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# 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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SCHOLARONE™  
Manuscripts

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4 **Rapamycin and inulin for third dose vaccine response stimulation (RIVASTIM) –**

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6 **Inulin: study protocol for a pilot, multicentre, randomized, double-blinded, controlled**  
7  
8 **trial of dietary inulin to improve SARS-CoV-2 vaccine response in kidney transplant**  
9 **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 26<sup>th</sup> 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.
Trial registration	Australia New Zealand Clinical Trials Registry: ACTRN12621001465842. Registered 26 <sup>th</sup> October 2021
Protocol version	28 <sup>th</sup> February 2022, version 4.0

Funding	No external funding
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Name and contact information for the trial sponsor	<p>Central Adelaide Local Health Network Incorporated          Royal Adelaide Hospital, Port Road, Adelaide, SA, Australia</p> <p>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</p> <p>Email: <a href="mailto:toby.coates@sa.gov.au">toby.coates@sa.gov.au</a></p>
Role of sponsor	<p>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.</p>

## 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)



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4 The emergence of several highly effective vaccines which target the SARS-CoV-2  
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6 spike protein have been critical in reducing disease burden and retarding the  
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8 development severe COVID-19 disease among the general population. However,  
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11 KTRs are known to exhibit suboptimal vaccine responses (4, 5), and the efficacy of  
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13  
14 standard 2-dose COVID-19 vaccination schedules in KTRs is poor.(6)  
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18  
19 To address the inadequate vaccine response observed in KTRs, and in other  
20  
21 immunocompromised groups, additional doses of mRNA vaccine have been  
22  
23 recommended.(7) Whilst a randomised controlled trial demonstrated that a third dose  
24  
25 of mRNA vaccine increased the proportion of KTRs achieving protective levels of  
26  
27 neutralising antibodies to 60%(5), this and other recent studies clearly indicate that a  
28  
29 substantial minority of KTRs remain inadequately protected from COVID-19 despite  
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31 a third vaccination.(8, 9)  
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40 Reducing or altering immunosuppression in KTRs is an attractive strategy to  
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42 augment vaccine responses, yet must be balanced against the risk of enhanced allo-  
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44 immunity and subsequent organ rejection. Whilst this approach may be suitable for  
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46 some KTRs, it may be declined by others who are not prepared to accept an  
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48 increased risk of acute rejection, or would prefer to remain stable on their long-term  
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50 immunosuppression regime.  
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4 Improving the gut microbiome may be another way to improve the vaccine response.

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6 The commensal microorganisms that reside in the gastrointestinal tract have wide-  
7  
8 reaching effects on systemic immunity and are critical in the development and  
9  
10 licensing of immune cells, and in maintaining adequate immune responses to  
11  
12 encountered antigens, including those encountered through vaccination.(10-13) In  
13  
14 KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common  
15  
16 occurrence and contributes to the observed immune dysfunction in this group.(14,  
17  
18 15) Normalisation of the gut microbiome may be achieved by prebiotic dietary  
19  
20 interventions which promote the growth of beneficial microbiota.(16) Inulin, a  
21  
22 naturally occurring, non-digestible fibre promotes the selective growth of beneficial  
23  
24 short chain fatty acid (SCFA) producing species, such as *Bifidobacteria*, occurring at  
25  
26 the expense of microbiota which lack the metabolic machinery necessary for fibre  
27  
28 fermentation.(16-18) Importantly, *Bifidobacteria sp.* and SCFAs have been  
29  
30 independently associated with improved virus-specific antibody responses and  
31  
32 increased reactivity to parental vaccines(19, 20), including those directed against  
33  
34 COVID-19.(21) Whether the microbiota of prevalent KTRs are amenable to dietary  
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36 interventions, and whether correction of dysbiosis can promote the immunogenicity  
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38 of COVID-19 vaccines are therefore important research questions.  
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52 RIVASTIM-Inulin will assess the efficacy and tolerability of dietary inulin  
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54 supplementation to enhance vaccine response in KTRs who have failed to develop  
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56 vaccine-induced protective immunity to COVID-19, prior to a third vaccination. We  
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4 hypothesise that dietary fibre supplementation will enhance the abundance of key  
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6 microbiota species and improve the immune response to a third vaccine dose with  
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8 an mRNA COVID-19 vaccine. The data generated from this pilot trial will inform the  
9  
10 design and viability of larger clinical trials to assess the efficacy of dietary prebiotics  
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12 to improve vaccine responses.  
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## 17 18 Methods and analysis

### 19 20 21 Trial design

22  
23 RIVASTIM-inulin is a pilot multicentre, parallel-arm, randomised, placebo-controlled,  
24  
25 double-blinded, exploratory trial, examining the effect of dietary inulin on the immune  
26  
27 response to a third dose of mRNA COVID-19 vaccine in kidney transplant recipients  
28  
29 who have failed to demonstrate protective immunity following a two-dose vaccine  
30  
31 schedule. KTRs who have received 2-doses of a COVID-19 vaccine will be enrolled  
32  
33 and their immune response to vaccination assessed by measurement of the anti-  
34  
35 RBD IgG titre. Those with a satisfactory immune response (anti-RBD IgG  $\geq$  100  
36  
37 U/mL) will exit the study and be advised to receive a third mRNA COVID-19  
38  
39 vaccination as per recommended guidelines. KTRs who fail to demonstrate  
40  
41 protective immunity (anti-RBD IgG  $<$ 100 U/mL) will proceed to randomisation.  
42  
43  
44 Randomisation will occur at a participant level with an allocation ratio of 1:1, stratified  
45  
46 by study site and the magnitude of immune response following 2 doses of vaccine  
47  
48 (anti-RBD IgG titre; non-responder:  $<$  0.4 U/mL; low responder: 0.4 – 99 U/mL). An  
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50 outline of the trial is shown in Figure 1. Following randomisation patients will receive  
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3 a dietary supplement in the form of a white, soluble, and largely flavourless powder  
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6 consisting of inulin (active arm) or maltodextrin (control arm). Participants will  
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9 consume 10 grams of supplement dissolved in 200ml water daily, escalating to 10  
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11 grams twice daily after one week. Following a 4-week lead in period, participants will  
12  
13  
14 receive a third dose of mRNA COVID-19 vaccine, with the subsequent antibody  
15  
16  
17 response measured at 4-6 weeks post vaccination. Participants will continue the  
18  
19  
20 dietary supplement up until the time of antibody assessment.

21  
22 The first study participant was enrolled on the 8<sup>th</sup> November 2021 and recruitment is  
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24  
25 anticipated to continue until March 2022, with the final study visit of the last recruited  
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27  
28 patient expected to occur in May 2022.

### 32 33 Study setting

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35 The trial will be conducted at the renal transplant units of two tertiary referral  
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38 hospitals in Australia; (1) The Royal Adelaide, Hospital, Adelaide, South Australia;  
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40  
41 and (2) The Royal Prince Alfred Hospital, Sydney, New South Wales.

### 43 44 Eligibility criteria

45  
46 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 $\geq 18$ 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.

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4 3. Potential participants may also indicate their interest in trial participation by  
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6 responding to a QR code displayed during the Transplant Australia COVID  
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8 Vaccination Update Webinar, broadcast in November 2021.  
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14 Prior to enrolling, patients will be provided with written information regarding the  
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16 rationale behind the trial, the potential risk and benefits of participation, and the  
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18 personal commitment involved. Patient's will be enroled by trained research staff and  
19  
20 consented for trial participation, the collection of data, and the storage of biological  
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22 samples. Recruitment will continue until target recruitment is fulfilled, or until  
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24 recruitment of dual-vaccinated transplant recipients is no longer feasible, or if  
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26 delaying a third vaccination becomes no longer ethically permissible due to clinical  
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28 urgency. Participants will not receive payment for participation.  
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### 34 35 Randomisation

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38 Participants are randomised 1:1 to either inulin or maltodextrin (control).  
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41 Randomisation will occur via computer-generated stratified block randomisation with  
42  
43 randomly permuted block sizes of 2, 4 and 6. Stratification will occur by site and the  
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45 response to a two dose COVID-19 vaccine schedule (low responder anti-RBD IgG  
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47 0.4 - 99 U/mL; or non-responder, anti-RBD IgG < 0.4 U/mL).  
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### 51 Allocation concealment

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54 The allocation sequence is generated by an independent and blinded statistician,  
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56 and administered centrally through an external web-based randomisation module  
57  
58 contained within a purpose built Research Electronic Data Capture (REDCap) data  
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3 management platform.(22) The randomisation algorithm and treatment allocation are  
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5 not accessible to study investigators or research staff. Trained study investigators  
6  
7 will enrol participants, whilst at each study site, an un-blinded administrative  
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9 assistant who is not a member of the study team will perform randomisation via the  
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11 web-based platform and assign the concealed intervention to each participant.  
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## 16 Blinding

17  
18 The study participant and their treating nephrologist, in addition to investigators,  
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20 research staff, and outcome assessors will be blinded to treatment allocation.  
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22 Unblinding is not permitted during the trial except in the occurrence of a serious  
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24 adverse event. In such an event, the principal investigator will decide whether  
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26 unblinding is required, and if deemed necessary, will direct the un-blinded  
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28 administrative assistant to contact the participant's treating healthcare professional  
29  
30 to discuss their treatment allocation. Wherever possible, trial staff will remain  
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32 blinded, and the participant will continue with trial follow-up and their study treatment.  
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## 41 Trial Interventions

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43 Inulin is a naturally occurring non-digestible dietary fibre that may be extracted from  
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45 natural sources, such as chicory root, or produced synthetically in commercial  
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47 quantities by enzymatic processes.(23) Accessible in powdered form, inulin is readily  
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49 soluble in water with a neutral unflavoured taste, and is widely used as a food  
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51 additive and dietary supplement. Upon ingestion, inulin functions as a prebiotic by  
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53 trafficking undigested to the colon where it is metabolised by fibre-fermenting  
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3 bacteria to release SCFAs, and promote the growth of SCFA producing genera.(24)  
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6 Inulin exerts a dose-dependent response on the gut microbiota, with a dose of  
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9 20g/day sufficient to significantly alter the composition of the microbiome whilst  
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11 limiting untoward gastrointestinal adverse effects. (25-27) Adverse gastrointestinal  
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13 symptoms are dose-related and commonly occur with initial ingestion.(28) A dose  
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15 escalation strategy (10 grams/daily for one week, increasing to 10 grams twice daily  
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17 for the remainder of the study), is employed to minimise the risk of adverse  
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19 gastrointestinal symptoms. Participants who experience mild adverse effects (bowel  
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21 discomfort, bloating, flatulence) will be instructed to reduce the dose to 10  
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23 grams/day. Study subjects who develop persistent gastrointestinal or other  
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25 symptoms will be asked to discontinue the study intervention and continue with trial  
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27 follow-up.  
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53 Maltodextrin is a polysaccharide which is easily soluble in water and rapidly  
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55 absorbed in the upper gastrointestinal tract, leading to negligible interaction with  
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57 colonic microbiota. In addition to its inert effect on the gut microbiota, maltodextrin  
58  
59 was selected as a placebo due to its similar physical appearance and taste to inulin.  
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### 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.



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4 (Cosucra group, Warcoing, Belgium) or

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6 2. Maltodextrin (Bulkpowders, Braeside, Victoria, Australia)

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11 The study products are prepared and packaged by Bulk Powders Pty Ltd (Braeside,  
12 VIC, Australia) specifically for the trial in identical sealed, opaque, and numbered 1kg  
13 bags with an accompanying 10-gram measuring scoop. Participants will be  
14 instructed to consume 10 grams (1 level scoop) dissolved in approximately 200mL of  
15 water each morning for 7 days, increasing to 10 grams each morning and night (20  
16 grams/daily) as tolerated, for the remainder of the trial period. Participants will be  
17 provided with the study supplement in a sealed bag, which will be weighed prior to  
18 allocation. At the final trial visit, participants will return any unused supplement, with  
19 the bag again weighed to determine the total weight of supplement consumed during  
20 the study.  
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40 All trial participants will receive a third dose of a COVID-19 mRNA vaccine, either  
41 Pfizer-BioNTech BNT162b2 (30 µg, IM) or Moderna mRNA-1273 (50 µg, IM)  
42 determined by local practice and vaccine availability. Study participants will receive  
43 written pre-vaccination information on the benefits and potential risks and harms of  
44 the COVID-19 vaccine and be screened for contraindications to immunisation such  
45 as serious adverse events attributable to a previous dose of a mRNA COVID-19  
46 vaccine. All patients will be advised of the need to continue with additional public  
47 health measures (e.g. physical distancing, hand washing, wearing a face mask, and  
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4 COVID-19 testing and isolation as required).

## 5 6 Concomitant care and interventions

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9 All participants will continue with usual transplant management as per local standard  
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11 of care and at the discretion of their treating nephrologist. Any changes to  
12  
13 medications will be recorded. Participants will be asked to continue with their usual  
14  
15 diet and medications but abstain from dietary supplements (including non-study pre-  
16  
17 and pro-biotics) for the duration of the study. The trial Sponsor has indemnity to  
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19 compensate those who suffer from potential harm resulting from their participation in  
20  
21 the trial.  
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## 27 28 Management of COVID-19 positive participants during the trial

29  
30 Study participants who return a positive COVID-19 result during the trial will be  
31  
32 managed in consultation with their treating transplant unit as per local best practice.  
33  
34 Participants who contract COVID-19 following randomisation but prior to a third  
35  
36 vaccination may have their third vaccine dose delayed. Where possible, participants  
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38 will be asked to continue with their allocated treatment regimens and attend study  
39  
40 visits and follow-up.  
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## 47 48 Outcomes

### 49 50 Primary outcome measure

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53 The primary outcome is the proportion of participants in each trial arm that achieve  
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55 protective serological neutralisation of live SARS-CoV-2 virus (Wuhan). Protective  
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57 neutralisation is defined as 20.2% of the mean neutralisation level of a standardised  
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3 cohort of COVID-19 convalescent individuals, and correlates with 50% protection  
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6 from infection with SARS-CoV-2 (Wuhan) in healthy individuals.(29)  
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## 10 11 Secondary outcome measures: 12

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14 The secondary outcome measures include the following:  
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- 16  
17 1. Tolerance of dietary inulin determined by the change in gastrointestinal  
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19 symptom rating scale (GSRS) at baseline, week 4, and the final trial visit.  
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21 Adherence to the intervention will be assessed by the weight of unconsumed  
22  
23 supplement returned at the final study visit.  
24  
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- 26  
27 2. The proportion of participants who generate a serological response 4-6 weeks  
28  
29 following a third COVID-19 vaccination. A serological response is defined as  
30  
31 reaching a threshold of anti-receptor-binding domain antibody (anti-RBD IgG)  
32  
33  $\geq 100$  units/mL (measured with an Elecsys Anti-SARS-CoV-2 immunoassay  
34  
35 [Roche]). This RBD antibody threshold was chosen on the basis of pre-clinical  
36  
37 and clinical studies (30, 31), and is consistent with the reported outcomes in  
38  
39 published COVID-19 clinical vaccine trials.(5, 32)  
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- 42  
43 3. Changes in the community structure, relative abundance, and functional  
44  
45 characteristics of the gut microbiome following 4 weeks of dietary intervention,  
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47 determined by 16S-rRNA metagenomic sequencing of participant stool  
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49 samples.  
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- 52  
53 4. The development of COVID-19 following randomisation, determined by:  
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- 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 IFN $\gamma$  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.<sup>(10, 21)</sup> 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. <sup>(33, 34)</sup> 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

1  
2  
3 study. Interested patients were geographically diverse. To provide an opportunity for  
4  
5 regional patients to participate, recruitment for the study occurred during outreach  
6  
7 clinics in South Australia and New South Wales.  
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9

## 10 11 12 13 14 Data collection and management 15

16  
17 Clinical and safety laboratory tests (haematology, chemistry, urinalysis) for  
18  
19 enrolment and end-point criteria (anti-RBD IgG) are performed at the hospital  
20  
21 laboratory of each study site.  
22  
23

24  
25 All study data are collected by trained research staff and entered directly onto study-  
26  
27 specific electronic data capture forms (eDCF) created and housed within a secure,  
28  
29 web-based data management tool (REDCap). The data capture forms contain inbuilt  
30  
31 protections to promote data quality, including range checks for numerical data  
32  
33 values, restrictions on alphanumeric entries, and prevention of duplicate records.  
34  
35

36  
37 The RIVASTIM REDCap database is stored on secure servers in an on-site limited  
38  
39 access data centre at the Royal Prince Alfred Hospital and operated behind the  
40  
41 Sydney Local Health District (SLHD) firewall. All electronic information and  
42  
43 transmissions are protected via Secure Sockets Layer (SSL) encryption. Access to  
44  
45 the RIVASTIM REDCap database is limited to approved research staff, with  
46  
47 individual user authentication and logging of all data entry and modification, and  
48  
49 access to restricted modules (randomisation, scheduling, and data export) privileged.  
50  
51

52  
53 The database is maintained by the SHLD Information and Communication  
54  
55 Technology (ICT) Services with regular back-up processes in place.  
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## 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 IFN $\gamma$  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<sup>+</sup> 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

1  
2  
3 diet will be captured using a 4-day food diary, completed at the time stool samples  
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5  
6 are collected.  
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8  
9 Validated questionnaires are completed by participants to capture adverse  
10  
11 gastrointestinal symptoms (gastrointestinal symptom rating scale, GSRS (35, 36)),  
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13  
14 and health-related quality of life (EQ-5D) information.(37)  
15

16  
17 All biological specimens will be deidentified and labelled with the participants unique  
18  
19 study identifier. Stool and blood samples will be stored and maintained in access-  
20  
21 restricted laboratory freezers at their corresponding trial site (Adelaide Health and  
22  
23 Medical Sciences building, University of Adelaide, or the Transplant Institute, RPA,  
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26 Sydney).  
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## 32 Confidentiality

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35 Prior to study enrolment, participants will consent to research staff accessing their  
36  
37 electronic medical record to obtain baseline and demographic information, and the  
38  
39 results of laboratory assessments. The privacy and confidentiality of screened and  
40  
41 enrolled participants will be preserved with all study data stored in the RIVASTIM  
42  
43 REDCap database under a unique numerical study identifier. No identifying  
44  
45  
46 information or individually identifiable participant data will be reported in publications,  
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49 presentations, or in any report arising from this study.  
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## 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  $\geq 100$  U/mL in both groups using the chi-square test. An unadjusted

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4 and adjusted relative risk (RR) will be calculated. For the adjusted RR estimate, the  
5  
6 primary outcome of a threshold SARS-CoV-2 anti-RBD IgG titre will be analysed  
7  
8 using a log-binomial regression model. The initial immune response to a two-dose  
9  
10 vaccine schedule (anti-RBD IgG titre; low responder: 0.4 – 99 U/mL; or non-  
11  
12 responder: <0.4 U/mL) will be included in the model as a fixed effect, with study site  
13  
14 as a random effect.  
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18  
19 Secondary outcomes will be analysed using univariate and multivariate methods  
20  
21 dependant on the outcome type. Baseline characteristics and demographic data will  
22  
23 be reported as mean  $\pm$  SD for normally distributed data and median  $\pm$  IQR for non-  
24  
25 normally distributed data, with categorical variables reported as frequencies. All  
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27 statistical analyses will be described in detail with arising publications. A two-sided  
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29 significance level of 5% will be used for all analyses.  
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### 34 35 Oversight and monitoring

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37 The coordinating trial centre is located at the Royal Adelaide Hospital. The Trial  
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39 Steering committee (TSC) is co-chaired by the Principal Investigator (PI) at each  
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41 study site and includes the trial associate investigators. The TSC is responsible for  
42  
43 the study conception, drafting and completion of the study protocol and associated  
44  
45 documents, recruitment plan, data monitoring and integrity, end point adjudication,  
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47 and approving publications arising from the study.  
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53 Following publication of all study results, deidentified participant level data may be  
54  
55 made available upon reasonable request to the principal investigator, or in the case  
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57 of published works, through the corresponding author.  
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## 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

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4 (approval numbers: X21-0411 and 2021/STE04280). Written informed consent to  
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6 participate will be obtained from all participants.  
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9 The results of the RIVASTIM-inulin trial will be published in peer-reviewed academic  
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11 journals and presented at national and international scientific meetings. Additionally,  
12  
13 a lay summary containing the study aim, salient findings, conclusions, and a take  
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15 home message will be prepared and distributed to trial participants, research staff,  
16  
17 and interested members of the transplant community. Datasets and results  
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19 generated as part of this study will be jointly owned by Central Adelaide Local Health  
20  
21 Network, the University of Adelaide, and the Royal Prince Alfred Hospital (RPA,  
22  
23 SLHD). Deidentified participant data may be made available from the corresponding  
24  
25 author of published works upon reasonable request and submission of a research  
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27 plan of appropriate scientific merit and ethical standing.  
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## 39 Discussion

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42 Interventions that improve the efficacy of COVID-19 vaccinations are urgently  
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44 required to reduce the burden of disease in at risk groups such as KTRs. Additional  
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46 vaccine doses are recommended for this purpose, yet many KTRs fail to achieve  
47  
48 protective immunity after a third (5), or even fourth vaccination.(38) Vaccine  
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50 hyporesponsiveness in this cohort is driven by deficiencies in the underlying immune  
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52 response, and although immunosuppressive medications are likely the greatest  
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54 contributor(39), dysregulation of the gut microbiota adds to the observed immune  
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3 dysfunction. Strategies that address the underlying immune deficits in KTRs  
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5 therefore offer an attractive pathway to restore vaccine responsiveness, but are not  
6  
7 without risk. Maintaining graft function remains a priority for both patients and  
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9 clinicians(40), and strategies that enhance vaccine responses must be demonstrated  
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11 not to significantly enhance allo-immunity, lest organ rejection occur. The RIVASTIM  
12  
13 trials, consisting of sister studies RIVASTIM-Sirolimus and RIVASTIM-Inulin, directly  
14  
15 investigate two strategies to enhance the cellular and humoral response to a third  
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17 vaccine dose in differing groups of KTRs. Whilst RIVASTIM-sirolimus will examine  
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19 the efficacy and safety of immunosuppression modification in KTRs, RIVASTIM-  
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21 inulin assesses the response to and efficacy of a dietary prebiotic to improve vaccine  
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23 immunogenicity.  
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35 As the primary outcome, RIVASTIM-inulin will assess in vitro neutralization titres  
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37 following a third vaccine dose, which are highly predictive of immune protection from  
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39 symptomatic COVID-19. Additionally, SARS-CoV-2 RBD IgG, which offers a close  
40  
41 correlate of the efficacy of serum to neutralise SARS-CoV-2 and widely available in  
42  
43 clinical practice, will be measured as a secondary outcome.(30, 31) However,  
44  
45 antibody titres, whilst clinically significant, do not offer a complete explanation for the  
46  
47 divergent vaccine responses observed across the cohort of KTRs. Despite  
48  
49 recognition that COVID-19 vaccine efficacy in immunosuppressed individuals  
50  
51 remains suboptimal, robust examination of the cellular and humoral immune  
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53 responses in those with sufficient, partial, or negligible vaccine responses are  
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4 lacking. Through sophisticated immunophenotyping, RIAVSTIM-inulin will examine  
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6 the adaptive immune responses prior to and following a third COVID-19 vaccination,  
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8 to quantify which immune deficits contribute to vaccine hyporesponsiveness in  
9  
10 KTRs, and whether these are impacted by an improvement in gut health. Detailed  
11  
12 examination of the gut metagenome will comprehensively evaluate the relationship  
13  
14 between the gut microbiota and vaccine response, and examine whether a response  
15  
16 to targeted prebiotics can shift vaccine immunogenicity.  
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21 At the time of trial registration, Australia had low community transmission in a largely  
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23 SARS-CoV-2 naïve population and was uniquely placed to assess interventions to  
24  
25 improve vaccine efficacy. However, the subsequent emergence of variants of clinical  
26  
27 significance such as Delta and Omicron have led to COVID-19 surges in Australia.  
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29 Such surges may impact trial conduct, but also serve to highlight the need for  
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31 emergent strategies to boost vaccine responsiveness for at-risk groups such as  
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33 KTRs.  
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41 Dietary interventions designed to modulate the gut microbiota may offer an adjuvant  
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43 approach to improve vaccine efficacy, however robust clinical trials in this field are  
44  
45 thus far lacking. Results of the RIVASTIM-inulin trial will seek to inform vaccine  
46  
47 policy, and may provide evidence for a meaningful, inexpensive, scalable, and  
48  
49 accessible intervention by which vaccine responses may be enhanced. Such  
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51 discoveries would address our current unmet need to protect at risk populations from  
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53 COVID-19 related morbidity and mortality and would hence be of global interest.  
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## Trial status

Protocol version	3.0
Protocol date	3 <sup>rd</sup> October 2021
Recruitment start date	8 <sup>th</sup> November 2021
Anticipated recruitment end date	15 <sup>th</sup> 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.

## References

1. Geneva: World Health Organization. WHO COVID-19 Dashboard 2020 [17th January 2022]. Available from: <https://covid19.who.int/>.

- 1  
2  
3 2. Phanish M, Ster IC, Ghazanfar A, Cole N, Quan V, Hull R, et al. Systematic review and meta-analysis of  
4 COVID-19 and kidney transplant recipients, the South West London Kidney Transplant Network experience.  
5 Kidney international reports. 2020.  
6  
7
- 8  
9 3. Kremer D, Pieters TT, Verhaar MC, Berger SP, Bakker SJ, van Zuilen AD, et al. A systematic review and  
10 meta-analysis of COVID-19 in kidney transplant recipients: lessons to be learned. American Journal of  
11 Transplantation. 2021;21(12):3936-45.  
12  
13
- 14 4. Boyarsky BJ, Werbel WA, Avery RK, Tobian AA, Massie AB, Segev DL, et al. Antibody response to 2-  
15 dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. Jama. 2021;325(21):2204-6.  
16  
17
- 18 5. Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized trial of a third  
19 dose of mRNA-1273 vaccine in transplant recipients. New England Journal of Medicine. 2021;385(13):1244-6.  
20  
21
- 22 6. Callaghan CJ, Mumford L, Curtis RM, Williams SV, Whitaker H, Andrews N, et al. Real-world  
23 Effectiveness of the Pfizer-BioNTech BNT162b2 and Oxford-AstraZeneca ChAdOx1-S Vaccines Against SARS-  
24 CoV-2 in Solid Organ and Islet Transplant Recipients. Transplantation. 2022.  
25  
26
- 27 7. Australian Technical Advisory Group on Immunisation (ATAGI). Recommendations on the use of a 3rd  
28 primary dose of COVID-19 vaccine in individuals who are severely immunocompromised [18th January 2022].  
29 Available from: [www.health.gov.au/resources/publications/](http://www.health.gov.au/resources/publications/).  
30  
31
- 32 8. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three Doses of an mRNA Covid-19  
33 Vaccine in Solid-Organ Transplant Recipients. New England Journal of Medicine. 2021;385(7):661-2.  
34  
35
- 36 9. Benotmane I, Gautier G, Perrin P, Olagne J, Cognard N, Fafi-Kremer S, et al. Antibody response after a  
37 third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic  
38 response to 2 doses. Jama. 2021;326(11):1063-5.  
39  
40
- 41 10. Lynn DJ, Benson SC, Lynn MA, Pulendran B. Modulation of immune responses to vaccination by the  
42 microbiota: implications and potential mechanisms. Nature Reviews Immunology. 2021:1-14.  
43  
44
- 45 11. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre M-L, et al. The commensal microbiome is  
46 associated with anti-PD-1 efficacy in metastatic melanoma patients. Science. 2018;359(6371):104-8.  
47  
48
- 49 12. Lynn DJ, Pulendran B. The potential of the microbiota to influence vaccine responses. Journal of  
50 Leukocyte Biology. 2018;103(2):225-31.  
51  
52
- 53 13. Chen J, Vitetta L, Henson JD, Hall S. The intestinal microbiota and improving the efficacy of COVID-19  
54 vaccinations. Journal of Functional Foods. 2021;87:104850.  
55  
56  
57  
58  
59  
60



14. Fricke W, Maddox C, Song Y, Bromberg J. Human microbiota characterization in the course of renal transplantation. *American Journal of Transplantation*. 2014;14(2):416-27.
15. Lee JR, Magruder M, Zhang L, Westblade LF, Satlin MJ, Robertson A, et al. Gut microbiota dysbiosis and diarrhea in kidney transplant recipients. *American Journal of Transplantation*. 2019;19(2):488-500.
16. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. *British Journal of Nutrition*. 2015;113(S1):S1-S5.
17. Sonnenburg JL, Bäckhed F. Diet–microbiota interactions as moderators of human metabolism. *Nature*. 2016;535(7610):56-64.
18. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. 2012;489(7415):220.
19. Zhao T, Li J, Fu Y, Ye H, Liu X, Li G, et al. Influence of gut microbiota on mucosal IgA antibody response to the polio vaccine. *NPJ vaccines*. 2020;5(1):1-9.
20. Huda MN, Lewis Z, Kalanetra KM, Rashid M, Ahmad SM, Raqib R, et al. Stool microbiota and vaccine responses of infants. *Pediatrics*. 2014;134(2):e362-e72.
21. Ng SC, Peng Y, Zhang L, Mok CKP, Zhao S, Li A, et al. Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events. *Gut*. 2022:gutjnl-2021-326563.
22. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*. 2009;42(2):377-81.
23. Niness KR. Inulin and Oligofructose: What Are They? *The Journal of Nutrition*. 1999;129(7):1402S-6S.
24. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, et al. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *The American journal of clinical nutrition*. 2018;107(6):965-83.
25. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, et al. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut*. 2017;66(11):1968-74.
26. Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati Jrm, Pochart P, et al. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *The Journal of nutrition*. 1999;129(1):113-6.

- 1  
2  
3 27. Bruhwylter J, Carreer F, Demanet E, Jacobs H. Digestive tolerance of inulin-type fructans: a double-  
4 blind, placebo-controlled, cross-over, dose-ranging, randomized study in healthy volunteers. *Int J Food Sci*  
5 *Nutr.* 2009;60(2):165-75.  
6  
7  
8  
9 28. Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal tolerance of chicory inulin products.  
10 *Journal of the American Dietetic Association.* 2010;110(6):865-8.  
11  
12  
13 29. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels  
14 are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Medicine.*  
15 2021;27(7):1205-11.  
16  
17  
18 30. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of  
19 protection against SARS-CoV-2 in rhesus macaques. *Nature.* 2021;590(7847):630-4.  
20  
21  
22 31. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels  
23 are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature medicine.* 2021:1-  
24 7.  
25  
26  
27 32. Peled Y, Ram E, Lavee J, Segev A, Matezki S, Wieder-Finesod A, et al. Third dose of the BNT162b2  
28 vaccine in heart transplant recipients: immunogenicity and clinical experience. *The Journal of Heart and Lung*  
29 *Transplantation.* 2021.  
30  
31  
32 33. Teare MD, Dimairo M, Shephard N, Hayman A, Whitehead A, Walters SJ. Sample size requirements to  
33 estimate key design parameters from external pilot randomised controlled trials: a simulation study. *Trials.*  
34 2014;15(1):1-13.  
35  
36  
37 34. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised  
38 trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome  
39 variable. *Statistical methods in medical research.* 2016;25(3):1057-73.  
40  
41  
42 35. Svedlund J, Sjödin I, Dotevall G. GSRS—a clinical rating scale for gastrointestinal symptoms in patients  
43 with irritable bowel syndrome and peptic ulcer disease. *Digestive diseases and sciences.* 1988;33(2):129-34.  
44  
45  
46 36. Kleinman L, Faull R, Walker R, Prasad GR, Ambuehl P, Bahner U, editors. Gastrointestinal-specific  
47 patient-reported outcome instruments differentiate between renal transplant patients with or without GI  
48 complications. *Transplantation proceedings;* 2005: Elsevier.  
49  
50  
51 37. Cleemput I, Kesteloot K, Moons P, Vanrenterghem Y, Van Hooff JP, Squifflet J-P, et al. The construct  
52 and concurrent validity of the EQ-5D in a renal transplant population. *Value in Health.* 2004;7(4):499-509.  
53  
54  
55 38. Caillard S, Thauinat O, Benotmane I, Masset C, Blancho G. Antibody Response to a Fourth Messenger  
56 RNA COVID-19 Vaccine Dose in Kidney Transplant Recipients: A Case Series. *Annals of internal medicine.* 2022.  
57  
58  
59  
60

1  
2  
3 39. Duni A, Markopoulos GS, Mallioras I, Pappas H, Pappas E, Koutlas V, et al. The Humoral Immune  
4 Response to BNT162b2 Vaccine Is Associated With Circulating CD19+ B Lymphocytes and the Naïve CD45RA to  
5 Memory CD45RO CD4+ T Helper Cells Ratio in Hemodialysis Patients and Kidney Transplant Recipients.  
6 Frontiers in immunology. 2021;12:760249-.

7  
8  
9  
10 40. Sautenet B, Tong A, Manera KE, Chapman JR, Warrens AN, Rosenbloom D, et al. Developing  
11 consensus-based priority outcome domains for trials in kidney transplantation: a multinational Delphi survey  
12 with patients, caregivers and health professionals. Transplantation. 2017;101(8):1875.  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
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For peer review only

## 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

1. Kidney transplant recipients aged  $\geq 18$ 

AND

2. Have received 2 doses of a COVID-19 vaccine  
AND3. Demonstrated a suboptimal COVID-19 vaccine  
immune response (anti-RBD IgG  $< 100$  U/mL)**Study  
Population**

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)

**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

4-week  
lead-in4 week  
treatment  
phase**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

**Outcome  
Measures**

	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
<b>ENROLMENT:</b>					
Eligibility screen	X				
Informed consent	X				
Baseline characteristics	X				
Allocation		X			
<b>INTERVENTIONS:</b>					
<i>Inulin (active)</i>		←————→			
<i>Maltodextrin (placebo control)</i>		←————→			
<i>COVID-19 mRNA Vaccine</i>			X		
<b>ASSESSMENTS:</b>					
<i>Anti-RBD IgG titre</i>	X			X	
<i>Routine biochemistry</i>	X			X	
<i>Blood draw for cellular and humoral immune assays</i>		X		X	
<i>Faecal microbiota assessment</i>		X	X		
<i>4-day food diary</i>		X	X		
<i>Medication Review</i>	X		X	X	
<i>GSRS</i>		X	X	X	
<i>Adherence Assessment</i>		X	X	X	
<i>EQ-5D</i>		X	X	X	
<i>AE/SAE</i>			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 SPIRIT reporting guidelines, and cite them as:

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

1	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	3
2	responsibilities:			
3	sponsor contact			
4	information			
5				
6				
7				
8	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	3
9	responsibilities:		collection, management, analysis, and interpretation of data;	
10	sponsor and funder		writing of the report; and the decision to submit the report for	
11			publication, including whether they will have ultimate authority	
12			over any of these activities	
13				
14				
15				
16	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating centre,	NA
17	responsibilities:		steering committee, endpoint adjudication committee, data	
18	committees		management team, and other individuals or groups overseeing the	
19			trial, if applicable (see Item 21a for data monitoring committee)	
20				
21				
22				
23	<b>Introduction</b>			
24				
25	Background and	<a href="#">#6a</a>	Description of research question and justification for undertaking	4-5
26	rationale		the trial, including summary of relevant studies (published and	
27			unpublished) examining benefits and harms for each intervention	
28				
29				
30	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	9
31	rationale: choice of			
32	comparators			
33				
34				
35				
36	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	10-11
37				
38	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel	6
39			group, crossover, factorial, single group), allocation ratio, and	
40			framework (eg, superiority, equivalence, non-inferiority,	
41			exploratory)	
42				
43				
44				
45	<b>Methods:</b>			
46	<b>Participants,</b>			
47	<b>interventions, and</b>			
48	<b>outcomes</b>			
49				
50				
51				
52	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic, academic	6
53			hospital) and list of countries where data will be collected.	
54			Reference to where list of study sites can be obtained	
55				
56				
57	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable,	7
58			eligibility criteria for study centres and individuals who will	
59				
60				



		perform the interventions (eg, surgeons, psychotherapists)	
1			
2	Interventions:	<a href="#">#11a</a> Interventions for each group with sufficient detail to allow	8-9
3	description	replication, including how and when they will be administered	
4			
5	Interventions:	<a href="#">#11b</a> Criteria for discontinuing or modifying allocated interventions for a	9
6	modifications	given trial participant (eg, drug dose change in response to harms,	
7		participant request, or improving / worsening disease)	
8			
9	Interventions:	<a href="#">#11c</a> Strategies to improve adherence to intervention protocols, and any	9
10	adherence	procedures for monitoring adherence (eg, drug tablet return;	
11		laboratory tests)	
12	Interventions:	<a href="#">#11d</a> Relevant concomitant care and interventions that are permitted or	10
13	concomitant care	prohibited during the trial	
14			
15	Outcomes	<a href="#">#12</a> Primary, secondary, and other outcomes, including the specific	10-11
16		measurement variable (eg, systolic blood pressure), analysis metric	
17		(eg, change from baseline, final value, time to event), method of	
18		aggregation (eg, median, proportion), and time point for each	
19		outcome. Explanation of the clinical relevance of chosen efficacy	
20		and harm outcomes is strongly recommended	
21	Participant timeline	<a href="#">#13</a> Time schedule of enrolment, interventions (including any run-ins	Figure 2
22		and washouts), assessments, and visits for participants. A	
23		schematic diagram is highly recommended (see Figure)	
24			
25	Sample size	<a href="#">#14</a> Estimated number of participants needed to achieve study	12
26		objectives and how it was determined, including clinical and	
27		statistical assumptions supporting any sample size calculations	
28			
29	Recruitment	<a href="#">#15</a> Strategies for achieving adequate participant enrolment to reach	7
30		target sample size	
31			
32			
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44			
45	<b>Methods: Assignment</b>		
46	<b>of interventions (for</b>		
47	<b>controlled trials)</b>		
48			
49			
50	Allocation: sequence	<a href="#">#16a</a> Method of generating the allocation sequence (eg, computer-	8
51	generation	generated random numbers), and list of any factors for	
52		stratification. To reduce predictability of a random sequence,	
53		details of any planned restriction (eg, blocking) should be provided	
54		in a separate document that is unavailable to those who enrol	
55		participants or assign interventions	
56			
57			
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60			

1	Allocation concealment	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence (eg, central	8
2	mechanism		telephone; sequentially numbered, opaque, sealed envelopes),	
3			describing any steps to conceal the sequence until interventions are	
4			assigned	
5				
6				
7				
8	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol	8
9	implementation		participants, and who will assign participants to interventions	
10				
11	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg, trial	8
12			participants, care providers, outcome assessors, data analysts), and	
13			how	
14				
15				
16				
17	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is permissible,	8
18	emergency unblinding		and procedure for revealing a participant's allocated intervention	
19			during the trial	
20				
21				
22	<b>Methods: Data</b>			
23	<b>collection,</b>			
24	<b>management, and</b>			
25	<b>analysis</b>			
26				
27				
28				
29	Data collection plan	<a href="#">#18a</a>	Plans for assessment and collection of outcome, baseline, and other	13
30			trial data, including any related processes to promote data quality	
31			(eg, duplicate measurements, training of assessors) and a	
32			description of study instruments (eg, questionnaires, laboratory	
33			tests) along with their reliability and validity, if known. Reference	
34			to where data collection forms can be found, if not in the protocol	
35				
36				
37				
38				
39	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete follow-up,	13
40	retention		including list of any outcome data to be collected for participants	
41			who discontinue or deviate from intervention protocols	
42				
43				
44	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage, including any	13
45			related processes to promote data quality (eg, double data entry;	
46			range checks for data values). Reference to where details of data	
47			management procedures can be found, if not in the protocol	
48				
49				
50				
51	Statistics: outcomes	<a href="#">#20a</a>	Statistical methods for analysing primary and secondary outcomes.	14
52			Reference to where other details of the statistical analysis plan can	
53			be found, if not in the protocol	
54				
55				
56	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and adjusted	14
57	analyses		analyses)	
58				
59				
60				

1	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-	14
2	population and missing		adherence (eg, as randomised analysis), and any statistical methods	
3	data		to handle missing data (eg, multiple imputation)	
4				
5				
6	<b>Methods: Monitoring</b>			
7				
8	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC); summary of its	NA
9	formal committee		role and reporting structure; statement of whether it is independent	
10			from the sponsor and competing interests; and reference to where	
11			further details about its charter can be found, if not in the protocol.	
12			Alternatively, an explanation of why a DMC is not needed	
13				
14	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping guidelines,	NA
15	interim analysis		including who will have access to these interim results and make	
16			the final decision to terminate the trial	
17				
18	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and managing solicited	14
19			and spontaneously reported adverse events and other unintended	
20			effects of trial interventions or trial conduct	
21				
22	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct, if any, and	NA
23			whether the process will be independent from investigators and the	
24			sponsor	
25				
26				
27				
28	<b>Ethics and</b>			
29	<b>dissemination</b>			
30				
31	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee / institutional review	16
32	approval		board (REC / IRB) approval	
33				
34	Protocol amendments	<a href="#">#25</a>	Plans for communicating important protocol modifications (eg,	NA
35			changes to eligibility criteria, outcomes, analyses) to relevant	
36			parties (eg, investigators, REC / IRBs, trial participants, trial	
37			registries, journals, regulators)	
38				
39	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from potential trial	7
40			participants or authorised surrogates, and how (see Item 32)	
41				
42	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use of participant	NA
43	ancillary studies		data and biological specimens in ancillary studies, if applicable	
44				
45	Confidentiality	<a href="#">#27</a>	How personal information about potential and enrolled participants	14
46			will be collected, shared, and maintained in order to protect	
47			confidentiality before, during, and after the trial	
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1	Declaration of interests	<a href="#">#28</a>	Financial and other competing interests for principal investigators for the overall trial and each study site	19
2				
3				
4				
5	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
6				
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9				
10	Ancillary and post trial care	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
11				
12				
13				
14	Dissemination policy: trial results	<a href="#">#31a</a>	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
15				
16				
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20				
21	Dissemination policy: authorship	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of professional writers	NA
22				
23				
24	Dissemination policy: reproducible research	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
25				
26				
27				
28	<b>Appendices</b>			
29				
30				
31	Informed consent materials	<a href="#">#32</a>	Model consent form and other related documentation given to participants and authorised surrogates	NA
32				
33				
34				
35	Biological specimens	<a href="#">#33</a>	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
36				
37				
38				
39				

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# 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.**

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-062747.R1
Article Type:	Protocol
Date Submitted by the Author:	22-Aug-2022
Complete List of Authors:	Singer, Juilan; The University of Sydney, Kidney Node, Charles Perkins Centre; Royal Prince Alfred Hospital, Department of Renal Medicine Tunbridge, Matthew; Royal Adelaide Hospital, Central and Northern Adelaide Renal and Transplantation Service (CNARTS), Perkins, Griffith; The University of Adelaide, School of Biological Sciences; SA Pathology Salehi, Tania; Royal Adelaide Hospital, Central and Northern Adelaide Renal and Transplantation Service (CNARTS), Ying, Tracey; The University of Sydney, Kidney Node, Charles Perkins Centre; Royal Prince Alfred Hospital, Department of Renal Medicine Wu, Huiling; The University of Sydney, Kidney Node Laboratory, Charles Perkins Centre; Royal Prince Alfred Hospital, Department of Renal Medicine Coates, P.; Royal Adelaide Hospital, Central and Northern Adelaide Renal and Transplantation Service (CNARTS), ; The University of Adelaide, Discipline of Medicine, Adelaide Medical School Chadban, Steve; The University of Sydney, Kidney Node, Charles Perkins Centre; Royal Prince Alfred Hospital, Department of Renal Medicine
<b>Primary Subject Heading</b>:	Renal medicine
Secondary Subject Heading:	Immunology (including allergy)
Keywords:	COVID-19, Transplant medicine < INTERNAL MEDICINE, Renal transplantation < NEPHROLOGY, VIROLOGY, IMMUNOLOGY, NUTRITION & DIETETICS

SCHOLARONE™  
Manuscripts

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4 **Rapamycin and inulin for third dose vaccine response stimulation (RIVASTIM) –**

5  
6 **Inulin: study protocol for a pilot, multicentre, randomized, double-blinded, controlled**  
7  
8 **trial of dietary inulin to improve SARS-CoV-2 vaccine response in kidney transplant**  
9 **recipients.**  
10  
11  
12  
13  
14  
15

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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



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3  
4 diversity and differential abundance of gut microbiota, and vaccine-specific immune  
5  
6 cell populations and responses.  
7

8  
9 **Ethics and dissemination:** Ethics approval was obtained from the Central Adelaide  
10  
11 Local Health Network (CALHN) Human Research Ethics Committee (HREC)  
12  
13 (approval number: 2021/HRE00354) and the Sydney Local Health District (SHLD)  
14  
15 HREC (approval numbers: X21-0411 and 2021/STE04280). Results of this trial will  
16  
17 be published following peer-review and presented at scientific meetings and  
18  
19 congresses.  
20  
21  
22

23  
24 **Trial registration:** Australia New Zealand Clinical Trials Registry:  
25

26  
27 ACTRN12621001465842. Registered 26<sup>th</sup> October 2021  
28

### 29 30 **Keywords**

31  
32 Kidney transplantation, randomized controlled trial, gut microbiome, SARS-CoV-2,  
33  
34 COVID-19, vaccination, diet  
35  
36  
37

## 38 39 **Administrative information**

40  
41  
42 Rapamycin and inulin for third-dose vaccine response stimulation (RIVASTIM) –  
43  
44 Inulin: study protocol for a pilot, multicentre, randomized, double-blinded, controlled  
45  
46 trial of dietary inulin to improve SARS-CoV-2 vaccine response in kidney transplant  
47  
48 recipients. The trial was registered on the 26<sup>th</sup> October 2021 with the Australia New  
49  
50 Zealand Clinical Trials Registry: ACTRN12621001465842. RIVASTIM is an  
51  
52 investigator-initiated research trial with the coordinating trial center, the Central  
53  
54 Adelaide Local Health Network, as the study sponsor. No funding is provided  
55  
56  
57  
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1  
2  
3 externally. The principal and associate investigators are solely responsible for the  
4  
5  
6 conception, execution, analysis, and dissemination of the research work.  
7  
8  
9

## 10 11 12 13 Strengths and limitations of this study

- 14  
15  
16 - RIVASTIM-Inulin takes advantage of a largely COVID-19 naïve population to  
17  
18 assess the efficacy of a third COVID-19 vaccination in KTRs who have failed  
19  
20 to adequately respond to a standard two-dose vaccine schedule.  
21  
22
- 23  
24 - A broad inclusion criterion is employed to promote equitable access and the  
25  
26 generalizability of trial results across a diverse patient population.  
27  
28
- 29  
30 - Blinding of the study participants, investigators, and outcome assessors to  
31  
32 treatment allocation will reduce the risk of bias.  
33  
34
- 35  
36 - Habitual diet will be assessed via a 4-day food diary to account for variation in  
37  
38 baseline fibre and macronutrient intake.  
39  
40
- 41  
42 - The continued emergence of COVID-19 variants of clinical significance may  
43  
44 alter the clinical landscape and limit trial recruitment via an emergent demand  
45  
46 for booster vaccinations.  
47  
48

## 49 50 51 Introduction

52  
53 At the beginning of 2022, the number of deaths worldwide caused by the coronavirus  
54  
55 disease 2019 (COVID-19) pandemic exceeded 5.5 million.(1) Immunosuppressed  
56  
57 populations such as kidney transplant recipients (KTRs) are at high-risk for COVID-  
58  
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1  
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3  
4 19 related adverse events. In Australia alone, more than 13,000 people currently live  
5  
6 with a kidney transplant.(2) Meta-analyses of cohort studies in this at-risk population  
7  
8 report mortality rates approaching 25%, and high risks of hospitalisation, acute  
9  
10 kidney injury, and graft loss among survivors.(3, 4)  
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12  
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16 The emergence of several highly effective vaccines which target the SARS-CoV-2  
17  
18 spike protein have been critical in reducing disease burden and retarding the  
19  
20 development severe COVID-19 disease among the general population. However,  
21  
22 KTRs are known to exhibit suboptimal vaccine responses (5, 6), and the efficacy of  
23  
24 standard 2-dose COVID-19 vaccination schedules in KTRs is poor.(7)  
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32 To address the inadequate vaccine response observed in KTRs, and in other  
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34 immunocompromised groups, additional doses of mRNA vaccine have been  
35  
36 recommended.(8) Whilst a randomised controlled trial demonstrated that a third dose  
37  
38 of mRNA vaccine increased the proportion of KTRs achieving protective levels of  
39  
40 neutralising antibodies to 60%(6), this and other recent studies clearly indicate that a  
41  
42 substantial minority of KTRs remain inadequately protected from COVID-19 despite  
43  
44 a third vaccination.(9, 10)  
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52 Reducing or altering immunosuppression in KTRs is an attractive strategy to  
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54 augment vaccine responses, yet must be balanced against the risk of enhanced allo-  
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56 immunity and subsequent organ rejection. Whilst this approach may be suitable for  
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3 some KTRs, it may be declined by others who are not prepared to accept an  
4  
5 increased risk of acute rejection, or would prefer to remain stable on their long-term  
6  
7 immunosuppression regime.  
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13  
14 Improving the gut microbiome may be another way to improve the vaccine response.

15  
16 The commensal microorganisms that reside in the gastrointestinal tract have wide-  
17  
18 reaching effects on systemic immunity and are critical in the development and  
19  
20 licensing of immune cells, and in maintaining adequate immune responses to  
21  
22 encountered antigens, including those encountered through vaccination.(11-14) In  
23  
24 KTRs, disruption of the gut microbial landscape, termed dysbiosis, is a common  
25  
26 occurrence and contributes to the observed immune dysfunction in this group.(15,  
27  
28 16) Normalisation of the gut microbiome may be achieved by prebiotic dietary  
29  
30 interventions which promote the growth of beneficial microbiota.(17) Inulin, a  
31  
32 naturally occurring, non-digestible fibre promotes the selective growth of beneficial  
33  
34 short chain fatty acid (SCFA) producing species, such as *Bifidobacteria*, occurring at  
35  
36 the expense of microbiota which lack the metabolic machinery necessary for fibre  
37  
38 fermentation.(17-19) Importantly, *Bifidobacteria sp.* and SCFAs have been  
39  
40 independently associated with improved virus-specific antibody responses and  
41  
42 increased reactivity to parental vaccines(20, 21), including those directed against  
43  
44 COVID-19.(22) Whether the microbiota of prevalent KTRs are amenable to dietary  
45  
46 interventions, and whether correction of dysbiosis can promote the immunogenicity  
47  
48 of COVID-19 vaccines are therefore important research questions.  
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6 RIVASTIM-Inulin will assess the efficacy and tolerability of dietary inulin  
7  
8  
9 supplementation to enhance vaccine response in KTRs who have failed to develop  
10  
11 vaccine-induced protective immunity to COVID-19, prior to a third vaccination. We  
12  
13 hypothesise that dietary fibre supplementation will enhance the abundance of key  
14  
15 microbiota species and improve the immune response to a third vaccine dose with  
16  
17 an mRNA COVID-19 vaccine. The data generated from this pilot trial will inform the  
18  
19 design and viability of larger clinical trials to assess the efficacy of dietary prebiotics  
20  
21 to improve vaccine responses.  
22  
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27

## 28 **Methods and analysis**

29  
30  
31 The RIVASTIM clinical trials represent protocols for parallel studies designed  
32  
33 to investigate the effect of either rapamycin (RIVASTIM-rapamycin)(23)or  
34  
35 inulin supplementation (RIVASTIM-inulin) on the immune response to a third  
36  
37 SARS-CoV2 vaccination in kidney transplant recipients. The trials were  
38  
39 conceived, designed, and authored, and will be conducted concurrently by the  
40  
41 RIVASTIM authors. To improve research quality and efficiency, the trial  
42  
43 protocols share common methodologies in participant screening and  
44  
45 enrolment, data collection and management, and outcome assessments.  
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57 RIVASTIM-inulin is a pilot multicentre, parallel-arm, randomised, placebo-controlled,  
58  
59 double-blinded, exploratory trial, examining the effect of dietary inulin on the immune  
60

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3  
4 response to a third dose of mRNA COVID-19 vaccine in kidney transplant recipients  
5  
6 who have failed to demonstrate protective immunity following a two-dose vaccine  
7  
8 schedule. KTRs who have received 2-doses of a COVID-19 vaccine will be enrolled  
9  
10 and their immune response to vaccination assessed by measurement of the anti-  
11  
12 RBD IgG titre. Those with a satisfactory immune response (anti-RBD IgG  $\geq$  100  
13  
14 U/mL) will exit the study and be advised to receive a third mRNA COVID-19  
15  
16 vaccination as per recommended guidelines. KTRs who fail to demonstrate  
17  
18 protective immunity (anti-RBD IgG  $<$ 100 U/mL) will proceed to randomisation.  
19  
20  
21 Randomisation will occur at a participant level with an allocation ratio of 1:1, stratified  
22  
23 by study site and the magnitude of immune response following 2 doses of vaccine  
24  
25 (anti-RBD IgG titre; non-responder:  $<$  0.4 U/mL; low responder: 0.4 – 99 U/mL). An  
26  
27 outline of the trial is shown in Figure 1. Following randomisation patients will receive  
28  
29 a dietary supplement in the form of a white, soluble, and largely flavourless powder  
30  
31 consisting of inulin (active arm) or maltodextrin (control arm). Participants will  
32  
33 consume 10 grams of supplement dissolved in 200ml water daily, escalating to 10  
34  
35 grams twice daily after one week. Following a 4-week lead in period, participants will  
36  
37 receive a third dose of mRNA COVID-19 vaccine, with the subsequent antibody  
38  
39 response measured at 4-6 weeks post vaccination. Participants will continue the  
40  
41 dietary supplement up until the time of antibody assessment.  
42  
43  
44 The first study participant was enrolled on the 8<sup>th</sup> November 2021 and recruitment is  
45  
46 anticipated to continue until March 2022, with the final study visit of the last recruited  
47  
48 patient expected to occur in May 2022.  
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## Study setting

The trial will be conducted at the renal transplant units of two tertiary referral hospitals in Australia; (1) The Royal Adelaide, Hospital, Adelaide, South Australia; and (2) The Royal Prince Alfred Hospital, 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 $\geq 18$ 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



1  
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3  
4 personal commitment involved. Patient's will be enrolled by trained research staff and  
5  
6 consented for trial participation, the collection of data, and the storage of biological  
7  
8 samples (see supplementary file 1). Recruitment will continue until target recruitment  
9  
10 is fulfilled, or until recruitment of dual-vaccinated transplant recipients is no longer  
11  
12 feasible, or if delaying a third vaccination becomes no longer ethically permissible  
13  
14 due to clinical urgency. Participants will not receive payment for participation.  
15  
16  
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18

### 19 Randomisation

20  
21  
22 Participants are randomised 1:1 to either inulin or maltodextrin (control).  
23  
24  
25 Randomisation will occur via computer-generated stratified block randomisation with  
26  
27 randomly permuted block sizes of 2, 4 and 6. Stratification will occur by site and the  
28  
29 response to a two dose COVID-19 vaccine schedule (low responder anti-RBD IgG  
30  
31 0.4 - 99 U/mL; or non-responder, anti-RBD IgG < 0.4 U/mL).  
32  
33  
34

### 35 Allocation concealment

36  
37  
38 The allocation sequence is generated by an independent and blinded statistician,  
39  
40 and administered centrally through an external web-based randomisation module  
41  
42 contained within a purpose built Research Electronic Data Capture (REDCap) data  
43  
44 management platform.(24) The randomisation algorithm and treatment allocation are  
45  
46 not accessible to study investigators or research staff. Trained study investigators  
47  
48 will enrol participants, whilst at each study site, an un-blinded administrative  
49  
50 assistant who is not a member of the study team will perform randomisation via the  
51  
52 web-based platform and assign the concealed intervention to each participant.  
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## 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.<sup>(25)</sup> 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.<sup>(26)</sup> 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 whilst limiting untoward gastrointestinal adverse effects. <sup>(27-29)</sup> Adverse gastrointestinal symptoms are dose-related and commonly occur with initial ingestion.<sup>(30)</sup> A dose

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3  
4 escalation strategy (10 grams/daily for one week, increasing to 10 grams twice daily  
5  
6 for the remainder of the study), is employed to minimise the risk of adverse  
7  
8 gastrointestinal symptoms. Participants who experience mild adverse effects (bowel  
9  
10 discomfort, bloating, flatulence) will be instructed to reduce the dose to 10  
11  
12 grams/day. Study subjects who develop persistent gastrointestinal or other  
13  
14 symptoms will be asked to discontinue the study intervention and continue with trial  
15  
16 follow-up.  
17  
18  
19  
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23

24 Maltodextrin is a polysaccharide which is easily soluble in water and rapidly  
25  
26 absorbed in the upper gastrointestinal tract, leading to negligible interaction with  
27  
28 colonic microbiota. In addition to its inert effect on the gut microbiota, maltodextrin  
29  
30 was selected as a placebo due to its similar physical appearance and taste to inulin.  
31  
32  
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37

### 38 Intervention description

39  
40 Participants will be randomly allocated and blinded to their inclusion in one of two  
41  
42 groups:  
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44

- 45 1. Inulin – Fibruline Instant, a soluble dietary fibre extracted from chicory roots.  
46  
47 (Cosucra group, Warcoing, Belgium) or  
48  
49
- 50 2. Maltodextrin (Bulkpowders, Braeside, Victoria, Australia)  
51  
52  
53  
54  
55

56 The study products are prepared and packaged by Bulk Powders Pty Ltd (Braeside,  
57  
58 VIC, Australia) specifically for the trial in identical sealed, opaque, and numbered 1kg  
59  
60

1  
2  
3 bags with an accompanying 10-gram measuring scoop. Participants will be  
4  
5 instructed to consume 10 grams (1 level scoop) dissolved in approximately 200mL of  
6  
7 water each morning for 7 days, increasing to 10 grams each morning and night (20  
8  
9 grams/daily) as tolerated, for the remainder of the trial period. Participants will be  
10  
11 provided with the study supplement in a sealed bag, which will be weighed prior to  
12  
13 allocation. At the final trial visit, participants will return any unused supplement, with  
14  
15 the bag again weighed to determine the total weight of supplement consumed during  
16  
17 the study.  
18  
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27 All trial participants will receive a third dose of a COVID-19 mRNA vaccine, either  
28  
29 Pfizer-BioNTech BNT162b2 (30 µg, IM) or Moderna mRNA-1273 (50 µg, IM)  
30  
31 determined by local practice and vaccine availability. Study participants will receive  
32  
33 written pre-vaccination information on the benefits and potential risks and harms of  
34  
35 the COVID-19 vaccine and be screened for contraindications to immunisation such  
36  
37 as serious adverse events attributable to a previous dose of a mRNA COVID-19  
38  
39 vaccine. All patients will be advised of the need to continue with additional public  
40  
41 health measures (e.g. physical distancing, hand washing, wearing a face mask, and  
42  
43 COVID-19 testing and isolation as required).  
44  
45  
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### 51 Concomitant care and interventions

52  
53 All participants will continue with usual transplant management as per local standard  
54  
55 of care and at the discretion of their treating nephrologist. Any changes to  
56  
57 medications will be recorded. Participants will be asked to continue with their usual  
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4 diet and medications but abstain from dietary supplements (including non-study pre-  
5  
6 and pro-biotics) for the duration of the study. The trial Sponsor has indemnity to  
7  
8 compensate those who suffer from potential harm resulting from their participation in  
9  
10 the trial.  
11  
12

### 13 14 Management of COVID-19 positive participants during the trial 15

16  
17 Study participants who return a positive COVID-19 result during the trial will be  
18  
19 managed in consultation with their treating transplant unit as per local best practice.  
20  
21 Participants who contract COVID-19 following randomisation but prior to a third  
22  
23 vaccination may have their third vaccine dose delayed. Where possible, participants  
24  
25 will be asked to continue with their allocated treatment regimens and attend study  
26  
27 visits and follow-up.  
28  
29  
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32

## 33 34 Outcomes 35

### 36 37 Primary outcome measure 38

39  
40 The primary outcome is the proportion of participants in each trial arm that achieve  
41  
42 protective serological neutralisation of live SARS-CoV-2 virus (Wuhan). Protective  
43  
44 neutralisation is defined as 20.2% of the mean neutralisation level of a standardised  
45  
46 cohort of COVID-19 convalescent individuals, and correlates with 50% protection  
47  
48 from infection with SARS-CoV-2 (Wuhan) in healthy individuals.(31)  
49  
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52  
53

### 54 55 Secondary outcome measures: 56

57  
58 The secondary outcome measures include the following:  
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60

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3  
4 1. Change in the median magnitude of the SARS-CoV-2 spike-specific, antiviral  
5  
6 T cell response prior to and at 4-6 weeks following vaccination, determined as  
7  
8 the frequency of cells that secrete IFN $\gamma$  in response to stimulation with spike-  
9  
10 protein (Wuhan)-derived peptides by ELISpot.  
11  
12
- 13  
14 2. Adverse events following immunisation (AEFI) including adverse events of  
15  
16 special interest (AESI) will be assessed via phone consultation at 1 week, and  
17  
18 again at 4-6 weeks post-vaccination during the final follow-up visit, and  
19  
20 include:  
21  
22
  - 23 a. Changes in kidney allograft function, determined by serum creatinine,  
24  
25 eGFR (CKD-EPI equation), and proteinuria.  
26  
27
  - 28 b. The occurrence of biopsy proven acute allograft rejection.  
29  
30
  - 31 c. The recurrence of primary kidney disease.  
32  
33
  - 34 d. Patient reported quality of life as recorded by the EQ-5D questionnaire.  
35  
36
- 37  
38 3. Tolerance of dietary inulin determined by the change in gastrointestinal  
39  
40 symptom rating scale (GSRS) at baseline, week 4, and the final trial visit.  
41  
42 Adherence to the intervention will be assessed by the weight of unconsumed  
43  
44 supplement returned at the final study visit.  
45  
46
- 47  
48 4. The proportion of participants who generate a serological response 4-6 weeks  
49  
50 following a third COVID-19 vaccination. A serological response is defined as  
51  
52 reaching a threshold of anti-receptor-binding domain antibody (anti-RBD IgG)  
53  
54  $\geq 100$  units/mL (measured with an Elecsys Anti-SARS-CoV-2 immunoassay  
55  
56  $\geq 100$  units/mL (measured with an Elecsys Anti-SARS-CoV-2 immunoassay  
57  
58 [Roche]). This RBD antibody threshold was chosen on the basis of pre-clinical  
59  
60

1  
2  
3 and clinical studies (32, 33), and is consistent with the reported outcomes in  
4  
5  
6 published COVID-19 clinical vaccine trials.(6, 34)  
7

- 8  
9 5. Changes in the community structure, relative abundance, and functional  
10  
11 characteristics of the gut microbiome following 4 weeks of dietary intervention,  
12  
13 determined by 16S-rRNA metagenomic sequencing of participant stool  
14  
15  
16 samples.  
17  
18  
19 6. The development of COVID-19 following randomisation, determined by:  
20  
21 a. Positive SARS-CoV-2 PCR test, or rapid antigen test in the setting of  
22  
23 symptomatic disease  
24  
25  
26 b. Detection of SARS-CoV-2 anti-nucleocapsid antibodies at the time of  
27  
28  
29 primary outcome assessment.  
30  
31

32 Following trial registration, additional secondary outcome numbers 4 to 6, were  
33  
34 approved by the HREC, and added to the trial protocol. These outcomes are  
35  
36 exploratory in nature and will seek to inform the design and scope of larger clinical  
37  
38 trials.  
39  
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47

## 48 Participant timeline

49 Participants are followed from the time of enrolment through until study close-out, 1-  
50  
51 week following their final assessment visit. The schedule of enrolment,  
52  
53 randomisation, interventions, and assessments is shown in Figure 2.  
54  
55  
56  
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## 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%  $\alpha$ -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

1  
2  
3 access to restricted modules (randomisation, scheduling, and data export) privileged.

4  
5  
6 The database is maintained by the SHLD Information and Communication

7  
8  
9 Technology (ICT) Services with regular back-up processes in place.

## 10 11 12 13 14 Collection and evaluation of biological samples

15  
16  
17 Blood samples will be taken from participants for immunological assessment at  
18  
19 randomisation and at 4-6 weeks following vaccination. Blood will be drawn from  
20  
21 participants by clinical research staff and collected in 7 x 9mL EDTA and 1 x 5mL  
22  
23 CAT serum separator vacutainer tubes. Peripheral blood mononuclear cells  
24  
25 (PBMCs) will be isolated from whole blood by density gradient centrifugation in  
26  
27 Ficoll-Paque and aliquoted and cryopreserved in liquid nitrogen for batch testing,  
28  
29 with sera aliquoted and stored at -80°C. Phenotypic and functional assessments of  
30  
31 vaccine-specific T-cell responses will be initially assessed by IFN $\gamma$  release assay  
32  
33 (ELISpot) following stimulation with overlapping peptides spanning the length of  
34  
35 SARS-CoV-2 spike protein, with any notable change triggering a more in-depth  
36  
37 investigation. For example, the assessment of spike-specific circulating T follicular  
38  
39 helper cells based on the frequency and phenotype of CD4<sup>+</sup> T cells expressing  
40  
41 CD40L following stimulation with spike protein-derived peptides may be analysed via  
42  
43 FACS. Using participant serum, the titres of SARS-CoV-2 spike-protein-specific IgG  
44  
45 and the capacity of participant serum to neutralise SARS-CoV-2 viral entry into ACE<sup>+</sup>  
46  
47 cells will be assessed. The capacity of pre- and post-immunisation serum to induce  
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4 spike-protein-specific antibody-dependent innate immune responses will be  
5  
6 measured.

7  
8 Isolation and sequencing of the faecal metagenome will occur on self-collected stool  
9  
10 samples placed in a DNA stabilising solution (OMNIgene GUT OM-200, DNA  
11  
12 Genotek, Canada). Stool samples will be aliquoted and stored at -80°C until batch  
13  
14 testing. Analysis of the faecal metagenome will be performed by comparative  
15  
16 sequencing of 16S-rRNA amplicons (V4 region). Estimation of participants habitual  
17  
18 diet will be captured using a 4-day food diary, completed at the time stool samples  
19  
20 are collected.  
21  
22  
23  
24  
25

26  
27 Validated questionnaires are completed by participants to capture adverse  
28  
29 gastrointestinal symptoms (gastrointestinal symptom rating scale, GSRS (38, 39)),  
30  
31 and health-related quality of life (EQ-5D) information.(40)  
32  
33

34  
35 All biological specimens will be deidentified and labelled with the participants unique  
36  
37 study identifier. Stool and blood samples will be stored and maintained in access-  
38  
39 restricted laboratory freezers at their corresponding trial site (Adelaide Health and  
40  
41 Medical Sciences building, University of Adelaide, or the Transplant Institute, RPA,  
42  
43 Sydney).  
44  
45  
46  
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49

## 50 51 Confidentiality

52  
53 Prior to study enrolment, participants will consent to research staff accessing their  
54  
55 electronic medical record to obtain baseline and demographic information, and the  
56  
57 results of laboratory assessments. The privacy and confidentiality of screened and  
58  
59  
60

1  
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3  
4 enroled participants will be preserved with all study data stored in the RIVASTIM  
5  
6 REDCap database under a unique numerical study identifier. No identifying  
7  
8 information or individually identifiable participant data will be reported in publications,  
9  
10 presentations, or in any report arising from this study.  
11  
12  
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## 18 Statistical methods

19  
20  
21 The primary analysis will be by intention-to-treat, with participants assessed  
22  
23 according to their treatment allocation. Participants who develop a positive SARS-  
24  
25 CoV-2 PCR result during the study will be excluded from the primary analysis to  
26  
27 avoid confounding. A per-protocol analysis will also be reported, with participants  
28  
29 who failed to adhere or tolerate the dietary intervention and consumed < 80% of the  
30  
31 prescribed supplement, and participants who withdrew or were lost to follow-up  
32  
33 excluded from the analysis. A sensitivity analyses adjusting for potential confounding  
34  
35 may be performed should significant imbalances in baseline characteristics between  
36  
37 the treatment groups occur. Multiple imputation will be used to handle data missing  
38  
39 at random from baseline characteristics. Data missing at random for the primary and  
40  
41 secondary outcome will not be imputed, with these cases excluded from ITT  
42  
43 analysis. If > 10% of the primary outcome data is determined to be missing not at  
44  
45 random, a best-worst and worst-best case sensitivity analyses will be performed.  
46  
47  
48 Subgroup analyses will be performed to examine for statistical interaction between  
49  
50 treatment arm and; (1) the initial response to 2-dose vaccine schedule (non-  
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3 responder or low-responder), (2) the duration between previous vaccine dose (less  
4 than, or greater than 6 weeks) and randomisation. Patients who develop primary  
5  
6 COVID-19 infection during the study period will have both primary and secondary  
7  
8 outcomes analysed as a pre-specified subgroup analysis.  
9  
10

11  
12  
13 The primary endpoint is the proportion of participants in each trial arm that achieve  
14  
15 in-vitro neutralisation of live SARS-CoV-2 virus using the chi-square test. An  
16  
17 unadjusted and adjusted relative risk (RR) will be calculated. For the adjusted RR  
18  
19 estimate, the primary outcome of a threshold SARS-CoV-2 anti-RBD IgG titre will be  
20  
21 analysed using a log-binomial regression model. The initial immune response to a  
22  
23 two-dose vaccine schedule (anti-RBD IgG titre; low responder: 0.4 – 99 U/mL; or  
24  
25 non-responder: <0.4 U/mL) will be included in the model as a fixed effect, with study  
26  
27 site as a random effect.  
28  
29  
30  
31  
32  
33

34  
35 Secondary outcomes will be analysed using univariate and multivariate methods  
36  
37 dependant on the outcome type. Baseline characteristics and demographic data will  
38  
39 be reported as mean  $\pm$  SD for normally distributed data and median  $\pm$  IQR for non-  
40  
41 normally distributed data, with categorical variables reported as frequencies.  
42  
43  
44

45  
46 Changes in the differential abundance of key bacterial species will be approached  
47  
48 using analytical methods such as DESeq2,(41) ANCOM,(42) MaAslin2,(43) or Linear  
49  
50 Discriminant Analysis Effect Size (LEfSe),(44) depending on the data characteristics.  
51  
52 We anticipate a 2-4 fold increase in Bifidobacterium species in response to inulin  
53  
54 supplementation. (45, 46)  
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4 All statistical analyses will be described in detail with arising publications. A two-  
5  
6 sided significance level of 5% will be used for all analyses.  
7

### 8 9 Oversight and monitoring

10  
11 The coordinating trial centre is located at the Royal Adelaide Hospital. The Trial  
12  
13 Steering committee (TSC) is co-chaired by the Principal Investigator (PI) at each  
14  
15 study site and includes the trial associate investigators. The TSC is responsible for  
16  
17 the study conception, drafting and completion of the study protocol and associated  
18  
19 documents, recruitment plan, data monitoring and integrity, end point adjudication,  
20  
21 and approving publications arising from the study.  
22  
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25

26  
27 Following publication of all study results, deidentified participant level data may be  
28  
29 made available upon reasonable request to the principal investigator, or in the case  
30  
31 of published works, through the corresponding author.  
32  
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### 38 39 Adverse event reporting and harms

40  
41 All protocol deviations and adverse events (AEs) will be documented, regardless of  
42  
43 their potential relationship to the study intervention. Adverse events will be recorded  
44  
45 using an adaptation of the National Institute of Health's Common Terminology  
46  
47 Criteria for Adverse Events by a study team member on an eDCF. Screening for  
48  
49 adverse events will occur during each study visit and during scheduled clinical  
50  
51 follow-up with their treating nephrologist, and will be captured up to 7 days following  
52  
53 the final study visit. Adverse events following immunisation (AEFIs) with the  
54  
55 exception of mild and/or short-lived symptoms, will be reported to the Therapeutic  
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4 Goods Administration (TGA). Serious adverse events (SAEs) will be reported to the  
5  
6 trial sponsor with 24-hours of the study team being made aware of the event.  
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## 10 Ethics and Dissemination

11  
12

13 The study is conducted in accordance with the *National Statement on Ethical*  
14  
15 *Conduct in Human Research* (2018), the *CPMP/ICH Note for Guidance on Good*  
16  
17 *Clinical Practice* and consistent with the principles that have their origin in the  
18  
19 Declaration of Helsinki. Compliance with these standards provides assurance that  
20  
21 the rights, safety, and well-being of trial participants are respected.  
22  
23  
24

25  
26 Ethics approval for the RIVASTM trials was obtained from the Central Adelaide Local  
27  
28 Health Network (CALHN) Human Research Ethics Committee (HREC) (approval  
29  
30 number: 2021/HRE00354) and the Sydney Local Health District (SHLD) HREC  
31  
32 (approval numbers: X21-0411 and 2021/STE04280). Written informed consent to  
33  
34 participate will be obtained from all participants.  
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38  
39 The results of the RIVASTIM-inulin trial will be published in peer-reviewed academic  
40  
41 journals and presented at national and international scientific meetings. Additionally,  
42  
43 a lay summary containing the study aim, salient findings, conclusions, and a take  
44  
45 home message will be prepared and distributed to trial participants, research staff,  
46  
47 and interested members of the transplant community. Datasets and results  
48  
49 generated as part of this study will be jointly owned by Central Adelaide Local Health  
50  
51 Network, the University of Adelaide, and the Royal Prince Alfred Hospital (RPA,  
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59  
60 SLHD). Deidentified participant data may be made available from the corresponding

1  
2  
3 author of published works upon reasonable request and submission of a research  
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6 plan of appropriate scientific merit and ethical standing.  
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## 11 Discussion

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15  
16 Interventions that improve the efficacy of COVID-19 vaccinations are urgently  
17  
18 required to reduce the burden of disease in at risk groups such as KTRs. Additional  
19  
20 vaccine doses are recommended for this purpose, yet many KTRs fail to achieve  
21  
22 protective immunity after a third (6), or even fourth vaccination.(47) Vaccine  
23  
24 hyporesponsiveness in this cohort is driven by deficiencies in the underlying immune  
25  
26 response, and although immunosuppressive medications are likely the greatest  
27  
28 contributor(48), dysregulation of the gut microbiota adds to the observed immune  
29  
30 dysfunction. Strategies that address the underlying immune deficits in KTRs  
31  
32 therefore offer an attractive pathway to restore vaccine responsiveness, but are not  
33  
34 without risk. Maintaining graft function remains a priority for both patients and  
35  
36 clinicians(49), and strategies that enhance vaccine responses must be demonstrated  
37  
38 not to significantly enhance allo-immunity, lest organ rejection occur. The RIVASTIM  
39  
40 trials, consisting of sister studies RIVASTIM-Sirolimus and RIVASTIM-Inulin, directly  
41  
42 investigate two strategies to enhance the cellular and humoral response to a third  
43  
44 vaccine dose in differing groups of KTRs. Whilst RIVASTIM-sirolimus will examine  
45  
46 the efficacy and safety of immunosuppression modification in KTRs, RIVASTIM-



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4 inulin assesses the response to and efficacy of a dietary prebiotic to improve vaccine  
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6 immunogenicity.  
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10  
11 As the primary outcome, RIVASTIM-inulin will assess in vitro neutralization titres  
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13 following a third vaccine dose, which are highly predictive of immune protection from  
14  
15 symptomatic COVID-19. Additionally, SARS-CoV-2 RBD IgG, which offers a close  
16  
17 correlate of the efficacy of serum to neutralise SARS-CoV-2 and widely available in  
18  
19 clinical practice, will be measured as a secondary outcome.(32, 33) However,  
20  
21 antibody titres, whilst clinically significant, do not offer a complete explanation for the  
22  
23 divergent vaccine responses observed across the cohort of KTRs. Despite  
24  
25 recognition that COVID-19 vaccine efficacy in immunosuppressed individuals  
26  
27 remains suboptimal, robust examination of the cellular and humoral immune  
28  
29 responses in those with sufficient, partial, or negligible vaccine responses are  
30  
31 lacking. Through sophisticated immunophenotyping, RIAVSTIM-inulin will examine  
32  
33 the adaptive immune responses prior to and following a third COVID-19 vaccination,  
34  
35 to quantify which immune deficits contribute to vaccine hyporesponsiveness in  
36  
37 KTRs, and whether these are impacted by an improvement in gut health. Detailed  
38  
39 examination of the gut metagenome will comprehensively evaluate the relationship  
40  
41 between the gut microbiota and vaccine response, and examine whether a response  
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43 to targeted prebiotics can shift vaccine immunogenicity.  
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55 At the time of trial registration, Australia had low community transmission in a largely  
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57 SARS-CoV-2 naïve population and was uniquely placed to assess interventions to  
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3 improve vaccine efficacy. However, the subsequent emergence of variants of clinical  
4 significance such as Delta and Omicron have led to COVID-19 surges in Australia.  
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8 Such surges may impact trial conduct, but also serve to highlight the need for  
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11 emergent strategies to boost vaccine responsiveness for at-risk groups such as  
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13  
14 KTRs.  
15

16 Dietary interventions designed to modulate the gut microbiota may offer an adjuvant  
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18 approach to improve vaccine efficacy, however robust clinical trials in this field are  
19  
20 thus far lacking. Results of the RIVASTIM-inulin trial will seek to inform vaccine  
21  
22 policy, and may provide evidence for a meaningful, inexpensive, scalable, and  
23  
24 accessible intervention by which vaccine responses may be enhanced. Such  
25  
26  
27 discoveries would address our current unmet need to protect at risk populations from  
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29  
30 COVID-19 related morbidity and mortality and would hence be of global interest.  
31  
32  
33

### 34 35 Trial status

36  
37 Protocol version 3.0, dated 3<sup>rd</sup> October 2021. Recruitment commenced November  
38  
39 2021, with anticipated recruitment end date 15<sup>th</sup> March 2022.  
40  
41

### 42 43 Authors' contributions

44  
45 PTC and SJC conceived the study. PTC, SJC, JS, GBP, MT, TS, HW and TY  
46  
47 designed the study methodology. MT and JS wrote the first draft of the protocol, and  
48  
49 JS prepared the study manuscript and constructed the figures and tables. All authors  
50  
51 contributed to the protocol development and read and approved the final manuscript.  
52  
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## Competing interests

The authors declare that they have no competing interests.

## References

1. Geneva: World Health Organization. WHO COVID-19 Dashboard 2020 [17th January 2022]. Available from: <https://covid19.who.int/>.
2. ANZDATA Registry. 43rd Report, Chapter 7: Transplantation. 2020;<https://www.anzdata.org.au>
3. Phanish M, Ster IC, Ghazanfar A, et al. Systematic review and meta-analysis of COVID-19 and kidney transplant recipients, the South West London Kidney Transplant Network experience. *Kidney international reports*. 2020
4. Kremer D, Pieters TT, Verhaar MC, et al. A systematic review and meta-analysis of COVID-19 in kidney transplant recipients: lessons to be learned. *American Journal of Transplantation*. 2021;21(12):3936-45
5. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA*. 2021;325(21):2204-6
6. Hall VG, Ferreira VH, Ku T, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. *New England Journal of Medicine*. 2021;385(13):1244-6
7. Callaghan CJ, Mumford L, Curtis RM, et al. Real-world Effectiveness of the Pfizer-BioNTech BNT162b2 and Oxford-AstraZeneca ChAdOx1-S Vaccines Against SARS-CoV-2 in Solid Organ and Islet Transplant Recipients. *Transplantation*. 2022
8. Australian Technical Advisory Group on Immunisation (ATAGI). Recommendations on the use of a 3rd primary dose of COVID-19 vaccine in individuals who are severely immunocompromised [18th January 2022]. Available from: [www.health.gov.au/resources/publications/](http://www.health.gov.au/resources/publications/).
9. Kamar N, Abravanel F, Marion O, et al. Three Doses of an mRNA Covid-19 Vaccine in Solid-Organ Transplant Recipients. *New England Journal of Medicine*. 2021;385(7):661-2 DOI:10.1056/NEJMc2108861.

10. Benotmane I, Gautier G, Perrin P, et al. Antibody response after a third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic response to 2 doses. *JAMA*. 2021;326(11):1063-5
11. Lynn DJ, Benson SC, Lynn MA, Pulendran B. Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms. *Nature Reviews Immunology*. 2021:1-14
12. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*. 2018;359(6371):104-8
13. Lynn DJ, Pulendran B. The potential of the microbiota to influence vaccine responses. *Journal of Leukocyte Biology*. 2018;103(2):225-31 DOI:<https://doi.org/10.1189/jlb.5MR0617-216R>.
14. Chen J, Vitetta L, Henson JD, Hall S. The intestinal microbiota and improving the efficacy of COVID-19 vaccinations. *Journal of Functional Foods*. 2021;87:104850
15. Fricke W, Maddox C, Song Y, Bromberg J. Human microbiota characterization in the course of renal transplantation. *American Journal of Transplantation*. 2014;14(2):416-27
16. Lee JR, Magruder M, Zhang L, et al. Gut microbiota dysbiosis and diarrhea in kidney transplant recipients. *American Journal of Transplantation*. 2019;19(2):488-500
17. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. *British Journal of Nutrition*. 2015;113(S1):S1-S5
18. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature*. 2016;535(7610):56-64
19. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. 2012;489(7415):220
20. Zhao T, Li J, Fu Y, et al. Influence of gut microbiota on mucosal IgA antibody response to the polio vaccine. *NPJ vaccines*. 2020;5(1):1-9
21. Huda MN, Lewis Z, Kalanetra KM, et al. Stool microbiota and vaccine responses of infants. *Pediatrics*. 2014;134(2):e362-e72
22. Ng SC, Peng Y, Zhang L, et al. Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events. *Gut*. 2022:gutjnl-2021-326563 DOI:10.1136/gutjnl-2021-326563.
23. Tunbridge M, Perkins GB, Singer J, et al. Rapamycin and inulin for booster vaccine response stimulation (RIVASTIM)—rapamycin: study protocol for a randomised, controlled trial of immunosuppression

1  
2  
3 modification with rapamycin to improve SARS-CoV-2 vaccine response in kidney transplant recipients. *Trials*.  
4 2022;23(1):1-12  
5

6  
7 24. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)—A metadata-driven  
8 methodology and workflow process for providing translational research informatics support. *Journal of*  
9 *Biomedical Informatics*. 2009;42(2):377-81 DOI:<https://doi.org/10.1016/j.jbi.2008.08.010>.  
10  
11

12 25. Niness KR. Inulin and Oligofructose: What Are They? *The Journal of Nutrition*. 1999;129(7):1402S-6S  
13 DOI:10.1093/jn/129.7.1402S.  
14  
15

16 26. So D, Whelan K, Rossi M, et al. Dietary fiber intervention on gut microbiota composition in healthy  
17 adults: a systematic review and meta-analysis. *The American Journal of Clinical Nutrition*. 2018;107(6):965-83  
18  
19

20 27. Vandeputte D, Falony G, Vieira-Silva S, et al. Prebiotic inulin-type fructans induce specific changes in  
21 the human gut microbiota. *Gut*. 2017;66(11):1968-74 DOI:10.1136/gutjnl-2016-313271.  
22  
23

24 28. Bouhnik Y, Vahedi K, Achour L, et al. Short-chain fructo-oligosaccharide administration dose-  
25 dependently increases fecal bifidobacteria in healthy humans. *The Journal of nutrition*. 1999;129(1):113-6  
26  
27

28 29. Bruhwiler J, Carreer F, Demanet E, Jacobs H. Digestive tolerance of inulin-type fructans: a double-  
29 blind, placebo-controlled, cross-over, dose-ranging, randomized study in healthy volunteers. *Int J Food Sci*  
30 *Nutr*. 2009;60(2):165-75 DOI:10.1080/09637480701625697.  
31  
32

33 30. Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal tolerance of chicory inulin products.  
34 *Journal of the American Dietetic Association*. 2010;110(6):865-8  
35  
36

37 31. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune  
38 protection from symptomatic SARS-CoV-2 infection. *Nature Medicine*. 2021;27(7):1205-11  
39  
40  
41  
42  
43 DOI:10.1038/s41591-021-01377-8.

44 32. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus  
45 macaques. *Nature*. 2021;590(7847):630-4  
46  
47

48 33. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune  
49 protection from symptomatic SARS-CoV-2 infection. *Nature medicine*. 2021:1-7  
50  
51

52 34. Peled Y, Ram E, Lavee J, et al. Third dose of the BNT162b2 vaccine in heart transplant recipients:  
53 immunogenicity and clinical experience. *The Journal of Heart and Lung Transplantation*. 2021  
54  
55

56 35. Teare MD, Dimairo M, Shephard N, et al. Sample size requirements to estimate key design  
57 parameters from external pilot randomised controlled trials: a simulation study. *Trials*. 2014;15(1):1-13  
58  
59  
60

- 1  
2  
3 36. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised  
4 trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome  
5 variable. *Statistical methods in medical research*. 2016;25(3):1057-73  
6  
7  
8  
9 37. Schrezenmeier E, Rincon-Arevalo H, Stefanski A-L, et al. B and T Cell Responses after a Third Dose of  
10 SARS-CoV-2 Vaccine in Kidney Transplant Recipients. *Journal of the American Society of Nephrology*.  
11 2021;32(12):3027-33 DOI:10.1681/asn.2021070966.  
12  
13  
14 38. Svedlund J, Sjödin I, Dotevall G. GSRS—a clinical rating scale for gastrointestinal symptoms in patients  
15 with irritable bowel syndrome and peptic ulcer disease. *Digestive diseases and sciences*. 1988;33(2):129-34  
16  
17  
18 39. Kleinman L, Faull R, Walker R, et al., editors. Gastrointestinal-specific patient-reported outcome  
19 instruments differentiate between renal transplant patients with or without GI complications. *Transplantation*  
20 *proceedings*; 2005: Elsevier.  
21  
22  
23  
24 40. Cleemput I, Kesteloot K, Moons P, et al. The construct and concurrent validity of the EQ-5D in a renal  
25 transplant population. *Value in Health*. 2004;7(4):499-509  
26  
27  
28 41. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data  
29 with DESeq2. *Genome biology*. 2014;15(12):550  
30  
31  
32 42. Mandal S, Van Treuren W, White RA, et al. Analysis of composition of microbiomes: a novel method  
33 for studying microbial composition. *Microbial ecology in health and disease*. 2015;26(1):27663  
34  
35  
36 43. Mallick H, Rahnavard A, McIver LJ, et al. Multivariable association discovery in population-scale meta-  
37 omics studies. *PLoS computational biology*. 2021;17(11):e1009442  
38  
39  
40 44. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome*  
41 *biology*. 2011;12(6):1-18  
42  
43  
44 45. Ramirez-Farias C, Slezak K, Fuller Z, et al. Effect of inulin on the human gut microbiota: stimulation of  
45 *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *British Journal of Nutrition*. 2008;101(4):541-50  
46  
47  
48 46. Le Bastard Q, Chapelet G, Javaudin F, et al. The effects of inulin on gut microbial composition: a  
49 systematic review of evidence from human studies. *European Journal of Clinical Microbiology & Infectious*  
50 *Diseases*. 2020;39(3):403-13  
51  
52  
53  
54 47. Caillard S, Thauinat O, Benotmane I, Masset C, Blancho G. Antibody Response to a Fourth Messenger  
55 RNA COVID-19 Vaccine Dose in Kidney Transplant Recipients: A Case Series. *Annals of internal medicine*. 2022  
56  
57  
58 48. Duni A, Markopoulos GS, Mallioras I, et al. The Humoral Immune Response to BNT162b2 Vaccine Is  
59 Associated With Circulating CD19+ B Lymphocytes and the Naïve CD45RA to Memory CD45RO CD4+ T Helper  
60

1  
2  
3 Cells Ratio in Hemodialysis Patients and Kidney Transplant Recipients. *Frontiers in immunology*.  
4 2021;12:760249-  
5

6  
7 49. Sautenet B, Tong A, Manera KE, et al. Developing consensus-based priority outcome domains for trials  
8 in kidney transplantation: a multinational Delphi survey with patients, caregivers and health professionals.  
9 *Transplantation*. 2017;101(8):1875  
10  
11  
12  
13  
14  
15  
16  
17  
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20  
21  
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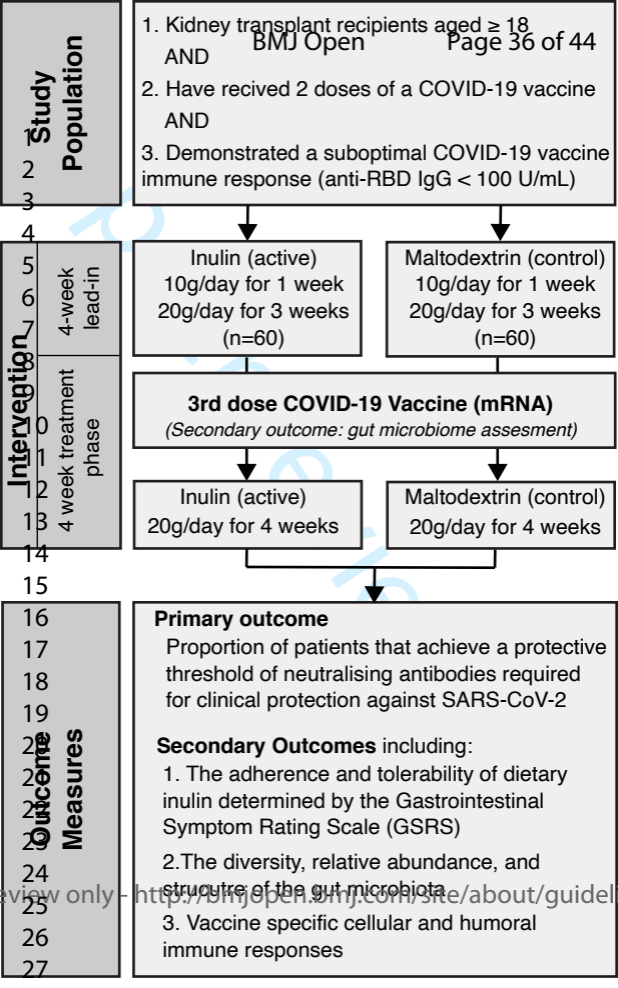
For peer review only

## 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

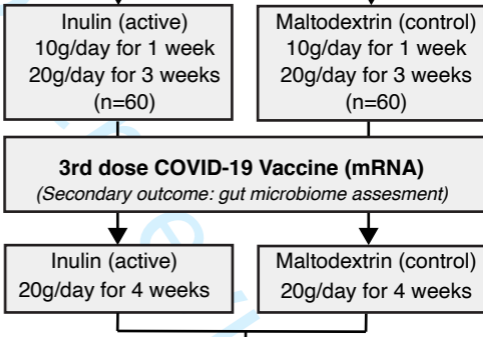




Study Population

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

Interventions



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
<b>ENROLMENT:</b>					
Eligibility screen	X				
Informed consent	X				
Baseline characteristics	X				
Allocation		X			
<b>INTERVENTIONS:</b>					
<i>Inulin (active)</i>		←————→			
<i>Maltodextrin (placebo control)</i>		←————→			
<i>COVID-19 mRNA Vaccine</i>			X		
<b>ASSESSMENTS:</b>					
<i>Anti-RBD IgG titre</i>	X			X	
<i>Routine biochemistry</i>	X			X	
<i>Blood draw for cellular and humoral immune assays</i>		X		X	
<i>Faecal microbiota assessment</i>		X	X		
<i>4-day food diary</i>		X	X		
<i>Medication Review</i>	X		X	X	
<i>GSRS</i>		X	X	X	
<i>Adherence Assessment</i>		X	X	X	
<i>EQ-5D</i>		X	X	X	
<i>AE/SAE</i>			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 SPIRIT reporting 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
<b>Administrative information</b>			
Title	<a href="#">#1</a>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	<a href="#">#2b</a>	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	<a href="#">#3</a>	Date and version identifier	3
Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	19
Roles and responsibilities: contributorship	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	1 + 19

1	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	3
2	responsibilities:			
3	sponsor contact			
4	information			
5				
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8	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	3
9	responsibilities:		collection, management, analysis, and interpretation of data;	
10	sponsor and funder		writing of the report; and the decision to submit the report for	
11			publication, including whether they will have ultimate authority	
12			over any of these activities	
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16	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating centre,	NA
17	responsibilities:		steering committee, endpoint adjudication committee, data	
18	committees		management team, and other individuals or groups overseeing the	
19			trial, if applicable (see Item 21a for data monitoring committee)	
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23	<b>Introduction</b>			
24				
25	Background and	<a href="#">#6a</a>	Description of research question and justification for undertaking	4-5
26	rationale		the trial, including summary of relevant studies (published and	
27			unpublished) examining benefits and harms for each intervention	
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30	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	9
31	rationale: choice of			
32	comparators			
33				
34				
35				
36	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	10-11
37				
38	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel	6
39			group, crossover, factorial, single group), allocation ratio, and	
40			framework (eg, superiority, equivalence, non-inferiority,	
41			exploratory)	
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45	<b>Methods:</b>			
46	<b>Participants,</b>			
47	<b>interventions, and</b>			
48	<b>outcomes</b>			
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52	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic, academic	6
53			hospital) and list of countries where data will be collected.	
54			Reference to where list of study sites can be obtained	
55				
56				
57	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable,	7
58			eligibility criteria for study centres and individuals who will	
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		perform the interventions (eg, surgeons, psychotherapists)	
1			
2	Interventions:	<a href="#">#11a</a> Interventions for each group with sufficient detail to allow	8-9
3	description	replication, including how and when they will be administered	
4			
5	Interventions:	<a href="#">#11b</a> Criteria for discontinuing or modifying allocated interventions for a	9
6	modifications	given trial participant (eg, drug dose change in response to harms,	
7		participant request, or improving / worsening disease)	
8			
9	Interventions:	<a href="#">#11c</a> Strategies to improve adherence to intervention protocols, and any	9
10	adherence	procedures for monitoring adherence (eg, drug tablet return;	
11		laboratory tests)	
12	Interventions:	<a href="#">#11d</a> Relevant concomitant care and interventions that are permitted or	10
13	concomitant care	prohibited during the trial	
14			
15	Outcomes	<a href="#">#12</a> Primary, secondary, and other outcomes, including the specific	10-11
16		measurement variable (eg, systolic blood pressure), analysis metric	
17		(eg, change from baseline, final value, time to event), method of	
18		aggregation (eg, median, proportion), and time point for each	
19		outcome. Explanation of the clinical relevance of chosen efficacy	
20		and harm outcomes is strongly recommended	
21	Participant timeline	<a href="#">#13</a> Time schedule of enrolment, interventions (including any run-ins	Figure 2
22		and washouts), assessments, and visits for participants. A	
23		schematic diagram is highly recommended (see Figure)	
24			
25	Sample size	<a href="#">#14</a> Estimated number of participants needed to achieve study	12
26		objectives and how it was determined, including clinical and	
27		statistical assumptions supporting any sample size calculations	
28			
29	Recruitment	<a href="#">#15</a> Strategies for achieving adequate participant enrolment to reach	7
30		target sample size	
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45	<b>Methods: Assignment</b>		
46	<b>of interventions (for</b>		
47	<b>controlled trials)</b>		
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50	Allocation: sequence	<a href="#">#16a</a> Method of generating the allocation sequence (eg, computer-	8
51	generation	generated random numbers), and list of any factors for	
52		stratification. To reduce predictability of a random sequence,	
53		details of any planned restriction (eg, blocking) should be provided	
54		in a separate document that is unavailable to those who enrol	
55		participants or assign interventions	
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1	Allocation concealment	<a href="#">#16b</a>	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
2	mechanism			
3				
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8	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
9	implementation			
10				
11	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
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17	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
18	emergency unblinding			
19				
20				
21				
22	<b>Methods: Data</b>			
23	<b>collection,</b>			
24	<b>management, and</b>			
25	<b>analysis</b>			
26				
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29	Data collection plan	<a href="#">#18a</a>	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
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39	Data collection plan:	<a href="#">#18b</a>	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
40	retention			
41				
42				
43				
44	Data management	<a href="#">#19</a>	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
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51	Statistics: outcomes	<a href="#">#20a</a>	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
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56	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
57	analyses			
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1	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-	14
2	population and missing		adherence (eg, as randomised analysis), and any statistical methods	
3	data		to handle missing data (eg, multiple imputation)	
4				
5				
6	<b>Methods: Monitoring</b>			
7				
8	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC); summary of its	NA
9	formal committee		role and reporting structure; statement of whether it is independent	
10			from the sponsor and competing interests; and reference to where	
11			further details about its charter can be found, if not in the protocol.	
12			Alternatively, an explanation of why a DMC is not needed	
13				
14	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping guidelines,	NA
15	interim analysis		including who will have access to these interim results and make	
16			the final decision to terminate the trial	
17				
18	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and managing solicited	14
19			and spontaneously reported adverse events and other unintended	
20			effects of trial interventions or trial conduct	
21				
22	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct, if any, and	NA
23			whether the process will be independent from investigators and the	
24			sponsor	
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28	<b>Ethics and</b>			
29	<b>dissemination</b>			
30				
31	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee / institutional review	16
32	approval		board (REC / IRB) approval	
33				
34	Protocol amendments	<a href="#">#25</a>	Plans for communicating important protocol modifications (eg,	NA
35			changes to eligibility criteria, outcomes, analyses) to relevant	
36			parties (eg, investigators, REC / IRBs, trial participants, trial	
37			registries, journals, regulators)	
38				
39	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from potential trial	7
40			participants or authorised surrogates, and how (see Item 32)	
41				
42	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use of participant	NA
43	ancillary studies		data and biological specimens in ancillary studies, if applicable	
44				
45	Confidentiality	<a href="#">#27</a>	How personal information about potential and enrolled participants	14
46			will be collected, shared, and maintained in order to protect	
47			confidentiality before, during, and after the trial	
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1	Declaration of interests	<a href="#">#28</a>	Financial and other competing interests for principal investigators for the overall trial and each study site	19
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4	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
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10	Ancillary and post trial care	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10
11				
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14	Dissemination policy: trial results	<a href="#">#31a</a>	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
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21	Dissemination policy: authorship	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of professional writers	NA
22				
23				
24	Dissemination policy: reproducible research	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
25				
26				
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28	<b>Appendices</b>			
29				
30				
31	Informed consent materials	<a href="#">#32</a>	Model consent form and other related documentation given to participants and authorised surrogates	NA
32				
33				
34	Biological specimens	<a href="#">#33</a>	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
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 41 3.0. This checklist was completed on 18. January 2021 using <https://www.goodreports.org/>, a tool made by the  
 42 [EQUATOR Network](#) in collaboration with [Penelope.ai](#)  
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