Rapamycin and inulin for third-dose vaccine response stimulation (RIVASTIM): Inulin – study protocol for a pilot, multicentre, randomised, double-blinded, controlled trial of dietary inulin to improve SARS-CoV-2 vaccine response in kidney transplant recipients

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ABSTRACT

Introduction  Kidney transplant recipients (KTRs) are at an increased risk of hospitalisation and death from COVID-19. Vaccination against SARS-CoV-2 is our primary risk mitigation strategy, yet vaccine effectiveness in KTRs is suboptimal. Strategies to enhance vaccine efficacy are therefore required. Current evidence supports the role of the gut microbiota in shaping the immune response to vaccination. Gut dysbiosis is common in KTRs and is a potential contributor to impaired COVID-19 vaccine responses. We hypothesise that dietary fibre supplementation will attenuate gut dysbiosis and promote vaccine responsiveness in KTRs.

Methods and analysis  Rapamycin and inulin for third-dose vaccine response stimulation-inulin is a multicentre, randomised, prospective, double-blinded, placebo-controlled pilot trial examining the effect of dietary inulin supplementation prior to a third dose of COVID-19 vaccine in KTRs who have failed to develop protective immunity following a 2-dose COVID-19 vaccine schedule. Participants will be randomised 1:1 to inulin (active) or maltodextrin (placebo control), administered as 20 g/day of powdered supplement dissolved in water, for 4 weeks prior to and following vaccination. The primary outcome is the proportion of participants in each trial arm that achieve the proportion of participants in each trial arm that achieve in vitro neutralisation of live SARS-CoV-2 virus at 4 weeks following a third dose of COVID-19 vaccine. Secondary outcomes include the safety and tolerability of dietary inulin, the diversity and differential abundance of gut microbiota, and vaccine-specific immune cell populations and responses.

Ethics and dissemination  Ethics approval was obtained from the Central Adelaide Local Health Network (CALHN) Human Research Ethics Committee (HREC) (approval number: 2021/HRE00354) and the Sydney Local Health District (SHLD) HREC (approval numbers: X21-0411 and ACTRN12621001465842). Results of this trial will be published following peer-review and presented at scientific meetings and congresses.

Trial registration number  ACTRN12621001465842.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Rapamycin and inulin for third-dose vaccine response stimulation-inulin takes advantage of a largely COVID-19 naïve population to assess the efficacy of a third COVID-19 vaccination in kidney transplant recipients who have failed to adequately respond to a standard two-dose vaccine schedule.

⇒ A broad inclusion criterion is employed to promote equitable access and the generalisability of trial results across a diverse patient population.

⇒ Blinding of the study participants, investigators and outcome assessors to treatment allocation will reduce the risk of bias.

⇒ Habitual diet will be assessed via a 4-day food diary to account for variation in baseline fibre and macronutrient intake.

⇒ The continued emergence of COVID-19 variants of clinical significance may alter the clinical landscape and limit trial recruitment via an emergent demand for booster vaccinations.

INTRODUCTION

At the beginning of 2022, the number of deaths worldwide caused by the COVID-19 pandemic exceeded 5.5 million.1 Immunosuppressed populations such as kidney transplant recipients (KTRs) are at high-risk for COVID-19-related adverse events (AEs).
Reducing or altering immunosuppression in KTRs is an attractive strategy to augment vaccine responses, yet must be balanced against the risk of enhanced allo-immunity and subsequent organ rejection. While this approach may be suitable for some KTRs, it may be declined by others who are not prepared to accept an increased risk of acute rejection, or would prefer to remain stable on their long-term immunosuppression regimen.

Improving the gut microbiome may be another way to improve the vaccine response. The commensal microorganisms that reside in the gastrointestinal tract have wide-reaching effects on systemic immunity and are critical in the development and licensing of immune cells, and in maintaining adequate immune responses to encountered antigens, including those encountered through vaccination. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota. In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group. Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions, which promote the growth of beneficial microbiota.

In Australia alone, more than 13,000 people currently live with a kidney transplant. Meta-analyses of cohort studies in this at-risk population report mortality rates approaching 25%, and high risks of hospitalisation, acute kidney injury and graft loss among survivors.

The emergence of several highly effective vaccines which target the SARS-CoV-2 spike protein have been critical in reducing disease burden and retarding the development of COVID-19 disease among the general population. However, KTRs are known to exhibit suboptimal vaccine responses, and the efficacy of standard 2-dose COVID-19 vaccination schedules in KTRs is poor. To address the inadequate vaccine response observed in KTRs, and in other immunocompromised groups, additional doses of mRNA vaccine have been recommended.

While a randomised controlled trial demonstrated that a third dose of mRNA vaccine increased the proportion of KTRs achieving protective levels of neutralising antibodies to 60%, this and other recent studies clearly indicate that a substantial minority of KTRs remain inadequately protected from COVID-19 despite a third vaccination.

Figure 1 Outline of the RIVISTIM-inulin trial. RIVISTIM, rapamycin and inulin for third-dose vaccine response stimulation.
common methodologies in participant screening and enrolment, data collection and management, and outcome assessments.

RIVASTIM-inulin is a pilot multicentre, parallel-arm, randomised, placebo-controlled, double-blinded, exploratory trial, examining the effect of dietary inulin on the immune response to a third dose of mRNA COVID-19 vaccine in KTRs who have failed to demonstrate protective immunity following a two-dose vaccine schedule. KTRs who have received two doses of a COVID-19 vaccine will be enrolled and their immune response to vaccination assessed by measurement of the anti-RBD IgG titre. Those with a satisfactory immune response (anti-RBD IgG ≥100 U/mL) will exit the study and be advised to receive a third mRNA COVID-19 vaccination as per recommended guidelines. KTRs who fail to demonstrate protective immunity (anti-RBD IgG<100 U/mL) will proceed to randomisation.

Randomisation will occur at a participant level with an allocation ratio of 1:1, stratified by study site and the magnitude of immune response following two doses of vaccine (anti-RBD IgG titre; non-responder: <0.4 U/mL; low responder: 0.4–99 U/mL). An outline of the trial is shown in figure 1. Following randomisation, patients will receive a dietary supplement in the form of a white, soluble and largely flavourless powder consisting of inulin (active arm) or maltodextrin (control arm). Participants will consume 10 g of supplement dissolved in 200 mL water daily, escalating to 10 g twice daily after 1 week. Following a 4-week lead in period, participants will receive a third dose of mRNA COVID-19 vaccine, with the subsequent antibody response measured at 4–6 weeks postvaccination. Participants will continue the dietary supplement up until the time of antibody assessment.

The first study participant was enrolled on the 8 November 2021 and recruitment is anticipated to continue until March 2022, with the final study visit of the last recruited patient expected to occur in May 2022.

Table 1 Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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<tr>
<td>Recipients of a functioning kidney transplant from a living or deceased donor</td>
<td>Recipients of multiorgan transplants (eg, kidney-pancreas)</td>
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<tr>
<td>Individuals aged ≥18 who can give informed consent and are willing to participate and adhere to the requirements of the study</td>
<td>Documented prior infection with COVID-19</td>
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<tr>
<td>Recipient of 2 doses of a COVID-19 vaccine (either adenoviral vector or mRNA-based)</td>
<td>Individuals aged &lt;18 or &gt;80 years, or who are currently pregnant</td>
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<td>SARS-CoV-2 receptor binding domain antibody (anti-RBD IgG) below the threshold for clinical protection from COVID-19 (&lt;100 units/mL).</td>
<td>Underlying conditions predisposing to altered gut permeability and/or dysbiosis (including but not limited to: active or recent gastrointestinal infection, inflammatory bowel disease, short gut syndrome, coeliac disease, or the presence of a gastrointestinal stoma)</td>
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<td></td>
<td>Known intolerance, allergy or sensitivity to inulin or dietary fibre</td>
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<td></td>
<td>Inability or unwillingness of an individual or their legal guardian to give written and informed consent.</td>
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Study setting
The trial will be conducted at the renal transplant units of two tertiary referral hospitals in Australia: (1) The Royal Adelaide, Hospital, Adelaide, South Australia and (2) The Royal Prince Alfred Hospital (RPA), Sydney, New South Wales.

Eligibility criteria
The inclusion and exclusion criteria are in table 1.

Recruitment
Prospective participants will be identified through the following means:
1. Review of local transplant recipient databases at each trial site. (Only local site clinical staff will have access to identifying information for the purpose of recruitment.)
2. During routine clinical review with their treating nephrologist or transplant centre.
3. Potential participants may also indicate their interest in trial participation by responding to a QR code displayed during the Transplant Australia COVID-19 Vaccination Update Webinar, broadcast in November 2021.

Prior to enrolling, patients will be provided with written information regarding the rationale behind the trial, the potential risk and benefits of participation, and the personal commitment involved. Patient’s will be enrolled by trained research staff and consented for trial participation, the collection of data and the storage of biological samples (see online supplemental file 1). Recruitment will continue until target recruitment is fulfilled, or until recruitment of dual-vaccinated transplant recipients is no longer feasible, or if delaying a third vaccination becomes no longer ethically permissible due to clinical urgency. Participants will not receive payment for participation.

Randomisation
Participants are randomised 1:1 to either inulin or maltodextrin (control). Randomisation will occur via computer-generated stratified block randomisation with randomly
permuted block sizes of 2, 4 and 6. Stratification will occur by site and the response to a two dose COVID-19 vaccine schedule (low responder anti-RBD IgG 0.4–99 U/mL; or non-responder, anti-RBD IgG<0.4 U/mL).

Allocation concealment
The allocation sequence is generated by an independent and blinded statistician, and administered centrally through an external web-based randomisation module contained within a purpose built Research Electronic Data Capture (REDCap) data management platform. The randomisation algorithm and treatment allocation are not accessible to study investigators or research staff. Trained study investigators will enrol participants, while at each study site, an un-blinded administrative assistant who is not a member of the study team will perform randomisation via the web-based platform and assign the concealed intervention to each participant.

Blinding
The study participant and their treating nephrologist, in addition to investigators, research staff and outcome assessors will be blinded to treatment allocation. Unbinding is not permitted during the trial except in the occurrence of a serious AE (SAE). In such an event, the principal investigator (PI) will decide whether unblinding is required, and if deemed necessary, will direct the unblinded administrative assistant to contact the participant’s treating healthcare professional to discuss their treatment allocation. Wherever possible, trial staff will remain blinded and the participant will continue with trial follow-up and their study treatment.

Trial interventions
Inulin is a naturally occurring non-digestible dietary fibre that may be extracted from natural sources, such as chicory root, or produced synthetically in commercial quantities that may be extracted from natural sources, such as chicory roots. (Cosucra group, Warcoing, Belgium).

Maltodextrin is a polysaccharide which is easily soluble in water and rapidly absorbed in the upper gastrointestinal tract, leading to negligible interaction with colonic microbiota. In addition to its inert effect on the gut microbiota, maltodextrin was selected as a placebo due to its similar physical appearance and taste to inulin.

Intervention description
Participants will be randomly allocated and blinded to their inclusion in one of two groups:
1. Inulin—fibruline Instant, a soluble dietary fibre extracted from chicory roots. (Cosucra group, Warcoing, Belgium).
2. Maltodextrin (Bulkpowders, Braeside, Victoria, Australia).

The study products are prepared and packaged by Bulk Powders Pty Ltd (Braeside, VIC, Australia) specifically for the trial in identical sealed, opaque and numbered 1 kg bags with an accompanying 10 g measuring scoop. Participants will be instructed to consume 10 g (1 level scoop) dissolved in approximately 200 mL of water each morning for 7 days, increasing to 10 g each morning and night (20 g/daily) as tolerated, for the remainder of the trial period. Participants will be provided with the study supplement in a sealed bag, which will be weighed prior to allocation. At the final trial visit, participants will return any unused supplement, with the bag again weighed to determine the total weight of supplement consumed during the study.

All trial participants will receive a third dose of a COVID-19 mRNA vaccine, either Pfizer-BioNTech BNT162b2 (30 µg, IM) or Moderna mRNA-1273 (50 µg, IM) determined by local practice and vaccine availability. Study participants will receive written pre-vaccination information on the benefits and potential risks and harms of the COVID-19 vaccine and be screened for contraindications to immunisation such as SAEs attributable to a previous dose of a mRNA COVID-19 vaccine. All patients will be advised of the need to continue with additional public health measures (eg, physical distancing, hand washing, wearing a face mask and COVID-19 testing and isolation as required).

Concomitant care and interventions
All participants will continue with usual transplant management as per local standard of care and at the discretion of their treating nephrologist. Any changes to medications will be recorded. Participants will be asked to continue with their usual diet and medications but abstain from dietary supplements (including non-study prebiotics and probiotics) for the duration of the study. The trial Sponsor has indemnity to compensate those who suffer from potential harm resulting from their participation in the trial.

Management of COVID-19 positive participants during the trial
Study participants who return a positive COVID-19 result during the trial will be managed in consultation with...
their treating transplant unit as per local best practice. Participants who contract COVID-19 following randomisation but prior to a third vaccination may have their third vaccine dose delayed. Where possible, participants will be asked to continue with their allocated treatment regimens and attend study visits and follow-up.

Outcomes
Primary outcome measure
The primary outcome is the proportion of participants in each trial arm that achieve protective serological neutralisation of live SARS-CoV-2 virus (Wuhan). Protective neutralisation is defined as 20.2% of the mean neutralisation level of a standardised cohort of COVID-19 convalescent individuals, and correlates with 50% protection from infection with SARS-CoV-2 (Wuhan) in healthy individuals.

Secondary outcome measures
The secondary outcome measures include the following:
1. Change in the median magnitude of the SARS-CoV-2 spike-specific, antiviral T cell response prior to and at 4–6 weeks following vaccination, determined as the frequency of cells that secrete IFNγ in response to stimulation with spike-protein (Wuhan)-derived peptides by ELISpot.
2. AE(s) following immunisation (AEFI) including AEs of special interest (AESI) will be assessed via phone consultation at 1 week, and again at 4–6 weeks postvaccination during the final follow-up visit, and include:
a. Changes in kidney allograft function, determined by serum creatinine, eGFR (CKD-EPI equation) and proteinuria.
b. The occurrence of biopsy proven acute allograft rejection.
c. The recurrence of primary kidney disease.
d. Patient reported quality of life as recorded by the EuroQol 5 Dimensions (EQ-5D) questionnaire.
3. Tolerance of dietary inulin determined by the change in gastrointestinal symptom rating scale (GSRS) at baseline, week 4 and the final trial visit. Adherence to the intervention will be assessed by the weight of unconsumed supplement returned at the final study visit.
4. The proportion of participants who generate a serological response 4–6 weeks following a third COVID-19 vaccination. A serological response is defined as reaching a threshold of anti-receptor-binding domain antibody (anti-RBD IgG) ≥100 units/mL (measured with an Elecsys Anti-SARS-CoV-2 immunoassay (Roche)). This RBD antibody threshold was chosen on the basis of pre-clinical and clinical studies, and is consistent with the reported outcomes in published COVID-19 clinical vaccine trials.
5. Changes in the community structure, relative abundance and functional characteristics of the gut microbiome following 4 weeks of dietary intervention, determined by 16S-rRNA metagenomic sequencing of participant stool samples.
6. The development of COVID-19 following randomisation, determined by:
a. Positive SARS-CoV-2 PCR test, or rapid antigen test in the setting of symptomatic disease.
b. Detection of SARS-CoV-2 anti-nucleocapsid antibodies at the time of primary outcome assessment.
Following trial registration, additional secondary outcome numbers 4–6 were approved by the HREC, and added to the trial protocol. These outcomes are exploratory in nature and will seek to inform the design and scope of larger clinical trials.

Participant timeline
Participants are followed from the time of enrolment through until study close-out, 1-week following their final assessment visit. The schedule of enrolment, randomisation, interventions and assessments is shown in figure 2.

Sample size
The aim of this pilot study is to investigate whether prebiotic supplementation with dietary fibre sufficiently improves COVID-19 vaccine responses to warrant further investigation in a larger trial. Current evidence supports the role of gut microbial communities in modulating the

Figure 2 Participant timeline. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist. Enrolment, interventions and assessments. AE, adverse event; EQ-5D, EuroQol 5 Dimensions Questionnaire; GSRS, Gastrointestinal Symptom Rating Scale; SAE, serious AE.

Table 2 Study period

<table>
<thead>
<tr>
<th>TIMEPOINT</th>
<th>Enrolment</th>
<th>Randomisation</th>
<th>Post-allocation</th>
<th>Close-out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit window</td>
<td>≤5 days</td>
<td>0</td>
<td>7 days</td>
<td>4–6 weeks from vaccination</td>
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</table>

**Study Period**

- **Enrolment**: Eligibility screen, Informed consent, Baseline characteristics, Allocation
- **Interventions**: Inulin (active), Malondextrin (placebo control), COVID-19 mRNA Vaccine
- **Assessments**: Anti-RBD IgG titre, Routine biochemistry, Blood draw for cellular and humoral immune assessment, Faecal microbiota assessment, 4-day food diary, Medication Review, GRS, Adherence Assessment, EQ-5D, AE/SAE
immune response to vaccination, including COVID-19 vaccines. However, evidence supporting interventions which target the microbiota in this setting are lacking and estimates of effect size cannot be accurately generated.

In defining a target sample size, we considered the following: (1) the number of eligible KTRs across the two sites; (2) their current vaccination status; (3) the feasibility of conducting a trial within the contemporary resource setting; (4) local prevalence of COVID-19 and (5) the recommended sample size requirements for a pilot study. A recruitment target of approximately 60–120 participants was considered feasible, and sufficient to generate a meaningful estimate of effect size with adequate confidence. With full recruitment of 120 participants, and assuming a 25% virus neutralisation endpoint in the control group, we would require 54% virus neutralisation in the intervention group to demonstrate superiority using a one-sided hypothesis with 2.5% α-risk and 90% power.

**Patient and public involvement**

This trial was presented at the Transplantation Australia Webinar. Of 513 national attendees, 42 registered their interest to learn more and potentially enrol in the study. Interested patients were geographically diverse. To provide an opportunity for regional patients to participate, recruitment for the study occurred during outreach clinics in South Australia and New South Wales.

**Data collection and management**

Clinical and safety laboratory tests (haematology, chemistry, urinalysis) for enrolment and end-point criteria (anti-RBD IgG) are performed at the hospital laboratory of each study site.

All study data are collected by trained research staff and entered directly onto study-specific electronic data capture forms (eDCF) created and housed within a secure, web-based data management tool (REDCap). The DCFs contain inbuilt protections to promote data quality, including range checks for numerical data values, restrictions on alphanumeric entries and prevention of duplicate records. The RIVASTIM REDCap database is stored on secure servers in an on-site limited access data centre at the RPA Hospital and operated behind the Sydney Local Health District (SLHD) firewall. All electronic information and transmissions are protected via Secure Sockets Layer encryption. Access to the RIVASTIM REDCap database is limited to approved research staff, with individual user authentication and logging of all data entry and modification, and access to restricted modules (randomisation, scheduling and data export) privileged. The database is maintained by the SHLD Information and Communication Technology Services with regular back-up processes in place.

**Collection and evaluation of biological samples**

Blood samples will be taken from participants for immunological assessment at randomisation and at 4–6 weeks following vaccination. Blood will be drawn from participants by clinical research staff and collected in 7×9 mL EDTA and 1×5 mL CAT serum separator vacutainer tubes. Peripheral blood mononuclear cells will be isolated from whole blood by density gradient centrifugation in Ficoll-Paque and aliquoted and cryopreserved in liquid nitrogen for batch testing, with sera aliquoted and stored at −80°C. Phenotypic and functional assessments of vaccine-specific T-cell responses will be initially assessed by IFNγ release assay (ELISpot) following stimulation with overlapping peptides spanning the length of SARS-CoV-2 spike protein, with any notable change triggering a more in-depth investigation. For example, the assessment of spike-specific circulating T follicular helper cells based on the frequency and phenotype of CD4+ T cells expressing CD40L following stimulation with spike protein-derived peptides may be analysed via FACS. Using participant serum, the titres of SARS-CoV-2 spike-protein-specific IgG and the capacity of participant serum to neutralise SARS-CoV-2 viral entry into ACE2 cells will be assessed. The capacity of preimmunisation and postimmunisation serum to induce spike-protein-specific antibody-dependent innate immune responses will be measured.

Isolation and sequencing of the faecal metagenome will occur on self-collected stool samples placed in a DNA stabilising solution (OMNiGene GUT OM-200, DNA Genotek, Canada). Stool samples will be aliquoted and stored at −80°C until batch testing. Analysis of the faecal metagenome will be performed by comparative sequencing of 16S rRNA amplicons (V4 region). Estimation of participants habitual diet will be captured using a 4-day food diary, completed at the time stool samples are collected.

Validated questionnaires are completed by participants to capture adverse gastrointestinal symptoms (GSRS37 38), and health-related quality of life (EQ-5D) information.39

All biological specimens will be deidentified and labelled with the participants unique study identifier. Stool and blood samples will be stored and maintained in access-restricted laboratory freezers at their corresponding trial site (Adelaide Health and Medical Sciences building, University of Adelaide, or the Transplant Institute, RPA, Sydney).

**Confidentiality**

Prior to study enrolment, participants will consent to research staff accessing their electronic medical record to obtain baseline and demographic information, and the results of laboratory assessments. The privacy and confidentiality of screened and enrolled participants will be preserved with all study data stored in the RIVASTIM REDCap database under a unique numerical study identifier. No identifying information or individually identifiable participant data will be reported in publications, presentations, or in any report arising from this study.
Statistical methods

The primary analysis will be by intention-to-treat (ITT), with participants assessed according to their treatment allocation. Participants who develop a positive SARS-CoV-2 PCR result during the study will be excluded from the primary analysis to avoid confounding. A per-protocol analysis will also be reported, with participants who failed to adhere or tolerate the dietary intervention and consumed <80% of the prescribed supplement, and participants who withdrew or were lost to follow-up excluded from the analysis. A sensitivity analyses adjusting for potential confounding may be performed should significant imbalances in baseline characteristics between the treatment groups occur. Multiple imputation will be used to handle data missing at random from baseline characteristics. Data missing at random for the primary and secondary outcome will not be imputed, with these cases excluded from ITT analysis. If >10% of the primary outcome data is determined to be missing not at random, a best-worst and worst-best case sensitivity analyses will be performed.

Subgroup analyses will be performed to examine for statistical interaction between treatment arm and: (1) the initial response to two-dose vaccine schedule (non-responder or low responder), (2) the duration between previous vaccine dose (less than, or greater than 6 weeks) and randomisation. Patients who develop primary COVID-19 infection during the study period will have both primary and secondary outcomes analysed as a prespecified subgroup analysis.

The primary endpoint is the proportion of participants in each trial arm that achieve in-vitro neutralisation of live SARS-CoV-2 virus using the χ² test. An unadjusted and adjusted relative risk (RR) will be calculated. For the adjusted RR estimate, the primary outcome of a threshold SARS-CoV-2 anti-RBD IgG titre will be analysed using a log-binomial regression model. The initial immune response to a two-dose vaccine schedule (anti-RBD IgG titre; low responder: 0.4–99 U/mL or non-responder: <0.4 U/mL) will be included in the model as a fixed effect, with study site as a random effect.

Secondary outcomes will be analysed using univariate and multivariate methods dependant on the outcome type. Baseline characteristics and demographic data will be reported as mean±SD for normally distributed data and median±IQR for non-normally distributed data, with categorical variables reported as frequencies.

Changes in the differential abundance of key bacterial species will be approached using analytical methods such as DESeq², ANCOM, MaAslin² or linear discriminant analysis effect size depending on the data characteristics. We anticipate a 2–4 fold increase in Bifidobacterium species in response to inulin supplementation.

All statistical analyses will be described in detail with arising publications. A two-sided significance level of 5% will be used for all analyses.

Oversight and monitoring

The coordinating trial centre is located at the Royal Adelaide Hospital. The trial steering committee (TSC) is cochaired by the PI at each study site and includes the trial associate investigators. The TSC is responsible for the study conception, drafting and completion of the study protocol and associated documents, recruitment plan, data monitoring and integrity, end point adjudication and approving publications arising from the study.

Following publication of all study results, deidentified participant level data may be made available on reasonable request to the PI, or in the case of published works, through the corresponding author.

AE reporting and harms

All protocol deviations and AEs will be documented, regardless of their potential relationship to the study intervention. AEs will be recorded using an adaptation of the National Institute of Health’s Common Terminology Criteria for Adverse Events by a study team member on an eDCF. Screening for AEs will occur during each study visit and during scheduled clinical follow-up with their treating nephrologist, and will be captured up to 7 days following the final study visit. AEFIs with the exception of mild and/or short-lived symptoms, will be reported to the Therapeutic Goods Administration. SAES will be reported to the trial sponsor with 24 hours of the study team being made aware of the event.

Ethics and dissemination

The study is conducted in accordance with the National Statement on Ethical Conduct in Human Research (2018), the CPMP/ICH Note for Guidance on Good Clinical Practice and consistent with the principles that have their origin in the Declaration of Helsinki. Compliance with these standards provides assurance that the rights, safety and well-being of trial participants are respected.

Ethics approval for the RIVASTIM trials was obtained from the Central Adelaide Local Health Network (CALHN) Human Research Ethics Committee (HREC) (approval number: 2021/HRE00354) and the SHLD HREC (approval numbers: X21-0411 and 2021/STE04280). Written informed consent to participate will be obtained from all participants.

The results of the RIVASTIM trial will be published in peer-reviewed academic journals and presented at national and international scientific meetings. In addition, a lay summary containing the study aim, salient findings, conclusions and a take home message will be prepared and distributed to trial participants, research staff and interested members of the transplant community. Datasets and results generated as part of this study will be jointly owned by Central Adelaide Local Health Network, the University of Adelaide and the RPA Hospital (SLHD). Deidentified participant data may be made available from the corresponding author of published works on reasonable request and submission of a research plan of appropriate scientific merit and ethical standing.
DISCUSSION
Interventions that improve the efficacy of COVID-19 vaccinations are urgently required to reduce the burden of disease in at-risk groups such as KTRs. Additional vaccine doses are recommended for this purpose, yet many KTRs fail to achieve protective immunity after a third, or even fourth vaccination. Vaccine hyporesponsiveness in this cohort is driven by deficiencies in the underlying immune response, and although immunosuppressive medications are likely the greatest contributor, dysregulation of the gut microbiota adds to the observed immune dysfunction. Strategies that address the underlying immune deficits in KTRs therefore offer an attractive pathway to restore vaccine responsiveness, but are not without risk. Maintaining graft function remains a priority for both patients and clinicians, and strategies that enhance vaccine responses must be demonstrated not to significantly enhance alloimmunity, lest organ rejection occur. The RIVASTIM trials, consisting of sister studies RIVASTIM-sirolimus and RIVASTIM-inulin, directly investigate two strategies to enhance the cellular and humoral response to a third vaccine dose in differing groups of KTRs. While RIVASTIM-sirolimus will examine the efficacy and safety of immunosuppression modification in KTRs, RIVASTIM-inulin assesses the response to and efficacy of a dietary prebiotic to improve vaccine immunogenicity.

As the primary outcome, RIVASTIM-inulin will assess in vitro neutralisation titres following a third vaccine dose, which are highly predictive of immune protection from symptomatic COVID-19. In addition, SARS-CoV-2 RBD IgG, which offers a close correlate of the efficacy of serum neutralisation titres following a third vaccine dose, is highly predictive of immune protection from COVID-19-related morbidity and mortality and would hence be of global interest.

Trial status

Administrative information
RIVASTIM-inulin: study protocol for a pilot, multi-centre, randomised, double-blinded, controlled trial of dietary inulin to improve SARS-CoV-2 vaccine response in KTRs. The trial was registered on the 26 October 2021 with the Australia New Zealand Clinical Trials Registry: ACTRN12621001465842. RIVASTIM is an investigator-initiated research trial with the coordinating trial centre, the Central Adelaide Local Health Network, as the study sponsor. No funding is provided externally. The principal and associate investigators are solely responsible for the conception, execution, analysis and dissemination of the research work.

Open access

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Contributors PTC and SC conceived the study. PTC, SC, JS, GBP, MT, TS, HW and TY designed the study methodology. MT and JS wrote the first draft of the protocol, and JS prepared the study manuscript and constructed the figures and tables. All authors contributed to the protocol development and read and approved the final manuscript.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.
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REFERENCES


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CONSENT FORM

PROTOCOL NAME:
Rapamycin and Inulin for booster VAccine response STIMulation (RIVASTIM) – Inulin Study

INVESTIGATORS:

Co-ordinating Principal Investigator: Professor P. Toby H. Coates
RAH Co-Investigators: Dr Matthew Tunbridge, Dr Tania Salehi, A/Professor Pravin Hissaria, Mr Griffith Boord-Perkins, Dr Michael Collins, Mr Chris Drogemuller, A/Professor Phillip Clayton, Professor Simon Barry, Dr Beatrice Sim
RPAH Co-Investigators: Professor Steven Chadban, Professor Kate Wyburn, Dr Julian Singer, Dr Tracey Ying, Professor Laurence Macia, A/Professor Huiling Wu

1. I have read, or have had read to me in a language that I understand, this document and I understand the purposes, procedures and risks as described within it.

2. I understand the risks, and agree to take part, which involves receiving either a dietary fibre or sugar solution for a month, giving stool and blood samples, and receiving an additional COVID vaccine dose. Each blood sample will be approximately 100 mL.

3. I understand that I may not benefit from taking part in the trial.

4. I give consent for the results of medical tests, performed as part of my routine patient care, to be included in this study.

5. I give consent for data on my genetic/DNA sequence to be generated, with the understanding that this does not include any hereditary information with health implications such as disease risk variants.

6. I understand that, while information gained during the study may be published, I will not be identified and my personal results will remain confidential.

7. I understand that I can withdraw from the study at any stage and that this will not affect my medical care, now or in the future.

8. I have had the opportunity to discuss taking part in the investigation with a family member or friend.

9. I am over 18 years of age.

Name of Participant: ______________________________________  ______
Gender:  __________________                 D.O.B.:       _____________
Signed:   ________________________________________________

I certify that I have explained the study to the patient/volunteer and consider that he/she understands what is involved

Signed:   ________________________________________________

Dated:   ________________________________________________ (Investigator / Recruiting staff)

RIVASTIM – Inulin Study PICF       Version 2: 7th October 2021