PROTOCOL TITLE:

A randomized controlled clinical trial to determine if a combined screening /treatment programme can prevent premature failure of renal transplants due to chronic rejection in patients with HLA antibodies.

Short Title – 'Optimized TacrolimuS and MMF for HLA Antibodies after Renal Transplantation:' – "OuTSMART"

Trial Identifiers

EudraCT Number – 2012-004308-36
ISRCTN – 46157828
REC Number – 12/L0/1759

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Study Synopsis

Title of clinical trial	**A randomized controlled clinical trial to determine if a combined screening /treatment programme can prevent premature failure of renal transplants due to chronic rejection in patients with HLA antibodies.**
Protocol Short Title/Acronym	**Optimized TacrolimuS and MMF for HLA Antibodies after Renal Transplantation /OuTSMART**
Study Phase if not mentioned in title	**Phase IV**
Sponsor name	**King's College London / GST NHS Foundation Trust**
Chief Investigator	**Prof. Anthony Dorling**
Eudract number	**2012-004308-36**
REC number	**12/LO/1759:**
Medical condition or disease under investigation	**Premature allograft failure / Chronic rejection**
Purpose of clinical trial	**The overall objective is to test whether a structured screening programme to identify patients with a validated prognostic biomarker for kidney transplant failure, allied with an optimized immunosuppression treatment protocol, can reduce the time to graft failure at the primary endpoint (approximately 43 months post-randomisation).**
Primary objective	**Compare the time to graft failure in patients with HLA Ab who receive an optimized anti-rejection medication intervention ('treatment'), with that in a control group with HLA Ab who remain on their established immunotherapy and whose clinicians are not aware of their Ab status.**
Secondary objective (s)	**a) Determine the time to graft failure in patients randomized to 'unblinded' HLA Ab screening, compared to a control group randomized to 'blinded' HLA Ab screening.** **b) Determine whether treatment influences patient survival** **c) Determine whether 'treatment' influences the development of graft dysfunction as assessed by presence of proteinuria (Protein:Creatinine Ratio > 50 or Albumin:Creatinine Ratio > 35) and change in estimated Glomerular Filtration Rate (eGFR).**

	d) Determine whether 'treatment' influences the rates of acute rejection in these groups **e) Determine the adverse effect profiles of 'treatment' in this group, in particular whether they are associated with increased risk of infection, malignancy or DM.** **f) Determine the cost effectiveness of routine screening for HLA Ab and prolonging transplant survival using this screening/treatment protocol.** **g) Determine the impact of biomarker screening and "treatment" on the patients' adherence to drug therapy and their perceptions of risk to the health of the transplant.**
Trial Design	**A prospective, open labelled, randomised marker-based strategy (hybrid) trial design, with two arms stratified by biomarker (HLA Ab) status.** **Recruitment will take place in 13 renal transplant units, recruiting for minimum of 45 months with recruits followed up intensively for 32 months (maximum 64 months) and primary endpoint assessed by remote evaluation when approximately 43 months post-randomisation is achieved by all.**
Endpoints	**Primary: Time to graft failure in HLA Ab positive patients randomized to biomarker-led treatment groups vs. time to graft failure in HLA Ab positive patients randomized to the control (standard care) group. Graft failure will be defined as re-starting dialysis or requiring a new transplant.** **Secondary:** **Clinical:** **• time to graft failure in patients randomized to unblinded HLA Ab screening vs. blinded screening** **• patient survival.** **• graft dysfunction, as assessed by two separate measures; presence of proteinuria (Protein:Creatinine Ratio > 50 or Albumin:Creatinine Ratio > 35) and change in estimated Glomerular Filtration Rates over 32 months.** **• rates of biopsy-proven T cell-mediated or antibody-mediated rejection over 32 months.** **• rates of culture-positive infection, biopsy-proven malignancy and diabetes mellitus.** **• health economic analysis of outcomes in intervention vs. control groups.** **• analysis of adherence and perceptions of risk in biomarker led care vs standard care groups.**
Sample Size	**It is anticipated that approximately 2357 total patients will need to be recruited. Given the observed proportions of DSA participants, predicted drop outs and HLA Ab conversion rates, this will allow the target of 165 (~83 per group) DSA participants to be recruited. It is expected based on observed proportions, that this will result in approximately 824 (412 per group) non-DSA participants being recruited and 1368 (684 per group) participants remaining HLA Ab negative at the primary endpoint, exceeding the target numbers required for these groups.**

Summary of eligibility criteria	**Included: Renal transplant recipients aged 18-75, > 1 year post-transplantation, with estimated glomerular filtration rate (GFR) ≥30 by 4 variable MDRD.** **Excluded: Recipients of cross-match positive transplant requiring HLA desensitization to remove antibody, recipients of additional solid organ transplants (e.g. pancreas, heart etc), history of malignancy (with exclusions), recent acute rejection, recipients with hepatitis B, C or HIV, recipients known to have HLA antibody who have received specific treatment, known hypersensitivity to any of the IMPs, known hereditary disorders of carbohydrate metabolism, pregnancy, females who refuse to consent to using suitable contraception through trial, patients enrolled in any other studies involving administration of another IMP at time of recruitment.**
IMP, dosage and route of administration	**Oral Tacrolimus od or bd titrated to pre-dose levels of 4-8ng/ml.** **Oral Mycophenolate Mofetil or enteric coated mycophenolic acid bd, tds or qds given at highest tolerated daily dose or according to unit guidelines, with maximum dose determined by SmPC.** **Oral Prednisone od according to the following regime: 20mg od for 2 weeks tapering to 5mg od over 4 weeks.**
Active comparator product(s)	**None**
Maximum duration of treatment of a Subject	**HLA Ab-screening phase will last 45 months. For each recruit, the duration of study will be a minimum of 32 months and up to 64 months, as patients who initially tested negative for HLA Ab, but become HLA Ab positive in the final screening round will be followed up for a further 32 months from that point.**

CONTENTS

1. Background & Rationale

The problem addressed by this study is 'premature' transplant failure – kidney transplants do not last for the natural lifespan of most recipients. Premature in this context refers to the lifespan of the recipient[1]. Current death-censored 10-year transplant survival rates vary between 59 and 70%, so 30-40% of patients have their transplant for < 10 years [1]. Since 2000, a consistent annual attrition rate of around 3% of kidney transplants [2] means that approximately 700 patients return to dialysis each year in the UK. Attrition rates in the USA are similar [1], so this is a worldwide problem. Although many of these patients are eligible for a second transplant, the legacy of the first often makes it harder to find a well-matched second kidney. In addition, second (and any subsequent) transplants have a shorter lifespan than the original transplant, so the problem of premature failure becomes amplified. Of the various reasons why transplanted kidneys fail the single biggest cause is immune-mediated injury [3].

1.1 Existing Research

1.1.1 Using HLA Ab (a prognostic biomarker of premature kidney transplant failure) as a screening test:

Two types of study have linked antibodies (Ab) against human leukocyte antigens (HLA) to immune-mediated injury and premature graft failure. Case control studies have compared patients who have lost grafts with those in whom grafts are still working, performing retrospective analysis of prospectively collected serum samples. For instance, Mizutani et al [4] studied 39 patients with failed grafts due to 'chronic rejection' (CR) and 26 matched controls with functioning grafts. In the former group, 72% had IgG HLA Ab, compared to 46% of controls. Similar results were obtained from a different study of a separate population [5]. The surprising thing from these studies was the high incidence of HLA Ab in patients with working grafts. There are several potential explanations for this. First, it is possible that factors about the HLA Ab (such as complement fixing ability) or factors other than the Ab influence the progression of CR and thus the timing of eventual graft rejection. Our data supports the latter, by linking progression of renal dysfunction to activity of the cellular immune responses (see appendices). A second, related possibility, is that all patients with HLA Ab develop pathology, but progressing at different rates, such that patients showing up in the control groups in these studies are deteriorating more slowly. Evidence for this comes from Mizutani [4], who showed that their CR group with HLA Ab showed progressive deterioration of renal function prior to graft failure. The same progressive deterioration was seen in the control group of patients with HLA Ab, whose grafts did not fail. These data illustrate that CR is a time-dependent process in which progressive graft dysfunction precedes graft failure. Moreover, the time from development of HLA Ab to graft failure is highly variable in different people.
Separate studies have reported prospective follow-up of outcomes in those with HLA Ab. Terasaki et al [6] studied 2231 patients. In the group of 479 with HLA Ab, the two-year graft failure rate was 15%, compared to 6.8% in the 1753 with no HLA Ab. This trial noted that the patients who failed within two years had worse renal function on testing than those that didn't, consistent with the fact that CR is a progressive and time-dependent process and those that fail are at the end of this process. In another study, the same group [7] reported 4 year survival rates in 1329 patients, all with functioning transplants, of 58% in those with HLA Ab (158 patients) vs. 81% in those without (806). Lachmann et al [8] have performed the best study to date, of 1014 patients with stable kidney function (for the six months pre-recruitment) from a single centre in Berlin, on average 6 years post-transplantation, who were tested for HLA Ab and prospectively followed for 5½ years. Grafts failed in 37% of the 302 who had HLA Ab, but in only 17% of the 712 patients who tested negative for HLA Ab. Moreover, in this latter group, a subgroup of 195 patients had a repeat test performed 2 ½ years into the study; of these, 148 remained negative and only 6% of grafts failed in this group. In contrast, 47 had developed new HLA Ab since the beginning of the study and 21% of these suffered

1 In the transplantation literature, this problem is called 'late' allograft failure, in which 'late' refers to the lifespan of the transplanted organ. We have changed the term to shift emphasis onto the recipient.

graft failure, confirming that the development of new HLA Ab in the 'negative' group was predictive of future graft loss. Finally, this study identified a difference between the prognostic value of HLA Ab that were specific for the donor (donor specific antibodies – DSA, found in 33% of HLA Ab positive patients) and those that were not (non-DSA, found in 66%). Graft failure rates were 51% over 5.5 years in patients with DSA and 30% in patients with non-DSA. In subgroup of patients who had transplant biopsies, 78% of those with failed grafts and HLA Ab+ had changes consistent with CR. They concluded that grafts in patients with HLA Ab were >3x more likely to fail than those without, even when corrected for age, gender, year of transplant, estimated GFR and number of previous kidney transplants. These findings have been corroborated by a second study from the Netherlands [9], in which the risk of graft failure with HLA Ab was also shown to be independent of graft dysfunction and proteinuria.

1.1.2: The biology of HLA Ab and CR:

Although there is a widespread view in the literature is that HLA Ab *cause* CR, there is no evidence of this in humans. We have data indicating that T cell responses, via specific interaction with donor-specific B cells, drive the progressive deterioration in a subgroup of patients with CR and HLA Ab (see appendices).
Donor HLA are the primary target of the immune response against the transplanted kidney and it is for this reason that HLA matching pre-transplantation still underpins organ allocation, to maximise the similarities between donor and recipient HLA.
The prevailing view in the literature is that when HLA Ab are present, they cause the pathology that ultimately leads to graft dysfunction and kidney failure – indeed, one type of CR is called chronic antibody-mediated rejection (CAMR), exemplifying how Ab are thought to contribute to the process [10] (see appendix 1). This is easy to conceptualise when the HLA Ab detected in the circulation are donor specific (i.e. DSA), but less easy when only non-DSA are found, though conventional wisdom has it that the DSA in these patients are all deposited within the graft [11]. However, although Ab against the mouse equivalent of HLA have been shown to induce some of the features of CR in experimental models, there is no clinical evidence to support a causative role for HLA Ab in human CR.
In reality, the pathophysiology of human CR is likely to be complex. The presence of HLA Ab indicates that the recipient's immune system has recognised and reacted to donor HLA. Although the Abs are made by plasma cells, these arise from activated antigen-specific B cells through a process that involves intimate contact between the B cells and HLA-specific T cells. Thus multiple components of the recipient immune system, including T and B cells are sensitised against the donor in patients with HLA Ab and there are several mechanisms capable of damaging the transplanted organ that may be operating in these patients.
The chief investigator's group has been investigating patients with CR for several years. This work has been seeking the answers to two basic questions; which elements of the immune system are involved in the pathology of CR and what is driving the deterioration in kidney function? Data from these studies indicates that in patients with HLA Ab, the activity of T and B cells is most strongly associated with progression of CR. For example, 15 patients with early 'Ab-mediated' injury on protocol kidney biopsy were followed for 2.5 years, during which 6/15 developed clinical evidence of CR, manifesting as a progressively deteriorating creatinine or development of significant proteinuria. Examining the peripheral blood mononuclear cells of these patients, interferon-gamma (IFN-γ) production by T cells at the time of biopsy was most strongly associated with development of deteriorating function, compared to multiple other variables examined, including HLA Ab. In 75% of these people, B cells were helping CD4+ T cells to make the IFN-γ, consistent with hypothesis that the close interaction between antigen-specific T and B cells is at the centre of the mechanisms operating in the deteriorating transplants in these patients (see appendix 1).

1.1.3: Evidence supporting the use of optimized oral immunotherapy regime in patients with HLA Ab:

The data from the CI, referred to above suggests that it would be more rational to target underlying cellular immune responses (i.e. by T and B cells) to prevent CR, rather than focus on the Ab. Based on this, a strategy to enhance immunosuppression to target T and B cells was tested in a group of >30 patients with established CR, all of whom had deteriorating

transplant function. 18 of these, with relatively homogenous pathology on their renal biopsies, received 'optimized treatment' with tacrolimus (Tac), MMF and in a few, rituximab. After this intervention, 11/18 were assessed to have stabilised 1 year later. However, at 3 years, only 7/18 remained stable. Analysis of T and B cell activity at the time of biopsy indicates that donor-specific IFN-γ production by T cells strongly associated with long-term stability, indicating that these patients benefit most from the enhanced immunosuppression (appendix 1). In addition to this work, the CI is also running a randomized controlled trial (RituxiCAN-C4) in patients with advanced CR, and this has a run-in phase in which all patients get optimized on Tac and MMF. Though still ongoing, it is clear from the interim analyses that up to 30-50% of the patients stabilize during this run-in phase, some of them remaining stable for more than 3 years (appendix 2). The patients who appear to benefit are those with the least advanced CR, suggesting that, if caught early, optimized oral therapy to suppress T and B cells may be sufficient to control the underlying pathological process.

Research by others working in this area is mostly focussed on how to treat established CR. All reports contain small numbers of patients. Theruvath et al reported 12-month stabilisation of kidney function in ¾ patients with kidney dysfunction due to CR associated with HLA Ab after transfer onto Tac and MMF and a short course of prednisolone [12]. In addition, several studies have reported successful stabilization of CR using B cell depletional therapy [13-15], further supporting the hypothesis that targeting underlying cellular responses, rather than HLA Ab, is a rational approach to treatment in these patients.

In contrast to these, this trial will involve optimization of therapy in many patients before they have started to clinically deteriorate, to try and prevent the graft dysfunction associated with CR. As described above, the natural history of untreated CR is of progressive loss of function, usually at a predictable rate, leading eventually to complete loss of graft function. Depending on the starting creatinine, the time to graft loss will be variable, but graft loss rarely occurs without this period of progressive loss of function. Lachmann et al [8] reported a constant rate of graft loss from the start of their study, so we expect that the rate of graft loss in our study to be constant in the standard care group. We predict that optimised immunotherapy will change the natural history of the condition, and lead to stabilisation of function in a significant proportion (50%) of those with HLA Ab. This will be visible in our analyses of the secondary endpoints. Hopefully it will prevent the predicted graft loss in the first 3 years in this group and thus impact on the primary endpoint of the study.

Several other strands of evidence support the use of an optimized Tac and MMF regime to achieve this. First, both MMF [16] and Tac [17] are better at suppressing acute rejection than alternative agents and the combination of the two agents achieves better outcomes at 1 and 2 years compared to alternative regimes [18, 19]. Second, regimes containing MMF are associated with a lower prevalence of HLA Ab [20] and MMF specifically reduces the development of HLA Ab development during episodes of acute rejection [21]. Third, although these benefits have not always fed through to improvements in graft survival rates, one recent landmark study did show improved graft survival on the combination of Tac and MMF ([22]. For Tac, enhanced graft survival also emerged during a systematic Cochrane review comparing Tac with ciclosporin (CsA) [19]. For MMF, a retrospective analysis of US registry data revealed an association with significantly lower rates of premature allograft failure [23]. Finally, some studies have shown that conversion from CsA to Tac is beneficial in patients with deteriorating graft function [24], and that introduction of MMF has a similar effect [25]. Although other studies have reported contradictory results, [26] much of this literature is difficult to interpret because many studies do not distinguish between CR and other causes of chronic graft dysfunction [27].

In addition to Tac and MMF, we propose to use a short course of moderate-dose prednisolone followed by low dose steroid maintenance in this trial. There is no direct evidence from the transplant literature to support this intervention, but a similar treatment course is standard therapy in many situations where quick and effective suppression of immune responses is required, for example in acute asthma and in many types of autoimmune diseases.

1.2. Risks and Benefits

1.2.1: Risks:

Conversion to Tac from CsA is associated with an increased risk of diabetes mellitus (DM), reported in one study as occurring in 12.5% compared to 4.5% in those who remained on CsA [24]. Analysis of >130 trials comparing the two agents revealed that Tac is associated with a 5% higher rate of DM compared to CsA [19]. This risk is likely to be exacerbated by the prednisolone, although the DM induced by combined pred/Tac is only transient in approximately 50% of patients. Enhanced immunosuppression is associated with an increased incidence of infection, especially viral and with an increased risk of malignancy. The precise risks of both these in this trial are difficult to estimate. The incidence of DM, infection and malignancy will be monitored carefully on this trial.

1.2.2: Benefits:

Tac is associated with a better cholesterol profile [28] and lower blood pressures [19] than CsA.

1.3. Rationale for Current Study

Spending on kidney failure services is 3% of the total NHS budget. The National Service Framework recognizes transplantation as the most clinically and cost effective treatment for patients with kidney failure. To maximise rates of transplantation, efforts are focussed on increasing the number of donor organs by 50% in the next few years. In the words of the NHSBT, “The cost benefit of kidney transplantation compared to dialysis over a period of ten years is £241,000.” The problem of premature transplant failure undermines the ability of the NHS to capitalise maximally on improved transplantation rates and is an aspect hitherto ignored by health strategists.

Because CR is the single biggest cause of premature graft failure and because HLA Ab are an established prognostic biomarker for premature graft failure, there is a need to test whether treatment decisions based on the presence of the biomarker can alter prognosis. So this trial combines 2 elements, testing whether a routine screening programme for HLA Ab in all kidney transplant recipients is useful, and then, for those found to be HLA Ab +, testing whether the (randomized) introduction of a standard optimization treatment protocol can reduce graft failure rates.

Management in both the biomarker-led care (BLC) and standard care (SC) groups will involve control of hypertension, proteinuria and hypercholesterolaemia to defined target ranges using conventional agents. Clinicians will be blinded to the results of the screening tests in the ‘blinded’ groups. Patients who have no HLA Ab at their initial screening will be re-screened every 8 months for the first three years of the study. If found to be HLA Ab+, those in the ‘unblinded’ group (D) will enter the standardized anti-rejection optimized treatment protocol, whereas the treatment of those in the ‘blinded’ group (C) will be unchanged and clinicians will remain unaware of the change in HLA Ab status.

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2. Trial Objectives and Design

2.1. Trial Objectives

The overall objective is to test whether a structured screening programme to identify patients with a validated prognostic biomarker for kidney transplant failure, allied with an optimized immunosuppression treatment protocol, can reduce transplant failure rates over time.

Primary objective;
Determine the time to graft failure in patients testing positive for HLA Ab at baseline or within 32 months of randomization who receive an optimized anti-rejection medication intervention with prednisone, Tac and MMF ('treatment'), compared to a control group who test positive for HLA Ab at baseline or within 32 months post-randomization who remain on their established immunotherapy and whose clinicians are not aware of their Ab status. The primary endpoint will be assessed remotely when approximately 43 months post-randomisation has been achieved by all.

Secondary objectives;
a) Determine the time to graft failure in patients randomized to 'unblinded' HLA Ab screening, compared to a control group randomized to 'blinded' HLA Ab screening.
b) Determine whether 'treatment' influences patient survival
c) Determine whether 'treatment' influences the development of graft dysfunction as assessed by presence of proteinuria (Protein:Creatinine Ratio > 50 or Albumin:Creatinine Ratio > 35) and change in estimated Glomerular Filtration Rate (eGFR).
d) Determine whether 'treatment' influences the rates of acute rejection in these groups
e) Determine the adverse effect profiles of 'treatment' in this group, in particular whether they are associated with increased risk of infection, malignancy or DM.
f) Determine the cost effectiveness of routine screening for HLA Ab and prolonging transplant survival using this screening/treatment protocol.
g) Determine the impact of biomarker screening and "treatment" on the patients' adherence to drug therapy and their perceptions of risk to the health of the transplant.

2.1.1 Primary endpoints

The primary endpoint is time to graft failure in HLA Ab positive patients randomized to biomarker led care groups vs. time to graft failure in HLA Ab + patients randomized to standard care groups assessed at approximately 43 months post-randomisation achieved by all. A second capture of primary endpoint data will also be taken at 46-92 months post-randomisation to be used in a sensitivity analysis. Graft failure will be defined as re-starting dialysis or requiring a new transplant.

2.1.2 Secondary endpoints

The secondary clinical endpoints are:
• time to graft failure in patients randomized to blinded HLA Ab screening vs those randomized to unblinded screening. Graft failure will be defined as re-starting dialysis or requiring a new transplant.
The following endpoints will be assessed at end of intensive follow up (32 months):
• patient survival.

• graft dysfunction, as assessed by two separate measures proteinuria (Protein:Creatinine Ratio > 50 or Albumin:Creatinine Ratio > 35) and change in estimated Glomerular Filtration Rates, and the rate of progression to graft dysfunction.
• rates of biopsy-proven rejection.
• rates of culture- or polymerase chain reaction (PCR)-positive infection, biopsy-proven malignancy and DM.
• health economic analysis of outcomes in intervention vs. control groups.
• analysis of adherence and perceptions of risk in BLC groups.

2.2 Trial Design & Flowchart

This is a prospective, open labelled, randomised marker-based strategy (hybrid) trial design, with two arms stratified by biomarker (HLA Ab) status. Recruitment will take place in 13 renal transplant units, recruiting for 45 months with recruits followed up intensively for at least 32 months (maximum 64 months) and primary endpoint assessed by remote evaluation after approximately 43 months post-randomisation is achieved by all. The trial design is represented in the flow diagram in section 2.3, showing the number of patients anticipated to be in each group by the end of the trial, based on sample size calculations, consent rates, eligibility and estimated fall-out. Using the flow diagram (top-to-bottom) as a guide: recipients of cross-match negative transplants aged 18-75, > 1 year post-transplant with an eGFR ≥ 30 will consent to the screening/treatment process. The first stratification will result from blood test screening for HLA Ab. Approximately 35% will be HLA positive, with ~65% negative. The HLA Ab+ patients will be further screened with single antigen beads to determine whether DSA are present (~1/6 DSA and 5/6 non-DSA). Thus, biomarker stratification leads to three groups (DSA+, non-DSA+ and HLA Ab-neg). The second stratification will be based on current immunosuppression, to ensure balanced numbers already on Tac or MMF in each group. The final stratification will be by site. HLA Ab positive patients will be randomized 1:1 into either Blinded Standard Care or Unblinded Biomarker led-care. Patients in the former (groups A1 & A2 in the flow chart in 2.3) will be blind to their biomarker status and will remain on baseline immunotherapy, whereas patients in the latter (groups B1 and B2 in the flow chart) will know their HLA Ab status and will be offered “treatment”. HLA Ab-negative patients will remain on their existing immunotherapy and randomized 1:1 into either Blinded (group C) or Unblinded groups (D), with only the latter knowing their HLA Ab status. Both these groups will receive regular Ab status monitoring for the first 3 years. Those patients who become positive during subsequent screening rounds (~10% per year) will be moved to the appropriate HLA Ab positive groups (DSA+ or non-DSA+) for final data analysis. All patients in group D found to be positive on second or subsequent rounds will be offered the same “treatment” as those patients who were positive in the first screening round, and be intensively followed up for an additional 32 months from the time they become positive. Thus the maximum amount of time any single patient may remain in intensive follow up is 64 months[2]. New patients will be recruited to the study at each successive screening round.

[2] For example, a patient recruited at the beginning of the study into groups C or D, found to have developed HLA Ab on the final screening round, will transfer into groups A or B and remain in intensive follow up for another 32 months.

2.2.1 Table of events - Summary of study procedures

Phase	Peri-Randomization	Post-Randomization											
		Unblinded HLA Ab+ groups – Approximate times of assessment (+/- 1 week). Once stabilised, go to month 8 assessment						All Groups – Approximate times of assessment (+/- 3 months)					
Study Week/month	Day -56 to 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12	Month 8	Month 16	Month 24	Month 32	~ 43 months	Month 46-92
Informed consent	x												
Inclusion/Exclusion Criteria	x[3]												
Medical History inc. Drugs	x[4]												
Transplant / sensitisation Hx	x												
Registration / Demographics	x[5]												
Weight / BP	x							x	x	x	x		
Urine PCR or ACR	x							x	x	x	x		
Haematology[6]	x		X		x		x	x	x	x	x		
Biochemistry	x[7]		x[8]		x[8]		x[8]	x[9]	x[10]	x[9]	x[10]		
[Calcineurin inhibitor] trough	x	x[11]	X	x	x	x	x	x	x	x	x		

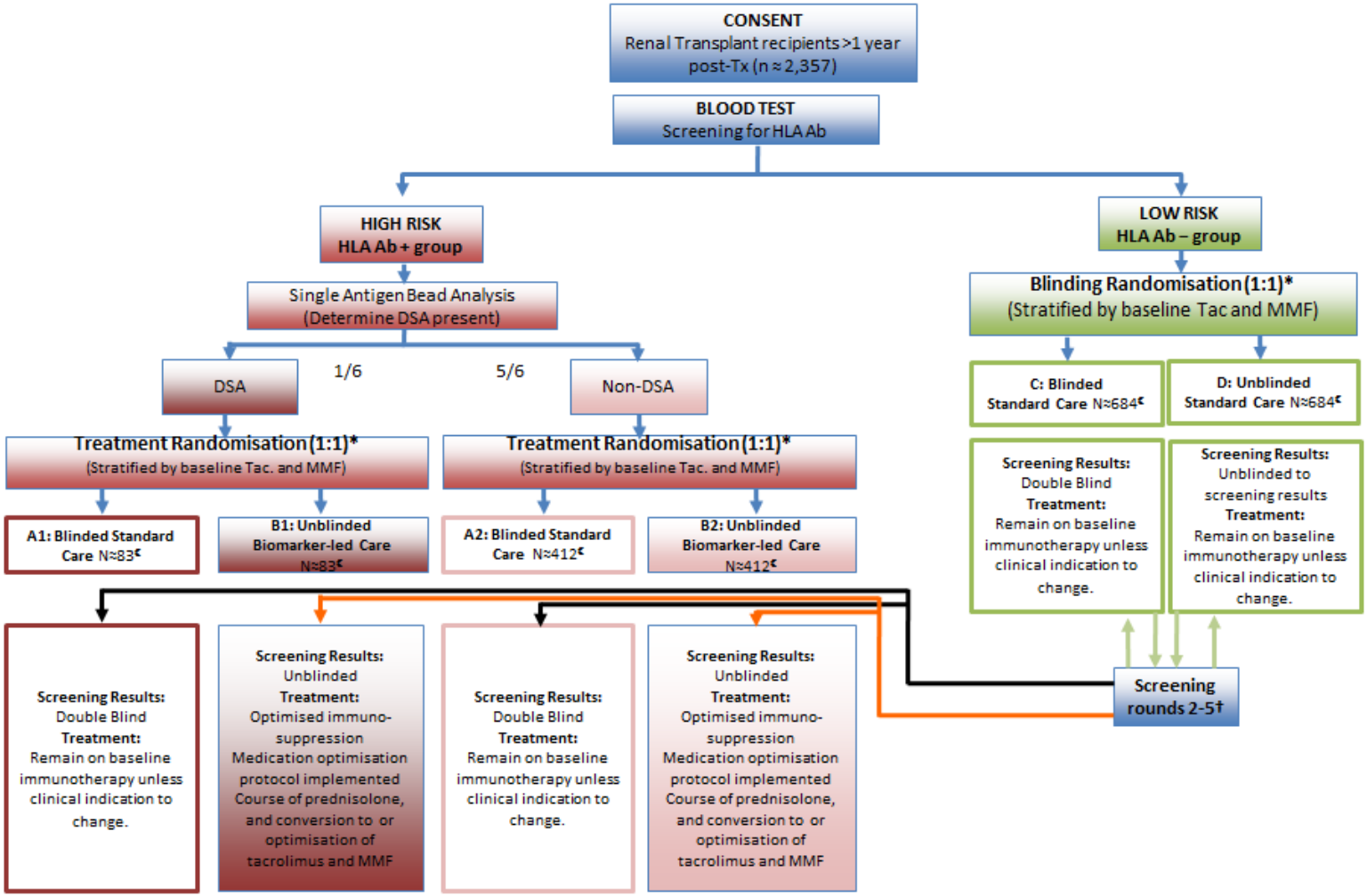
[3] Including virology and pregnancy testing where appropriate.
[4] For registration, need to know whether already on tacrolimus and / or MMF/myfortic.
[5] Do this prior to taking blood for HLA Ab screening
[6] Hb, WCC, platelet count at all time periods
[7] Creatinine, Na^+, K^+, bicarbonate, calcium, CRP, lipid profile, glucose, HbA1c.
[8] Creatinine, Na^+, K^+, glucose, HbA1c
[9] Creatinine, Na^+, K^+, bicarbonate, calcium, CRP, glucose, HbA1c
[10] As enrolment biochemistry
[11] In those patients having optimization of tacrolimus – continue until trough levels achieved

Total immunoglobulin (or IgG, IgM +/- IgA)	x								x		x		
HLA antibody screening	x[12]							x[12]	x[12]	x[12]	x[12]		
Apply optimized treatment protocol[13]		x	X	x	x	X	x						
See Trial-specific Nurse	x							x	x	x	x		
Record Medications	x							x	x	x	x		
Adverse Events Form		x	X	x	x	X	x	x	x	x	x		
Questionnaire for analysis of adherence / risk	x									x			
Questionnaire for health economics	x								x				
Primary Endpoint (remote data collection)												x	
Primary Endpoint (sensitivity analysis; remote data collection)													x

Note: where an “x” is contained within a field this denotes that the associated data will be collected at the identified time point.

[12] At enrollment, on everyone. Beyond enrollment, send sample from recruits in unblinded HLA Ab-negative group and ALL blinded patients.
[13] Ideally participant will see a physician once a month whilst being optimized. Visit details are recorded in an Optimisation Log and not in the eCRF.

2.3 Trial Flowchart



*Randomisation performed on results of a recruit's first screening test. Those with HLA Ab undergo no further screening as part of the trial (but serum will be stored for analysis of HLA Ab profiles later). †Those initially HLA Ab-negative undergo routine screening every 8 months. THERE IS NO SECOND RANDOMIZATION: If a recruit allocated to Blinded standard care (group C) becomes HLA Ab positive (black lines), he/she remains in Standard care group (group A1 or A2). If in unblinded standard care group (D), they change to unblinded biomarker-led treatment care (group B1 or B2) (orange lines). € Numbers in each group are those anticipated at the end of study.

3 Trial Medication

All treatments will be introduced on the basis that they will be tailored to the individual patient, according to compliance, tolerance and achievement of target levels (for Tac). Failure to tolerate one or more of the components of the protocol (or refusal to take any of the agents) will not be used as a reason for withdrawal from the study.

3.1 Investigational Medical Products and dosing regimen

The 'optimized treatment' protocol in the two groups (B_1, B_2 in section 2.3) with HLA Ab will be;
a) Mycophenolate mofetil bd, tds or qds, or enteric coated mycophenolic acid bd, with daily dose determined according to local unit guidelines. The patient will be stabilized on the maximum tolerated dose.
b) Tacrolimus od or bd, according to local unit preference, with dose titrated to achieve 12-hour post-dose levels of 4μg/L to 8μg/L (4-8 ng/ml). The patient will be stabilized on the maximum tolerated dose that achieves these levels.
c) Prednisolone od. Starting at 20mg for two weeks, then reducing by 5 mg od every two weeks down to their previous maintenance dose or 5mg od, if not previously taking.
After consultation with the MHRA, we have confirmed that all these medicines will be classed as IMPs, whereas all others will not. Mycophenolate mofetil/mycophenolic acid is being used outside of its Marketing Authorisation (which states that it should be used with ciclosporin). However, because it is now used so widely in combination of tacrolimus in most units in the UK, the two can be regarded as 'standard care'. We therefore propose that the three drugs will not require labelling in line with annex 13. This means the IMPs can be managed in the same way as normal i.e. GP or hospital prescription (as appropriate) and will not require special labelling/accountability/storage etc.

3.2 Drug Accountability

Not applicable

3.3 Subject Compliance

See section 6.2

3.4 Concomitant Medication

Patients in all groups will have blood pressure controlled and total cholesterol lowered, using agents according to local unit guidelines and working to unit-defined targets. All other medication and treatment will be determined by local unit guidelines.

4. Selection and Withdrawal of Subjects

4.1 Inclusion Criteria

• Sufficient grasp of English to enable written and witnessed informed consent to participate.
• Renal transplant recipients >1 year post-transplantation, male or female
• Aged 18-75 years
• Estimated glomerular filtration rate (eGFR by 4 variable MDRD) of ≥30 (within the previous 6 months of signing consent or taken at screening if not done in the previous 6 months).

4.2 Exclusion Criteria

• Recipient requiring HLA desensitisation to remove antibody for a positive XM transplant
• Recipient known already to have HLA antibody WHO HAS RECEIVED specific intervention for that antibody or for CAMR / chronic rejection
• Recipient of additional solid organ transplants (e.g. pancreas, heart, etc).

• History of malignancy in previous 5 years (excluding non-melanomatous tumours limited to skin)
• HBsAg+, HepC IgG+ or HIV+ recipient (on test performed within previous 5 years)
• History of acute rejection requiring escalation of immunosuppression in the 6 months prior to screening.
• Patient enrolled in any other studies involving administration of another IMP at time of recruitment
The following exclusion criteria are based on information contained within the SMPcs of the IMPs
• Known hypersensitivity to any of the IMPs
• Known hereditary disorders of carbohydrate metabolism
• Pregnancy or breastfeeding females (based on verbal history of recipient)
• Pre-menopausal females who refuse to consent to using suitable methods of contraception throughout the trial.

4.3 Selection of Participants

The local transplant clinic database will be used to identify patients meeting the baseline inclusion/exclusion criteria. At the start of the trial, the entire population of transplant clinic attendees who meet the eligibility criteria are potentially eligible for recruitment. On subsequent screening rounds, patients who reach 12 months post-transplantation after the start of the trial will become eligible and these will be recruited before the next screening round.
Informed consent – Potentially eligible patients will be approached at a routine clinic appointment by the PI or research nurses and given printed and verbal information about the trial. They will have the opportunity to return for a second consultation within a few days to give informed consent for recruitment into the study or to do this on their next routine appointment. Alternatively, eligible patients will be sent information about the study through the post, for discussion and consent at their next routine appointment. Following consent, full eligibility criteria will be reviewed. This may include testing for chronic viral disease (if no such test within last 5 years) or pregnancy (if history suggests possibility of pregnancy).

4.4 Randomisation Procedure / Code Break

Prior to randomisation but after consent, site staff will register all recruits online and each will be assigned a MACRO PIN. Samples from all recruits will be sent to the HLA laboratory, along with this PIN and a sample request form containing other information required for randomisation.

Lab staff will screen for HLA Ab and perform single antigen bead testing on positive screening samples to check for the presence of DSA. Once this information is known, the lab staff will access the randomisation system and randomise the patient, using the HLA Ab results and information on the sample form to stratify. In all sites, the PI's and nurses will be automatically emailed and the system will tell them whether the patient is in the blinded or unblinded groups. If in the unblinded group it will feed back HLA Ab status to the PI. Unblinded patients will be identified by blue stickers to be appended to the notes and all future clinical samples. The system will tell the trial staff to enter HLA Ab-negative patients into the subsequent 8 monthly screening rounds, and also whether the patients have been selected to provide future samples for 8 monthly scientific analysis (for transfer to the CIs laboratory). This information will be relayed using a 'star' on the blue labels, appended to their laboratory request forms thereafter.
Blinded patients will have green stickers/labels. HLA Ab status will not be fed back to the PIs or trial staff. A 'star' will be used to tell the trial staff which recruits have been selected to provide 8 monthly samples for transfer back to the CIs lab for scientific analysis. All these patients will have samples taken 8 monthly for HLA Ab screening. Once inside the lab, the lab staff will use their knowledge of the HLA status to determine those from HLA Ab-negative patients which will undergo screening. The samples from HLA Ab positive patients will be discarded.

On the second and subsequent HLA Ab screening rounds, the lab staff will update the randomisation system. The lab staff will have 52 days from the date the re-screen sample

was taken to perform the HLA Ab re-screen for the required participants. The results from patients in the unblinded groups only will be forwarded to the PI and lab staff, via email. This will indicate whether status has changed and trigger the initiation of the treatment protocol in those that have changed from HLA Ab negative to positive.

Randomisation will be via the online King's Clinical Trials Unit randomisation system. Laboratory staff at each recruiting site, with access to HLA Ab results, will be provided with a unique username and password to access the randomisation system. Password access must be authorised by the trial manager in all cases and request directly from sites will not be processed. Access to the system is via www.ctu.co.uk, clicking the 'randomisation – advanced' link and selecting the OuTSMART Trial.

There are no blinded study medications in the trial so no emergency code break is required. In the event that a study site clinician wishes to be made aware of blinded laboratory results, this must be discussed and agreed with the trial manager and the study Chief Investigator in all cases. It is not anticipated that unblinding in this manner will be required and only in extraordinary circumstances would this be agreed.

4.5 Withdrawal of Subjects

Withdrawal and stopping criteria: Individual recruits can withdraw at any time if they wish. Failure to tolerate one or more components of the 'treatment' will ***not*** be seen as a reason to withdraw an individual participant from the trial but is to be anticipated as an integral part of individualising therapy. The randomization process will be halted temporarily if any of the following are noted;

• A patient death attributable to 'treatment'.

• Unacceptable incidence of severe adverse events attributable to 'treatment' (if occurring in >10% patients). In both these instances, the trial will undergo urgent review by the Data Monitoring and Ethics and Trial Steering committees.

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, AEs, SAE's, SUSAR's, protocol violations, cure, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible. Should a patient withdraw from study drug only, efforts will be made to continue to obtain follow-up data, with the permission of the patient.

Participants who wish to withdraw from 'treatment' will be asked to confirm whether they are still willing to provide study specific data and samples for scientific laboratory analysis according to the trial protocol.

4.6 Expected Duration of Trial

The trial is expected to recruit for a minimum of 45 months. The recruitment target is to recruit 165 HLA Ab positive DSA patients, 269 HLA Ab positive non-DSA patients and 243 patients to remain HLA Ab negative until the end of follow up. The targets for DSA and non-DSA include HLA Ab negative participants who develop do-novo antibodies at the 8 monthly re-screening rounds and hence become either HLA Ab positive DSA or HLA Ab positive non-DSA. An estimated 237 patients will need to be recruited overall to recruit the target of 16 DSA patients. Because of this requirement to recruit sufficient DSA participants, recruitment to the other groups is likely to be greater than the specified targets.

Following recruitment to the trial, the patients will undergo 32 months of intensive follow up involving 8-monthly clinic visits post-randomisation, except in the following scenario; a patient in groups C or D who becomes Ab positive during the initial 32 months follow up will transfer to the relevant Ab+ group and undergo intensive follow up for a further 32 months from date of transfer. Therefore, the maximum amount of time that any single patient may remain in intensive follow up is 64 months. The secondary endpoints will be assessed at the end of the

intensive follow up period (32 months up to 64 months) and at this point trial procedures relating to the participants will finish. The participants will be informed that they no longer are required to attend research clinic visits. The original plan was for the primary endpoint to be assessed remotely when at least 43 months post-randomisation had been achieved by all. The last participant research clinic visit was expected to be in March 2020 with the assessment of the primary endpoint being performed during the final three months of the trial concluding at the end of June 2020 when the last participant recruited reaches 43 months post-randomisation.

Due to the coronavirus pandemic in the UK in 2020 most clinical trial activity, including this trial, was severely limited and so it was not possible to rely on the original plan to obtain the primary endpoint data between April and June 2020. For this reason, the best alternative was to obtain the primary endpoint data from patients' clinic notes. Evidence for graft failure or death will be taken from the participants' last hospital contact prior to March 16 2020. These data, which will reflect participants' pre-COVID status, will be used for the primary endpoint analysis.

Evidence of graft failure or death will also be taken from participants' notes from their most recent hospital contact at the point of a final assessment between September 1 2020 and November 30 2020. During this designated three-month window, endpoint data will be collected from each patients' notes only once. These data, reflecting status post-onset of COVID crisis, will be used for a sensitivity analysis. The trial will conclude on November 30 2020.

The end of trial for this study has been defined as the last follow up of primary outcome data.

5. Trial Procedures

Synopsis: A structured screening programme for IgG HLA Ab is proposed in patients who give consent. Results obtained will initially be blinded to the transplant clinicians and patients. DSA+, non-DSA+ and HLA Ab-neg groups will be randomized through the KCL CTU (stratified by current immunosuppression) into the groups as detailed. Patients in groups A1, A2 and C (see flow diagram section 2.3) will remain blinded to the results of their screening (as will their clinicians), whereas those in B1, B2 and D will learn whether they are HLA Ab+ or Ab-neg. All recruits will undergo a final test for HLA Ab status as they reach the end of the study. The 'optimized treatment' protocol in the two groups (B_1, B_2) with HLA Ab is outlined in section 3 above. Patients in all groups will have blood pressure controlled and total cholesterol lowered, according to local unit guidelines.
All treatments will be introduced on the basis that they will be tailored to the individual patient, according to compliance, tolerance and achievement of target levels (for Tac). Failure to tolerate one or more of the components of the protocol (or refusal to take any of the agents) will not be used as a reason for withdrawal from the study.

5.1 By Visit

Post consent, patients who have not been screened for HIV or hepatitis B/C within the last 5 years will need to have additional screening tests for these viruses. Female patients who report they may be pregnant will have a blood or urine test for beta-HCG levels. Once eligibility criteria have been met, the following baseline data will be recorded at recruitment. a) Weight and bp; b) Sex and ethnicity; c) Age and date of birth; d) HLA type and that of donor kidney (if known); e) Any significant past medical history, including history of diabetes mellitus, cause of renal failure, details of previous transplants and cause of graft loss, evidence of sensitisation pre-transplantation (PRA and antibody specificities if known); f) medication list and doses; g) Protein:Creatinine ratio on urine sample;
All patients will then have blood taken for;
i) Baseline clinical parameters: a) Full blood count (minimum Hb, WCC, platelets); b) Biochemical series (creatinine, Na^+, K^+, bicarbonate, calcium, CRP, lipid profile, glucose, HbA1C); c) MDRD eGFR on latest creatinine; d) current calcineurin inhibitor 12 hour trough

levels (as appropriate); e) Total immunoglobulin or IgG, IgM +/- IgA levels. If the participant has had blood taken for any of these tests, sampled for routine care up to one week prior to consent, use results present in hospital notes. If this is the case, then only take blood for trial-specific tests.
ii) Scientific analyses: 50-60 mls blood for separation of PBMC and 20mls for serum storage. For participants who require screening for HIV or hepatitis B/C for eligibility, baseline blood samples for scientific analyses will not be taken. Scientific blood samples from these participants will be taken on their next research clinic visit after eligibility has been confirmed. The collection of scientific blood samples will continue throughout the trial only as long as the resources and the capability of receiving, processing and storing samples are available.

iii) Analysis of HLA Ab status (10mls clotted blood), as described above, which will allow randomization to proceed.
All patients will be asked to complete questionnaires.

Subsequent intensive follow up visits.
HLA Ab+ participants in the unblinded group will have a discussion about the risks and prognostic significance of being antibody positive. The optimised treatment protocol will be introduced ideally within 3 months or as soon as possible thereafter in those HLA Ab+ patients allocated to this group (optimisation must be performed within 8 months of HLA Ab positive screening). The first optimisation visit can be performed by the physician either in the clinic or alternatively over the phone. Recruits will then be seen *up to* two weekly during this period (maximum of 6 extra clinic appointments are envisaged), though they should be on maintenance dose prednisolone 7 weeks after initiating optimisation. During this period they will have FBC (as above), creatinine, Na^+, K^+, glucose, calcineurin inhibitor trough levels and blood pressure monitored according to the schedule in 2.2.1. Optimisation visits will not be recorded in the eCRF but will be documented in an “Optimisation Log” at each site. Results from blood tests taken during the optimisation process will be recorded in the patients’ medical notes but not in the eCRF. Once stabilized, they will be seen at least 8 monthly in transplant clinic. Patients allocated to all other groups will be seen at least every 8 months in transplant clinic for formal study assessments. Trial-specific nurses will carry out all trial-related procedures with participants at follow up visits. Patients may be seen at other times during this period, according to clinical need, but study assessments should be done within the time parameters in Table 2.2.1. Ideally assessment times will be performed +/- 1 month of the study assessment month. However assessments can be performed up to 3 months prior and 3 months after the scheduled study visit without deviation to the protocol. If required, research nurses or clinic staff will contact participants up to a week before their allocated clinic appointment to ensure that the participant will be attending their appointment.

Once every 8 months the following will be recorded. a) Weight and bp; b) Full blood count (minimum Hb, WCC, platelets); c) Biochemical series (creatinine, Na^+, K^+, bicarbonate, calcium, CRP, glucose, HbA1C); d) MDRD eGFR on latest creatinine; e) calcineurin inhibitor trough levels; f) protein: creatinine ratio on urine sample; g) episodes of infection, malignancy or new DM; h) episodes considered to be adverse events; i) medications and doses participant is currently taking on the day of the follow up visit. We will not record medications taken for short courses during the 8 months prior to any study visit. Every 16 months total immunoglobulin or IgG, IgM +/- IgA levels will be measured and recorded in the eCRF. In addition, every 16 months a lipid profile will be performed. If research nurses are contacting participants by telephone to remind them of allocated appointments, and provided that the participant is in agreement, details regarding episodes of infection, malignancy or new DM and episodes considered to be adverse events will be collected during the phone call. These details will be updated on the appointment day and then recorded in the eCRF.

Assuming resources are available, on all patients with HLA Ab, and a small cohort of patients without, separate blood samples will be taken for separation and storage for future non-routine scientific analyses. Steps will be taken to ensure this sampling does not break the blinding of group allocations. Once every 8 months, HLA Ab-negative patients will undergo further screening for HLA Ab (see above). The aim of the study is to conduct all visit procedures, baseline and follow up, but as it is a Type A trial any procedure missing will not be considered a protocol deviation.

Upon completion of the 32- to 64-month intensive follow up, consisting of 8-monthly research visits, the participants will be told by the research nurses that they no longer need to attend to clinic for research visits but their regular clinic visits will continue. At the last intensive follow up visit a HLA Ab sample will be collected from all participants. Due to the changes required due to coronavirus pandemic described above (Section 4.6), data regarding the primary endpoint will be collected from patient notes for all participants. These data will be collected from the participants' last hospital contact prior to March 16 2020 (pre-COVID pandemic). A sensitivity analysis will also be performed by collecting data a second time from the participant's most recent hospital contact in the assessment period between September 1 2020 and November 30 2020, at which point participants' involvement in the study will be completed.

Recruits consented to the trial prior to Protocol Version 12 (01/12/2016) will be re-consented specifically to access their patient notes to assess primary endpoint data after completion of their intensive follow-up visits. This re-consenting process will be performed by research nurses during a research clinic visit prior to the completion of the intensive follow-up.

In recruits with living donors (known to them), the living donors will be invited to participate either by the recruit, or directly by the study team, following consent from the recruit to inform donors of their participation in the study. Donors will attend the clinic at their convenience, where consent will be taken. If donors are no longer being followed up at the hospital involved in the trial, they will be contacted by telephone to discuss the study and arrange a mutually convenient time for them to attend. As these participants are only needed to provide blood, consent will not need to be taken by a doctor. Donors who have not been screened for HIV, HepBSAg or HepC within the last 5 years will need to have additional screening tests for these viruses. Finally, blood (up to 80mls) will be taken for separation of PBMC which will be stored in the research laboratory, identified only as the donor of a particular study recruit. Donors may be asked to donate another aliquot of blood at another time within the next three years. The collection of living donor blood samples will continue throughout the trial as long as the resources and the capability of receiving, processing and storing samples are available.

5.2 Laboratory Tests

5.2.1 HLA Ab analysis:

Serum prepared from 10mls of blood will be used in the commercially available 'LABScreen' tests, containing fluorescently tagged beads coated with purified HLA antigens. All participating centres have 'Luminex' equipment for analysis of these tests and the skills to process samples and interpret results. Therefore, the tests will be performed in each of the centres. A sequential analysis of samples is planned, first to identify those with HLA Ab, using mixed class I & class II Ab 'screening' beads coated with multiple different types of HLA; To interpret these tests, the manufacturer's definition of a positive and negative test will be used. In those patients with positive results, the specificity of the HLA Ab will be determined by single antigen beads (SAB), coated with single HLA class I or class II antigens. As before, to interpret these tests, the manufacturer's definition of a positive and negative test will be used. Any patient with a positive test for HLA Ab identified by SAB will be regarded as HLA Ab+ positive for the trial if the mean fluorescence intensity (MFI) is ≥2000. If that HLA Ab is directed against a mismatched donor HLA antigen, this will be assigned as DSA+. The number of DSA with an MFI ≥2000 will be recorded to define the Ab 'burden' of an individual patient. In the final analysis, correlations between HLA Ab burden and outcomes will be sought. Patients with SAB positivity that is difficult to label as DSA/non DSA (because of insufficient data on donor mismatches, for instance), will be regarded as having non-DSA. Patient's with a positive reaction on screening but lacking reactivity with the SAB at the level described will be considered negative. Excess serum will be stored. The same screening will continue on the HLA Ab negative patients every 8 months, with the samples taken at a routine clinic appointment. Patients with HLA Ab allocated to the unblinded arm will be told the result (possibly be telephone) as soon as possible and invited to undergo optimisation.

Those in the blinded groups or in the unblinded HLA Ab negative arm will be told the result of their randomisation at their next clinic visit. All participants will be screened for HLA Abs at their last intensive follow up visit.

5.2.2 Routine biochemical, haematological and calcineurin inhibitor trough analysis:

These will be performed by the local clinical laboratories and results recorded as above.

5.2.3 Scientific laboratory analysis:

Serum and PBMC samples for future scientific analysis will be collected as long as resources allow; the precise nature of the analyses will be determined in future. Patients will also consent to allow analysis, for research purposes, of any stored serum, blood or tissues (such as transplant biopsies). This will apply for all existing and future samples taken for clinical reasons. In the case of future transplant biopsies, the patients will be asked to consent to having an extra core taken for transcriptome analysis. Subject to available funding, this will be stored in appropriate medium and transported to CI's laboratory for storage.

6 Assessment of Efficacy

6.1.1 Primary Efficacy Parameters

• Time to graft failure assessed by remote evaluation when approximately 43 months post-randomisation is achieved by all.

6.1.2 Secondary Efficacy Parameters

• Graft dysfunction, as assessed by two separate measures, presence of proteinuria (Protein:Creatinine Ratio > 50 or Albumin:Creatinine Ratio > 35) at 32 months and change in estimated Glomerular Filtration Rates over 32 months.
• Rates of acute rejection over 32 months.
• Health economic analysis of outcomes in intervention vs. control groups.
• Analysis of adherence and perceptions of risk in all biomarker led care groups.

6.2 Procedures for Assessing Efficacy Parameters

• Graft failure; will be defined as the return to long-term dialysis or re-transplantation. This will be measured from the date of recruitment and will be reported for failure due to all causes. The date of re-starting dialysis or of re-transplantation will be recorded on the CRF.

• Estimated Glomerular Filtration rates (eGFRs) will be calculated using the Modification of Diet in Renal Disease (MDRD) Study equation and recorded on the CRF. Mean eGFR slopes at 32 months will be compared between arms, using all available observations between baseline and 32 months using a test of interaction in a linear mixed model.

• Proteinuria; This will be defined by a Protein:Creatinine Ratio (PCR) > 50 or an Albumin:Creatinine Ratio (ACR) > 35 from a urine sample. The PCR or ACR will be recorded on the CRF.

• Acute rejection; will be defined by a combination of: a) acute rise in serum creatinine prompting a renal biopsy; b) any pathology on the biopsy which meets criteria for acute rejection, according to latest BANFF criteria. The number of biopsies and the appropriate biopsy reports will be recorded on the CRF.

• Health economic analysis; Effectiveness: A full economic evaluation will adopt a NHS perspective. 16 month outcomes rates of; (1) graft failure; (2) patient survival (3) graft

dysfunction (see defn. above); (4) acute rejection; (5) culture-positive infection, malignancy or diabetes. (6) EQ-5D, which is a patient-specific quality-adjusted life years (QALY) measurement. Cost-effectiveness: will use (1)-(5) where (1) is primary and the others are secondary outcomes. Cost-utility: will use (6) (EQ-5D questionnaires). Cost-benefit: Net benefit per patient calculated by multiplying QALY by assumed maximum willingness-to-pay for QALY (£20000 per QALY) and subtracting costs. Costs: of all interventions will be obtained from Guy's or estimated by identifying relevant categories of resource utilization OR measuring the volume of each category and multiplying by the average NHS resource costs ([30] BNF and NHS reference lists). Costs of intervention include the cost of screening beads and enhanced drug costs.

Measurement of adherence; Participants report of adherence behaviour will be assessed using the Medicines Adherence Report Scale (MARS) [31, 32], a valid and reliable scale that has been previously used to assess adherence in renal transplant recipients [33, 34]. Self-report measures have the advantage of being inexpensive and non-intrusive. However, it is known that self-report underestimates the true extent of nonadherence because of inherent self-presentational and recall biases. Self-presentational bias occurs when respondents may be reluctant to admit to nonadherence because they perceive a social contract where the expectation is one of high adherence. The MARS takes steps to diminish this bias by sanctioning and normalising reports of nonadherence. However, this does not totally remove the effect self-presentational and recall biases that are inherent in all self-report measures. For this reason we will apply a combined approach to adherence assessment, where initial categorisation of patients into high vs. low on the basis of self report is revised based on calcineurin inhibitor (CNI) blood monitoring (carried out in routine management for patients prescribed tacrolimus or ciclosporin) and tablet counts (conducted on a sample or participants). In this approach, reports of low adherence are accepted as self presentational biases act in the opposite direction (reports of low adherence are more reliable than reports of high adherence [35, 36]). Patients who report high adherence are reclassified to low adherence on the basis of CNI results (e.g if levels are undetectable then participant is assumed to be nonadherent) or tablet counts (e.g. if there is a greater than 20% discrepancy between the actual and expected tablet count then participant is reclassified as non adherent).

In order to explore the potential antecedents to participants' adherence behaviours, they will be also be asked to complete specially adapted versions of questionnaires relating to treatment intrusiveness (TIQ), symptoms associated with immunosuppressants (SAQ), beliefs about medicines (BMQ) [37], satisfaction with information about their medicines (SIMS) [38] and whether they are feeling anxious and/or depressed (HADS) [39]. Perceived risk will be measured on the basis of questionnaire-based approaches to qualifying perceptions of personal risk (IPQr) [40].

On the basis of their survey responses, a small number of participants will be purposively selected (e.g. positive and negative attitudes, high and low adherers) for qualitative interview to explore their perception of risk and adherence behaviours in more depth.

All patients taking part in the trial will be asked to complete all or some of the questionnaires at specified times (see table 2.2.1). Questionnaires will be administered electronically. Respondents will complete the survey online whilst in clinic, using an IPad or equivalent tablet device that is designated solely for this trial. Completed survey responses are stored on the Qualtrics secure servers and can only be accessed using a login/ password. Nothing will be recorded on the main trial CRF.

The questionnaires will be piloted in the first few participants recruited to the Guy's site. These respondents will be asked to undergo cognitive interviewing whilst completing the survey, a technique used to ensure the validity of questionnaire items [41]. On the basis of this pilot, the questionnaire items may undergo minor modification. The ease of utility of the online survey and tablet device will also be evaluated during the pilot.

7. Assessment of Safety

7.1 Specification, Timing and Recording of Safety Parameters.

The following safety parameters will be assessed as formal end-points for the trial:

• Patient survival.

• Rates of culture- or PCR-positive infection, biopsy-proven malignancy and diabetes mellitus. – these will be assessed at each formal study visit.

• Patient survival will be measured from day of recruitment. All deaths will be recorded, along with the cause and date, on the CRF.
• Infection; This will be defined as a positive microbiological culture or other test (such as PCR) confirming viral, bacterial or fungal replication in association with specific symptoms. Also, clinical episodes with classical presentations and signs (such as 'shingles' due to Herpes zoster) or episodes with confirmatory imaging of infection (for instance, consolidation on lung imaging) will be regarded as an infective episode and recorded.
• Malignancy; this will be defined by histopathological confirmation of malignancy on a biopsy of any suspicious lesion. The site of malignancy and the biopsy report (where available) will be filled in the CRF.
• Diabetes Mellitus; potential new cases of diabetes mellitus will be identified by elevated serum glucose and HbA1C measurements at study assessments and where possible, glucose measurements in between will be recorded on CRF. Standard WHO definitions for diagnosis of DM will be used to confirm diagnosis.

7.2 Procedures for Recording and Reporting Adverse Events

Recording of adverse events in the eCRF for OuTSMART will use the following definitions of expectedness reported below which are based on those listed in the SmPC for each IMP.

Adverse Event (AE): Any untoward medical occurrence in a subject to whom an IMP has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR): Any untoward and unintended response in a subject to an IMP which is related to any dose administered to that subject.
Unexpected Adverse Reaction (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the IMP in question set out in the summary of product characteristics (SmPC) for that product.
Serious Adverse Event (SAE): Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR): Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

Results in death;
Is life-threatening;
Required hospitalisation or prolongation of existing hospitalisation;
Results in persistent or significant disability or incapacity;
Consists of a congenital anomaly or birth defect.

This trial fulfils the criteria for a 'Type A' trial (i.e. risk no higher than that of standard care). Therefore, there will be reduced reporting of adverse events to the Sponsor and the MHRA. In this trial, SAE's will be reported on only those patients in who, medication is assigned IMP status i.e. those in the unblinded HLA Ab positive arm who have undergone optimisation. In addition, only serious adverse events that fulfil the following criteria will be reported to the sponsor and MHRA:

a) result in death
b) require hospitalisations resulting in kidney graft failure
c) are SAR's that would prompt yellow-card reporting in the blinded arm of the trial.

In the very unlikely event of pregnancy, study subjects will not be withdrawn from the treatment. The study IMP is used as per standard of care and treatment will be optimised specifically for the patient. Therefore the risk is not greater than that of standard of care. Thus, even though it is not a serious adverse event, any unplanned pregnancy in patients taking the IMP will be reported via the SAE reporting system as stated below.
We will not report important medical events (IME).

7.3 Reporting Responsibilities

King's College London and GSTT have delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partner's (KHP) Clinical Trials Office (CTO).

The PIs on each site will take responsibility for reporting all adverse events (and pregnancy) to the Chief Investigator. All SAEs, SARs and SUSARs (excepting those specified in this protocol as not requiring reporting – see above) will be reported by the local investigators on the SAE form provided by the KHP CTO to the Chief Investigator, *immediately they become aware,* and by the Chief Investigator to the KHP CTO in accordance with the current Pharmacovigilance Policy. SAEs will be reported up to the last intensive visit (i.e. either at 32-months or up to 64 months) of each recruit.

All deaths will be reported as SAEs. Those that occur as a result of disease progression and other events that are primary or secondary outcome measures will also be reported on the appropriate CRF.

Important Medical Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the SAE definition should also be considered serious. However, as stated, we will not report IMEs to the sponsor unless fulfilling the criteria for SAE reporting set out above.

The KHP CTO will report SUSARs to the regulatory authorities (MHRA, competent authorities of other EEA (European Economic Area) states in which the trial is taking place.
The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.

The Chief Investigator and KHP CTO (on behalf of the *co*-sponsors), will submit a Development Safety Update Report (DSUR) relating to this trial's IMPs, to the MHRA and REC annually.

7.3.1 Adverse events that do not require reporting

In the unblinded HLA Ab positive groups receiving IMPs:
• Events or reactions listed in the SmPC for each of the IMPs do not need reporting for this trial (see http://www.medicines.org.uk/emc), unless they are SAEs according to trial reporting guidelines above. A summary of AEs from the SmPCs are included in appendix 1.
• AE's not thought to be related to the IMP must be recorded in the eCRF but do not need reporting, unless they are SAE's according to trial reporting guidelines above.

In all other study (Non-IMP-receiving) groups:
• AEs and SAEs occurring in subjects not administered an IMP must be recorded in the eCRF but do not need reporting to the sponsor/MHRA.
• AR's in these subjects should be reported using the standard yellow form system

In all groups:
• Hospital admissions occurring as a result of a planned or elective admission for any reason will not be regarded as SAE according to trial reporting guidelines above unless the site PI decides they need reporting, in which case the procedure above should be followed. All adverse events should be recorded throughout the trial, by the PIs within 28 days of becoming aware.

7.4 Treatment Stopping Rules

The trial will stop recruiting once 165 HLA Ab positive DSA patients have been recruited, including patients who are HLA Ab positive with DSA antibodies at baseline and those who become HLA Ab positive DSA at the 8-monthly rescreening rounds.

The trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Monitoring & Ethics Committee / Trial Steering Committee regulatory authority or ethics committee concerned.

The trial may also be prematurely discontinued due to lack of recruitment or upon advice from a Trial Steering Committee who will advise on whether to continue or discontinue the study and make a recommendation to the sponsor. If the study is prematurely discontinued, active participants will be informed and no further participant data will be collected

Earlier termination *will be considered* by the Data Monitoring committee if there is a significant excess of adverse events in the intervention arm.

8. Statistics

8.1 Sample Size

The primary purpose of this trial is to demonstrate superior outcomes using a defined treatment strategy in biomarker (HLA Ab) positive patients, and at the same time demonstrate non-inferior outcomes when the unblinded screening strategy is applied to the entire patient population. Time to graft failure has been chosen as a clinically relevant primary outcome. As a reference for power calculations, we have used the observed failure rates reported by Lachmann et al. [8] for HLA Ab+ and HLA Ab-neg patients. Since failure rates differ between DSA+ and non-DSA+ patients, sample size calculations have been carried out separately for these groups. Following these calculations, we have estimated the number to be screened, based on expected drop out rates, expected screening results and eligibility criteria (see below).

We have based our estimates of the *differences* in primary outcome between groups on two things; first, the results of our preliminary data from patients with CR treated with a similar regime as used here; second, our assessment that large differences in primary outcome will be needed to make the screening programme cost-effective.

Hypotheses and power calculations: (group labels refer to flow diagram in section 2.3)

1. Superiority on Biomarker Positive Patients:

    1.1. $A_1 > B_1$: HLA Ab+ patients, with DSA, randomized to standard care (A_1) will show higher graft failure rates than patients randomized to biomarker-led care (B_1). We hypothesize that the experimental treatment will bring the failure rate in group B_1 down to that of non-DSA patients in standard care (A_2). Assuming that 30% of patients with DSA randomised to standard care (A_1) will have experienced chronic rejection (CR) by 3-years follow up, we expect treatment optimisation to reduce the rate of CR in DSA patients randomised to group B_1 down to 16% at 3-years follow up (rate observed in patients with non-DSA). This corresponds to a Hazard ratio (HR) of 0.489. The expectation is for 11% and 21% of CR among patients with DSA in in group A_1 at 1 and 2-years follow up respectively (as in [8]), and extrapolating based on a HR of .489, we expect BLC to reduce those CR to 5.5%, and 10.89% at 1 and 2-years. Using a variable follow up design assuming an average accrual monthly rate of 3.6 patients per month, and a follow up time of 43 months, recruiting 165 patients with DSA would allow us to observe 23/83 (28%) events of CR in patients under biomarker led care (B_1), and 39/82 (47%) in the standard care group (A_1). This would provide 80% power and 5% type I error, for a two-sided log-rank test.

1.2. $A_2>B_2$: HLA Ab+ patients, with non-DSA, randomized to standard care (A_2) will show higher graft failure rate than patients randomized to biomarker-led care (B_2). We hypothesize that the experimental treatment will bring the failure rate in group B_2 down to that of biomarker negative patients in standard care (C). Assuming that 16% of patients with NDSA randomised to SoC will have experienced chronic rejection (CR) by 3-years follow up, we expect treatment optimisation to reduce the rate of CR in NDSA patients randomised to BLC down to 6% at 3-years follow up (rate observed in patients without HLA antibodies). This corresponds to a Hazard ratio of 0.351. Based on Lachman et al. the expectation is for 3% and 11% of CR among patients with NDSA in SoC at 1 and 2-years follow up respectively, and extrapolating based on a HR of 0.351, we expect BLC to reduce those CR to 1.1%, and 4.1% at 1 and 2-years. Using a variable follow-up design (patients followed until failure, drop out or end of minimum follow up), assuming an average accrual monthly rate of 15.5 patients per month, and a minimum follow up time of 22.4 months, recruiting 296 patients with NDSA, would allow us to observe 8/149 (5.3%) events of CR in patients under BLC, and 21/147 (14%) in the SoC group (total duration = 41.5 months). This would provide 80% power to determine a statistically significant difference between SoC and BLC, using a log-rank test, with a 2-sided type-I error rate.

The numbers enrolled in groups A & B include those patients initially enrolled in groups C or D who become HLA Ab+ during re-screening.

2. Non-inferiority of all Unblinded patients compared to all Blinded patients:
   2.1. $A_1+A_2+C \geq B_1+B_2+D$: All patients randomized to unblinded screening will show equal or lower graft failure rates than all patients randomized to blinded screening, irrespective of biomarker status. At the end of the trial, we expect 58% of patients to be in the HLA Ab negative groups, 7% DSA+ groups and 35% non-DSA+ groups (after drop-outs). At the time of planning the OuTSMART study, we calculated that based on all assumptions above, all patients randomised to SoC combined would experience 13.9% of CR. We established a non-inferiority limit of 5% absolute difference in rate of CR at 3-years, so that the BLC group would be considered inferior to SoC with a CR rate of 18.9% or higher (expectation under the null hypothesis). This corresponds to a HR of 1.4 under the null hypothesis, and a HR of 0.63 under the alternative. Recruiting 672 patients over a period of 13.2 months, at an average accrual rate of 51 patients per month, and a minimum follow up of 18.21 months, would allow us to observe 22/337 (6.5%) events of CR in the SoC group, and 32/335 (9.5%) in the BLC group. This would provide 90% power to demonstrate non-inferiority with a one-sided 95% Confidence Interval of the HR estimated using a Cox regression model. Given the above proportions, this requires enrolling 336 patients in each of groups C&D and this should allow 423 total patients to reach the primary endpoint (i.e. remain negative (after dropouts) at the end of their three year follow-up).

An audit of potentially available patients within the 5 renal units was performed initially to determine the likelihood of the study being able to recruit all the required patients from the 5 centres. We considered the number of patients in each centre under annual follow-up and the numbers of new patients who will become eligible throughout the first three years of the study (i.e. those who reach >12 months post transplantation, which are those transplanted in the period between12 months prior to the study and the end of year two). We estimated that 60% of these will be potential recruits, the others having reasons for not being included. We assumed that 10% of those approached will refuse consent and 10% of HLA Ab+ patients would have no detectable single Class I or II on single bead analysis. Additionally, we expected 6% of initially Ab-neg patients to become Ab+ in each screening round.

Following 16 months of recruitment to the OuTSMART trial, the observed % of DSA patients (including those from re-screening rounds) was lower than expected, at 6.6%. The percentage of antibody positive patients at baseline was 35.1%, considerably higher than expected (25-30%). 5.8% of all patients had DSA at baseline (expected 9%). 300 Ab-neg patients had been re-screened as part of the Month-8 screening round, of whom 23 had

developed de-novo antibodies (7.6% - expected 6%). Five out of the 23 had DSA (1.6% of all –expected 2%).

Based on an overall expected proportion of 7% DSA participants (including from re-screening rounds) we will need to recruit 2357 patients overall to recruit the target of 165 DSA patients. Because of this requirement to recruit sufficient DSA participants, the recruits to the other groups are likely to be more than the minimum required for statistical power for the individual hypotheses.

8.2 Randomisation

Using the flow diagram (section 2.3, top-to-bottom) as a guide: renal transplant recipients aged 18-75, > 1 year post-transplant with an eGFR ≥ 30 will consent to the screening/treatment process. The first stratification will result from blood test screening for HLA Ab. Approximately 35% will be HLA positive, with ~65% negative. The HLA Ab+ patients will be further screened with single antigen beads to determine whether DSA are present (~1/6 DSA and 5/6 non-DSA). Thus, biomarker stratification leads to three groups (DSA+, non-DSA+ and HLA Ab-neg). The second stratification will be based on current immunosuppression, to ensure balanced numbers already on Tac or MMF in each group. The final stratification will be by site.

HLA Ab+ patients will be randomized 1:1 into either Blinded Standard Care or Unblinded Biomarker led-care. Patients in the former (groups A1 &A2) will be blind to their biomarker status and will remain on baseline immunotherapy, whereas patients in the latter (groups B1 and B2) will know their HLA Ab status and will be offered "treatment". HLA Ab- patients will be randomized 1:1 into either Blinded (group C) or Unblinded (group D) and remain on standard care, with only the latter knowing their HLA Ab status. All HLA Ab-negative patients (groups C & D) will receive regular (8 monthly) Ab status monitoring for the first 3 years. Those patients who become positive during subsequent screening rounds (~10% per year) will be moved to the appropriate HLA Ab positive groups (DSA+ or non-DSA+) for final data analysis. For details of the randomisation procedure see section 4.4.

8.3 Analysis

Statistical analysis will be on an intention to treat and treatment received-basis, to consider the patients who become positive during the follow up in the appropriate group (group labels refer to flow diagram in section 2.3).

The primary analysis will use data collected up until March 16 2020 and analyses will be conducted for each of the hypotheses as outlined below. A sensitivity analysis will be carried out for the primary outcome using, additionally, data from participants' most recent hospital contact as of the assessment period between September 1 2020 and November 30 2020. The sensitivity analysis will otherwise be carried out in exactly the same way for each of the hypotheses.

1. Superiority:

H_0: $h_{A1}(t) = h_{B1}(t)$ & $h_{A2}(t) = h_{B2}(t)$[14]
H_1: $h_{A1}(t) \neq h_{B1}(t)$ & $h_{A2}(t) \neq h_{B2}(t)$

In order to test superiority for the primary outcome in the Biomarker (HLA Ab) positive groups (Hypothesis 1.1 and 1.2), we will use Cox proportional hazards regression models to estimate the graft failure hazard ratio between the biomarker led care and standard care groups and test at the 5% level of significance. Results will be given as estimates and 95% confidence intervals (CIs). Within the model, we will adjust for previous immunosuppression regimen and

[14] Here $h_{A1}(t)$, $h_{B1}(t)$, etc. represent the graft failure hazard rates in each of the groups.

research site (as these are the randomisation stratification factors) for increased statistical efficiency.

We will check the proportional hazards assumption by examining Kaplan-Meier plots and by testing for an interaction between group (BLC or SC) and time to graft failure within the model.

2. . Non-inferiority:

H_0: $h_{Unblind}(t) / h_{Blind}(t) \geq \delta$
H_1: $h_{Unblind}(t) / h_{Blind}(t) < \delta$

In order to test for non-inferiority of the unblinded groups compared to the blinded groups (hypothesis 2.1), we will use Cox proportional hazards regression models to estimate the graft failure hazard ratio. We will adjust for the stratification factors in the model as outlined above and check the proportional hazards assumption by examining Kaplan Meier plots and by testing for an interaction between unblinded/blinded group and time to graft failure. We will conclude non-inferiority if H_0 gets rejected at 5% significance, and the corresponding upper bound of the 95% CI for the hazard ratio excludes the limit δ (hazard ratio of 1.4).

We will use a similar procedure using Cox proportional hazards regression for the analysis of secondary time to event (survival outcomes). Where numbers allow, secondary binary outcomes will be analysed using logistic regression with adjustment for stratification factors. Where numbers are too small for this, the z-test or Fisher's exact will be used. Results will be given as estimates (odds ratios or differences in proportions) and 95% CIs. For continuous secondary outcomes we will use linear regression (or a linear mixed model if accounting for repeated measures) with adjustment for stratification factors, transforming data where they are skew.

Economic Evaluation: Cost data is usually skew but will be analysed using arithmetic means so that total costs are preserved. Non-normality in errors will be allowed for by using generalized linear models with appropriate error structure (e.g. gamma distribution[42]). Incremental cost-effectiveness ratios (ICERs) or incremental cost-utility ratio (ICUR) will be presented where appropriate. Cost effectiveness acceptability curves will be plotted to summarize information on uncertainty in cost-effectiveness.

9. Trial Steering Committee

An independent Trial Steering Committee (TSC) will be convened in the post-award period. The membership will be decided by the CI and approved by the NIHR. The chair will be a senior transplantation physician or surgeon from the UK who is unconnected to the study. Members will include the CI, two other PIs from the trial, a representative of the GSTT Kidney Patients Association, one other senior independent renal/transplant physician/surgeon, and an independent senior HLA clinical scientist.
The TSC will meet at least annually during the study, approximately 2 weeks after the DMC. The TSC is an executive committee. Terms of reference of the TSC will be agreed and documented prior to start of recruitment. The Trial Manager will prepare reports to the TSC

10. Data Monitoring Committee

A Data Monitoring and Ethics Committee (DMC) will be established comprising a senior UK-based transplant physician/surgeon as chair, an HLA clinical scientist, a biostatistician and trials-experienced pharmacist. All the members will be independent of the trial.
The DMC will meet at least annually during the study, approximately 2 weeks prior to the TSC. The DMC is advisory to the TSC. The DMC charter will be drafted and agreed prior to recruitment. The Trial Statistician will prepare reports to the DMC.

11. Direct Access to Source Data and Documents

The investigators and the institutions will permit trial-related monitoring, audits, REC review, and regulatory inspections (where appropriate) by providing direct access to source data and other relevant documents.

12. Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to London-Hampstead Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation

The Chief Investigator will submit a final report at conclusion of the trial to the KHP CTO (on behalf of the Sponsor), the REC and the MHRA within the timelines defined in the Regulations.

13. Quality Assurance

Monitoring of this study to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained by KHP CTO. All samples will be anonymised before laboratory analysis. No patient-related data will be held in research laboratories. During the study, paper copies will be held in a locked filing cabinet in the chief investigators office and retained for a minimum of 5 years following the end of the study.
The investigators and the institutions will permit trial-related monitoring, audits, REC review, and regulatory inspections (where appropriate) by providing direct access to source data and other relevant documents (ie patients' case sheets, blood test reports, X-ray reports, histology reports etc).
All study data will be stored and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Clinical Trials Office Archiving SOP.
Record keeping will be the responsibility of the investigators.
The chief investigator will review all presentations and publications arising from this study and decide authorship in accordance with accepted guidelines.

14. Data Handling, Publication Policy and Finance

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

Patient data will be anonymised

- All anonymised data will be stored on a password protected computers.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act.

and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Clinical Trials Office Archiving SOP.

15. Data Handling

Research data will be collected at sites onto source data worksheets, which will form part of the NHS medical notes. Clinical and research data will be transcribed from the medical notes and source data worksheets to the study eCRF system, hosted at the King's Clinical Trials Unit, KCL. The eCRF (InferMed MACRO) is GCP and FDA 21 CFR Part 11 compliant with e-signatures for site PI confirmation of each eCRF at end of study. Data entry staff at site will be

provided with unique usernames and passwords to the system and will be trained in data entry by the trial manager. Study monitors will be given access to review data on the system, raise discrepancies and confirm source data verification checks. The study trial manager and data manager will have access to review data on the system and raise discrepancies. All requests for access to the data entry system must be authorised by the trial manager. All requests for data exports must be authorised by the trial statistician.

16. Publication Policy

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals.

17. Insurance / Indemnity

The study will be indemnified by King's College London for negligent and non-negligent harm. In addition, the Chief Investigator and local Principal Investigators (the clinicians) also have independent insurance with medical defence societies.

18. Financial Aspects

The NIHR have supported the study through an EME programme grant award. The scientific analyses of stored blood samples will be funded separately. The analysis of adherence and perceived risk will be funded separately via a PhD fellowship application by Professor Rob Horne.

19. Signatures

08/07/2020

_____ _____

Chief Investigator Date

Print name

Appendix 1

Adverse drug reactions mentioned in the SmPCs for the IMPS. These combine pre- and post-marketing experience. These do not require reporting as adverse events in OuTSMART unless resulting in an SAE.

MMF

Infections and infestations:[15]

Very common Sepsis, gastrointestinal candidiasis, urinary tract infection, herpes simplex, herpes zoster

Common Pneumonia, influenza, respiratory tract infection, respiratory moniliasis, gastrointestinal infection, candidiasis, gastroenteritis, infection, bronchitis, pharyngitis, sinusitis, fungal skin infection, skin candida, vaginal candidiasis, rhinitis

The most serious infections including meningitis, endocarditis, tuberculosis and atypical mycobacterial infection. Cases of BK virus associated nephropathy, as well as cases of JC virus associated progressive multifocal leukoencephalopathy (PML), have been reported in patients treated with immunosuppressants, including MMF.

Neoplasms:[16]

Common Skin cancer, benign neoplasm of skin

Blood and lymphatic system disorders:

Very common Leukopenia, thrombocytopenia, anaemia

Common Pancytopenia, leukocytosis

Agranulocytosis and neutropenia have been reported; therefore, regular monitoring of patients taking MMF is advised[17]. There have been reports of aplastic anaemia and bone marrow depression in patients treated with MMF, some of which have been fatal.

Cases of pure red cell aplasia (PRCA) have been reported in patients treated with MMF. Isolated cases of abnormal neutrophil morphology, including the acquired Pelger-Huet anomaly, have been observed in patients treated with MMF. These changes are not associated with impaired neutrophil function. These changes may suggest a 'left shift' in the maturity of neutrophils in haematological investigations, which may be mistakenly interpreted as a sign of infection in immunosuppressed patients such as those that receive MMF.

Uncommon Pseudolymphoma and bone marrow failure.

Metabolism and nutrition disorders:

Common Acidosis, hyperkalaemia, hypokalaemia, hyperglycaemia, hypomagnesaemia, hypocalcaemia, hypercholesterolaemia, hyperlipidaemia, hypophosphataemia, hyperuricaemia, gout, anorexia

Psychiatric disorders:

Common Agitation, confusional state, depression, anxiety, thinking abnormal, insomnia

Nervous system disorders:

Common Convulsion, hypertonia, tremor, somnolence, myasthenic syndrome, dizziness, headache, paraesthesia, dysgeusia

Cardiac disorders:

[15] NB these should be collected as end-points and not reported as adverse reactions

[16] As for infections and infestations

[17] Normal transplant clinic monitoring as per unit protocol

Common Tachycardia

Vascular disorders:
Common Hypotension, hypertension, vasodilatation, venous thrombosis
Uncommon Lymphocele

Respiratory, thoracic and mediastinal disorders:
Common Pleural effusion, dyspnoea, cough
There have been isolated reports of interstitial lung disease and pulmonary fibrosis in patients treated with MMF in combination with other immunosuppressants, some of which have been fatal. There have also been report of bronchiectasis in children and adults.

Gastrointestinal disorders:
Very common Vomiting, abdominal pain, diarrhoea, nausea, constipation, dyspepsia
Common Gastrointestinal haemorrhage, peritonitis, ileus, colitis, gastric ulcer, duodenal ulcer, gastritis, oesophagitis, stomatitis, flatulence, eructation, gingival hyperplasia, colitis including cytomegalovirus colitis[18], pancreatitis and intestinal villous atrophy.

Hepatobiliary disorders:
Common Hepatitis, jaundice, hyperbilirubinaemia

Skin and subcutaneous tissue disorders:
Common Skin hypertrophy, rash, acne, alopecia,

Musculoskeletal and connective tissue disorders:
Common Arthralgia

Renal and urinary disorders:
Common Renal impairment

General disorders and administration site conditions:
Oedema, including peripheral, face and scrotal oedema, was reported very commonly during the pivotal trials. Musculoskeletal pain such as myalgia, and neck and back pain were also very commonly reported.

Immune system disorders
Hypogammaglobulinaemia has been reported in patients receiving CellCept in combination with other immunosuppressants.

Investigations:
Common Hepatic enzyme increased, blood creatinine increased, blood lactate dehydrogenase increased, blood urea increased, blood alkaline phosphatase increased, weight decreased

Hypersensitivity:[19]
Hypersensitivity reactions, including angioneurotic oedema and anaphylactic reaction have been reported.

[18] As for infections and infestations
[19] Patients with known hypersensitivity to any of the IMPs are excluded from the trial

Tacrolimus

Infections and infestations:[20]

As is well known for other potent immunosuppressive agents, patients receiving tacrolimus are frequently at increased risk for infections (viral, bacterial, fungal, protozoal). The course of pre-existing infections may be aggravated. Both generalised and localised infections can occur. Cases of BK virus associated nephropathy, as well as cases of JC virus associated progressive multifocal leukoencephalopathy (PML), have been reported in patients treated with immunosuppressants, including tacrolimus.

Neoplasms:[21]

Patients receiving immunosuppressive therapy are at increased risk of developing malignancies. Benign as well as malignant neoplasms including EBV-associated lymphoproliferative disorders and skin malignancies have been reported in association with tacrolimus treatment.

Blood and lymphatic system disorders:

Common: anaemia, leukopenia, thrombocytopenia, leukocytosis, red blood cell analyses abnormal

Uncommon: coagulopathies, coagulation and bleeding analyses abnormal, pancytopenia, neutropenia

Rare: thrombotic thrombocytopenic purpura, hypo-prothrombinaemia, thrombotic microangiopathy

Not known: pure red cell aplasia, agranulocytosis, haemolytic anaemia.

Immune system disorders:[22]

Allergic and anaphylactoid reactions have been observed in patients receiving tacrolimus

Endocrine disorders:

Rare: hirsuitism

Metabolism and nutrition disorders:

Very common: hyperglycaemic conditions, diabetes mellitus[23], hyperkalaemia

Common: hypomagnesaemia, hypophosphataemia, hypokalaemia, hypocalcaemia, hyponatraemia, fluid overload, hyperuricaemia, appetite decreased, anorexia, metabolic acidoses, hyperlipidaemia, hypercholesterolaemia, hypertriglyceridaemia, other electrolyte abnormalities

Uncommon: dehydration, hypoproteinaemia, hyperphosphataemia, hypoglycaemia

Psychiatric disorders:

Very common: insomnia

Common: anxiety symptoms, confusion and disorientation, depression, depressed mood, mood disorders and disturbances, nightmare, hallucination, mental disorders

Uncommon: psychotic disorder

Nervous system disorders:

Very common: tremor, headache

Common: seizures, disturbances in consciousness, paraesthesias and dysaesthesias, peripheral neuropathies, dizziness, writing impaired, nervous system

[20] NB these should be collected as end-points and not reported as adverse reactions
[21] As for infections and infestations
[22] Patients with known hypersensitivity to any of the IMPs are excluded from the trial
[23] DM is one of the end-points and serum glucoses should be recorded on CRF

disorders
Uncommon: coma, central nervous system haemorrhages and cerebrovascular accidents, paralysis and paresis, encephalopathy, speech and language abnormalities, amnesia
Rare: hypertonia
Very rare: myasthenia

Eye disorders:
Common: vision blurred, photophobia, eye disorders
Uncommon: cataract
Rare: blindness
Not known: optic neuropathy

Ear and labyrinth disorders:
Common: tinnitus
Uncommon: hypoacusis
Rare: deafness neurosensory
Very rare: hearing impaired

Cardiac disorders:
Common: ischaemic coronary artery disorders, tachycardia
Uncommon: ventricular arrhythmias and cardiac arrest, heart failures, cardiomyopathies, ventricular hypertrophy, supraventricular arrhythmias, palpitations,
Rare: pericardial effusion
Very rare: *Torsades de Pointes*

Vascular disorders:
Very common: hypertension
Common: haemorrhage, thrombembolic and ischaemic events, peripheral vascular disorders, vascular hypotensive disorders
Uncommon: infarction, venous thrombosis deep limb, shock

Respiratory, thoracic and mediastinal disorders:
Common: dyspnoea, parenchymal lung disorders, pleural effusion, pharyngitis, cough, nasal congestion and inflammations
Uncommon: respiratory failures, respiratory tract disorders, asthma
Rare: acute respiratory distress syndrome

Gastrointestinal disorders:
Very common: diarrhoea, nausea
Common: gastrointestinal inflammatory conditions, gastrointestinal ulceration and perforation, gastrointestinal haemorrhages, stomatitis and ulceration, ascites, vomiting, gastrointestinal and abdominal pains, dyspeptic signs and symptoms, constipation, flatulence, bloating and distension, loose stools, gastrointestinal signs and symptoms
Uncommon: ileus paralytic, peritonitis, acute and chronic pancreatitis, blood amylase increased, gastrooesophageal reflux disease, impaired gastric emptying
Rare: subileus, pancreatic pseudocyst

Hepatobiliary disorders:
Common: cholestasis and jaundice, hepatocellular damage and hepatitis, cholangitis[24]

[24] As for infections and infestations

Rare: hepatitic artery thrombosis, venoocclusive liver disease
Very rare: hepatic failure, bile duct stenosis

Skin and subcutaneous tissue disorders:
Common: pruritus, rash, alopecias, acne, sweating increased
Uncommon: dermatitis, photosensitivity
Rare: toxic epidermal necrolysis (Lyell's syndrome)
Very rare: Stevens Johnson syndrome

Musculoskeletal and connective tissue disorders:
Common: arthralgia, muscle spasms, pain in extremity, back pain
Uncommon: joint disorders
Rare: mobility decreased

Renal and urinary disorders:
Very common: renal impairment
Common: renal failure, renal failure acute, oliguria, renal tubular necrosis, nephropathy toxic, urinary abnormalities, bladder and urethral symptoms
Uncommon: anuria, haemolytic uraemic syndrome
Very rare: nephropathy, cystitis haemorrhagic

Reproductive system and breast disorders:
Uncommon: dysmenorrhoea and uterine bleeding

General Disorders and administration site conditions:
Common: asthenic conditions, febrile disorders, oedema, pain and discomfort, body temperature perception disturbed
Uncommon: multi-organ failure, influenza like illness, temperature intolerance, chest pressure sensation, feeling jittery, feeling abnormal,
Rare: thirst, fall, chest tightness, ulcer
Very rare: fat tissue increased

Investigations:
common: hepatic enzymes and function abnormalities, blood alkaline phosphatase increased, weight increased
uncommon: amylase increased, ECG investigations abnormal, heart rate and pulse investigations abnormal, weight decreased, blood lactate dehydrogenase increased
very rare: echocardiogram abnormal, electrocardiogram QT prolonged

Description of selected adverse reactions:
Pain in extremity has been described in a number of published case reports as part of Calcineurin-Inhibitor Induced Pain Syndrome (CIPS). This typically presents as a bilateral and symmetrical, severe, ascending pain in the lower extremities and may be associated with supra-therapeutic levels of tacrolimus. The syndrome may respond to tacrolimus dose reduction. In some cases, it was necessary to switch to alternative immunosuppression.

Prednisolone

Infections and infestations:
Increased susceptibility and severity of infections[25] with suppression of clinical symptoms
and signs, opportunistic infections, recurrence of dormant tuberculosis.

Blood and lymphatic system disorders:
Leukocytosis

Immune system disorders:
Hypersensitivity including anaphylaxis, fatigue and malaise

Endocrine disorders:
Cushing syndrome, Cushingoid facies, weight gain, impaired carbohydrate tolerance with increased requirement for antidiabetic therapy, manifestation of latent diabetes mellitus, menstrual irregularity and amenorrhea.

Metabolism and nutrition disorders:
Sodium and water retention, hypokalaemic alkalosis, potassium loss, negative nitrogen and calcium balance.

Psychiatric disorders:
A wide range of psychiatric reactions including affective disorders (such as irritable, euphoric, depressed and labile mood, and suicidal thoughts), psychotic reactions (including mania, delusions, hallucinations, and aggravation of schizophrenia), marked euphoria leading to dependence; aggravation of epilepsy, behavioural disturbances, irritability, nervousness, anxiety, sleep disturbances, and cognitive dysfunction including confusion and amnesia have been reported. Reactions are common and may occur in both adults and children. In adults, the frequency of severe reactions has been estimated to be 5-6%. Psychological effects have been reported on withdrawal of corticosteroids; the frequency is unknown. Psychological dependence, dizziness, headache, vertigo.

Eye disorders:
Increased intra-ocular pressure, glaucoma, papilloedema, posterior subcapsular cataracts, central serous chorioretinopathy, exophthalmos, corneal or scleral thinning, scleral perforation, exacerbation of ophthalmic viral or fungal disease and vision, blurred.

Cardiac disorders:
Congestive heart failure in susceptible patients, hypertension.

Vascular disorders:
Thromboembolism

Gastrointestinal:
Dyspepsia, nausea, peptic ulceration with perforation and hemorrhage, abdominal distension, abdominal pain, increased appetite, oesophageal candidiasis, oesophageal ulceration, acute pancreatitis, perforation of the small bowel, particularly in patients with inflammatory bowel disease.

[25] NB these should be collected as end-points and not reported as adverse reactions

Skin and subcutaneous tissue disorders:
Hirsutism, skin atrophy, bruising, impaired healing, striae, telangiectasia, acne, increased sweating, may suppress reactions to skin tests, pruritis, rash, urticaria

Musculoskeletal and connective tissue disorders:
Proximal myopathy, osteoporosis, vertebral and long bone fractures , avascular osteonecrosis, tendon rupture, myalgia, muscle weakness, wasting and loss of muscle mass.

Renal and urinary disorders:
Nocturia, scleroderma renal crisis (Amongst the different subpopulations the occurrence of scleroderma renal crisis varies. The highest risk has been reported in patients with diffuse systemic sclerosis. The lowest risk has been reported in patients with limited systemic sclerosis (2%) and juvenile onset systemic sclerosis (1%))

General disorders and administration site conditions:
Impaired healing and withdrawal symptoms

Withdrawal symptoms:
Too rapid a reduction of corticosteroid dosage following prolonged treatment can lead to acute adrenal insufficiency, hypotension and death. A “withdrawal syndrome” seemingly unrelated to adrenocortical insufficiency may also occur following abrupt discontinuance of glucocorticoids. This syndrome includes symptoms such as: anorexia, nausea, vomiting, lethargy, headache, fever, joint pain, desquamation, myalgia, arthralgia, rhinitis, conjunctivitis, painful itchy skin, weight loss and or hypotension. These effects are thought to be due to the sudden change in glucocorticoid concentration rather than too low corticosteroid levels.

Additional side effects in children and adolescents:
Suppression of the hypothalamo-pituitary adrenal axis particularly in times of stress, as in trauma, surgery or illness, growth suppression in infancy, childhood and adolescence. Raised intracranial pressure with papilloedema (pseudotumor cerebri) in children, usually after treatment withdrawal.