BMJ Open

Multicentre randomised controlled trial protocol of urine CXCL10 monitoring strategy in kidney transplant recipients

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To cite: Ho J, Sharma A, Kroeker K, *et al*. Multicentre randomised controlled trial protocol of urine CXCL10 monitoring strategy in kidney transplant recipients. *BMJ Open* 2019;**9**:e024908. doi:10.1136/bmjopen-2018-024908

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-024908).

Received 20 June 2018
Revised 8 November 2018
Accepted 21 January 2019

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ABSTRACT

Introduction Subclinical inflammation is an important predictor of death-censored graft loss, and its treatment has been shown to improve graft outcomes. Urine CXCL10 outperforms standard post-transplant surveillance in observational studies, by detecting subclinical rejection and early clinical rejection before graft functional decline in kidney transplant recipients.

Methods and analysis This is a phase ii/iii multicentre, international randomised controlled parallel group trial to determine if the early treatment of rejection, as detected by urine CXCL10, will improve kidney allograft outcomes. Incident adult kidney transplant patients (n~420) will be enrolled to undergo routine urine CXCL10 monitoring postkidney transplant. Patients at high risk of rejection, defined as confirmed elevated urine CXCL10 level, will be randomised 1:1 stratified by centre (n=250). The intervention arm (n=125) will undergo a study biopsy to check for subclinical rejection and biopsy-proven rejection will be treated per protocol. The control arm (n=125) will undergo routine post-transplant monitoring. The primary outcome at 12 months is a composite of death-censored graft loss, clinical biopsy-proven acute rejection, de novo donor-specific antibody, inflammation in areas of interstitial fibrosis and tubular atrophy (Banff i-IFTA, chronic active T-cell mediated rejection) and subclinical tubulitis on 12-month surveillance biopsy. The secondary outcomes include decline of graft function, microvascular inflammation at 12 months, development of IFTA at 12 months, days from transplantation to clinical biopsy-proven rejection, albuminuria, EuroQol five-dimension five-level instrument, cost-effectiveness analysis of the urine CXCL10 monitoring strategy and the urine CXCL10 kinetics in response to rejection therapy.

Ethics and dissemination The study has been approved by the University of Manitoba Health Research Ethics Board (HS20861, B2017:076) and the local research ethics boards of participating centres. Recruitment commenced in March 2018 and results are expected to be published in 2023. De-identified data may be shared with other researchers according to international guidelines (International Committee of Medical Journal Editors [ICJME]).

Trial registration number NCT03206801; Pre-results.

Strengths and limitations of this study

- This is a large, international multicentre randomised controlled trial in incident adult renal transplant patients.
- This pragmatic clinical trial has broad inclusion criterion whose findings should be highly generalisable.
- The control arm will remain blinded relative to the low risk, non-randomised arm to prevent off-protocol increases to immunosuppression to minimise potential sources of bias.
- It is not possible to blind the intervention arm which is undergoing a study biopsy.
- The Canadian and Australian population demographics are multicultural in nature, but may have a lower frequency of African ancestry, and this may limit the generalisability of the findings.

INTRODUCTION

Maximising kidney transplant outcomes requires integrated strategies to improve patient and kidney allograft survival.[1] Current empiric approaches to kidney transplant management lack precision and lead to underimmunosuppression in some individuals and overimmunosuppression in others.[2] Indeed, optimising immunosuppression to balance the risk of rejection and graft loss from underimmunosuppression against the risk of infection/malignancy from overimmunosuppression is a major challenge in transplantation.[3 4] Improved precision medicine tools are needed to guide immunosuppression including sensitive, non-invasive tests for serial monitoring of allograft inflammation. These approaches could be used following immunosuppression minimisation/withdrawal protocols to detect subclinical inflammation prior to injury and to follow the response to antirejection treatment.[2 5 6]

Subclinical T-cell mediated rejection (TCMR) is a major rejection phenotype within the first-year post-transplant and early predictor of graft failure.[7–14] Two randomised, controlled trials of patients on cyclosporine-based therapy showed that early subclinical TCMR treatment led to

diminished histological injury and improved functional outcomes.[15 16] Furthermore, effective subclinical TCMR treatment in patients on modern immunosuppression resulted in similar long-term graft survival as patients without rejection.[13] Notably, subclinical and early clinical TCMR events have been linked with subsequent development of de novo donor-specific antibody (dnDSA), chronic antibody-mediated rejection (AMR) and graft loss.[13 17] As there are no effective therapies for chronic AMR, prevention strategies such as early TCMR treatment may help mitigate the long-term risks of developing dnDSA. Prevention strategies for dnDSA are key, as no effective therapies exist for chronic AMR. Taken together, these data show that subclinical TCMR is clinically significant and effective therapy is available to improve long-term graft outcomes.

Observational studies demonstrate that urine CXCL10 is a sensitive marker for kidney allograft rejection,[18–33] which rises prior to serum creatinine,[24 32] can detect borderline and subclinical tubulitis,[21 28–30 33] and decreases after treatment of rejection.[18 22 24 26 32] The population-based diagnostic performance of urine CXCL10 for subclinical TCMR is modest (area under curve (AUC) 0.69), but still outperforms standard monitoring, as it detects rejection not identified by graft functional decline.[28] Urine CXCL10 is elevated when there is graft inflammation of the tubulointerstitial compartment, but not vascular compartment. Therefore, urine CXCL10 is elevated with peritubular capillaritis, although not glomerulitis or isolated v-lesions.[27 33] Urine CXCL10 is also elevated with polyoma (BKV) viraemia/nephritis and urinary tract infections/pyelonephritis, but not infections such as cytomegalovirus (CMV) viraemia with inflammation outside the tubulointerstitial compartment.[30 33]

Elevated 1-month urinary CXCL10 is associated with increased interstitial fibrosis/tubular atrophy (IFTA)[34] and decreased kidney allograft function at 6 months.[32] Urine CXCL10 also has long-term prognostic significance, as elevated 6-month urine CXCL10 is associated with a composite outcome of death-censored graft loss, late acute rejection and decline in graft function.[35 36] Finally, using urine CXCL10 to direct surveillance biopsies may spare up to two-thirds of unnecessary biopsies, while still capturing significant subclinical inflammation in the remaining biopsies.[28]

Therefore, the primary objective of this trial is to determine if the early treatment of rejection, as detected by urine CXCL10 and confirmed by CXCL10-guided biopsy, will improve kidney allograft outcomes. The secondary goal is to use this multicentre prospective cohort to evaluate different novel diagnostic or prognostic markers and perform mechanistic analyses.

METHODS

Trial design

This is a phase ii/iii multicentre, international, randomised controlled parallel group trial to determine if the early treatment of rejection, as detected by urine CXCL10, will improve kidney allograft outcomes. The screening phase will enrol 420 incident adult kidney transplant patients to undergo routine urine CXCL10 monitoring from 2 weeks to 9 months post-transplant. Patients with elevated urine CXCL10 that is confirmed on repeat testing within 1 week, and are within the first 9 months post-transplant, will be deemed at high risk for rejection. This enriched patient population, at high risk of rejection based on urine CXCL10 testing, will be randomised 1:1 to a study biopsy or routine monitoring (figure 1). We anticipate based on prior studies that enrolling approximately 420 patients to urine CXCL10 screening will result in 250 patients who qualify for randomisation.

Participants

Study coordinators will approach participants for informed consent in hospital or post-transplant clinic. Patients will be eligible for the screening phase if they meet the following criteria: written informed consent; willingness and availability to comply with study procedures; incident adult (age ≥18 years) kidney transplant

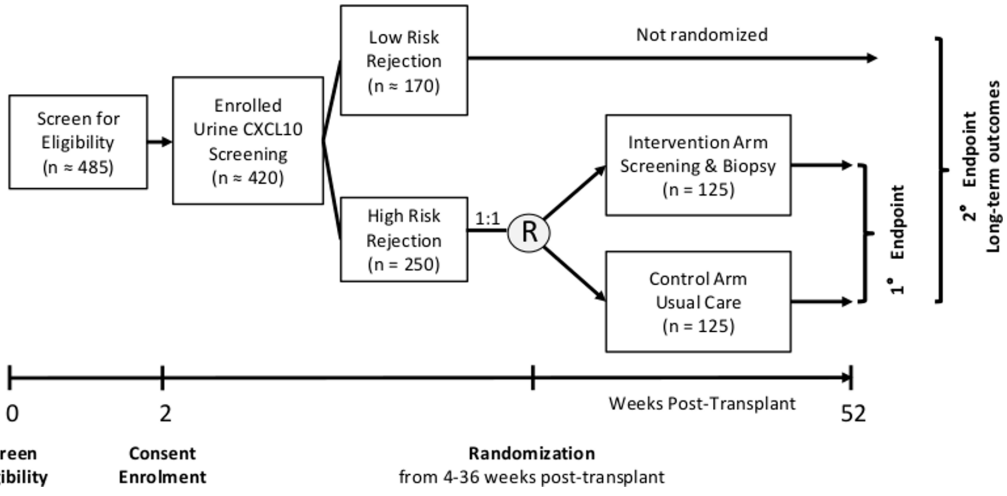

Figure 1 Trial design of a multicentre randomised controlled trial of urine CXCL10 monitoring postrenal transplant.

patients with a living or deceased donor kidney transplant. All ethnic and gender groups have equal access to participate. Exclusion criteria for the screening phase are: primary non-function; blood group ABO incompatible transplant; pretransplant DSA positive; HLA 0 DR +0 DQ mismatch; other cotransplanted organ or bone marrow/stem cell transplant; participation in other interventional drug trials; intention not to use maintenance immunosuppression with calcineurin inhibitor and antiproliferative agents; any condition that would pose a risk to the subject's safe participation, interfere with their ability to comply with the study protocol or impact the quality of data interpretation.

Patients will be eligible for the randomisation phase if they have an elevated urine CXCL10 level that is confirmed within 1 week, in the absence of a urinary tract infection or menses. Exclusion criteria for the randomisation phase are active infection at the time of randomisation that would preclude treatment of rejection. Cytomegalovirus and polyoma viraemia are not exclusion criteria.

Participating centres

Currently, there are six participating centres. The lead site is the University of Manitoba, Transplant Manitoba Adult Kidney Program (TMAKP), Winnipeg, Manitoba Canada. Participating centres include the Université Laval, L'Hôtel-Dieu de Québec, Quebec City, Quebec Canada; Western University, University Hospital, London, Ontario Canada; University of Toronto, Toronto General Hospital, Toronto, Ontario Canada; University of Ottawa, Ottawa General Hospital, Ottawa, Ontario Canada and the University of Adelaide, Royal Adelaide Hospital, Adelaide, South Australia Australia. Recruitment commenced as of March 2018.

Urine CXCL10 monitoring

Random, midstream clean catch urine samples will be obtained from all enrolled study participants at the following weeks after transplant: 2, 4, 6, 8, 12, 16, 20, 26, 36 and 52. Urine CXCL10 will be measured at the central laboratory (lead site) and results provided to the site coordinators, but not participants, physicians or site principal investigators. Elevated urine CXCL10 levels are defined as males (>13.0 pg/mL); and females (2 weeks to 5 months: >33.0 pg/mL and ≥6 months: >13.0 pg/mL). If a routine urine CXCL10 level is elevated at 4-weeks or beyond, a repeat sample will be done within 1 week to confirm it is elevated. Urine CXCL10 will also be measured bi-weekly for 4 weeks, following an episode of subclinical or clinical biopsy-proven rejection, to study the kinetics of CXCL10 in response to rejection treatment.

Interventions

Patients randomised to the intervention arm will undergo a study biopsy to check for subclinical rejection. All biopsies will be scored according to the Banff 2017 schema.[37]

Biopsy-proven rejection episodes will be treated with optimisation of baseline immunosuppression and:

- Borderline rejection: intravenous methylprednisolone 250 mg once daily for 3 days and no taper.
- Banff 1A/B rejection: intravenous methylprednisolone 250 mg once daily for 3 days, then prednisone taper over 2 weeks.
- Banff 2A/B, three rejection: intravenous methylprednisolone 250 mg once daily for 3 days, then prednisone taper over 2 weeks.
- Pure microvascular inflammation (defined as g+ptc ≥2 and i0 t0)—intravenous methylprednisolone 250 mg once daily for 3 days and no taper
- Acute or subacute AMR will be treated with plasmapheresis 4–6 exchanges, one plasma volume and intravenous immunoglobulin 2 g

A 4-week observational period will occur after treatment of subclinical or clinical biopsy-proven acute rejection, with urine CXCL10 evaluated but not triggering a biopsy, to avoid overbiopsying patients. However, after completion of this observational period, patients will recommence the urine CXCL10 screening which could trigger additional study biopsies in the Intervention Arm patients.

The incidence of subclinical Banff 2A/B or 3 is anticipated to be very low. However, risk mitigation strategies for these patients include:

- There will be no observational period following subclinical Banff 2A/B, 3 rejection. Weekly urine CXCL10 monitoring will recommence immediately and could trigger an early repeat study biopsy.
- Additional immunotherapy may be undertaken at the discretion of the treating physician but must be preceded by a clinical indication biopsy.
- Steroid non-responsive Banff 2A/B, 3 rejection will be treated with intravenous methylprednisolone 250–500mg once daily for 3 days and/or intravenous rabbit antithymocyte globulin (target dose 3–6 mg/kg, divided over 3–4 days as tolerated).

Immunosuppression

The use of induction immunosuppression and maintenance steroids is at the discretion of the treating physician, but there will be no steroid withdrawal. Mycophenolate mofetil target doses are 750–1000 mg two times a day. Tacrolimus trough levels will be measured and targets are 8±2 µg/L at 0–6 months and 6±2 µg/L at 6–12 months. Clinical biopsy-proven rejection episodes are treated as subclinical biopsy-proven rejection (above) with the exception of Banff 2A/B, 3 rejection. Acute or subacute AMR will be treated with plasmapheresis 4–6 exchanges, one plasma volume and intravenous immunoglobulin 2 g. Cytomegalovirus prophylaxis and management will be performed according to the 2018 International Consensus Guidelines on the Management of CMV in solid organ transplantation.[38] Polyomavirus screening and management and antibiotic prophylaxis for *Pneumocystis jirovecii* pneumonia will be done as per centre-specific protocols.

Participant discontinuation

Participants are free to withdraw at any time and for any reason; and may also be withdrawn if, in the opinion of the Investigator, it is in their best interests to do so. Participants may also be withdrawn for: lost to follow-up; newly developed or previously unrecognised exclusion criteria; unable or non-adherent with the protocol; any adverse event, medical condition or situation such that continued participation would not be in the best interest of the participant; individual safety stopping rule of two consecutive false positive CXCL10-directed renal biopsies and death.

Outcomes

The primary composite outcome at 12 months consists of death-censored graft loss; clinical biopsy-proven acute rejection; dnDSA development; subclinical chronic active TCMR or inflammation in areas of interstitial fibrosis and tubular atrophy (i-IFTA)[37]; or subclinical tubulitis. Subclinical chronic active TCMR and tubulitis will be evaluated on the 12-month study exit biopsy in all randomised patients. The rationale for the selecting the primary composite outcome include:

1. Death-censored graft loss is an Food and Drug Administration (FDA)-approved outcome.
2. Clinical biopsy-proven acute rejection is an FDA-approved outcome.
3. dnDSA at 12 months (2%) is a major risk factor for graft loss[17 39 40] and under consideration by The Transplant Society (TTS)-FDA as a surrogate marker.
4. i-IFTA at 12 months defined by Banff 2017.[37] IFTA accompanied by low-grade interstitial inflammation in atrophic (DeKAF iatr, Banff 2017 i-IFTA) and non-atrophic areas (IFTA+inflammation, Mayo criteria)[10 11 41–43] is associated with poor long-term graft outcomes.[44–46] These entities are associated with previous acute rejection episodes[45 46]; increased HLA mismatch[10 42]; rejection-like gene signatures[41 47] and graft loss.[10 11 44 46–49] A seminal sequential histology study showed i-IFTA is preceded by early TCMR/vascular rejection, and followed by transition to i-IFTA versus pure IFTA which has better long-term outcomes.[45] Overall, i-IFTA is a TCMR-related process associated with underimmunosuppression[45 46] that predicts graft loss independent of dnDSA[44] and AMR[45]; and the Banff 2017 schema now recognises i-IFTA as part of chronic active TCMR.[37]
5. Subclinical tubulitis at 12 months (10%) defined by Banff t-score. Patients with tubulitis (t>0) on 12-month protocol biopsies have an increased risk of graft loss compared with those with minor histological change,[11] and recurrent subclinical inflammation has been shown to result in worsening IFTA, declining graft function and increased graft loss.[49 50]

The principal secondary outcome is graft function evaluated by Modified Diet in Renal Disease estimated glomerular filtration rate (MDRD eGFR), defined by change in graft function (6–12 months, slope and delta) and absolute graft function (6 and 12 months). Additional secondary outcomes include subclinical microvascular inflammation at 12 months (Banff ptc, g, c4d and cg); development of IFTA from implantation to 12 months (delta Banff ci, ct, cv); days from transplantation to clinical biopsy-proven acute rejection (TCMR, AMR); albuminuria >300 mg/day (6 and 12 months); patient quality of life (EuroQol five-dimension five-level instrument, EQ-5D-5L); cost-effectiveness analysis and the kinetics of urine CXCL10 in response to immunotherapy.

Long-term outcomes

Administrative database linkages will be used to obtain long-term outcomes in this cohort, including death-censored graft loss and death. Canadian Organ Replacement Registry, Canadian Transplant Registry, Australia and New Zealand Dialysis and Transplant Registry (ANZDATA), provincial/state and medical record numbers will be used to enable probabilistic matching of administrative datasets.

Sample biobank

All enrolled participants will be approached with separate study consent for biobanking residual urine, serum and kidney tissue samples. All de-identified sample residuals will be stored from consenting patients in the central laboratory (lead site) and destroyed after 40 years. Mechanistic proteomics analyses are planned for these samples. Recipients of living and deceased donor kidney transplants have an allograft half-life of approximately 20 and 15 years, respectively.[51] Therefore, in order to link early biological signatures to late allograft outcomes, it is important to retain these samples for future analysis.

Sample size

Sample size calculation: randomisation phase

The TMAKP has a 2% cumulative incidence of death-censored graft loss and 13.6% clinical biopsy-proven acute rejection within the first-year post-transplant (censored for primary non-function and HLA 0 MM, 1999–2014), in a population that is not enriched for subclinical rejection by urine CXCL10. A conservative estimate of 13.6% clinical biopsy-proven acute rejection was used. The prevalence of dnDSA development varies in the literature, due to confounding by the inadequate exclusion of pretransplant DSA. In studies that exclude pretransplant DSA with a flow cross-match or solid phase assays, the prevalence of dnDSA ranges from 2.0% to 27% at 12 months after transplant,[17 43 52–55] therefore, we assumed a conservative estimate of 2%.

The literature demonstrates a range of IFTA + inflammation from 9.5% to 26%, depending on the patient population, time post-transplant and biopsy indication.[10 11 41–43 56] As 12-month surveillance biopsies showed a 20.2% prevalence of unfavourable prognostic features, including IFTA + inflammation, in patients on modern immunosuppression, this was the estimate we used[11] (table 1). In unselected populations, the prevalence of subclinical tubulitis at 12 months is 3.4%.[11] However,

Table 1 IFTA + inflammation prevalence estimates

Study	Population	Biopsy	Time post-Transplant	Prevalence	Definition	Ref
Park *et al*	LD recipients 2000–2007	Protocol	1 year	20/151 13%	Mayo	41
Cosio *et al*	LD and DD recipients 1998–2001	Protocol	1 year	53/292 18%	Mayo	10
Gago *et al*	LD and DD recipients 2000–2006	Protocol: 151/207, 73% Indication: 56/207, 27.1%	16.9±15.5 mos (1, 3, 5 years)	207/795 26%	Mayo	42
Gago *et al*	LD and DD recipients 2000–2006	Protocol*	1 year	14.4%	Mayo	42
Cosio *et al*	LD and DD recipients 1999–2010 LD=78%	Protocol	1 year	86/935 9.5%†	Mayo	11
Ho *et al*	LD and DD recipients	Protocol	2 years	28/111 25%‡	Mayo	56
Ho *et al*	LD and DD recipients	Protocol	6 mos	22/94 23.4%	Mayo	56
TMAKP	LD and DD recipients	Protocol	6 mos	48/222 21.6%	Mayo	
TMAKP	LD and DD recipients	Protocol	6 mo	Pure IFTA alone (ci+ct ≥2)§ 93/222 41.9%		
García-Carro *et al*	LD and DD recipients	Protocol	6 wks	108/598 18.1%	Oslo	43
García-Carro *et al*	LD and DD recipients	Protocol	1 year	125/588¶ 21.25%	Oslo	43

The most recent Banff classification has been revised to include i-IFTA as part of chronic active TCMR. Prevalence estimates for IFTA + inflammation were used, as prior to February 2018, there was no internationally accepted definition for IFTA + inflammation. The primary composite outcome for i-IFTA will be measured using the new Banff schema.[37]
*They did not explicitly state that the 1 year 14.4% reported IFTA + inflammation was from protocol biopsies, but based on their treatment protocols, it is assumed to be largely protocol biopsies.
†Reported as 9.5%, but calculated as 9.2%.
‡It is anticipated that later protocol biopsies would have higher rates of IFTA + inflammation, based on 1, 3, 5-year biopsy data from Gago *et al*.[42]
§Only 16 cases met the 'conservative criteria for IFTA + inflammation': ci +ct ≥2, i>0 (16/222, 7.2%). The whole table is otherwise scored with the Mayo clinic criteria for IFTA + inflammation.
¶Personal communication: Daniel Serón, Clara García-Carro and Anna Reisaeter.
DD, deceased donor; LD, living donor; mo, month; TCMR, T-cell mediated rejection; TMAKP, Transplant Manitoba Adult Kidney Program; wks, weeks.

patients with elevated urine CXCL10 have a 63% prevalence of subclinical tubulitis.[28] Therefore, a conservative estimate of 17.4% subclinical tubulitis was used, as the study design enriches for patients at high risk of rejection through elevated urine CXCL10. Therefore, a conservative estimate of the overall prevalence of the primary outcome in the control arm is 55%, allowing for some overlap in endpoints.

A 35.6% reduction in the primary composite outcome was considered clinically significant. The multicentre, interventional clinical transplant trials FKC008 and FKC014 had 82% and 78% of patients complete the study protocol, including 24-month protocol biopsies (Rush, personal communication). These were pharmacological interventional studies with serial surveillance biopsies and more intensive study protocols. This study protocol is much less intensive and a conservative 20% drop-out was assumed. Therefore, with an alpha error 0.05, power 0.80 and 20% drop-out rate, we will randomise 250 incident adult renal transplant patients. Sample size estimates were based on two-sample test of proportions using the power.prop.test function in R.[57] There will be no interim analyses.

Patient enrolment: screening phase, urine CXCL10

We anticipate approximately 420 patients will need to be enrolled for urine CXCL10 screening to obtain 250

patients at high risk of rejection for randomisation. TMAKP had 87% patients meet the eligibility criteria of adult incident kidney transplant patients on tacrolimus and mycophenolate mofetil with HLA ≥1 DR/DQ MM. We anticipate that approximately 40% patients will be persistently CXCL10 negative (low risk of rejection) on screening and will remain off-study, and 60% patients will have a confirmed elevated urine CXCL10 and undergo randomisation, based on observational studies.[28]

Randomisation

Participants will be randomly assigned to the intervention arm (n=125) or control arm (n=125). Randomisation will be stratified by centre to account for centre-specific effects, such as induction immunosuppression and steroids. Allocation concealment will be preserved using a central computer-generated randomisation, in randomly permuted blocks with an overall 1:1 allocation. Randomisation will be performed by the lead site, with the allocation provided by email from the multicentre study coordinator to the site coordinator.

Blinding

Due to the biopsy/interventional nature of this trial, randomisation to the intervention arm cannot be blinded. Study participants randomised to the control arm, their treating physicians and site investigators will remain blinded to their CXCL10 status compared with the non-randomised urine CXCL10 screening arm until the final 12-month study visit, to avoid an inadvertent increase in baseline immunosuppression. Control arm patients will be unblinded at 12 months as a study exit biopsy is required to determine the primary composite outcome. Histopathology will be reviewed centrally by a single, experienced transplant pathologist (IWG) and the dnDSA assessment performed centrally (CW). Each outcome assessor will be blinded to the randomisation arm.

Statistical methods

Primary analysis

The primary hypothesis is that the early treatment of rejection, as detected by urine CXCL10 and confirmed by biopsy, is superior to standard of care for improving kidney allograft outcomes in incident, adult kidney transplant patients on tacrolimus and mycophenolate mofetil. The primary analysis is a modified intention-to-treat, complete-case basis, in the intervention versus control arm. The initial analysis will be based on a binomial test of proportions for the primary composite outcome in the two treatment arms. In the event that there are a higher number of deaths than expected, a competing risk survival model for the primary outcome and death with function (unrelated to alloimmune inflammation) will be performed. However, patient survival from 1 to 12 months post-transplant is very high (TMAKP 1999–2014, 100%), so we anticipate that this will not significantly impact the analysis.

If missing outcome data are greater than anticipated we will impute them according to current FDA/NAS/NRC recommendations using multiple imputation with chained equations.[58–60] Specifically, multiple imputation creates 20–40 complete datasets with plausible values from the model-based predictive distributions, and pooled estimates and SEs are obtained through the use of Rubin's combining rules. These models are predicated on the assumption that the data are missing at random (MAR). Therefore, every effort will be made to minimise drop-out and ensure full follow-up of patients. Finally, the robustness of the inferences about treatment effects to violations of the MAR assumptions will be evaluated through a sensitivity analysis (eg, delta method).[60] Variable lengths of time from study entry to randomisation (or randomisation to 12-month outcome) will be accounted for as a covariate in the model. Additional covariates will be selected to account for any imbalances that occur in the groups despite randomisation. The covariates that will be considered include: induction immunosuppression; steroid exposure; tacrolimus trough levels; HLA eplet matching and serological matching; panel reactive antibody; previous transplant; recipient sex, age and race; delayed graft function; donor type, kidney donor profile index (KDPI) and living kidney donor profile index (LKDPI); and BKV viraemia.

Secondary outcomes

Principal secondary endpoint

A linear mixed effects regression model will be used to assess the temporal evolution of eGFR with adjustment for covariates (eg, age, gender, donor type, etc) and a random subject effect to account for the within-subject correlations (repeated measures).[61] A preliminary descriptive analysis will evaluate normality; non-normal data will be suitably transformed prior to modelling.

Secondary efficacy endpoints

Change in histological injury levels will be modelled using normal-theory linear analysis of covariance (ANCOVA) models for microvascular inflammation (Banff ptc, g, c4d, cg) and development of IFTA (Banff ci, ct, cv, ah) at 12 months. For each subject, the response will be the implantation/baseline biopsy to 12-month change (delta score). Time from transplantation to clinical biopsy-proven acute rejection will be summarised and modelled as rejection-free survival using Kaplan-Meier methods. Regression modelling may be used to adjust for potential confounders (eg, Cox proportional hazards regression or accelerated failure time models if the proportional hazards assumption is not satisfied). Albuminuria will be evaluated as the change in urine albumin: creatinine ratio from 2 weeks to 6 and 12 months, respectively. Temporal trends in the progression of urine albuminuria will be modelled using linear mixed model theory, with a random subject effect (random intercept) to account for the within-subject correlations due to repeated measures. This approach can easily accommodate additional

covariates if needed, and non-Gaussian outcomes can also be modelled after suitable variable transformations (eg, Box-Cox).

Given that the EQ-5D measures overall quality of life, the EQ-5D index score will be the primary measure used to determine whether the intervention has an impact on HRQOL. All participants who complete the EQ-5D baseline, 6 months and 12 months post-transplant will be included in the analysis of health-related quality of life data. A detailed cost analysis of the first-year post-transplant will be performed to determine the potential cost savings of a urine CXCL10-guided monitoring strategy by avoiding unnecessary biopsies with normal histology in programmes that use biopsies. Second, the early treatment of rejection may result in improved long-term allograft survival, therefore, a decision analysis model to compare the cost-effectiveness of a urine CXCL10 monitoring strategy on long-term allograft outcomes will be used to estimate the potential healthcare savings of potentially extending allograft longevity.

Urine CXCL10 kinetics after rejection therapy will be evaluated with descriptive statistics. Temporal trends in the evolution of urine CXCL10 will be modelled using linear mixed model theory with a random subject effect to account for the repeated measures; this approach can accommodate additional covariates and non-Gaussian outcomes can be modelled after suitable variable transformations (eg, Box-Cox). A time cut-off for the normalisation of chemokines in response to immunotherapy will be identified.

Planned subgroup analyses

Subgroup analyses may require adjustment for prognostic imbalances, as the effect of randomisation may not be preserved.

High immunological risk analysis

We postulate that patients who are at higher risk for rejection are more likely to benefit from the intervention (early study biopsy). Therefore, a subgroup analysis is planned in high versus low immunological risk patients. High immunological risk is defined as high HLA serological and/or eplet mismatch; panel reactive antibody ≥95% and history of a previous transplant. We will also adjust for individuals who develop delayed graft function.

On therapy analysis

Based on our data, we anticipate that approximately two-thirds of the intervention arm will have biopsy-proven subclinical rejection requiring therapy.[28] This estimate is based on subclinical rejection alone. However, urine CXCL10 may also identify early clinical rejection prior to a rise in serum creatinine,[20 24 32] suggesting that more patients may undergo a CXCL10-guided study biopsy. Therefore, we will determine the impact of those patients who received therapy for early and/or subclinical rejection (~⅔ intervention arm) versus control arm in a 1: many manner.

On protocol analysis

Non-adherence to transplant medications and clinic visits increases the risk of rejection, dnDSA and poor allograft outcomes, and is an important potential confounder.[40 62 63] Therefore, a prespecified analysis of the primary outcome will be performed 'on protocol', defined by study protocol adherence and transplant medication adherence, defined as adequate tacrolimus trough levels.

Ancillary analysis

The study sites consist of three surveillance biopsy programmes (University of Manitoba, Université Laval and Royal Adelaide Hospital) and three non-surveillance biopsy programmes (University of Ottawa, University of Toronto and Western University). The three surveillance biopsy programmes will perform a surveillance biopsy at 12 months post-transplant in the non-randomised, low-risk patients to determine the incidence of subclinical rejection in patients with persistently low urine CXCL10. A strictly observational comparison will be performed of the 12-month primary composite outcome of the non-randomised study population, the intervention and control arms.

Trial monitoring and safety

De-identified data will be recorded on a Research Electronic Data Capture (REDCap) database in a secure online research environment and data quality rules have been established. Trial monitoring will be conducted remotely via REDCap. The Canadian Donation and Transplant Research Program is providing an independent data safety monitoring board for ongoing safety monitoring and there are no planned interim analyses.

Patients and public involvement

Patients and the public were not directly engaged in the development of this study, however, preventing acute rejection and graft loss are consistently ranked as a top research priority in kidney transplantation by patients and caregivers.[64–66] Patients were not involved in the design, recruitment or conduct of this study. We will disseminate the findings to trial participants by mail outs or presentations to patient groups, for those that are interested.

ETHICS AND DISSEMINATION

Confidentiality

The study has been approved by the University of Manitoba Health Research Ethics Board (HS20861, B2017:076) and the local research ethics boards of participating centres (online supplementary appendix 1—consent form). A participant's privacy and confidentiality will be respected by all research team members. Measures will be taken to ensure that all data collected will remain confidential in accordance with each site's privacy legislation. The identity of the participants will not be revealed in any published data or presentation. Findings will be reported in aggregate.

Dissemination

Recruitment commenced in March 2018 and results are expected to be published in 2023.

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Acknowledgements The authors would like to gratefully acknowledge the generous in-kind contributions from the George and Fay Yee Centre for Healthcare Innovation, including assistance with project management and development (Brendon Foot, Alannah Baldwin); trial monitoring (Tarpan Mankad); biostatistical consulting (AS, KK) and data management (Dennis Bayomi). The authors would like to gratefully acknowledge the Canadian Donation and Transplant Research Program (CDTRP) for generously providing an in-kind, independent data safety monitoring board. The authors would like to acknowledge the staff at the Diagnostic Services of Manitoba for their assistance in translating the urine CXCL10 research assay into their clinical laboratory. Finally, we would like to acknowledge Daniel Serón, Clara García-Carro and Anna Reisaeter for their kind assistance in providing the 12-month prevalence of IFTA + inflammation in their cohort.

Contributors JH and PN are coprincipal investigators, designed the study and wrote the protocol. AS and KK performed the statistical analyses and contributed to the study design. IWG provided histopathological input. CW provided HLA eplet input. DNR is the medical monitor and provided participant safety input and PH-M provided urine CXCL10 input. RC, SDS, AJ, SJK and GK are site principal investigators and provided input on the trial design and protocol. All authors have reviewed and approved the manuscript.

Funding This investigator-initiated study is funded by Canadian Institutes of Health Research (CIHR) Project Grant 364003 (2016-2021). JH holds a CIHR New Investigator Salary award (2015-2020). PN holds the Flynn Family Chair in Renal Transplantation. SDS holds a Fonds de Recherche Québec Santé (FRQS) Clinician-Scientist Junior II Salary award (2018-2020).

Disclaimer The funding body (CIHR) has no input on the trial design, conduct or analysis.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval University of Manitoba Health Research Ethics Board.

Provenance and peer review Not commissioned; externally peer reviewed.

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