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Rapamycin and inulin for third dose vaccine response stimulation (RIVASTIM) – Inulin: study protocol for a pilot, multicentre, randomized, 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 coronavirus disease 2019 (COVID-19). Vaccination against SARS-CoV-2 is our primary risk mitigation strategy, yet vaccine effectiveness in kidney transplant recipients 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 kidney transplant recipients 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: RIVASTIM-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 kidney transplant recipients 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 20g/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 in-vitro neutralisation of live SARS-CoV-2 virus at 4 weeks following a third dose of COVID 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 2021/STE04280).

Trial registration: Australia New Zealand Clinical Trials Registry: ACTRN12621001465842. Registered 26th October 2021

Keywords

Kidney transplantation, randomized controlled trial, gut microbiome, SARS-CoV-2, COVID-19, vaccination, diet

Administrative information

Title	Rapamycin and inulin for third-dose vaccine response stimulation (RIVASTIM) – Inulin: study protocol for a pilot, multicentre, randomized, double-blinded, controlled trial of dietary inulin to improve SARS-CoV-2 vaccine response in kidney transplant recipients.
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Name and contact information for the trial sponsor	Central Adelaide Local Health Network Incorporated Royal Adelaide Hospital, Port Road, Adelaide, SA, Australia Principal Investigator: Professor P. Toby H Coates Director of Kidney and Islet Transplantation, Central and Northern Adelaide Renal and Transplantation Service Royal Adelaide Hospital, Adelaide, SA Australia Email: toby.coates@sa.gov.au
Role of sponsor	RIVASTIM is an investigator-initiated research trial with the coordinating trial center as the study sponsor. The principal and associate investigators are solely responsible for the conception, execution, analysis, and dissemination of the research work.

Strengths and limitations of this study

- RIVASTIM-Inulin takes advantage of a largely COVID-19 naïve population to assess the efficacy of a third COVID-19 vaccination in KTRs who have failed to adequately respond to a standard two-dose vaccine schedule.
- The trial will provide the first evidence in support of dietary interventions which target the microbiome to enhance COVID-19 vaccine responses.
- A broad inclusion criterion is employed to promote equitable access and the generalizability of trial results across a diverse patient population.
- The continued emergence of COVID-19 variants of clinical significance may alter the clinical landscape and trial conduct, whilst highlighting the emergent need for adjuvant strategies to enhance vaccine efficacy in vulnerable populations.

Introduction

At the beginning of 2022, the number of deaths worldwide caused by the coronavirus disease 2019 (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. Meta-analyses of cohort studies of KTRs reported mortality rates approaching 25%, and high risks of hospitalisation, acute kidney injury, and graft loss among survivors.(2, 3)

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 severe COVID-19 disease among the general population. However, KTRs are known to exhibit suboptimal vaccine responses (4, 5), and the efficacy of standard 2-dose COVID-19 vaccination schedules in KTRs is poor.(6)

To address the inadequate vaccine response observed in KTRS, and in other immunocompromised groups, additional doses of mRNA vaccine have been recommended.(7) Whilst 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%(5), this and other recent studies clearly indicate that a substantial minority of KTRs remain inadequately protected from COVID-19 despite a third vaccination.(8, 9)

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. Whilst 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 regime.

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.(10-13) In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group.(14, 15) Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions which promote the growth of beneficial microbiota.(16) Inulin, a naturally occurring, non-digestible fibre promotes the selective growth of beneficial short chain fatty acid (SCFA) producing species, such as *Bifidobacteria*, occurring at the expense of microbiota which lack the metabolic machinery necessary for fibre fermentation.(16-18) Importantly, *Bifidobacteria sp.* and SCFAs have been independently associated with improved virus-specific antibody responses and increased reactivity to parental vaccines(19, 20), including those directed against COVID-19.(21) Whether the microbiota of prevalent KTRs are amenable to dietary interventions, and whether correction of dysbiosis can promote the immunogenicity of COVID-19 vaccines are therefore important research questions.

RIVASTIM-Inulin will assess the efficacy and tolerability of dietary inulin supplementation to enhance vaccine response in KTRs who have failed to develop vaccine-induced protective immunity to COVID-19, prior to a third vaccination. We

hypothesise that dietary fibre supplementation will enhance the abundance of key microbiota species and improve the immune response to a third vaccine dose with an mRNA COVID-19 vaccine. The data generated from this pilot trial will inform the design and viability of larger clinical trials to assess the efficacy of dietary prebiotics to improve vaccine responses.

Methods and analysis

Trial design

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 kidney transplant recipients who have failed to demonstrate protective immunity following a two-dose vaccine schedule. KTRs who have received 2-doses of a COVID-19 vaccine will be enroled 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 2 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 grams of supplement dissolved in 200ml water daily, escalating to 10 grams twice daily after one 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 post vaccination. Participants will continue the dietary supplement up until the time of antibody assessment.

The first study participant was enrolled on the 8th 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.

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, Sydney, New South Wales.

Eligibility criteria

The inclusion and exclusion criteria are in Table 1.

Inclusion criteria	Exclusion criteria
Recipients of a functioning kidney transplant from a living or deceased donor	Documented prior infection with COVID-19
Individuals aged ≥ 18 who can give informed consent and are willing to	Underlying conditions predisposing to altered gut permeability and/or dysbiosis

participate and adhere to the requirements of the study	(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)
Recipient of 2 doses of a COVID-19 vaccine (either adenoviral vector or mRNA-based)	Known intolerance, allergy, or sensitivity to inulin or dietary fibre
SARS-CoV-2 receptor binding domain antibody (anti-RBD IgG) below the threshold for clinical protection from COVID-19 (< 100 units/mL).	Inability or unwillingness of an individual or their legal guardian to give written and informed consent.

Table 1: Inclusion and exclusion criteria

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 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 enroled by trained research staff and consented for trial participation, the collection of data, and the storage of biological samples. 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.(22) The randomisation algorithm and treatment allocation are not accessible to study investigators or research staff. Trained study investigators will enrol participants, whilst 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. Unblinding is not permitted during the trial except in the occurrence of a serious adverse event. In such an event, the principal investigator will decide whether unblinding is required, and if deemed necessary, will direct the un-blinded 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 by enzymatic processes.(23) Accessible in powdered form, inulin is readily soluble in water with a neutral unflavoured taste, and is widely used as a food additive and dietary supplement. Upon ingestion, inulin functions as a prebiotic by trafficking undigested to the colon where it is metabolised by fibre-fermenting

bacteria to release SCFAs, and promote the growth of SCFA producing genera.(24) Inulin exerts a dose-dependent response on the gut microbiota, with a dose of 20g/day sufficient to significantly alter the composition of the microbiome whist limiting untoward gastrointestinal adverse effects. (25-27) Adverse gastrointestinal symptoms are dose-related and commonly occur with initial ingestion.(28) A dose escalation strategy (10 grams/daily for one week, increasing to 10 grams twice daily for the remainder of the study), is employed to minimise the risk of adverse gastrointestinal symptoms. Participants who experience mild adverse effects (bowel discomfort, bloating, flatulence) will be instructed to reduce the dose to 10 grams/day. Study subjects who develop persistent gastrointestinal or other symptoms will be asked to discontinue the study intervention and continue with trial follow-up.

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) or

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 1kg bags with an accompanying 10-gram measuring scoop. Participants will be instructed to consume 10 grams (1 level scoop) dissolved in approximately 200mL of water each morning for 7 days, increasing to 10 grams each morning and night (20 grams/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 serious adverse events 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 (e.g. 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 pre- and pro-biotics) 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.(29)

Secondary outcome measures:

The secondary outcome measures include the following:

1. 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.
2. 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 (30, 31), and is consistent with the reported outcomes in published COVID-19 clinical vaccine trials.(5, 32)
3. 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.
4. 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.

5. 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.
6. Phenotypic and functional characterisation of T and B lymphocyte populations.
7. Adverse events following immunisation (AEFI) including adverse events of special interest (AESI) will be assessed via phone consultation at 1 week, and again at 4-6 weeks post-vaccination 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 EQ-5D questionnaire.

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 pre-biotic 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 immune response to vaccination, including COVID-19 vaccines.(10, 21) 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 kidney transplant recipients 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. (33, 34) A recruitment target of approximately 60-120 participants was considered feasible, and sufficient to generate a meaningful estimate of effect size with adequate confidence.

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 data capture forms 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 Royal Prince Alfred Hospital and operated behind the Sydney Local Health District (SLHD) firewall. All electronic information and transmissions are protected via Secure Sockets Layer (SSL) 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 (ICT) 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 x 9mL EDTA and 1 x 5mL CAT serum separator vacutainer tubes. Peripheral blood mononuclear cells (PBMCs) 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 and B-cell responses will be determined using a variety of laboratory techniques including but not limited to; cytometric analysis with intracellular cytokine staining and activation-induced marker (AIM) assays, and IFNγ enzyme-linked immunosorbent spot (ELISpot) assays. 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 ACE^+ cells will be assessed. The capacity of pre- and post-immunisation 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 (gastrointestinal symptom rating scale, GSRS (35, 36)), and health-related quality of life (EQ-5D) information.(37)

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 enroled 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, 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 2-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 pre-specified subgroup analysis.

The primary endpoint is the proportion of patients who achieved a post-intervention anti-RBD titre of ≥ 100 U/mL in both groups using the chi-square 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. 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 co-chaired by the Principal Investigator (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 upon reasonable request to the principal investigator, or in the case of published works, through the corresponding author.

Adverse event reporting and harms

All protocol deviations and adverse events (AEs) will be documented, regardless of their potential relationship to the study intervention. Adverse events 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 adverse events 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. Adverse events following immunisation (AEFIs) with the exception of mild and/or short-lived symptoms, will be reported to the Therapeutic Goods Administration (TGA). Serious adverse events (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 RIVASTM trials 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 2021/STE04280). Written informed consent to participate will be obtained from all participants.

The results of the RIVASTIM-inulin trial will be published in peer-reviewed academic journals and presented at national and international scientific meetings. Additionally, 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 Royal Prince Alfred Hospital (RPA, SLHD). Deidentified participant data may be made available from the corresponding author of published works upon 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 (5), or even fourth vaccination.(38) Vaccine hyporesponsiveness in this cohort is driven by deficiencies in the underlying immune response, and although immunosuppressive medications are likely the greatest contributor(39), 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(40), and strategies that enhance vaccine responses must be demonstrated not to significantly enhance allo-immunity, 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. Whilst 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 neutralization titres following a third vaccine dose, which are highly predictive of immune protection from symptomatic COVID-19. Additionally, SARS-CoV-2 RBD IgG, which offers a close correlate of the efficacy of serum to neutralise SARS-CoV-2 and widely available in clinical practice, will be measured as a secondary outcome.(30, 31) However, antibody titres, whilst clinically significant, do not offer a compete explanation for the divergent vaccine responses observed across the cohort of KTRs. Despite recognition that COVID-19 vaccine efficacy in immunosuppressed individuals remains suboptimal, robust examination of the cellular and humoral immune responses in those with sufficient, partial, or negligible vaccine responses are

lacking. Through sophisticated immunophenotyping, RIAVSTIM-inulin will examine the adaptive immune responses prior to and following a third COVID-19 vaccination, to quantify which immune deficits contribute to vaccine hyporesponsiveness in KTRs, and whether these are impacted by an improvement in gut health. Detailed examination of the gut metagenome will comprehensively evaluate the relationship between the gut microbiota and vaccine response, and examine whether a response to targeted prebiotics can shift vaccine immunogenicity.

At the time of trial registration, Australia had low community transmission in a largely SARS-CoV-2 naïve population and was uniquely placed to assess interventions to improve vaccine efficacy. However, the subsequent emergence of variants of clinical significance such as Delta and Omicron have led to COVID-19 surges in Australia. Such surges may impact trial conduct, but also serve to highlight the need for emergent strategies to boost vaccine responsiveness for at-risk groups such as KTRs.

Dietary interventions designed to modulate the gut microbiota may offer an adjuvant approach to improve vaccine efficacy, however robust clinical trials in this field are thus far lacking. Results of the RIVASTIM-inulin trial will seek to inform vaccine policy, and may provide evidence for a meaningful, inexpensive, scalable, and accessible intervention by which vaccine responses may be enhanced. Such discoveries would address our current unmet need to protect at risk populations from COVID-19 related morbidity and mortality and would hence be of global interest.

Trial status

Protocol version	3.0
Protocol date	3rd October 2021
Recruitment start date	8th November 2021
Anticipated recruitment end date	15th March 2022

Authors' contributions

PTC and SJC conceived the study. PTC, SJC, JS, GBP, MT 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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Competing interests

The authors declare that they have no competing interests.

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Figure Legends

Figure 1: Outline of the RIVISTIM-inulin trial.

Figure 2. Participant timeline. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist. Enrolment, interventions, and assessments. GSRS, gastrointestinal symptom rating scale; EQ-5D, EuroQol five dimensions questionnaire; AE, adverse events; SAE, serious adverse events

Study Population

1. Kidney transplant recipients aged ≥ 18
AND
2. Have recived 2 doses of a COVID-19 vaccine
AND
3. Demonstrated a suboptimal COVID-19 vaccine immune response (anti-RBD IgG < 100 U/mL)

Intervention

4-week lead-in

Inulin (active)
10g/day for 1 week
20g/day for 3 weeks
(n=60)

Maltodextrin (control)
10g/day for 1 week
20g/day for 3 weeks
(n=60)

4 week treatment phase

3rd dose COVID-19 Vaccine (mRNA)
(Secondary outcome: gut microbiome assesment)

Inulin (active)
20g/day for 4 weeks

Maltodextrin (control)
20g/day for 4 weeks

Outcome Measures

Primary outcome

Proportion of patients that achieve a protective threshold of neutralising antibodies required for clinical protection against SARS-CoV-2

Secondary Outcomes including:

1. The adherence and tolerability of dietary inulin determined by the Gastrointestinal Symptom Rating Scale (GSRS)
2. The diversity, relative abundance, and structure of the gut microbiota
3. Vaccine specific cellular and humoral immune responses

	STUDY PERIOD				
	Enrolment	**Randomisation**	**Post-allocation**		**Close-out**
TIMEPOINT	***- 7 days***	**0**	***Day 28***	***Day 56 -70***	***+ 7 days***
Visit window	***+/-5 days***		***+ 7 days***	***4-6 weeks from vaccination***	***+/- 5 days***
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Baseline characteristics	X				
Allocation		X			
INTERVENTIONS:					
Inulin (active)		←	—	→	
Maltodextrin (placebo control)		←	—	→	
COVID-19 mRNA Vaccine			X		
ASSESSMENTS:					
Anti-RBD IgG titre	X			X	
Routine biochemistry	X			X	
Blood draw for cellular and humoral immune assays		X		X	
Faecal microbiota assessment		X	X		
4-day food diary		X	X		
Medication Review	X		X	X	
GSRS		X	X	X	
Adherence Assessment		X	X	X	
EQ-5D		X	X	X	
AE/SAE			X	X	X

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	19
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 + 19

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	3
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	9
Objectives	#7	Specific objectives or hypotheses	10-11
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	7

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

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

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Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	COVID-19, Transplant medicine < INTERNAL MEDICINE, Renal transplantation < NEPHROLOGY, VIROLOGY, IMMUNOLOGY, NUTRITION & DIETETICS

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

Authors

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Abstract

Introduction: Kidney transplant recipients (KTRs) are at an increased risk of hospitalisation and death from coronavirus disease 2019 (COVID-19). Vaccination against SARS-CoV-2 is our primary risk mitigation strategy, yet vaccine effectiveness in kidney transplant recipients 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 kidney transplant recipients 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: RIVASTIM-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 kidney transplant recipients 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 20g/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 in-vitro neutralisation of live SARS-CoV-2 virus at 4 weeks following a third dose of COVID 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 2021/STE04280). Results of this trial will be published following peer-review and presented at scientific meetings and congresses.

Trial registration: Australia New Zealand Clinical Trials Registry: ACTRN12621001465842. Registered 26th October 2021

Keywords

Kidney transplantation, randomized controlled trial, gut microbiome, SARS-CoV-2, COVID-19, vaccination, diet

Administrative information

Rapamycin and inulin for third-dose vaccine response stimulation (RIVASTIM) – Inulin: study protocol for a pilot, multicentre, randomized, double-blinded, controlled trial of dietary inulin to improve SARS-CoV-2 vaccine response in kidney transplant recipients. The trial was registered on the 26th October 2021 with the Australia New Zealand Clinical Trials Registry: ACTRN12621001465842. RIVASTIM is an investigator-initiated research trial with the coordinating trial center, 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.

Strengths and limitations of this study

- RIVASTIM-Inulin takes advantage of a largely COVID-19 naïve population to assess the efficacy of a third COVID-19 vaccination in KTRs 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 generalizability 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 coronavirus disease 2019 (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. In Australia alone, more than 13,000 people currently live with a kidney transplant.(2) 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.(3, 4)

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 severe COVID-19 disease among the general population. However, KTRs are known to exhibit suboptimal vaccine responses (5, 6), and the efficacy of standard 2-dose COVID-19 vaccination schedules in KTRs is poor.(7)

To address the inadequate vaccine response observed in KTRS, and in other immunocompromised groups, additional doses of mRNA vaccine have been recommended.(8) Whilst 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%(6), this and other recent studies clearly indicate that a substantial minority of KTRs remain inadequately protected from COVID-19 despite a third vaccination.(9, 10)

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. Whilst 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 regime.

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.(11-14) In KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common occurrence and contributes to the observed immune dysfunction in this group.(15, 16) Normalisation of the gut microbiome may be achieved by prebiotic dietary interventions which promote the growth of beneficial microbiota.(17) Inulin, a naturally occurring, non-digestible fibre promotes the selective growth of beneficial short chain fatty acid (SCFA) producing species, such as *Bifidobacteria*, occurring at the expense of microbiota which lack the metabolic machinery necessary for fibre fermentation.(17-19) Importantly, *Bifidobacteria sp.* and SCFAs have been independently associated with improved virus-specific antibody responses and increased reactivity to parental vaccines(20, 21), including those directed against COVID-19.(22) Whether the microbiota of prevalent KTRs are amenable to dietary interventions, and whether correction of dysbiosis can promote the immunogenicity of COVID-19 vaccines are therefore important research questions.

RIVASTIM-Inulin will assess the efficacy and tolerability of dietary inulin supplementation to enhance vaccine response in KTRs who have failed to develop vaccine-induced protective immunity to COVID-19, prior to a third vaccination. We hypothesise that dietary fibre supplementation will enhance the abundance of key microbiota species and improve the immune response to a third vaccine dose with an mRNA COVID-19 vaccine. The data generated from this pilot trial will inform the design and viability of larger clinical trials to assess the efficacy of dietary prebiotics to improve vaccine responses.

Methods and analysis

The RIVASTIM clinical trials represent protocols for parallel studies designed to investigate the effect of either rapamycin (RIVASTIM-rapamycin)(23)or inulin supplementation (RIVASTIM-inulin) on the immune response to a third SARS-CoV2 vaccination in kidney transplant recipients. The trials were conceived, designed, and authored, and will be conducted concurrently by the RIVASTIM authors. To improve research quality and efficiency, the trial protocols share 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 kidney transplant recipients who have failed to demonstrate protective immunity following a two-dose vaccine schedule. KTRs who have received 2-doses of a COVID-19 vaccine will be enroled 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 2 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 grams of supplement dissolved in 200ml water daily, escalating to 10 grams twice daily after one 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 post vaccination. Participants will continue the dietary supplement up until the time of antibody assessment.

The first study participant was enrolled on the 8th 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.

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, Sydney, New South Wales.

Eligibility criteria

The inclusion and exclusion criteria are in Table 1.

Inclusion criteria	Exclusion criteria
Recipients of a functioning kidney transplant from a living or deceased donor	Recipients of multi-organ transplants (e.g. kidney-pancreas)
Individuals aged ≥ 18 who can give informed consent and are willing to participate and adhere to the requirements of the study	Documented prior infection with COVID-19
Recipient of 2 doses of a COVID-19 vaccine (either adenoviral vector or mRNA-based)	Individuals aged < 18 or > 80 years, or who are currently pregnant
SARS-CoV-2 receptor binding domain antibody (anti-RBD IgG) below the threshold for clinical protection from COVID-19 (< 100 units/mL).	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)
	Known intolerance, allergy, or sensitivity to inulin or dietary fibre
	Inability or unwillingness of an individual or their legal guardian to give written and informed consent.

Table 1: Inclusion and exclusion criteria

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 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 enroled by trained research staff and consented for trial participation, the collection of data, and the storage of biological samples (see supplementary 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.(24) The randomisation algorithm and treatment allocation are not accessible to study investigators or research staff. Trained study investigators will enrol participants, whilst 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. Unblinding is not permitted during the trial except in the occurrence of a serious adverse event. In such an event, the principal investigator will decide whether unblinding is required, and if deemed necessary, will direct the un-blinded 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 by enzymatic processes.(25) Accessible in powdered form, inulin is readily soluble in water with a neutral unflavoured taste, and is widely used as a food additive and dietary supplement. Upon ingestion, inulin functions as a prebiotic by trafficking undigested to the colon where it is metabolised by fibre-fermenting bacteria to release SCFAs, and promote the growth of SCFA producing genera.(26) Inulin exerts a dose-dependent response on the gut microbiota, with a dose of 20g/day sufficient to significantly alter the composition of the microbiome whist limiting untoward gastrointestinal adverse effects. (27-29) Adverse gastrointestinal symptoms are dose-related and commonly occur with initial ingestion.(30) A dose

escalation strategy (10 grams/daily for one week, increasing to 10 grams twice daily for the remainder of the study), is employed to minimise the risk of adverse gastrointestinal symptoms. Participants who experience mild adverse effects (bowel discomfort, bloating, flatulence) will be instructed to reduce the dose to 10 grams/day. Study subjects who develop persistent gastrointestinal or other symptoms will be asked to discontinue the study intervention and continue with trial follow-up.

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) or
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 1kg

bags with an accompanying 10-gram measuring scoop. Participants will be instructed to consume 10 grams (1 level scoop) dissolved in approximately 200mL of water each morning for 7 days, increasing to 10 grams each morning and night (20 grams/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 serious adverse events 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 (e.g. 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 pre- and pro-biotics) 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.(31)

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. Adverse events following immunisation (AEFI) including adverse events of special interest (AESI) will be assessed via phone consultation at 1 week, and again at 4-6 weeks post-vaccination 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 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 (32, 33), and is consistent with the reported outcomes in published COVID-19 clinical vaccine trials.(6, 34)

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 to 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 pre-biotic 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 immune response to vaccination, including COVID-19 vaccines.(11, 22) 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 kidney transplant recipients 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. (35, 36) 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(6, 37), 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 data capture forms 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 Royal Prince Alfred Hospital and operated behind the Sydney Local Health District (SLHD) firewall. All electronic information and transmissions are protected via Secure Sockets Layer (SSL) 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 (ICT) 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 x 9mL EDTA and 1 x 5mL CAT serum separator vacutainer tubes. Peripheral blood mononuclear cells (PBMCs) 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 ACE^+ cells will be assessed. The capacity of pre- and post-immunisation 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 (gastrointestinal symptom rating scale, GSRS (38, 39)), and health-related quality of life (EQ-5D) information.(40)

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

enroled 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, 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 2-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 pre-specified 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 chi-square 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,(41) ANCOM,(42) MaAslin2,(43) or Linear Discriminant Analysis Effect Size (LEfSe),(44) depending on the data characteristics. We anticipate a 2-4 fold increase in Bifidobacterium species in response to inulin supplementation. (45, 46)

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 co-chaired by the Principal Investigator (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 upon reasonable request to the principal investigator, or in the case of published works, through the corresponding author.

Adverse event reporting and harms

All protocol deviations and adverse events (AEs) will be documented, regardless of their potential relationship to the study intervention. Adverse events 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 adverse events 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. Adverse events following immunisation (AEFIs) with the exception of mild and/or short-lived symptoms, will be reported to the Therapeutic

Goods Administration (TGA). Serious adverse events (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 RIVASTM trials 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 2021/STE04280). Written informed consent to participate will be obtained from all participants.

The results of the RIVASTIM-inulin trial will be published in peer-reviewed academic journals and presented at national and international scientific meetings. Additionally, 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 Royal Prince Alfred Hospital (RPA, SLHD). Deidentified participant data may be made available from the corresponding

author of published works upon 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 (6), or even fourth vaccination.(47) Vaccine hyporesponsiveness in this cohort is driven by deficiencies in the underlying immune response, and although immunosuppressive medications are likely the greatest contributor(48), 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(49), and strategies that enhance vaccine responses must be demonstrated not to significantly enhance allo-immunity, 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. Whilst 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 neutralization titres following a third vaccine dose, which are highly predictive of immune protection from symptomatic COVID-19. Additionally, SARS-CoV-2 RBD IgG, which offers a close correlate of the efficacy of serum to neutralise SARS-CoV-2 and widely available in clinical practice, will be measured as a secondary outcome.(32, 33) However, antibody titres, whilst clinically significant, do not offer a compete explanation for the divergent vaccine responses observed across the cohort of KTRs. Despite recognition that COVID-19 vaccine efficacy in immunosuppressed individuals remains suboptimal, robust examination of the cellular and humoral immune responses in those with sufficient, partial, or negligible vaccine responses are lacking. Through sophisticated immunophenotyping, RIAVSTIM-inulin will examine the adaptive immune responses prior to and following a third COVID-19 vaccination, to quantify which immune deficits contribute to vaccine hyporesponsiveness in KTRs, and whether these are impacted by an improvement in gut health. Detailed examination of the gut metagenome will comprehensively evaluate the relationship between the gut microbiota and vaccine response, and examine whether a response to targeted prebiotics can shift vaccine immunogenicity.

At the time of trial registration, Australia had low community transmission in a largely SARS-CoV-2 naïve population and was uniquely placed to assess interventions to

improve vaccine efficacy. However, the subsequent emergence of variants of clinical significance such as Delta and Omicron have led to COVID-19 surges in Australia. Such surges may impact trial conduct, but also serve to highlight the need for emergent strategies to boost vaccine responsiveness for at-risk groups such as KTRs.

Dietary interventions designed to modulate the gut microbiota may offer an adjuvant approach to improve vaccine efficacy, however robust clinical trials in this field are thus far lacking. Results of the RIVASTIM-inulin trial will seek to inform vaccine policy, and may provide evidence for a meaningful, inexpensive, scalable, and accessible intervention by which vaccine responses may be enhanced. Such discoveries would address our current unmet need to protect at risk populations from COVID-19 related morbidity and mortality and would hence be of global interest.

Trial status

Protocol version 3.0, dated 3rd October 2021. Recruitment commenced November 2021, with anticipated recruitment end fate 15th March 2022.

Authors' contributions

PTC and SJC conceived the study. PTC, SJC, 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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Competing interests

The authors declare that they have no competing interests.

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Figure Legends

Figure 1: Outline of the RIVISTIM-inulin trial.

Figure 2. Participant timeline. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist. Enrolment, interventions, and assessments. GSRS, gastrointestinal symptom rating scale; EQ-5D, EuroQol five dimensions questionnaire; AE, adverse events; SAE, serious adverse events

Study Population

1. Kidney transplant recipients aged ≥ 18
   AND
2. Have recived 2 doses of a COVID-19 vaccine
   AND
3. Demonstrated a suboptimal COVID-19 vaccine immune response (anti-RBD IgG < 100 U/mL)

Intervention

4-week lead-in

Inulin (active)
10g/day for 1 week
20g/day for 3 weeks
(n=60)

Maltodextrin (control)
10g/day for 1 week
20g/day for 3 weeks
(n=60)

4 week treatment phase

3rd dose COVID-19 Vaccine (mRNA)
(Secondary outcome: gut microbiome assesment)

Inulin (active)
20g/day for 4 weeks

Maltodextrin (control)
20g/day for 4 weeks

Outcome Measures

Primary outcome

Proportion of patients that achieve a protective threshold of neutralising antibodies required for clinical protection against SARS-CoV-2

Secondary Outcomes including:

1. The adherence and tolerability of dietary inulin determined by the Gastrointestinal Symptom Rating Scale (GSRS)
2. The diversity, relative abundance, and structure of the gut microbiota
3. Vaccine specific cellular and humoral immune responses

	STUDY PERIOD				
	Enrolment	**Randomisation**	**Post-allocation**		**Close-out**
TIMEPOINT	***- 7 days***	**0**	***Day 28***	***Day 56 -70***	***+ 7 days***
Visit window	***+/-5 days***		***+ 7 days***	***4-6 weeks from vaccination***	***+/- 5 days***
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Baseline characteristics	X				
Allocation		X			
INTERVENTIONS:					
Inulin (active)		←	—	→	
Maltodextrin (placebo control)		←	—	→	
COVID-19 mRNA Vaccine			X		
ASSESSMENTS:					
Anti-RBD IgG titre	X			X	
Routine biochemistry	X			X	
Blood draw for cellular and humoral immune assays		X		X	
Faecal microbiota assessment		X	X		
4-day food diary		X	X		
Medication Review	X		X	X	
GSRS		X	X	X	
Adherence Assessment		X	X	X	
EQ-5D		X	X	X	
AE/SAE			X	X	X

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)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	19
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 + 19

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	3
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	9
Objectives	#7	Specific objectives or hypotheses	10-11
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	7

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA