Reducing the frequency of Autoimmune adverse events in the treatment of Multiple sclerosis with alemtuzumab using B-celL dEpletion (RAMBLE): a phase II/III, randomised, placebo-controlled clinical trial.

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Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulations of this protocol, the conditions of the ethics committee approval, the NHMRC *National Statement on Ethical Conduct in Human Research (2007) – Updated 2018*, and the NHMRC and Universities Australia *Australian Code for the Responsible Conduct of Research (2018)*. As a clinical trial, the study will also comply with the *Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95)*.

Signed ……. Date ……/……/…….

STUDY INVESTIGATORS

Principal Investigator:	Name: Prof Simon Broadley Institution: Griffith University & Gold Coast University Hospital Department: Department of Neurology Address: 1 Hospital Boulevard, Southport QLD 4215 Tel: 07 5678 0174 Email: simon.broadley@griffith.edu.au Role in Study: Principal Investigator
Co-Investigator:	Name: Prof Sonya Marshall-Gradisnik Institution: Griffith University Department: Menzies Health Institute Queensland Address: Parklands Drive, Southport QLD 4215 Tel: 07 5678 0725 Email: s.marshall-gradisnik@griffith.edu.au Role in Study: Neuroimmunologist
Co-Investigator:	Name: Prof Pamela McCombe Institution: Royal Brisbane and Women's Hospital Department: Department of Neurology Address: Butterfield Street, Herston QLD 4029 Tel: 07 3646 8111 Email: pamela.mccombe@uq.edu.au Role in Study: Site Investigator
Co-Investigator:	Name: Prof Donald Staines Institution: Griffith University Department: Menzies Health Institute Queensland Address: Parklands Drive, Southport QLD 4215 Tel: 07 5678 0725 Email: don.staines@griffith.edu.au Role in Study: Public Health Physician
Co-Investigator:	Name: Prof Michael Barnett Institution: University of Sydney Department: Brain and Mind Centre Address: 94 Mallett Street, Camperdown NSW 2050 Tel: 02 9351 0774 Email: michael@sydneyneurology.com.au Role in Study: Independent MRI Analyser

Co-Investigator:	Name: A/Prof Jing Sun Institution: Griffith University Department: Menzies Health Institute Queensland Address: Parklands Drive, Southport QLD 4215 Tel: 07 5678 0924 Email: j.sun@griffith.edu.au Role in Study: Statistician
Co-Investigator:	Name: Dr Stefan Blum Institution: Mater Hospital Department: Department of Neurology Address: Raymond Terrace, South Brisbane QLD 4101 Tel: 07 3163 8111 Email: stefan.blum@brisbaneneurology.com.au Role in Study: Site Investigator
Co-Investigator:	Name: Dr Laura Clarke Institution: Princess Alexandra Hospital Department: Department of Neurology Address: 199 Ipswich Road, Woolloongabba QLD 4102 Tel: 07 3176 2111 Email: lauradaintreeclarke@gmail.com Role in Study: Site Investigator
Co-Investigator:	Name: Dr Joshua Barton Institution: Sunshine Coast University Hospital Department: Department of Neurology Address: 6 Doherty Street, Birtinya QLD 4575 Tel: 07 5202 0000 Email: joshua.barton@health.qld.gov.au Role in Study: Site Investigator
Co-Investigator:	Name: Dr Mike Boggild Institution: Townsville Hospital and Health Service Department: Department of Neurology Address: 100 Angus Smith Drive, Douglas QLD 4814 Tel: 07 4433 1111 Email: mike.boggild@health.qld.gov.au Role in Study: Site Investigator
Co-Investigator	Name: Dr Zara Ioannides Institution: Royal Brisbane and Women's Hospital Department: Department of Neurology Address: Butterfield Street, Herston QLD 4029 Tel: 07 3646 8111 Email: z.ioannides@gmail.com Role in Study: Site Investigator

Co-investigator	Name: Dr Andrew Swayne Institution: Mater Hospital Department: Department of Neurology Address: Raymond Terrace, South Brisbane QLD 4101 Tel: 07 3163 8111 Email: andrew.swayne@health.qld.gov.au Role in Study: Site Investigator
Co-investigator	Name: Dr Simon Arnett Institution: Griffith University Department: School of Medicine and Dentistry Address: Gold Coast Campus, Griffith University QLD 4222 Tel: 0407 428 389 Email: simon.arnett@griffithuni.edu.au Role in Study: Site Investigator
Co-investigator	Name: Ms Sofia Jimenez Sanchez Institution: Griffith University Department: School of Medicine and Dentistry Address: Gold Coast Campus, Griffith University QLD 4222 Tel: 0449 042 952 Email: s.jimenezsanchez@griffith.edu.au Role in Study: Clinical Trial Coordinator
Co-investigator	Name: Ms Victoria Cottam Institution: Gold Coast University Hospital Department: Clinical Trials Unit Address: 1 Hospital Boulevard, Southport QLD 4215 Tel: 07 5687 6395 Email: victoria.cottam@health.qld.gov.au Role in Study: Clinical Trial Nurse
Co-investigator	Name: Ms Rebecca Ward Institution: Gold Coast University Hospital Department: Department of Neurology Address: 1 Hospital Boulevard, Southport QLD 4215 Tel: 07 56870260 Email: Rebecca.ward2@health.qld.gov.au Role in Study: MS Nurse
Co-investigator	Name: Ms Michelle Prosser Institution: Gold Coast University Hospital Department: Department of Neurology Address: 1 Hospital Boulevard, Southport QLD 4215 Tel: 07 5687 4775 Email: michelle.prosser@health.qld.gov.au Role in Study: MS Nurse

Co-investigator	Name: Ms Fiona Marple-Clark Institution: Gold Coast University Hospital Department: Pharmacy Address: 1 Hospital Boulevard, Southport QLD 4215 Tel: 07 5687 0636 Email: fiona.marple-clark@health.qld.gov.au Role in Study: Pharmacist
Co-investigator	Name: Ms Michelle Ntiamoah Institution: Griffith University Department: School of Medicine and Dentistry Address: Gold Coast Campus, Griffith University QLD 4222 Tel: 07 5678 0702 Email: michelle.ntiamoah@griffithuni.edu.au Role in Study: Pharmacist
Co-investigator	Name: Mr Christopher Camarda Institution: Griffith University Department: School of Medicine and Dentistry Address: Gold Coast Campus, Griffith University QLD 4222 Tel: 07 5678 0702 Email: christopher.camarda@griffithuni.edu.au Role in Study: Database Manager

Steering Committee

Professor Simon Broadley – Chair

Professor Helmut Butzkueven (Monash University) – Academic Neurologist

Dr Michael Levy (Harvard University) – Academic Neurologist

Safety Review Board

Professor Bruce Taylor (University of Tasmania) – Academic Neurologist – Chair

Associate Professsor Sean Riminton (University of Sydney) – Academic Immunologist

Dr Heidi Beadnall (University of Sydney) – Academic Neurologist

STUDY SYNOPSIS

Please provide a brief summary of the information provided in the Protocol.

Title:	Reducing the frequency of Autoimmune adverse events in the treatment of Multiple sclerosis with alemtuzumab using B-celL dEpletion (RAMBLE)
Short Title:	RAMBLE Trial
Study Sites:	Gold Coast University Hospital Royal Brisbane and Women's Hospital Mater Hospital Princess Alexandra Hospital Sunshine Coast University Hospital Townsville Hospital and Health Service
Study Aims/Objectives/Hypothesis:	To reduce the occurrence of autoimmune adverse events from the treatment of multiple sclerosis (MS) with alemtuzumab through the subsequent targeted use of rituximab. The hypothesis to be tested is that rituximab therapy following alemtuzumab treatment for MS will reduce the frequency of autoimmune adverse events.
Study Design:	This is a multicentre, randomised, double-blind, placebo-controlled Phase II clinical trial.
Study Outcome Measures:	The occurrence of autoimmune disease adverse events, in particular autoimmune thyroid disease, idiopathic thrombocytopenia and anti-GBM renal disease.
Study Population:	People with relapsing remitting MS undergoing therapy with alemtuzumab for the treatment of their disease.
Number of participants:	80
Translation to Clinical Practice:	If this strategy proves to be safe and is effective in preventing or significantly reducing the frequency of autoimmune adverse events in the treatment of MS with alemtuzumab, then this approach could be adopted immediately in a large number of pwMS with an immediate reduction in co-morbidity.
Key Ethical and Safety Considerations:	Is the consecutive administration of alemtuzumab and rituximab safe?

Glossary of Abbreviations, Terms, and Acronyms

Abbreviation, Term, Acronym	Definition (using lay language)
AAN	American Academy of Neurology
ANZAN	Australian and New Zealand Association of Neurologists
BSA	Body Surface Area
CARE-MS	Comparison of Alemtuzumab and Rebif® Efficacy in Multiple Sclerosis
CNS	Central Nervous System
CONSORT	Consolidated Standard of Reporting Trials
CRO	Contract Research Organisation
CTN	Clinical Trial Notification
ECTRIMS	European Committee for Treatment and Research in Multiple Sclerosis
EDSS	Expanded disability status scale
eCRF	Electronic Case Report Form
EUC	Electrolytes Urea and Creatinine
FBC	Full Blood Count
HADS	Hospital Anxiety and Depression Scale
βhCG	Beta-human Chorionic Gonadotropin
ICH-GCP	International Conference on Harmonization – Good Clinical Practice
ieMR	Integrated Electronic Medical Record
HREC	Human Research Ethics Committee
HSV2	Herpes Simplex Virus Type 2
LFT	Liver Function Tests
LTFU	Loss to Follow Up
MedDRA	Medical Dictionary for Regulatory Activities
MRFF	Medical Research Future Fund

MRI	Magnetic Resonance Imaging
MS	Multiple sclerosis
MSIS-29	Multiple Sclerosis Impact Scale-29
MSRA	Multiple Sclerosis Research Australia
NHMRC	National Health and Medical Research Council
od	*Omne in Die* (Once Daily)
PBS	Pharmaceutical Benefits Scheme
PICF	Patient Information and Consent Form
PIDN	Patient Identification Number
PO	Per Oral (Taken Orally)
pwMS	People with Multiple Sclerosis
QML	Queensland Medical Laboratories
RRMS	Relapse Remitting Multiple Sclerosis
SAE	Serious Adverse Event
TFT	Thyroid Function Test
TSH	Thyroid Stimulating Hormone
UACR	Urinary Albumin/Creatinine Ratio

1. Background

Multiple sclerosis (MS) is the commonest cause of neurological disability in young adults, and particularly affects women.[1] Left untreated, MS causes marked difficulty walking or inability to walk in more than half after 20-30 years.[2] This dire prognosis has been radically altered by the advent of a variety of effective therapies, including three highly effective infusion therapies.[3]

Alemtuzumab is a highly effective therapy for the treatment of MS. In pivotal phase III clinical trials (CARE-MS I and II) alemtuzumab reduced the risk of relapse by 51-55% (Class I evidence) and sustained disability by 30-42% (Class I evidence) when compared against a proven, active-comparator, therapy in the form of interferon beta 1a.[4 5] This novel therapy is administered as two courses, the first over 5 days and the second course over 3 days, 12 months later. In the majority (53%) of people with MS (pwMS) treated with alemtuzumab no further therapy was required over 8 years of follow up.[6] The annual cost of alemtuzumab is similar to other highly effective therapies for MS (around $30,000 per year). Because only two years of therapy is required in most pwMS and rarely are more than three courses given, the recurring costs of this therapy are considerably less than other similarly efficacious therapies (natalizumab and ocrelizumab) which have to be administered on a recurring basis seemingly indefinitely. However, the use of alemtuzumab as a therapy in pwMS has been relatively limited and this is in large part because of a significant risk of autoimmune adverse events. Autoimmune thyroid disease occurs in approximately one-third of patients and idiopathic thrombocytopenia (2%) and anti-GBM renal disease (0.5%) are seen less commonly, together with a variety of other occasional autoimmune diseases that have been described.[7] It has been noted that this phenomenon is specifically seen in MS and does not occur as frequently in other indications for this therapy.

Alemtuzumab is a monoclonal antibody against CD52 which is a cell surface marker of lymphocytes of unknown function.[8] The antibody is lytic to lymphocytes and causes a profound temporary lymphopenia following intravenous administration. Bone marrow derived lymphocytes begin to re-emerge almost immediately and by 3 months are up to 70% of pre-treatment levels.[9] One of the features of this lymphocyte repopulation is that B-cells re-emerge faster than T-cells. It has therefore been postulated that the extra-thymic self-tolerisation of B-cells in the absence of regulatory T-cells might explain the frequent emergence of autoimmune disease following treatment with alemtuzumab.[10] It has further been suggested that one strategy to ameliorate the emergence of autoimmunity following alemtuzumab, B-cell depleting agents could be used to quell their early re-emergence. B-cell depleting agents have been used to successfully treat autoimmune disease following alemtuzumab.[11]

A recent phase I, open label, single arm clinical trial of rituximab therapy administered whenever B-cell counts reached 50% of baseline levels following treatment of MS with alemtuzumab demonstrated that this approach is safe and potentially completely effective in mitigating the risk of autoimmunity (Class V evidence).[12]

In view of this preliminary data, it is proposed that a phase II, placebo-controlled clinical trial be undertaken with the aim of assessing the effectiveness of B-cell depletion in mitigating the risk of autoimmune disease following treatment with alemtuzumab in pwMS.

2. Study Objectives

a. Research Question and Aims/objectives

- *Primary Objective*
  - *To demonstrate a reduction or complete abrogation of the occurrence of autoimmune disease with administration of rituximab following treatment with alemtuzumab for MS.*
- *Secondary Objectives*
  - *To assess the safety and efficacy (MS disease activity related) of this therapeutic approach.*
  - *To assess the profile of the immune repertoire of T and B-cells that re-emerge with this therapeutic approach.*
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b. Hypotheses

- *The following hypotheses will be tested:*
  - *1) Consecutive administration of alemtuzumab and rituximab is associated with a lower risk of autoimmune adverse events than when alemtuzumab is administered with placebo.*
  - *2) Consecutive administration of alemtuzumab and rituximab is associated with similar efficacy in MS disease control when compared to the administration of alemtuzumab with placebo.*
  - *3) Consecutive administration of alemtuzumab and rituximab is safe – adverse events will be no more common than when alemtuzumab is administered with placebo.*
  - *4) The early re-emergence of B-cells following alemtuzumab therapy is suppressed by the administration of rituximab when compared to placebo.*

3. Methods

a. Methodological Approach

- *This will be a 3-year, multi-centre, double-blinded, randomised (2:1), placebo-controlled trial of rituximab versus placebo in pwMS being treated with alemtuzumab.*
- *Participants will be recruited from one of six hospitals across Queensland, Gold Coast University Hospital, Royal Brisbane and Women's Hospital, Mater Hospital Brisbane, Princess Alexandra Hospital, Sunshine Coast University Hospital and Townsville Hospital and Health Service.*
- *The study would aim to recruit patients over 24 months and follow individual participants for a minimum of 2 years.*

b. Study Sites/Settings

- *This is a multi-centre study based in Queensland. The following clinical sites have been selected:*
  - *Gold Coast University Hospital*
  - *Royal Brisbane and Women's Hospital*
  - *Mater Hospital Brisbane*
  - *Princess Alexandra Hospital*
  - *Sunshine Coast University Hospital*
  - *Townsville Hospital and Health Service*
- *Each of these sites runs a dedicated MS Clinic led by a neurologist with sub-specialist training, expertise and experience in the management of pwMS. The service at each site is very similar and all have experience in treating MS with anti-CD52 (alemtuzumab) and anti-CD20 (rituximab) therapies. There is no plan to compare outcomes between sites although the randomisation strategy will include stratification by site.*
- *All treatment, study visits and magnetic resonance imaging (MRI) will be conducted at the study sites. Blood tests will be conducted both at the study sites and private pathology facilities (QML).*
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c. Study Population

- *The study will aim to recruit 80 pwMS who are due to commence treatment with alemtuzumab.*
- *Inclusion criteria*
  - *Aged 18 – 55 years (inclusive)*
  - *Diagnosed with relapsing remitting MS (RRMS)[13] by a neurologist*
  - *Diagnosis of MS meeting 2017 McDonald criteria[14]*
  - *Diagnosed with MS within the previous 10 years*
  - *Expanded disability status scale (EDSS)[15] score < 5.0*
  - *English speaking or non-English speaking who can ensure external interpreter assistance (e.g. relative or friend) to attend all visits for the duration of the clinical trial.*
  - *Available to attend clinic visits*
  - *Willing to sign up for and comply with Bloodwatch monitoring program*
  - *Fully vaccinated against COVID-19 (2 standard doses plus booster)*
- *Exclusion criteria*
  - *Known or suspected prior autoimmune disease (other than MS)*
  - *Any other serious co-morbidity that in the view of the investigator would preclude participation in the study*
  - *Pregnant (if female)*
  - *Currently lactating (if female)*

- *Unwilling or unable to use appropriate contraception for the treatment phase of the study (2 years) – male or female*
- *Recent or current history of major depression, bipolar disorder, psychosis or suicidality*
- *Currently or recently taking any illicit substances (including any cannabis product)*
- *Allergy to valaciclovir*
- *Allergy to Bactrim, Trimethoprim or Sulphur based antibiotics*

d. Recruitment/ Selection

- *Potential participants will be recruited through the MS or CNS inflammatory disease clinics of the six participating hospitals with a cap of 20 participants at any single centre. Participants will not be financially compensated for their involvement in this trial but will be reimbursed for any reasonable costs of participation (e.g. parking costs).*
- *Participants will be recruited by neurologists at each site, who having identified a patient who is about to commence treatment with alemtuzumab, will invite the pwMS to participate in the trial. Potential participants will be provided with an introduction and overview of the study by their treating neurologist. Those who are interested in participating will be provided with the Patient Information and Consent Form (PICF) to take away and read and a further visit with one of the study investigators will be arranged at a later date.*
- *Participants will be enrolled in the study at the baseline/screening visit provided they:*
    - *1. Have given written informed consent*
    - *2. Meet all the inclusion criteria*
    - *3. Meet none of the exclusion criteria*
- *Randomisation: Participants will be randomised at enrolment to treatment with rituximab or placebo at a 2:1 ratio. Randomisation will be performed using the ranomisation module of the REDCap database based on a random number sequence produced in Excel®, Microsoft (Seattle, CA, USA) stratified by site, sex and age. This randomization sheet will be generated by Assoc/Prof Jing Sun. There will a cap of 20 participants for any single site.*
- *Blinding and allocation concealment: This will be a double-blinded study with both patient and treating physician blinded as to whether the participant is receiving active treatment or placebo. At the screening visit, participants will be assigned a unique participant Screening Number. At the baseline/enrolment visit, after eligibility is confirmed and the full consent form has been signed, a Participant Identification Number (PIDN) will be assigned. PIDNs will be randomly assigned to intervention or placebo groups according to the randomisation code provided by the randomisation sheet. Randomisation*

codes will only be visible through REDCap to site pharmacists who will make up the active treatment and placebo. Both rituximab and 0.9% sodium chloride (placebo) are colourless fluids, so will be indistinguishable to treating physician, participants and other staff.

- *Breaking of study blinding: The randomisation code for an individual participant may only be unblinded in emergency situations, where the Site Investigator decides a participant cannot be adequately treated without knowing the identity of their treatment allocation. The Site Investigator may contact the site pharmacy to obtain the treatment identity. If possible, such emergencies should be discussed with the Principal Investigator before breaking the blind. The randomisation code will be stored electronically by the Griffith University randomisation service. If the blind is broken for a participant, the time, date, participant number and reason for opening must be documented.*
- *On completion of the study: Unblinded study data will only be available once all data collected have been entered into the REDCap study database for every participant and the database has been finalized (locked), except in the case of an emergency, as detailed above.*

e. Consent

- *The PICF provides outline details of the clinical trial and details the expectations for both the participant and those conducting the trial. In particular, the PICF makes it clear that participation is voluntary and that participants can withdraw at any time. It is also indicated that any decision to participate or not, will in no way affect a pwMS's subsequent treatment at that facility.*
- *At the subsequent visit prior to any study-related activity, the site investigator will review the details of the study, assess the pwMS understanding of the information in the PICF and having asked the potential participant if they have any questions, answer those questions. Having established that the potential participant is fully informed and wishes to proceed in the study, the participant will provide written informed consent using the PICF. Study staff will ensure that an adequate explanation is provided to the participant and their family about the aims, the requirements of the study that need to be adhered to strictly, and any potential known and unknown risks and benefits of the study. Study staff will ensure that all questions about the study are answered adequately and that the participant understands the information provided about the study. The study staff conducting the informed consent discussion will ensure that consent is voluntary and free from coercion. The staff member that conducts the consent discussion will also sign the informed consent form. A copy of the PICF will be provided to the participant to keep.*

- *Consent will be specific for this study only.*

f. Risk Mitigation Procedures

- *Treatment with alemtuzumab is associated with a risk (6-8%) of reactivation of HSV2 infection during the first month[4] and a much lower level risk of listeria meningitis over the same time period.[16] As is our usual practice all participants will receive valaciclovir 500 mg once daily for 30 days and Bactrim DS 960mg three times per week for 4 weeks commencing on the first day of therapy.*
- *In view of the potential additive risk of rituximab and alemtuzumab causing an increased risk of infection all participants will be advised to report any symptoms of fever, headache, neck stiffness, photophobia, shortness of breath, cough, abdominal pain or skin lesions to site investigators immediately.*
- *In view of the known risk of autoimmune adverse events with alemtuzumab (e.g. 30% risk of autoimmune thyroid disease) all participants will be monitored with monthly blood and urine tests through the Bloodwatch program. This is PBS mandated online monitoring program for all pwMS undergoing therapy with alemtuzumab which is sponsored by the manufacturer. This is standard of care for this therapy and includes monthly urine tests for red cells and protein, monthly blood tests for full blood count, urea and electrolytes and 3-monthly blood tests for TSH levels.*
- *Regular lymphocyte subset counts will be monitored and rituximab administration will be delayed if B-cell counts do not exceed 30% of pre-treatment levels.*

g. Participant Withdrawal Procedures

- *Screen Failure: Participants who do not meet the requirements for enrolment will be deemed to be screen failures. As such they will not be enrolled in the study and will not be randomised to any intervention or placebo. They will be given a unique trial ID and listed in the enrolment log as a screen failure. A minimal set of information (age, sex, date, reason for screen failure) will be recorded in the eRCF in order to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements,[17] respond to queries from regulatory authorities and to ensure transparent reporting of screen failure participants.*
- *Lost to follow up: Investigational staff will go to all necessary lengths possible to minimise loss to follow-up (LTFU) of participants by active and continued follow-up of participants who fail to attend study visits or who are otherwise not contactable. A participant will not be considered LTFU until all routes of contact have been exhausted. If a previously lost participant is*

retrieved within 2 weeks of the relevant study visit, they will be brought into the relevant study visit as soon as possible for the relevant measures such as primary endpoint determination. If a participant wishes to drop out due to study burden, the trial coordinator and site nurse will negotiate with the participant to reduce trial duties with a focus on continued collection of data relating to the primary outcome. If the participant wishes to drop out for health reasons (such as intolerable adverse events), the final assessment visit will be brought forward.

- *Stopping Rules: Participants will be able to withdraw from the study at any time by their own volition with no impact on any future care they may require from the recruitment site. Participants may also by their own volition choose to cease the study treatment at any time but remain in the trial with continued follow-up until the end of the study; this will have no impact on any future care they may require in the participating clinics. If the participant experiences a serious adverse event (SAE) that is suspected to be linked to the study medication or compromises the participant's ability to adhere to the intervention, the intervention will be ceased; however, the participant will remain in the trial and will be followed-up through to the end of the study.*

h. Study Procedure

- *Treatment Arms*
- *Arm 1: Intervention*
- *Arm 2: Placebo*
- *Intervention Description, Dosage and Route of Administration*
- *The investigational product is rituximab, which is a monoclonal antibody against CD20 that cause lysis of B-lymphocytes.[18] The antibody is a humanised mouse antibody and is administered intravenously via an infusion over a period of time that varies according to the dosage (see below). The dosage to be used is 100 mg/m2 of estimated body surface area (BSA). BSA will be estimated based on height (H) and weight (W) according to the Du Bois formula:[19]*
- *BSA = 0.007184 x W0.425 x H0.725*
- *With height measured in cm and weight measured in kg.*
- *Dosage will be rounded down to the nearest 10 mg.*
- *Study drug will be provided as a solution of 100 mg in 10 ml vials.*
- *Infusions will be delivered at set times of 10 weeks and 30 weeks after administration of alemtuzumab in combination with 100 mg intravenous methylprednisolone to reduce the risk of infusion reactions.*
- *Placebo*

- *Placebo will be 0.9% sodium chloride, which as a colourless solution is indistinguishable from diluted rituximab.*
- *Pre-medication*
- *Prior to administration of the study drug/placebo all trial participants (intervention and placebo arms) will be administered loratadine 10 mg orally and 100 mg intravenous methylprednisolone made up in 100 ml 0.9% sodium chloride and infused over 30 minutes.*
- *Preparation and Administration of Study Drug/Placebo*
- *Study drug and placebo will be made up by non-blinded onsite pharmacists according to the randomisation schedule product information sheet for rituximab. Specifically, the calculated dose of study drug will be aseptically withdrawn from vials and diluted in 100 ml of 0.9% sodium chloride ensuring that the calculated concentration is between 1 mg/ml and 4 mg/ml. To ensure adequate mixing the bag of diluted study drug/placebo will be gently inverted to avoid foaming. The prepared drug products will be visually inspected for particulate matter or discolouration.*
- *Rituximab/placebo will be administered according to Tables 1 and 2 below:*

Table 1: First Dose administration rate of Rituximab/Placebo

Period	1-30 mins			31-60 mins			61-90 mins			91-120 mins			Total		
Infusion Rate	50 mg/hr			100 mg/hr			150 mg/hr			200 mg/hr					
BSA (m^2)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)
1.5	30	25	16.7	30	50	33.3	30	75	50.0	0	0	0.0	90	150	100.0
1.6	30	25	15.6	30	50	31.3	30	75	46.9	3	10	6.3	93	160	100.0
1.7	30	25	14.7	30	50	29.4	30	75	44.1	6	20	11.8	96	170	100.0
1.8	30	25	13.9	30	50	27.8	30	75	41.7	9	30	16.7	99	180	100.0
1.9	30	25	13.2	30	50	26.3	30	75	39.5	12	40	21.1	102	190	100.0
2	30	25	12.5	30	50	25.0	30	75	37.5	15	50	25.0	105	200	100.0
2.1	30	25	11.9	30	50	23.8	30	75	35.7	18	60	28.6	108	210	100.0
2.2	30	25	11.4	30	50	22.7	30	75	34.1	21	70	31.8	111	220	100.0
2.3	30	25	10.9	30	50	21.7	30	75	32.6	24	80	34.8	114	230	100.0
2.4	30	25	10.4	30	50	20.8	30	75	31.3	27	90	37.5	117	240	100.0
2.5	30	25	10.0	30	50	20.0	30	75	30.0	30	100	40.0	120	250	100.0

Table 2: Subsequent dose administration rate of Rituximab/Placebo

Period	1-30 mins			31-60 mins			61-90 mins			Total		
Infusion Rate	100 mg/hr			200 mg/hr			300 mg/hr					
BSA (m^2)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)	Time (m)	Dose (mg)	Vol (ml)
1.5	30	50	33.3	30	100	66.7	0	0	0.0	60	150	100.0
1.6	30	50	31.3	30	100	62.5	2	10	6.3	62	160	100.0
1.7	30	50	29.4	30	100	58.8	4	20	11.8	64	170	100.0
1.8	30	50	27.8	30	100	55.6	6	30	16.7	66	180	100.0
1.9	30	50	26.3	30	100	52.6	8	40	21.1	68	190	100.0
2	30	50	25.0	30	100	50.0	10	50	25.0	70	200	100.0
2.1	30	50	23.8	30	100	47.6	12	60	28.6	72	210	100.0
2.2	30	50	22.7	30	100	45.5	14	70	31.8	74	220	100.0
2.3	30	50	21.7	30	100	43.5	16	80	34.8	76	230	100.0
2.4	30	50	20.8	30	100	41.7	18	90	37.5	78	240	100.0
2.5	30	50	20.0	30	100	40.0	20	100	40.0	80	250	100.0

- *First infusions will typically run over 90 – 120 minutes and subsequent infusions will run over 60 – 80 minutes.*
- *Pulse and blood pressure will be monitored every 30 minutes from the commencement methylprednisolone and until 30 minutes after the completion of rituximab/placebo and for no less than a minimum of 2 hours.*
- *Infusion rates should only be increased to the next phase provided there are no adverse events and that if there are symptoms of a mild infusion reaction the rate should be reduced to the previously tolerated level or if symptoms are more significant (shortness of breath, tachycardia, low blood pressure) the infusion should be discontinued and the PI notified immediately.*

- *Study Drug Accountability*
- *A nominated pharmacist at each participating site will maintain an inventory of receipt, use, return and collection of study drug.*
- *Control of Supplies*
- *The nominated site pharmacist at each site is responsible for recording the dispensing of the product to participants on an investigational product dispensing log which will be recorded in the eCRF. Accurate records will be maintained demonstrating dates and amount of product received, to whom dispensed and accounts of any product accidentally or deliberately destroyed. Clinical trial materials will not be loaned or dispensed to another investigator or trial centre or used for any purpose other than the trial without the prior approval of the trial coordinator and Site Investigator.*
- *Return of Supplies*
- *At the conclusion of the trial, the Principal Investigator or nominee will perform a final inventory. If any supplies cannot be accounted for, this will be documented on the product accountability form together with an explanation of the discrepancy. The originals of the product accountability and dispensing logs must be sent to the trial coordinator. The Principal Investigator will retain copies of these logs on file.*
- *Retention of Samples*
- *The Principal Investigator or delegate will maintain accurate records demonstrating dates and amount of product received, to whom dispensed and accounts of any product accidentally or deliberately destroyed. It will be the responsibility of the Principal Investigator or delegate to ensure that adequate samples of all trial doses are retained in accordance with the relevant regulatory guidelines. For example, for trials conducted under the CTN scheme in Australia, at least a sample from each batch of product used in the trial should be retained for one year longer than the shelf-life of the product, and to comply with ICH-GCP requirements, sufficient quantities of the product(s)*

should be retained either until the analyses of the trial data are complete or as required by the applicable regulatory requirements, whichever represents the longer retention period.

- *Study Procedures and Visits*
- *Participants will be required to undergo the following study procedures and visits.*
- *Screening Visit (-30 - -7 days)*
    - *Informed consent will be obtained*
    - *Completion of eligibility criteria checklist*
    - *Comprehensive review of medical history including; details of MS history, other past medical and surgical history, past and current medications, family history, social history and allergies*
    - *Comprehensive medical and neurological examination*
    - *EDSS*
    - *HADS[20]*
    - *Baseline/enrolment Visit (-7 - -4 days)*
    - *Informed consent will be re-obtained*
    - *Review of neurological symptoms*
    - *Review of medications*
    - *EDSS*
    - *MSIS29[21]*
    - *Blood tests – FBC, EUC, LFT, TFT, Lymphocyte subsets, bHCG (in women)*
    - *Urine screen – red cell count and albumin*
    - *UACR*
- *Alemtuzumab therapy visits (Year 1, Day 1 – 5; Year 2, Day 1-3) – part of normal care*
    - *Loratidine 10 PO od (continued for 10 days)*
    - *Valaciclovir 500 mg PO od*
    - *Bactrim DS (960 mg) PO 3 times per week (Mon, Weds, Fri)*
    - *0.9% Sodium Chloride IV 1L over 8 hours*
    - *Methylprednisolone 500 mg IV over 30 minutes*
    - *Alemtuzumab 12 mg IV over 4 hours*
    - *Hourly temperature, pulse and blood pressure*
    - *First month (Day 6 – 30) – part of normal care*
    - *Valaciclovir 500 mg PO od*
    - *Bactrim DS (960 mg) PO 3 times per week (Mon, Weds, Fri)*
    - *Bloodwatch pathology tests (Every month) – part of normal care*
    - *FBC/lymphocyte subsets (4 ml) – lymphocyte subsets will be performed at weeks -1, 4, 9, 13, 26, 35, 50, 57, 61, 65, 78, 83 and 104.*
    - *EUC/LFTs (10 ml)*
    - *Urine red cell count*

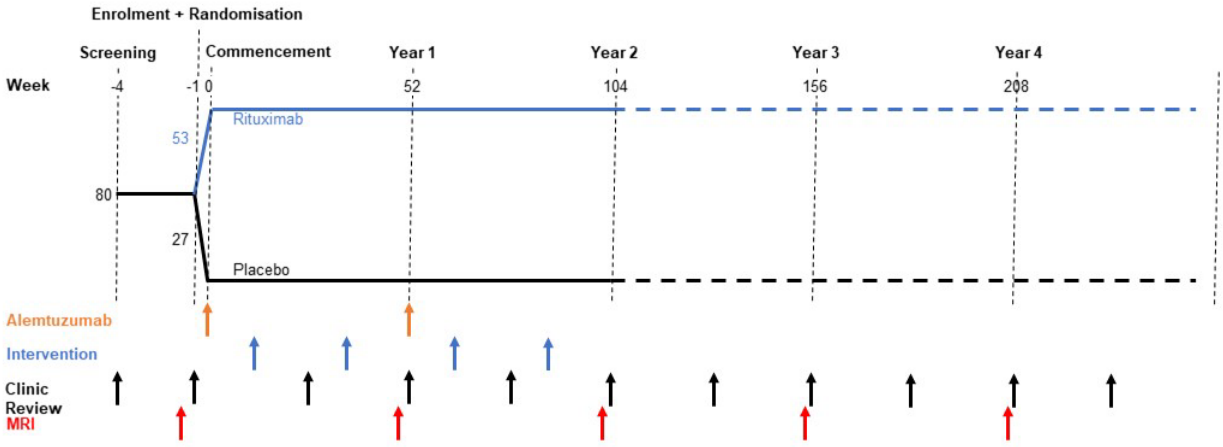
    - *Urine albumin*
    - *UACR*
    - *TSH (every 3rd month)*
- *Intervention visits (At weeks 10 and 30 provided B-cell count >30% of pre-treatment level.*
    - *Loratidine 10 mg PO 30 minutes prior to rituximab.*
    - *Methylprednisolone 100 mg IV over 30 minutes, commencing 30 minutes prior to rituximab.*
    - *Rituximab 100 mg per m^2 or placebo IV over 1 hour*
- *Monitoring visits (Every 6 months – weeks 25, 51, 77, 103, 129)*
    - *Review of relapse history*
    - *Review of adverse events*
    - *Review of intercurrent illness*
    - *Review of concomitant medications*
    - *EDSS*
    - *Monitoring visits may be conducted remotely via telehealth whether for reasons of COVID-lockdowns or remoteness of the participant0*
- *MRI (Every 12 months – Baseline, Weeks 51, 103, 155)*
    - *MRI Brain*
    - *Closeout visit (Week 155 or earlier at close of study)*
    - *Review of relapse history*
    - *Review of adverse events*
    - *Review of intercurrent illness*
    - *Review of concomitant medications*
    - *EDSS*
    - *MSIS-29*
- *The timeline of participant involvement and total time commitment per patient in the trial is outlined in Table 3. Participants will also undergo the following procedures as part of normal care for someone receiving alemtuzumab. Admissions to day infusion centre for 8 hours on 5 days in year 1 and 3 days in year 2 for administration of alemtuzumab. They will also undergo monthly blood and urine tests as listed about through any private pathology firm convenient to them as part of the Bloodwatch® (RxMx®, Australia) monitoring program. The additional lymphocyte subset counts will be undertaken as part of the same blood draw and will not involve any additional tubes or time.*
- *Time constraint tolerance on study related procedures will be as follows:*
- *Screening visit, Enrolment, Review visit, EDSS and Screening bloods will be +/- 1 week.*
- *MRI +/- 2 weeks.*
- *Rituximab administration +/- 3 days.*

- *If for clinical reasons the timing of alemtuzumab infusions must be adjusted, the timing of rituximab administration will be adjusted accordingly but the timing of all other study related procedures will remain unaltered.*

Table 3. Summary of patient participation

Procedure	Screen	MRI Brain 1	Enrolment	Course 1	Rituximab 1	6 Month Rev	Rituximab 2	MRI Brain 2	12 Month Rev	Course 2	Rituximab 3	18 Month Rev	Rituximab 4	NRI Brain 3	24 Month Rev	30 Month Rev	MRI Brain 4	36 Month Rev	Time (Hrs)	Total Time (Hrs)
Time (wks)	-4	-1	-1	0	10	26	30	48	50	52	62	78	82	100	104	130	152	154		
Screening Visit	1																		1	1
Clinical Rev			1			1			1			1			1	1		1	0.5	3.5
EDSS	1		1			1			1			1			1	1		1	0.5	4
MRI		1						1						1			1		1	4
Screen bloods	1																		0.25	0.25
Rituximab					1		1				1		1						4	16
															Total participant hours					28.8

Figure 1. RAMBLE Trial Design



Following screening participants will be randomized 2:1 to receive either rituximab or placebo at weeks 10 and 30 after each dose of alemtuzumab. Follow up will be for a minimum of 2 years and a maximum of 4 years. Alemtuzumab administered at Week 1 and Week 53 (yellow arrows). Rituximab/placebo administered at Weeks 10 and 30 in each of Years 1 and 2 (blue arrows). Clinic reviews at -4 and -1 weeks and then every 6 months (black arrows). MRI every year (red arrows).

i. Outcome Measures

- *Primary outcome measure: the frequency of autoimmune adverse events (autoimmune thyroid disease, idiopathic thrombocytopenia, anti-GBM renal disease and any other autoimmune diseases).*
- *Secondary outcome measures:*
  - *Other adverse events, in particular infections*
  - *Measures of alemtuzumab treatment efficacy*
    - *Time to first relapse or new disease activity on MRI*
    - *Time to first relapse*
    - *Annualised relapse rate*
    - *EDSS*
    - *MSIS-29*
    - *Sustained disability progression*
    - *Number of new T2 lesions on MRI*
    - *Number of new Gd-enhancing lesions on MRI*
  - *Measure of lymphocyte reconstitution*
    - *Total lymphocyte count*
    - *CD4, CD8, CD20 counts*
    - *B-cell subpopulation counts.*

j. Data Collection

- *Data will be collected in an eCRF created using a specifically created REDCap databse. This database will be held on Servers maintained by Griffith University and access to the database online will be restricted to study personal at the various sites. Access to the data will be further restricted according to utilisation requirements and for maintaining blinding.*

k. Data Storage and Confidentiality

- *Participants' privacy and confidentiality will be protected through the following:*
- *Participant information and consent forms will be held in dedicated files for each participant at participating sites. These files will be held in a secure location (locked room, which only clinical staff will have access to).*
- *Patient specific data will be stored within medical case note files within medical records departments or in electronic medical record systems at each site. Medical records departments at state health facilities have restricted access that only permits those with requisite authority to have access to the files. Queensland Health uses a statewide integrated electronic medical record system (ieMR, IBM Inc) which is password protected.*

- *This is a clinical trial and for safety reasons (accurate identification of participants) it will be essential that all clinically related records are identifiable. However, all centrally recorded patient information (e.g. eCRF) and correspondence with CRO and regulatory bodies will be de-identified. All such records will use a unique participant identification code. Data connecting participant identifying information (participant folders and medical records) will also carry this code ensuring that all participants are potentially re-identifiable at the individual site. These identifiers will not be removed at each site at any point in case of the urgent need to re-identify a participant for safety reasons (e.g. unblinding of treatment allocation). All safety and statistical analyses will be conducted on fully de-identified data.*
- *In accordance with International publication and regulatory requirements fully de-identified aggregate data and where necessary raw data will be made available for independent verification of results and regulatory processes. These data will be held on servers at Griffith University in password protected files and only released to third parties if required for regulatory purposes or if requested from other researchers after approval by local human research ethics committee (HREC).*
- *Under medical practice guidelines and laws participants will have access to their own clinical data as held in the medical records at each participating site.*

- *All records will be kept for a minimum of 15 years. Paper and electronic documents will be held at each site as part of existing medical record protocols. Trial related documents (participant folders) will be stored locally using secure storage facilities. All central electronic data will be stored on Griffith University servers in password protected files.*
- *There are no plans for secondary use of data or information (e.g. data banking/sharing).*

I. Data Analysis and Statistical Considerations

- *The primary outcome measure for this study will be frequency of autoimmune adverse events. Because the duration of follow up will not be uniform for all participants and in order to make the maximal use of data the frequency of autoimmune adverse events will be modelled using a Cox Proportional Hazards[22] time to event analysis (with onset of autoimmune adverse event as the event of interest). Although randomisation will be stratified for clinical site and sex, these factors as well as age and disease severity (MSSS) will be included in the model. Analysis will be performed on an intention-to-treat basis.*
- *Secondary outcome measures will be analysed as follows:*
    - *Other adverse events (MedDRA[23] terms) will be compared using frequencies and chi-squared statistics.*

- *Efficacy in terms of MS outcomes will be analysed using time to event analysis (Cox Proportional Hazards method) for annualized relapse rate and sustained disability progression (6 months), including the factors listed above for the primary outcome analysis in the model.*
- *Other secondary outcomes (e.g. change in EDSS, new T2 and Gd-enhancing MR imaging lesions) at 1 and 2 years will be assessed using linear regression analysis provided assumptions are met (in which case non-parametric methods will be used).*
- *Any effect of laboratory measures (lymphocyte subsets,) and baseline characteristics will be explored using regression analysis.*

- *All statistical analyses will be conducted using STATA® v16 software (StatCorp®, US). The primary outcome of this trial will be met if the hazard ratio of autoimmune adverse events in the treatment (rituximab) arm is significantly lower than that seen in the placebo arm. The study is unlikely to have sufficient power to identify any significant differences in the frequency of other adverse events or MS disease outcome measures, but these will be looked at carefully to ensure that these outcomes are similar in the two groups.*

Statistical power calculation

- *A previously published sample size calculation based on a Phase I study suggests that a trial of 80 participants (40 in each arm) would have 80% power with $p<0.05$ to detect a reduction in autoimmune adverse events from 40% down to 10%.*[12]
- *Primary analysis will be based on observed data only but a sensitivity analysis where missing data is imputed using maximum likelihood imputation methods will be undertaken.*
- *All statistical analyses will be conducted by our in-house statisticians, Prof Robert Ware and A/Prof Jing Sun.*

4. Translation to Changes in Clinical Practice

- *A successful outcome to this clinical trial would pave the way to more widespread use of alemtuzumab for the treatment of multiple sclerosis. If a significant reduction in the frequency of autoimmune adverse events is seen with the co-administration of rituximab with alemtuzumab this would remove the one major barrier to the use of this highly effective therapy. Such an outcome would remove or significantly reduce the morbidity and risk of serious adverse outcomes associated with autoimmune adverse events. It might even lead to the removal of or a less onerous form of ongoing monitoring for autoimmune adverse events after alemtuzumab administration. More widespread use of alemtuzumab would have considerable benefits to pwMS and healthcare providers. Treatment with alemtuzumab involves two brief courses of treatment 12 months apart and then for many nothing ever again. This compares favourably with all other approved forms of therapy for MS*

which are essentially indefinite or at least until a significantly advanced age is reached (e.g. age 65 years). This means that issues of medication compliance, venous access, treatment fatigue and the time and resource commitments are all reduced. It also means that pwMS can get on with the rest of their life, have a family and not have to worry about ongoing treatment. For health services the overall costs of alemtuzumab are considerably less. The lifetime costs of treatment with the currently approved therapies for MS are compared in Table 4, assuming a mean age of onset of 32 years for relapse-remitting MS and treatment to age 65 years.

Table 4. Comparison of Projected MS treatment costs (data from PBS, Australia)

Treatment	Annual cost	Years of therapy	Total cost
Alemtuzumab	$54,121.50/$32,472.90	2	$86,594.40
Ocrelizumab	$35,161.48	33	$1,160,328.84
Natalizumab	$16,088.16	33	$530,9009.28
Fingolimod	$26,633.40	33	$878,902.20
Siponimod	$26,633.40	23	$612,568.20
Cladribine*	$27,153.60	2 (+?)	$54,307.20
Dimethyl fumarate	$15,500.88	33	$511,529.04
Beta-interferon	$12,018.36	33	$396,605.88
Copaxone	$10,739.76	33	$354,412.08

* = based on 70 kg person

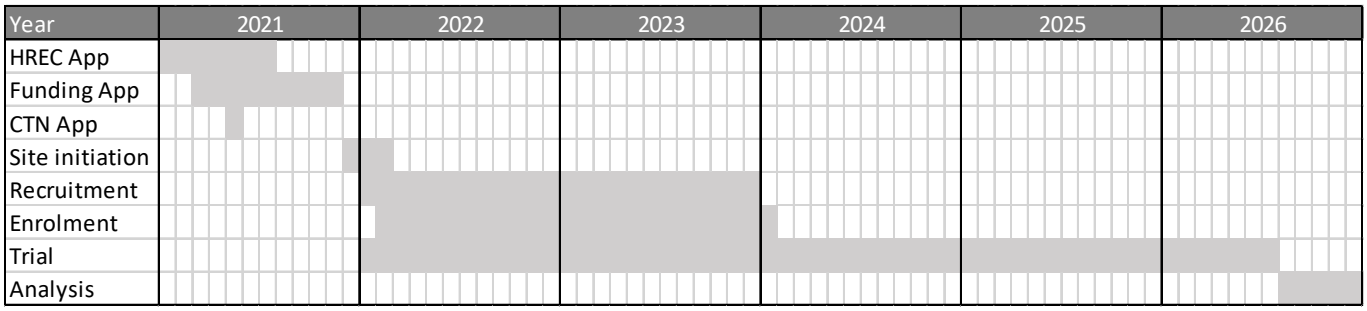
- *This would make alemtuzumab arguably the most effective therapy for MS and the cheapest. Only cladribine is cheaper for the first two years of therapy, but further relapses and additional treatment is required over a 3-year time frame in 60% of patients. Additional treatment with alemtuzumab is required in 30-40% of cases but this treatment is provided for free by the manufacturer in Australia. When compared to all other treatments for MS, across the 25,000 pwMS in Australia this would amount to a cost saving of between $133 and $536 million annually, assuming that 2/3 of pwMS are on treatment.*
- *The outcomes from this research will be disseminated in the following ways:*
- *It is anticipated that the results of this clinical trial will be published in a leading neurology or general medical journal.*

- *CIA is the leading author of the Australian and New Zealand treatment guidelines for MS which are periodically updated. The outcomes of this study will be incorporated into those guidelines.*
- *This trial will be registered with the Australia and New Zealand Clinical Trials Registry and as a Clinical Trial Notification with the Therapeutics Goods Administration.*
- *Findings from this study will likely be presented at local (ANZAN) and International (AAN and ECTRIMS) scientific meetings.*
- *Outcomes from this trial will be presented in lay form through the Griffith University Facebook page.*

5. Timeline

- *It is proposed that this clinical trial will be conducted over a 5-year time span. All necessary regulatory approvals, HREC approval and funding will be sought in 2021, with a proposed start date for the trial in January 2022. Recruitment of participants will span the first 2 years and follow will continue for a minimum of 2 years and 6 months (maximum of 4 years and 6 months). The analysis will be undertaken in the final 5 months. Please see Gant chart of proposed timeline in Table 5.*

Table 5. Gant chart of proposed timeline.



6. Funding and Resources

- *Funding for this project is to be sought in 3 ways:*
- *An application for NMHRC MRFF grant funding to cover the entire project.*
- *Applications to MSRA for both a project grant and a PhD fellowship to fund at least part of the project.*
- *Applications to local funding bodies (e.g. Gold Coast Foundation).*
- *Possible combinations of the above and local hospital funding applications.*
- *Several funding envelopes are to be considered but a minimum of $102,114.16 would be required to enroll 20 participants.*

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