring and Ethics Committee (DMEC). The DMEC will review the confidential data and make the recommendation of continuing the trial as planned, proposing or commenting on proposed protocol changes, or early termination etc. to the Trial Steering Committee (TSC).

16.3 SAMPLE SIZE ESTIMATION

The trial will enrol approximately 170 participants, randomised in a ratio of 1:1 between the two trial arms. With a total of approximately 170 participants recruited in 40 months and a further follow-up of 4 years (or a total of 104 allograft survival failures observed), the trial will have a 95% power (two-sided 5% type I error rate) to detect an improvement in 4-year allograft survival from 35% in the SOC arm to 60% in the SOCR arm, a hazard ratio = 0.49 (assuming exponential distribution). Allowing for 20% withdrawal or loss to follow-up, a total of 170 x 80% = 136 participants, the trial will have a power of 90% (with a total of 84 allograft survival failures) to detect a hazard ratio of 0.49 (see APPENDIX 7 for sample size estimation output).

16.3.1 Predicted Effect Size

The above effect and sample size estimates assume a 30% allograft survival after 4 years in the SOC arm. This was based on a retrospective analysis of 160 patients in the UK treated for acute AMR (53). Four-year allograft survival, defined as return to dialysis, was 37%, whilst incorporation of patients who have allografts where the function was <15 mL/min/1.73 m^2 resulted in a survival of <30%. Given that patients with an eGFR <15 mL/min/1.73 m^2 will be eligible for enrolment (with randomisation stratified for baseline eGFR) and that these data represent contemporary UK clinical practice, it is reasonable to expect allograft survival of 30% in the SOC arm.

Data on allograft survival with rituximab are varied, as outlined earlier. For SOCR, using a similar protocol to that proposed here, Lefaucheur *at al.* reported a 3-year allograft survival of 91.7%, over 40% higher than a group receiving IVIg alone (45). A separate study also

showed a 30% increase in allograft survival after 2 years using rituximab in addition to PEX (46).

Given the above estimates, we believe that the SOC arm and the SOCR arm allograft survival rates of 30% and 60% respectively are rational. Nevertheless, the trial is powered to detect a difference between the two arms with 95% power even if graft survival is as high as 35% in the SOC arm.

16.4 PROCEDURE TO ACCOUNT FOR MISSING OR SPURIOUS DATA

We do not anticipate a substantial rate of missing data. Missing data from participants who have consented to linkage with the UK NHSBT registry can be obtained from the registry for analysis of the primary outcome measure.

16.5 ECONOMICS EVALUATION

The economic analysis will comprise economic evaluation of SOCR versus SOC, and a value of information analysis at the interim and final analysis points.

The economic evaluation will report both within-trial and decision model based incremental cost per QALY gained from the perspective of the NHS.

At the outset, a decision model will be developed predicting the long-term costs and outcomes of the three treatment strategies based on current information (i.e. evidence from systematic review of the literature and/or expert belief on the effectiveness of, and AEs associated with the treatments, without any evidence from the trial). The model structure will be adapted from pre-existing models of treatments for ESKD, e.g. (12), and will focus on risk of return to maintenance dialysis, mortality and side effects from treatments.

At the interim analysis point, results of the trial to date will be combined with the prior information and inserted into the model. Whilst this will generate an early estimate of the cost-effectiveness of the different options, the focus will be on uncertainty in the point estimate. This will be used in a value of information (VOI) analysis to predict the return on investment from continuing with the trial as is, halting one or more of the arms, or other modifications including continuing full data collection out to 4 years versus a reduced dataset. This is a somewhat experimental approach (use of value of information analysis within an adaptive clinical trial design) and as such the economic recommendations will be considered as non-binding feasibility criteria. They will however be included as part of the decision-making process for trial adaptation. In particular, should the investigators agree to pursue a design that contradicts the VOI recommendations, this will be documented and the reasons for pursuing a less efficient design justified.

The economic analysis and value of information analysis will follow established methods and good practice guidelines in conduct and reporting (54-56), including reporting point estimate, increments +/- 95% credibility interval costs and health gain, cost-effectiveness acceptability curves and the expected value of perfect and sample information and expected net gain of sampling.

16.6 DEFINITION OF THE END OF THE TRIAL

The trial will close to recruitment after approximately 170 participants have been randomised. The trial will close at the last patient last visit, 4 years after the last participant has been recruited.

17 DATA HANDLING AND RECORD KEEPING

17.1 CRF

All protocol-required data will be transferred into a Case Report Form (CRF), which is the printed, optical or electronic document designed to record the information to be reported to the CI for each trial participant. The CRF will be labelled with the participants' trial ID number and partial date of birth. The partial date of birth is required in addition to the trial ID number to ensure the integrity of the data. All trial data in the CRF must be extracted from and be consistent with the relevant source documents. The CRFs must be completed, dated and signed by the investigator or designee in a timely manner. It remains the responsibility of the investigator for the timing, completeness, legibility and accuracy of the CRF pages. The CRF will be accessible to trial coordinators, data managers, the investigators, Clinical Trial Monitors, Auditors and Inspectors as required.

Completed CRF data should be entered into the database. Sites not performing data entry should scan and email completed originals of the CRFs to the trial coordination centre (add-tr.target1trial@nhs.net) in a timely manner.

The investigator will retain all original completed CRFs in the relevant sections of their Investigator Site File with any required background information from the medical records as required, labelled with the participants' trial ID number and partial date of birth.

The investigators must ensure that the CRFs and other trial related documentation sent to the trial coordination centre contains no participant identifiable data.

All completed CRF pages must be clear, legible and completed in black ink. Any errors should be crossed with a single stroke so that the original entry can still be seen. Corrections should be inserted and the change dated and initialled by the investigator or designee. If it is not clear why the change has been made an explanation should be written next to the change. Typing correction fluid must not be used.

17.2 SOURCE DATA

To enable peer review, monitoring, audit and/or inspection the investigator must agree to keep records of all participating participants (sufficient information to link records e.g. CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages.

Source documents include, but are not limited to:

- Patient medical records
- Trial-specific records
- Original signed consent forms
- Blood/urine/renal biopsy test results
- Questionnaires
- NHSBT Registry datasets

17.3 DATA PROTECTION & PARTICIPANT CONFIDENTIALITY

All investigators and trial site staff involved in this trial must comply with the requirements of the EU General Data Protection Regulation (GDPR) 2016/679, the Data Protection Act 2018 and Trust Policy with regards to the collection, storage, processing, transfer and disclosure of personal information and will uphold the Act's core principles.

Strict patient confidentiality will be observed throughout all aspects of the trial. Local research and/or clinical teams will have access to participants' patient identifiable data for the purposes of contacting the participants about the trial and to provide clinical care. Trial participants will provide explicit consent to the use of patient identifiable data for the purposes of the conduct of the trial. The trial coordination centre (CCTU) will hold patient identifiable data on all trial participants including name, data of birth and NHS number or equivalent. Patient identifiable data will be stored separately from link-anonymised trial data on a secure encrypted server with restricted access. Permission to access to this data may be given to the statistical team within Cambridge CTU, clinical trial monitors, auditors and inspectors as required. It is necessary to perform verification of NHS numbers (or equivalent) with the NHS Blood and Transplant Registry for the purposes of accurate linkage for long-term follow-up.

17.3.1 NHS Blood and Transplant (NHSBT) Registry

Applications will be made to the NHSBT Registry to access outcome data routinely collected by them (section 11.7). This may include transplant date, eGFR, date of dialysis and mortality information. The application and resulting data will be managed by the trial coordination centre Cambridge CTU.

17.3.2 Identifiable Data Transfer from Local Participating Sites to Trial Coordinating Centre

All identifiable data will be securely sent to the trial coordination centre (Cambridge CTU) via NHS encrypted email (i.e. from a @nhs.net account to add-tr.target1trial@nhs.net) and stored in a separate, password-encrypted database in compliance with the General Data Protection Regulation (EU GDPR) 2016/679 and the Data Protection Act 2018, with permission for access restricted to delegated trial staff. Consent will be sought for the transfer of identifiable information.

18 TRIAL MANAGEMENT

The trial will be conducted in accordance with the principles of Good Clinical Practice (GCP) and relevant local, national and international regulations.

18.1 DATA MONITORING AND ETHICS COMMITTEE

An independent Data Monitoring and Ethics Committee (DMEC) will be established for the trial. The DMEC will consist of an expert in transplantation, a statistician and a trials methodologist. The DMEC will meet annually to review data on the safety of participants in the trial and progress of the trial. There is no formal stopping rule, but as a guideline the DMEC will be asked to consider a significance level of 0.001 as an indication that the data would be sufficiently persuasive to change clinical practice. The DMEC will make recommendations on the continuation of the trial to the TSC.

18.2 TRIAL STEERING COMMITTEE

An independent Trial Steering Committee (TSC) will be established for the trial. The TSC will include an expert in trials methodology, a clinician, a statistician and a patient group representative. The TSC will meet at least annually and will provide overall supervision of the trial. The TSC will receive reports from CCTU, the TMG and the DMEC.

18.3 TRIAL MANAGEMENT GROUP

The trial will be coordinated by staff from the Cambridge Clinical Trials Unit (CCTU) in conjunction with Chief Investigator (Imperial). The trial management group (including the CI and staff from CCTU) will meet regularly and as required.

19 ETHICAL & REGULATORY CONSIDERATIONS

19.1 ETHICS APPROVAL

Before the start of the trial or implementation of any amendment approval of the trial protocol, protocol amendments, informed consent forms and other relevant documents e.g. advertisements and GP information letters if applicable, will be obtained from the REC. All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.

Annual reports will be submitted to the REC in accordance with national requirements. It is the CI's responsibility to produce the annual reports as required.

The trial must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the trial or any research activity is carried out.

19.2 CONSENT

All participants will undergo informed consent prior to enrolment in the trial in accordance with GCP guidelines. In paediatric cases, the parent or legal guardian will be asked for consent and the participants will be asked for their assent. Patients will be given written information about the trial, together with having the opportunity to ask questions to the local research team. Patients will be given sufficient time to decide on whether they would like to participate. This time will depend upon treatment already received at the time of screening, but is estimated to be a maximum of 7 days (up to 2 weeks post biopsy and must be before final PEX treatment).

19.3 REGULATORY COMPLIANCE

The trial will not commence until a Clinical Trial Authorisation from the Medicine and Healthcare Regulatory Agency (MHRA) has been obtained prior to the start of the trial. The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.

Development Safety Update Reports (DSURs) will be submitted to the MHRA in accordance with national requirements. It is the CI's responsibility to produce the annual reports as required.

19.4 PROTOCOL AMENDMENTS

Protocol amendments must be reviewed and agreement received from the Sponsor for all proposed amendments prior to submission to the HRA, REC and/or MHRA.

The only circumstance in which an amendment may be initiated prior to HRA, REC and/or MHRA approval is where the change is necessary to eliminate apparent, immediate risks to the participants (Urgent Safety Measures). In this case, accrual of new participants will be halted until the HRA, REC and/or MHRA approval has been obtained.

19.5 PEER REVIEW

The research proposal, from which the protocol was developed, was peer reviewed by the Funding Board from NIHR and KRUK, who are funding this trial.

19.6 DECLARATION OF HELSINKI AND GOOD CLINICAL PRACTICE

The trial will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of Good Clinical Practice, the protocol and applicable local regulatory requirements and laws.

19.6.1 GCP Training

All trial staff must hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this trial. This training should be updated every 2 years or in accordance with the local Trust's policy.

19.6.2 Protocol-Specific Training

Prior to initiation of the trial at any participating site, the participating site clinic staff will be trained in the methods of the trial.

20 SPONSORSHIP, FINANCIAL AND INSURANCE

The trial is sponsored by Imperial College London. This trial has received funding from the National Institute of Health Research Health Technologies Assessment Programme (NIHR HTA) and Kidney Research UK (KRUK).

Imperial College London, as Sponsor, holds negligent harm and non-negligent harm insurance policies which apply to this trial.

21 MONITORING, AUDIT & INSPECTION

Imperial College London will be responsible for the monitoring of the trial. Prior to the initiation of the trial at any participating site, a representative of the Sponsor will confirm that the participating site has adequate facilities and resources to carry out the trial (and if considered necessary a site visit will be undertaken). The Sponsor's monitoring frequency will be determined by an initial risk assessment performed prior to the start of the trial. A detailed monitoring plan will be generated detailing the frequency and scope of the monitoring for the trial. Throughout the course of the trial, the risk assessment will be reviewed and the monitoring frequency adjusted as necessary. The trial has been identified as high risk and as such will require monitoring as stipulated in the monitoring plan. During the conduction of the trial, any of the trial sites that are identified as having outlying data or quality control will undergo additional on-site monitoring. The monitor will be informed of any outlier data by the trial coordinator at CCTU. The Sponsor's monitor will have regular meetings with the coordinating centre.

The investigator must make all trial documentation and related records available should an MHRA Inspection occur. Should a monitoring visit or audit be requested, the investigator must make the trial documentation and source data available to the Sponsor's representative. All participant data must be handled and treated confidentially.

21.1 TRAINING AND MONITORING AT LOCAL SITE

Representatives from the Sponsor will visit all participating sites as required by the needs of the centre and monitoring. All paperwork will be provided by the CCTU. Provision of equipment e.g. blood sample tubes for research samples, will be coordinated by the CCTU, as described in the trial manual. Participating sites will undergo on-site monitoring on a periodic basis. The purpose of these visits will be to help participating site staff to resolve any local problems with the trial, to ensure that the trial is conducted according to the protocol, and to review trial records and data quality. A report of each visit will be prepared by the trial monitor and reviewed by the Sponsor. A close out visit to each of the sites will also be performed.

22 PROTOCOL COMPLIANCE AND BREACHES OF GCP

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used.

Protocol deviations, non-compliances, or breaches are departures from the approved protocol. They can happen at any time, but are not planned. They must be adequately documented on the relevant forms and reported to the CI and Sponsor immediately.

Deviations from the protocol which are found to occur constantly again and again will not be accepted and will require immediate action and could potentially be classified as a serious breach.

Any potential/suspected serious breaches of GCP must be reported immediately to the Sponsor without any delay.

23 PUBLICATION POLICY

The writing committee will approve all publications which incorporate the data from this trial. The writing committee will be composed of all the PIs, CCTU statistical staff, the trial group and relevant investigators involved in the mechanistic studies associated with the trial. Publications will adhere to the NIHR publications policy.

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25 APPENDICES

25.1 APPENDIX 1

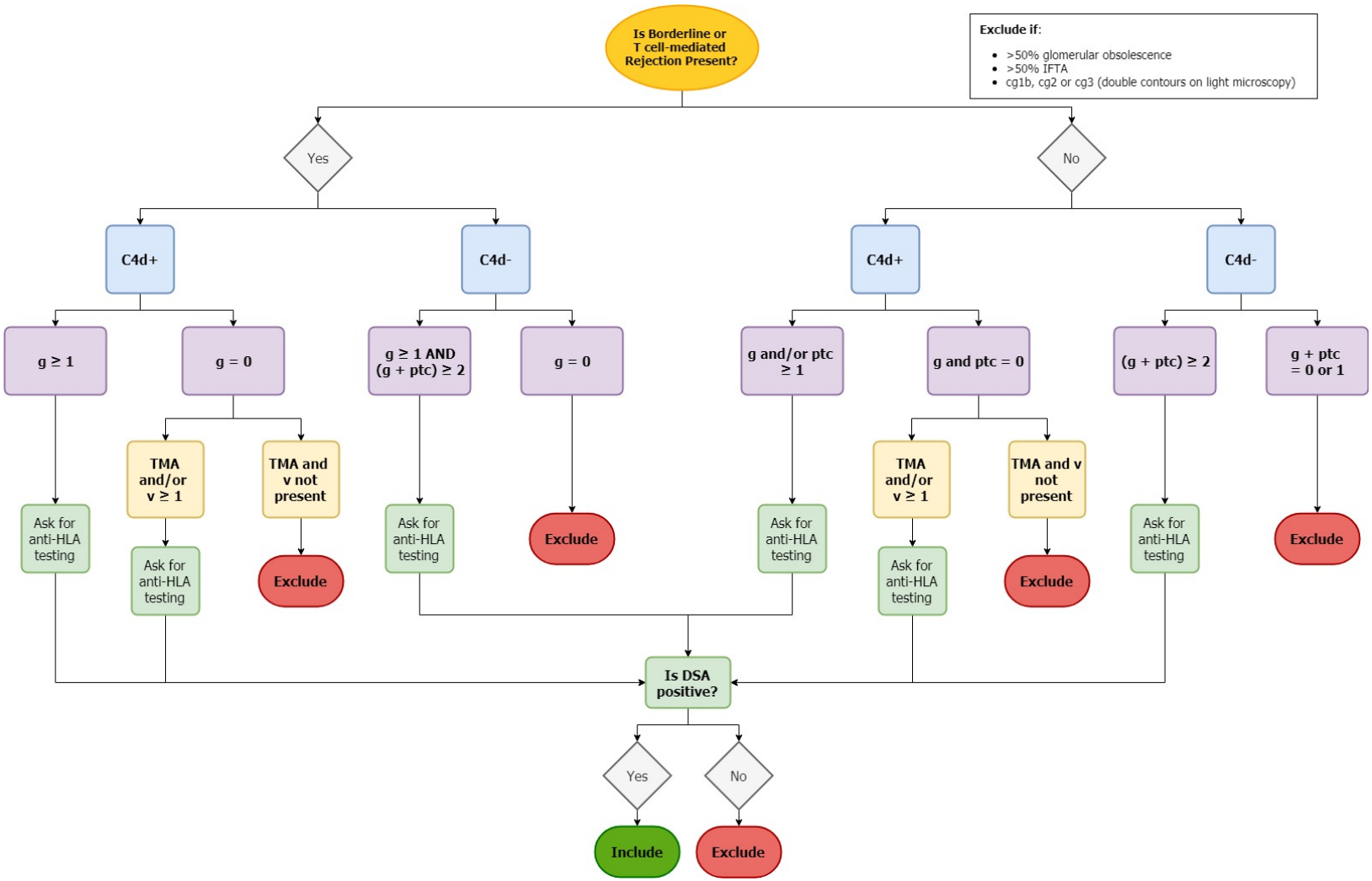


Figure 2 - Histological Flowchart for Inclusion Criteria

25.2 APPENDIX 2

Table 3 – Trial Visit and Procedures Schedule

Visits				1			2	3	4	5	6	7	8
Procedures	Presentation of AMR	SOC Treatment	Recruitment and Screening	Baseline	Randomisation (Time 0)	IMP Treatment	4 Weeks (±1 week)	3 Months (±2 weeks)	6 Months (±3 weeks)	1 Year (±3 weeks)	2 Years (±6 weeks)	3 Years (±8 weeks)	4 Years (±8 weeks)
Renal Transplant Biopsy[1]	X												
Donor Specific Antibodies[1]	X							X		X			
Methylprednisolone		X											
PEX		X											
IVIg		X											
Written Informed Consent			X										
PID for linkage to NHSBT			X										
Demography			X										
Pregnancy Test[2]			X										
Physical Examination			X	X[3]			X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]
Histopathology of biopsy			X										
Histocompatibility and Immunogenetics			X										
Eligibility Assessments			X	X			X	X	X	X	X	X	X
eGFR			X	X			X	X	X	X	X	X	X
Creatinine			X	X			X	X	X	X	X	X	X
UPCR			X	X			X	X	X	X	X	X	X
AE Review[4]			X	X			X	X	X	X	X	X	X
Concomitant Medications				X			X	X	X	X	X	X	X
Medical History				X									
FBC				X			X	X	X	X	X	X	X
Biochemistry[5]				X			X	X	X	X	X	X	X
Serum Immunoglobulins*				X			X						
Lymphocyte subsets*				X				X		X			
Optional samples[6]				X			X	X	X	X			
EQ-5D-5L[7]				X				X		X	X	X	X
Randomisation					X								
Rituximab[8]						X							

[1] Samples also sent to Imperial Biobank if additional biopsy/DSA testing is clinically-required during the trial duration

[2] Pregnancy tests only required for female participants of child-bearing potential

[3] Physical examination, including height and weight, must be performed at screening. Only performed if clinically required at subsequent visits.

[4] AEs to be recorded and reported as appropriate from the time of informed consent

[5] Biochemistry tests include CRP, albumin, HbA1c and others as clinically required

[6] For more information on optional research samples, please see the TAR:GET-1 Laboratory Manual

[7] EQ-5D-Y questionnaire for paediatric participants and EQ-5D-Y proxy questionnaire for the parent/legal guardian of the paediatric participant

[8] Two doses of rituximab 14 days (± 2 days) apart

*Optional for patients randomised to SOC arm.

25.3 APPENDIX 3

SOC and Rituximab Treatments

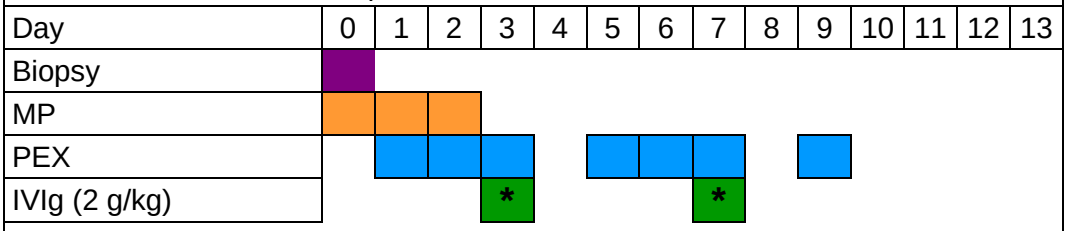
Table 4 – Treatment Doses and Schedules

Treatment Arm	Participant Age	Treatment	Dose	Number of doses	Notes
All Participants (SOC + SOCR)	Adult	Methylprednisolone	500 mg	3 doses on subsequent days	Permissible before the biopsy, if not should be given post biopsy
	Paediatric		600 mg/m^2		
	Adult	Plasma exchange (PEX)	60 ml/kg, to a maximum of 4l	7	Replacement fluid in line with local guidelines Anti-coagulation in line with local guidelines Premedication in line with local guidelines
	Paediatric		1 – 1.5 plasma volumes		
	Adult	Intravenous Immunoglobulin (IVIg)	100 mg/kg post each PEX or 2 g/kg in total	High dose IVIg may be divided into 2-4 doses	
	Paediatric				
SOCR Participants Only	Adult	Rituximab	375 mg/m², up to 1 g maximum	2 doses	2 weeks (± 2 days) apart, the first to be given within a week of the last PEX.
	Paediatric				

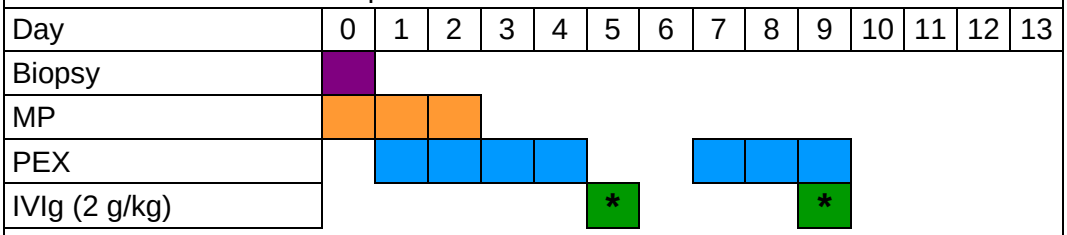
25.4 APPENDIX 4

Examples of SOC Treatment Schedules

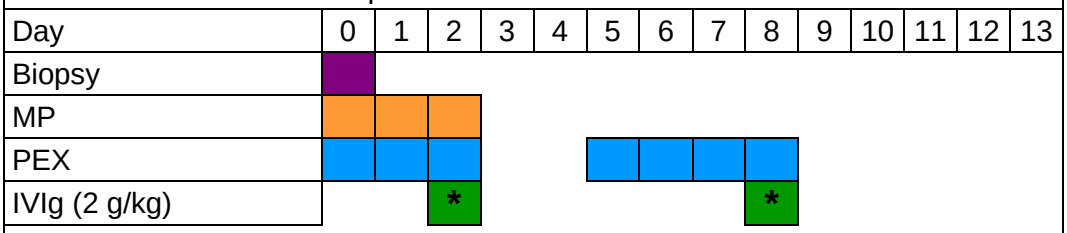
Treatment Schedule: Example 1



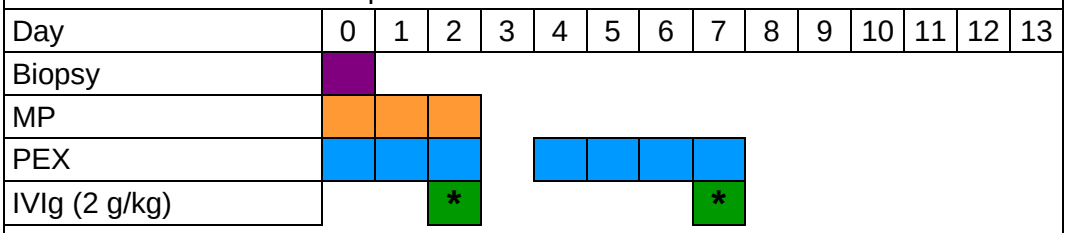
Treatment Schedule: Example 2



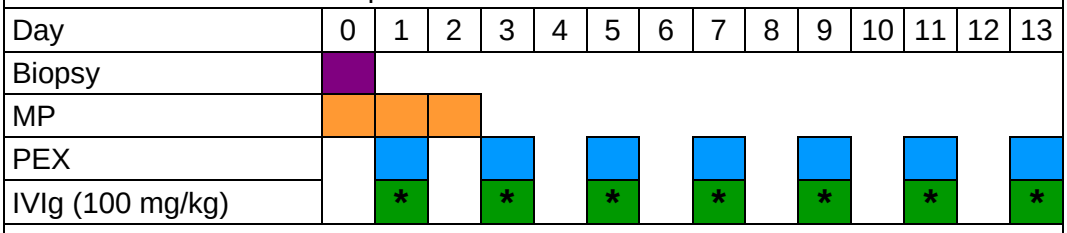
Treatment Schedule: Example 3



Treatment Schedule: Example 4



Treatment Schedule: Example 5



*****IVIg given post PEX

MP: Methylprednisolone (500 mg)

PEX: Plasma exchange (60 ml/kg, 4L max) - must be given at least 24 hours after IVIg

IVIg: Intravenous Immunoglobulin

Figure 3 – Possible Treatment Schedule

25.5 APPENDIX 5

Safety Reporting

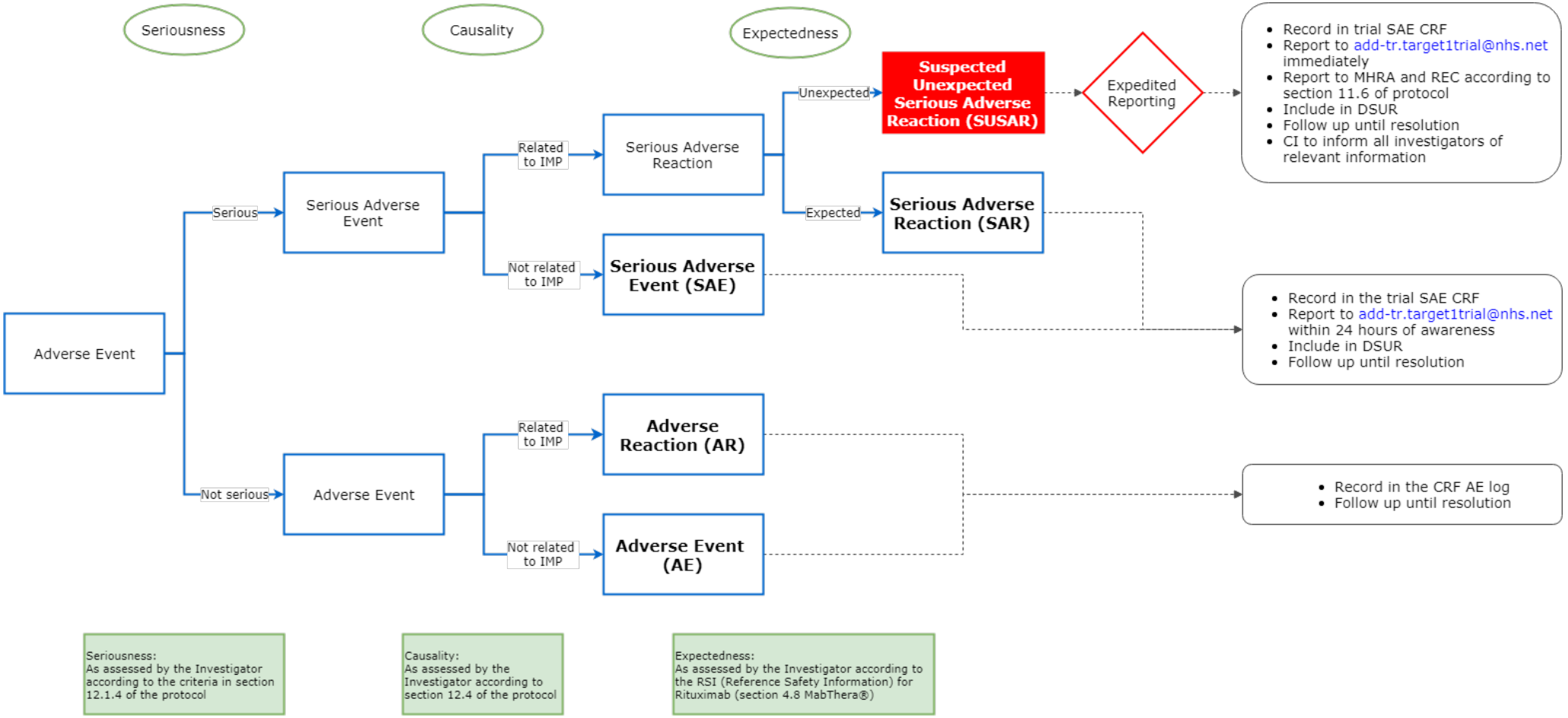


Figure 4 – Safety Reporting Flow Chart

25.6 APPENDIX 6

Trial Analysis Timeline

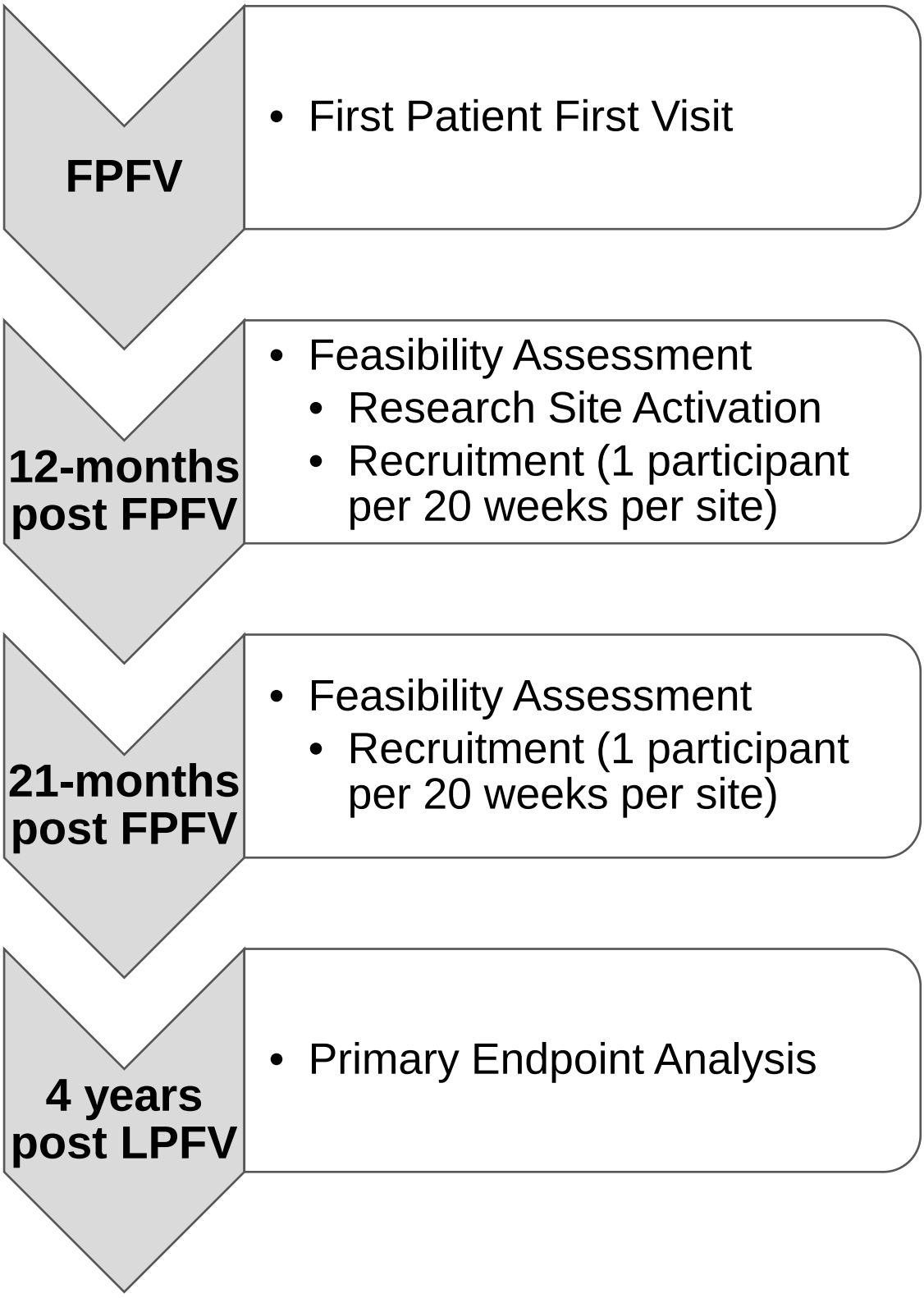


Figure 5 – Trial Analyses Flow Diagram

25.7 APPENDIX 7

Output of Sample Size Estimation

25.7.1 5% Significance Level, 95% power

Recruitment: 40 months recruitment duration with a weight of (0.25 0.5 0.75 1 1 1 1 1 1 1) using 4 months as a period.

Control arm data: 4-year survival = 35% in the control arm (group 1)

Difference to be detected: HR = 0.49, 4-year survival in the research arm (group 2) = 59.8% (~ 60%).

Follow-up: 4 years

Period = 4 months

. artsurv, method(l) nperiod(22) ngroups(2) fp(0) edf0(0.35, 12) hratio(1, 0.49) alpha(0.05) power(0.95) aratios(1 1) recrt(10 0, 0.25 0.5 0.75 1 1 1 1 1 1 1 , 0) distant(0) detail(0) onesided(0) ni(0) tunit(7) trend(0)

ART - ANALYSIS OF RESOURCES FOR TRIALS (version 1.1.0, 10 December 2013)

A sample size program by Abdel G Babiker, Patrick Royston & Friederike Barthel,

MRC Clinical Trials Unit at UCL, London WC2B 6NH, UK.

Type of trial	Superiority - time-to-event outcome
Statistical test assumed	Unweighted logrank test (local)
Number of groups	2
Allocation ratio	Equal group sizes
Total number of periods	22
Length of each period	Unspecified
Survival probs per period (group 1)	0.916 0.839 0.769 0.705 0.646 0.592
	0.542 0.497 0.455 0.417 0.382 0.350
	0.321 0.294 0.269 0.247 0.226 0.207
	0.190 0.174 0.159 0.146
Survival probs per period (group 2)	0.958 0.918 0.879 0.842 0.807 0.773
	0.741 0.710 0.680 0.651 0.624 0.598
	0.573 0.549 0.526 0.504 0.483 0.462

	0.443 0.424 0.406 0.389
Number of recruitment periods	10
Number of follow-up periods	12
Method of accrual	Uniform
Recruitment period-weights	0.25 0.5 0.75 1 1 1 1 1 1 1 0 0 0 0
	0 0 0 0 0 0 0 0
Hazard ratios as entered (groups 1,2)	1, 0.49
Alpha	**0.050 (two-sided)**
Power (designed)	**0.950**
Total sample size (calculated)	**165**
Expected total number of events	**104**

25.7.2 5% Significance Level, 90% power

Recruitment: 40 months recruitment duration with a weight of (0.25 0.5 0.75 1 1 1 1 1 1 1) using 4 months as a period.

Control arm data: 4-year survival = 35% in the control arm (group 1)

Difference to be detected: HR = 0.49, 4-year survival in the research arm (group 2) = 59.8% (~ 60%).

Follow up: 4 years

Period = 4 months

. artsurv, method(l) nperiod(22) ngroups(2) fp(0) edf0(0.35, 12) hratio(1, 0.49) alpha(0.05) power(0.90) aratios(1 1) recrt(10 0, 0.25 0.5 0.75 1 1 1 1 1 1 1 , 0) distan

> t(0) detail(0) onesided(0) ni(0) tunit(7) trend(0)

ART - ANALYSIS OF RESOURCES FOR TRIALS (version 1.1.0, 10 December 2013)

A sample size program by Abdel G Babiker, Patrick Royston & Friederike Barthel,

MRC Clinical Trials Unit at UCL, London WC2B 6NH, UK.

Type of trial	Superiority - time-to-event outcome
Statistical test assumed	Unweighted logrank test (local)

Number of groups	2
Allocation ratio	Equal group sizes
Total number of periods	22
Length of each period	Unspecified
Survival probs per period (group 1)	0.916 0.839 0.769 0.705 0.646 0.592 0.542 0.497 0.455 0.417 0.382 0.350 0.321 0.294 0.269 0.247 0.226 0.207 0.190 0.174 0.159 0.146
Survival probs per period (group 2)	0.958 0.918 0.879 0.842 0.807 0.773 0.741 0.710 0.680 0.651 0.624 0.598 0.573 0.549 0.526 0.504 0.483 0.462 0.443 0.424 0.406 0.389
Number of recruitment periods	10
Number of follow-up periods	12
Method of accrual	Uniform
Recruitment period-weights	0.25 0.5 0.75 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0
Hazard ratios as entered (groups 1,2)	**1, 0.49**
Alpha	**0.050 (two-sided)**
Power (designed)	**0.900**
Total sample size (calculated)	**134**
Expected total number of events	**84**

25.8 APPENDIX 8

Table 5 – Protocol Amendment History

Version Number	Date	Amendment
1.1	15.02.2019	Changes made as requested by MHRA: • Addition of specific sections describing the paediatric population of participants: o 6.1.1.1 o 6.1.2.1 o 6.2.1.1 o 7.1.2.1 o 8.3.1 o 9.1 • Rationale of rituximab dose o Section 10.3.2.4 • Rationale of treatment doses for paediatric participants o Section 10.3.1.1.1 o Section 10.3.1.2.1 o Section 10.3.1.3.1 o Section 10.3.2.5 • Reference safety information for the adult and paediatric participant populations o Section 6.2.2.1 o Section 10.5.5.1 o Section 12.1.6 o Section 12.5.2.1 o Section 12.6
1.2	04.04.2019	Changes made as requested by the REC: • Clarification of the trial flow diagram o Figure 1 (section 5) • Trial data identification o Section 9.3 o Section 12.6 o Section 17.1 • Clarification about the linkage with the NHSBT registry o Section 11.7 o Section 17 • Likelihood of rituximab's potential side effects o Section 10.3.2.6 • Contraception o Section 11.9 Change made as requested by NIHR: • Correction to peer review statement o Section 19.5 Typographical errors
2.0	11.07.2019	Changes made:

		• Identification references updated o Title page • Updated NIHR logo o Title page • Contact details completed o Section 2.1 o Section 2.2 • Inclusion Criteria – Removal of qualifying criteria for the renal transplant biopsy o Section 4 o Section 9.1 • Exclusion Criteria – Alteration of suspected HIV to active HIV infection o Section 4 o Section 9.2 • Clarification of the primary endpoint o Section 8.6.1 o Section 16.1.2 • Clarification to definition of DSA positivity o Section 9.3.1.1 • Clarification of biopsy central review process o Section 9.3.3 • Clarification of the system and required data for randomisation o Section 9.4 • Correction to oral corticosteroids o Section 10.3.1.3 • Rituximab premedication requirements modified to be consistent with local guidelines o Section 10.3.2.4 • Specification of the RSI and update to known drug reactions o Section 10.3.2.6 o Section 12.1.6 • Removal of requirement for prophylactic hepatitis B treatment o Section 10.7.1 • Clarification of trial assessments o Section 11.3.2 o Section 11.4 o Section 11.5 o Section 11.5.1 o Appendix 2 • Update to storage temperature for samples o Section 15 Typographical errors

3.0	18May2020	Changes made: • Change to title of trial: the words ‘safety and’ were removed. The title is now ‘A multicentre randomised controlled trial to assess the efficacy of adding rituximab to standard of care in treating acute antibody-mediated rejection in kidney transplantation’. • Exclusion criteria; (1) to specify that only those active malignancies ‘which would pose a contraindication to any of the trial interventions’ are exclusion criteria; (2) to add a further exclusion criterion: ‘Any other reason which, in the opinion of the PI, renders the patient unsuitable for the trial’. o Section 4 o Section 9.2 • Clarification of the primary endpoint and analyses o Section 8.6.1 o Section 16.1.2 o Section 16.1.2.1 o Section 16.5 • Clarification of sample procedures. o Section 9.3.1.2 • To describe the use of the paper-based back-up randomisation system. o Section 9.4 o Section 9.4.1 • Clarification of oral corticosteroids dose. o Section 10.3.1.3 o Section 10.3.1.3.1 • To specify that some tests are optional for participants randomised to the SOC arm. o Section 11.5 o Section 11.6.3 o Section 11.6.4 o Section 11.6.6 o APPENDIX 2 • Information on scanning biopsy slides and clarification of sample storage conditions o Section 15 • Clarification of statistical details o Section 16.1.2 o Section 16.1.2.1 • Correction of typographical error in the Histological Flowchart for Inclusion Criteria. o APPENDIX 1 Typographical errors corrected.