Phase III, multicentre, randomised, double-blinded, placebo-controlled, MAMS trial of SpironolacTone and famciclOvir in the treatment of Progressive MS to prevent disability progression (STOP-MS)

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Sponsor: Griffith University

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).

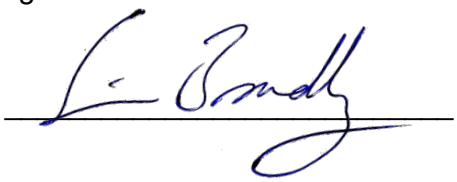
Signature Page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirements.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Principal Investigator:

Signature:  Date: 27 / JUN / 2024

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PLATYPUS Platform Governance

The above team are conducting three nationwide clinical trials in MS. To provide efficiencies of scale and expertise we have determined that a combined Governance structure will be appropriate. We have therefore proposed the "PLATYPUS Platform", which will be administered by MS Australia. We propose that the three clinical trials (PLATYPUS, STOP-MS and FIRMS-EBV) will be overseen by the following committees.

Steering Committee

This committee will provide strategic direction and advice to the Coordinating Chief Investigators (Professor Simon Broadley and Professor Todd Hardy). Several prominent national and international authorities on MS clinical trials have already agreed to be members of this committee.

Professor Pamela McCombe has kindly agreed to chair this committee. Please see Appendix 1 for the terms of reference for this committee.

Data Safety Monitoring Board (DSMB)

This board will review safety reports and adverse event reports for the three trials.

The chair and members for this committee are yet to be selected. Please see Appendix 2 for the terms of reference for this board.

Consumer Engagement Committee

This committee will provide strategic advice on consumer engagement and external representation.

This committee will be constituted wholly by people with MS who will advise on matters relating to participants and external engagement. Professor David Tscharke has kindly agreed to chair this committee. Please see Appendix 3 for the terms of reference for this committee.

Recruitment and Retention Committee

This committee will review recruitment and retention progress in the trials.

This committee will be constituted by both people with MS and others with expertise in MS clinical trials and the MS community. Mr Andrew Potter has kindly agreed to chair this committee. Please see Appendix 4 for the terms of reference for this committee.

Study Synopsis

Title:	Phase III, multicentre, randomised, double-blinded, placebo-controlled, MAMS trial of SpironolacTone and famciclOvir in the treatment of Progressive Multiple Sclerosis to prevent disability progression (STOP-MS)
Short Title:	STOP-MS
Study Sites:	Gold Coast University Hospital, QLD Royal Brisbane and Women's Hospital, QLD Princess Alexandra Hospital, QLD Mater Hospital Brisbane, QLD Sunshine Coast Hospital, QLD Royal Hobart Hospital, TAS Launceston General Hospital, TAS Brain and Mind Centre, NSW Concord Hospital Sydney NSW St Vincent's Hospital Sydney, NSW Royal North Shore Hospital, NSW Westmead Hospital, NSW Liverpool Hospital, NSW John Hunter Hospital, NSW Royal Melbourne Hospital, VIC Monash Medical Centre, VIC Alfred Hospital, VIC Austin Hospital, VIC Box Hill Hospital, VIC Flinders Medical Centre, SA Royal Adelaide Hospital, SA Perron Institute, WA
Study Aims/ Objectives/Hypothesis:	Stage 1: to demonstrate that spironolactone or famciclovir plus SOC reduce the frequency of EBV DNA being present in saliva and/or reduce EBNA1 antibody titres in people with progressive MS when compared to placebo plus SOC. Stage 2: to demonstrate that spironolactone or famciclovir plus SOC reduce the likelihood of 6mCDP in people with progressive MS when compared to placebo plus SOC. Secondary aims - to demonstrate that spironolactone or famciclovir plus SOC: a. are safe when used to treat people with progressive MS b. reduce the rate of brain atrophy at 3 years compared to placebo plus SOC c. reduce the numbers of new/expanded T2/ FLAIR and Gd-enhancing lesions on MRI brain compared to placebo plus SOC d. reduce the level of whole brain atrophy on MRI brain compared to placebo plus SOC e. improve PROMs of disease impact compared to placebo plus SOC. f. are cost-effective.

Study Design:	This is a multicentre, randomised, double-blinded, placebo-controlled, MAMS phase III clinical trial.
Study Outcome Measures:	Stage 1: co-primary outcome measures of salivary EBV DNA detection (viral shedding) and serum EBNA1 antibody titres. Stage 2: time to 6mCDP using a composite of EDSS, T25FW and 9-HPT. Secondary outcome measures: Clinical – time to first relapse, time to 6mCDP using EDSS only, MSFC Score. MRI – new and enlarging lesion counts. PROMs – MSIS-29, MSWS-12, Neuropathic Pain Scale and FSMCF. Health economics – EQ-5D-5L.
Study Population:	People with progressive MS (primary progressive or secondary progressive) either on current DMT or on no treatment.
Number of participants:	Stage 1: n = 150 (3 x 50 per arm) Stage 2: n = 200 (2 x 100 per arm – additional) Total = 350
Translation to Clinical Practice:	If spironolactone or famciclovir prove to be effective in reducing disability progression in people with progressive MS, the research team will apply to the Therapeutic Goods Administration for a new indication (progressive MS) for the relevant drug and prepare new treatment guidelines.
Key Ethical and Safety Considerations:	People with progressive MS are a vulnerable group both in terms of disability level and potential cognitive impairment. This combined with a current paucity of effective treatment options can lead to a sense of desperation. It will be essential to ensure that the study design incorporates safeguards against unrealistic expectations and overly burdensome procedures, as well as ensuring the consent process is informed and inclusive. The two therapies (spironolactone and famciclovir) are considered to be safe, but it will be necessary to monitor their safety in this population over a prolonged period of time. Specific risks include hyperkalaemia, renal failure and gynaecomastia. Both drugs should be avoided in pregnancy and whilst breast feeding.

Glossary of Abbreviations, Terms, and Acronyms

Abbreviation/Acronym	Definition
6mCDP	6-month Confirmed Disability Progression
9-HPT	9-Hole Peg Test
AE	Adverse Event
AI	Associate Investigator
CD8	Cluster of Differentiation 8
CDP	Confirmed Disability Progression
CI	Chief Investigator
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
CTN	Clinical Trial Notification
CV	Curriculum Vitae
DMT	Disease Modifying Therapy
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
EBNA1	Epstein-Barr Nuclear Antigen-1
EBV	Epstein-Barr Virus
eCRF	Electronic Cade Report Form
EDSS	Expanded Disability Status Scale
eGFR	Estimated Glomerular Filtration Rate
EQ-5D-5L	EuroQol - 5 Domains – 5 Levels
EUC	Electrolytes, Urea and Creatinine
FBC	Full Blood Count
FIRMS-EBV	Fatigue In Relapsing Multiple Sclerosis – Epstein Barr Virus
FLAIR	Fluid Attenuated Inversion Recovery
FSMCF	Fatigue Scale Motor and Cognitive Functions
Gd	Gadolinium
HADS	Hospital Anxiety and Depression Scale
HLA	Human Leukocyte Antigen
HREC	Human Research Ethics Committee
ICH-GCP	International Conference on Harmonisation – Good Clinical Practice
IM	Infectious Mononucleosis
IMP	Investigational Medicinal Product
LFT	Liver Function Tests
LTFU	Lost To Follow Up
MAMS	Multi-Arm, Multi-Stage
MRI	Magnetic Resonance Imaging
MRFF	Medical Research Future Fund
MS	Multiple Sclerosis
MSFC	Multiple Sclerosis Functional Composite
MSIS-29	Multiple Sclerosis Impact Scale-29
MSWS-12	Multiple Sclerosis Walking Scale-12
NPS	Neuropathic Pain Scale

PCR	Polymerase Chain Reaction
PI	Principal Investigator
PICF	Participant Information and Consent Form
PIDN	Participant Identification Number
PLATYPUS	PLatform Adaptive Trial for remYelination and neuroProtection in mUltiple Sclerosis
PROM	Participant Reported Outcome Measure
pwMS	Person with Multiple Sclerosis
QALY	Quality Adjusted Life Year
RCN	Randomisation Code Number
SAE	Serious Adverse Event
SDMT	Symbol Digit Modalities Test
SOC	Standard Of Care
SOP	Standard Operating Procedure
T25FW	Timed 25-Foot Walk
TNF	Tumour Necrosis Factor
UK	United Kingdom
US	United States

1. Background

1.1. The Problem

Multiple sclerosis (MS) is a complex autoimmune and neurodegenerative condition which manifests differently in individuals over time. Without treatment, for a staggering 70% of people diagnosed with MS, their symptoms will become increasingly disabling.[1] The commonest form of MS is relapsing remitting (90% at disease onset), where recurrent bouts of symptoms punctuate periods of relative normality.[2] After 10-30 years 60% of these cases will transition to secondary progressive MS, where deterioration occurs regardless of relapses. In around 10% of cases the disease is progressive from the outset, which is termed primary progressive MS. These latter two forms are collectively termed progressive MS. Progressive MS has been likened to terminal metastatic cancer, chronic kidney disease, and severe heart disease due to its substantial impact on an individuals' quality of life.[3] A person with progressive MS can experience many significant symptoms which can greatly impact their quality of life. These include issues with mobilisation and use of legs and/or arms, severe pain, bladder and bowel incontinence, sensory issues, balance issues, loss of vision, spasticity, and issues with cognition. These symptoms can lead to dependency on others for care, social isolation and increased mortality. As people transition from no disability to severe disability, the annual per-person cost for MS more than triples from $30,581 to $114,813, which highlights that disease progression accounts for much of Australia's healthcare expenditure for MS (~$1.75 billion each year). Progressive MS currently affects over 20,000 Australians, and 1.4 million individuals globally. For these people, slowing, halting, or even reversing disability is an urgent priority which – unfortunately – current therapies fail to address.

Dishearteningly, over 40 traditional phase II and phase III clinical trials evaluating putative therapies for progressive MS in the last three decades have yielded mostly negative or mixed results, or been constrained by the toxicity and side-effects of the tested drugs. Further, standard routes to develop new therapies take 10 to 20 years to reach the market – a length of time that people living with progressive MS simply do not have. Importantly, this is not just a generational problem: the total number of people with progressive MS may reduce over time due to improved access to anti-inflammatory therapies early on. However, 15-50% of the newly diagnosed will still experience disability progression, either because they do not respond to current treatments or because they fail to access them early.[4] There is an urgent need for an innovative and rapid new approach to identify and translate safe and effective drugs to treat progressive MS, now and in the future.

1.2. Comprehensive understanding of the cause of MS

MS is known to arise as the result of a combination of genetic and environmental factors. Certain genes including human leukocyte antigen (HLA)-DR1501 and over 200 other genetic loci are known to increase the risk of MS. Relative vitamin D deficiency, lack of sunlight prior to age 15, smoking, obesity and a diet high in saturated fat are all environmental factors that increase the risk of MS. A history of infectious mononucleosis (IM) is more common in people with MS than controls[5] and MS is associated with a later age of IM infection.[6] There is a near linear relationship between age of IM and age of onset of MS with a latency of 10-20 years.[7] Evidence of infection with Epstein-Barr virus (EBV), the cause of IM, is essentially universal in people with MS, compared to being seen in 90% of the general adult

population.[8] MS is associated with higher titres of antibodies to Epstein-Barr nuclear antigen-1 (EBNA1).[9] Recent studies have shown that MS only occurs in people who acquire EBV prior to the onset of their disease.[10] Thus, it appears that EBV is "essential, but not sufficient", to cause MS. This has led to the conclusion that EBV is the primary driver of MS pathology and disease activity. Many members of this experienced clinical trial team have been at the forefront of demonstrating the roles of genes, latitude, vitamin D, diet and EBV in the pathophysiology of MS. This understanding underpins the proposed clinical translation.

Many existing therapies for MS can be postulated to work by either removing EBV-infected B cells (ocrelizumab, alemtuzumab, teriflunomide, cladribine), blocking their entry into the central nervous system (CNS) (natalizumab), trapping them in lymphoid tissue (fingolimod, siponimod, ozanimod, ponesimod), or through upregulating anti-viral responses via γ-receptors (β-interferons). Recent studies of teriflunomide have demonstrated significant reductions in salivary EBV deoxyribonucleic acid (DNA)[11] and EBNA1 antibody titres[12] in people with MS. Importantly this study demonstrated that this effect was seen within 3-6 months of commencing teriflunomide. It is therefore logical to consider existing therapies with known efficacy against EBV as potential treatments for MS.

1.3. The Solution

Our project will harness the potential of removing or reducing the underlying cause of MS (EBV) and deliver on the need for treatments for progressive MS by driving innovation across two core domains of traditional MS research. Firstly, by adopting a pioneering multi-arm, multi-stage (MAMS), design for clinical trials that generates robust results more quickly and cheaply. Secondly, selecting drugs for testing and the trial's outcome measures in a rigorous and systematic way enhances confidence in positive trial outcomes and facilitates their rapid translation into practice. These innovations promise to transform the status quo of research and clinical care for progressive MS.

1.3.1. Innovative and informed approach to trial design

We have formed a group of national and international experts in neurology, virology, immunology, EBV biology, pharmacology, statistics clinical trial design and health economics to optimise the chance of a successful trial. The proposed protocol has been reviewed by international experts in MS clinical trials. We have consulted with a group of people living with progressive MS on the trial design and as a consequence, this will be an "add-on trial" where existing proven therapies for progressive MS will be permitted as standard of care (SOC), ensuring that participants entering the study will not be denied existing proven therapies.

1.3.2. Rationale for adaptive trial design

Despite the identified clear unmet clinical need for effective neuroprotection, which has been prioritised by consumer and professional groups, comparatively few clinical trials aim to modify the disease course of progressive MS. Novel approaches to evaluating multiple treatments concurrently, which incorporate adaptive elements such that they evolve over time to address the most current, relevant questions (sometimes termed 'platform' trial designs) have been highly successful in speeding up the evaluation of therapies in other

disease settings, such as the STAMPEDE trial in prostate cancer[13] and the RECOVERY trial for the treatments of COVID-19.[14] These have led to practice-changing advances.

MAMS adaptive platform designs offer flexible features, which can provide efficiencies at various levels, especially in a setting where there are numerous candidate drugs, which require evaluation. These include:

- simultaneous evaluation of multiple treatments against a common control arm (with efficiencies in terms of both time and the numbers of control participants)
- the ability to add new treatments as they become relevant, reducing the set-up time for new interventions, and dropping treatments that are not showing sufficient promise allowing redirection of resources

In Stage 1, participants are randomised as indicated to investigational product (IMP) or control arms and then each followed for 24 weeks with the primary outcome being measures of EBV activity. Once a participant has had their week 24 visit, they move into Stage 2, until the analysis of Stage 1 is reported. Then a decision will be made whether to continue a trial arm into Stage 2 follow-up or to terminate that arm. Once all participants have been recruited into Stage 1, further participants will be recruited into Stage 2, until at analysis a trial arm continues or is terminated. For Stage 2 clinical outcome measures of progression will be used.

The scientific integrity can be maintained as the overall hypothesis will be consistent through adaptations and the objectives unchanged, with arms being added and dropped on the basis of pre-specified criteria. Utilising an adaptive trial design has significant potential for delivering trials as a rolling programme. On an operational level, this maximises the use of infrastructure established at the start of the trial, thus reducing cost and set-up times, which would be associated with multiple individual trials and would further delay time to results. It also avoids issues of managing competing trials. As such, a MAMS design will provide a structure through which re-purposed and novel anti-viral drugs can be evaluated in a time- and cost-efficient manner in people with progressive MS.

1.4. EBV biology

EBV is a herpes virus that following acute infection (referred to as IM when this occurs in older children and adults), enters a latent phase within B cells that persists for life. It has been demonstrated that EBV-infected B cells can be found in the brains of people with MS[15] and that progressive MS is associated with enrichment of these cells in cortical lesions.[16] EBNA1 antibodies are also known to cross-react with a specific region of GlialCAM[17] and myelin,[18] leading to the generation of autoantibodies directly against these components of the CNS in people with MS. EBV gains access to human tissue via epithelial cell and B cell-specific antigens (gp350, gH/gL, gp42 and gB). The protein gp350 engages with complement receptor 2 on B cells to gain entry. Once EBV has infected B cells the virus goes through a cycle of phases but ultimately ends up in a latent state where additional antigens (including EBNA1) are produced. This latent phase assists EBV in evading the immune system indefinitely. It is known that people with MS have higher levels of antibodies to EBNA1, indicating that T cell responses (the normal mechanism for clearance of intracellular pathogens) may be suboptimal in people with MS. Elevated EBNA1 levels are associated with the risk genotype of several MS risk loci.[19] A significant proportion of MS risk loci exhibit a genotype-dependent expression pattern in EBV-infected B cells[20] and are enriched in the genome binding locations of the EBV-encoded transcription factor EBNA2.[21]

There is evidence that autoreactive, EBV-infected, B cells sequestered within lymphoid tissue within the CNS (pial surface of the grey matter in particular) are a primary driver of continuing low grade inflammation which leads to progressive disability in the absence of relapses in MS.[16] This appears to occur through a combination of slow-burn inflammation and failure of repair mechanisms, mediated via cytokines and chemo-attractants released from activated B cells that are immortalised as a result of latent EBV infection. Imagine then an intervention for progressive MS that targets the primary driver of continued CNS inflammation, degeneration and failure of repair. A number of existing anti-viral and other agents are known to have in vitro and clinical activity against EBV.[22] A key element to any putative therapy in this population will be CNS penetration. The blood-brain barrier is a multi-layered filtering system that normally protects the brain from the passage of large molecules and immune cells. For efficacy against the pial lymphoid tissue, high CNS and blood-brain barrier penetrance would be desirable.

Animal studies have established that HLA-DR15 (the genetic locus with the highest association with MS susceptibility) results in higher viral load.[23] In MS there is evidence for a relative deficiency of CD8+ T cells,[24] cells that are crucial in targeting intracellular pathogens such as EBV. Levels of EBNA1 antibodies correlate with disease activity in MS and appear up to 5 years prior to onset.[10]

A comprehensive review of MS and EBV biology was undertaken in 2022 by this research team (see Appendix 5).

1.5. Systematic approach to drug selection

We have conducted a peer-reviewed process for assessing putative anti-EBV therapies focusing on safety, CNS penetrance and evidence for efficacy against both the lytic and latent phases of the EBV life cycle. Using published reviews of putative agents, we have generated “drug CV’s” (see Appendices 6 and 7) for potential candidates and reviewed with in our Drug Selection Committee which included international experts as well as a group of 6 people with MS. A scoring system incorporating clinical and pre-clinical evidence of efficacy against EBV and in MS, route of administration, brain penetrance, toxicity and tolerability (as judged by people with MS) was used to produce a shortlist of final candidates (manuscript in preparation – see Appendix 8).

The team are aware of drugs specifically targeting the latent phase of EBV (e.g. EBNA1 inhibitors), but none of these are at a stage of development suitable for evaluation in a phase III clinical trial.

1.6. Evidence-based outcome measures

Stage 1 will use EBNA1 antibody titres and frequency of salivary EBV DNA as the co-primary outcome measures. These have been determined to be the best measures of EBV activity.[25] The UK MS Society Expert Consortium for Progression in MS Clinical Trials has undertaken a systematic review of clinical trial design and outcome measures for progressive MS showing that confirmed disability progression (CDP) was the best clinical outcome measure[26] and this will serve as our Stage 2 primary outcome measure. CDP is determined from a validated clinical composite score, derived at 6-monthly intervals using Expanded Disability Status Scale (EDSS),[27] the Timed 25-Foot Walk (T25FW)[28] and the 9-Hole Peg Test (9-HPT).[29] These outcome measures were influenced by both our community

advisory group and prior reviews of progressive MS outcomes undertaken for the UK OCTOPUS trial (see Appendices 9 and 10). In addition, we will use a web-based application (MSReactor) as an exploratory outcome measure (https://msreactor.com/).[30] MSReactor is an Australian developed application that has shown promise as a more sensitive marker of both physical and cognitive impairment using a simple reaction time test which takes only minutes to perform. This will be an optional component of the trial.

MS community engagement: - People with lived experience of MS have been involved in every stage of the trial design for this project via formal consumer advocacy pathways (please see Appendix 11). The research team includes two people with MS (one PI and one Associate Investigator (AI)). Our design meetings have been regularly attended by several people with MS and their input has been actively sought. As outlined above our drug selection committee included people with MS and a subcommittee of just people with MS was established to review tolerability profiles of the drugs being considered. Their input contributed 50% of the drug scores used to establish the final shortlist. People with MS were also consulted in the selection of outcome measures by our UK colleagues.

1.7. Impact

If either agent is effective in reducing the accrual of disability in progressive MS the use of such inexpensive and safe therapies could be transformative for the clinical care of people with progressive MS. In addition, confirmation of the role of EBV in progressive MS would pave the way for a wide range of therapeutic strategies targeting EBV in all forms of MS (e.g. anti-virals, vaccines, T cell therapy) that could drastically change the long-term prognosis for MS.

We propose to run this innovative adaptive MAMS phase III clinical trial to evaluate the effectiveness of promising therapies (spironolactone and famciclovir) which act against Epstein-Barr virus (EBV) in the treatment of progressive multiple sclerosis (MS). This study has been designed in active collaboration with people with MS and facilitates the prospect of rapidly and efficiently repurposing existing approved therapies for the treatment of MS.

The STOP-MS trial meets several aims:

- The STOP-MS trial can be rapidly translated to clinical care as the agents being tested are currently approved for use in Australia and elsewhere (for indications other than MS). We would aim to repurpose these agents for the treatment of MS.
- The STOP-MS trial will provide an opportunity for people with progressive MS to participate in a clinical trial designed specifically for them – this is an opportunity that has barely existed in the past.
- People with MS have been involved in all stages of planning for the STOP-MS, have actively contributed to the design and will be involved in the ongoing running and monitoring of the study.
- We have built upon established collaborations with trial design experts in the US and UK.

If successful the STOP-MS trial will bring a completely new class of therapeutic intervention (anti-EBV therapy) to address the single greatest unmet need for people with MS, namely effective treatments for progressive MS. Additionally, if anti-EBV therapy proves to be effective in progressive MS there is every likelihood that it would be effective in other forms of MS.

1.8. Proposed Trial

We propose a phase III, multicentre, randomised, double-blinded, placebo-controlled, MAMS trial of SpironolacTone and famciclOvir in the treatment of Progressive MS to prevent disability progression (STOP-MS).

Targeting progressive MS addresses the current single biggest unmet need in MS therapy and anti-EBV therapy potentially strikes at the root-cause of progressive disease (EBV-infected B cells in CNS lymphoid tissue).[16] The MAMS trial design aims to efficiently test putative anti-EBV therapies purely on their ability to reduce measures of EBV activity in stage 1 to determine the most likely clinically effective agent. The most effective therapy will then be tested in stage 2 using standard clinical measures of disability as the primary outcome measure. This protocol has been developed in accordance with the Consolidated Standards of Reporting Trials (CONSORT) checklist (Appendix 12).

The trial design has been developed by a large groups of Australian MS clinical and basic science researchers, including input from prominent international collaborators. A summary of the steps in developing this protocol is included in Appendix 13. The trial was also reviewed through the NHMRC granting process and was granted an MRFF grant (see Appendix 14).

Drug selection: - From the final shortlist of tenofovir alafenamide, maribavir, famciclovir and spironolactone from our international expert panel review (see Appendix 15) we have chosen spironolactone and famciclovir for this study in people with progressive MS. Mirabavir and tenofovir alafenamide were excluded based on cost given the long duration of this study.

Spironolactone has been shown to reduce tumour necrosis factor-α (TNFα) levels of lipopolysaccharide-activated microglia by >50%.[31] Spironolactone inhibits EBV replication in the late lytic phase by blocking SM protein.[32] This occurs as a result of inhibition of xeroderma pigmentosum group B-complementing protein, a component of human transcription factor II H which EBV recruits in the transcription of several late lytic antigens.[33] In a case report spironolactone was effective in controlling EBV in a case of non-human immunodeficiency virus, acquired immunodeficiency syndrome.[34] In a pilot study of 9 people with MS (6 with progressive MS) a combination of spironolactone and aldosterone was effective in improving symptoms.[35]

Famciclovir is a prodrug of penciclovir which is then phosphorylated to the active metabolite penciclovir triphosphate. Penciclovir is phosphorylated by herpesvirus thymidine kinase, so this only occurs in herpesvirus infected cells. Penciclovir inhibits EBV replication in cell culture[36] and brain concentrations in rats were 41.5% of the concentration in muscle.[37] The related drug aciclovir has been used for the treatment of severe acute-EBV infection[38] and famciclovir has been successfully used to treat a case of overwhelming IM.[39] Randomised, double-blind, placebo-controlled trials of valaciclovir (the prodrug of aciclovir) have shown a reduction in the number of new active lesions on magnetic resonance imaging (MRI) in those with active disease,[40] and trends towards a reduction in annualised relapse rate and EDSS progression.[41] A randomised double-blind, placebo-controlled trial of aciclovir showed a trend towards reduction in annualised relapse rate which when dichotomised to low or high relapse rate became statistically significant.[42] We have chosen famciclovir on the basis of its greater bioavailability, higher intracellular concentration and greater persistence in infected cells.[43]

Pharmacodynamic and Pharmacokinetic Data

The key pharmacodynamic and pharmacokinetic properties of the two drugs are given in Table 1.

Table 1. Pharmacodynamic Data for Spironolactone and Famciclovir

Measure	Spironolactone	Famciclovir
EBV EC_{50}		1.5 µg/mL[44] (penciclovir)
EBV IC_{50}	0.87 µg/mL[32] (spironolactone) 1.16 µg/mL[32] (canrenone)	5.1 µg/mL[36] (penciclovir)
t_{max}	2.6 hrs[45] (spironolactone) 4.3 hrs[45] (canrenone)	0.9 hrs (penciclovir)
C_{max}		
100 mg PO Daily	0.21 µg/mL[45] (spironolactone) 0.43 µg/mL[46] (canrenone)	
200 mg PO Daily	0.67 µg/mL[47] (canrenone)	
250 mg PO Daily		1.6 µg/mL (penciclovir)
500 mg PO Daily		3.3 µg/mL (penciclovir)
Half-life	12 hrs (canrenone)	2.3 hrs (penciclovir)
Protein binding	90% (canrenone)	<20% (penciclovir)
Excretion	50% Urine, 15% Bile	Urinary
Molecular weight	416.57 g/mol (spironolactone) 340.46 g/mol (canrenone)	321.34 g/mol (famciclovir) 253.26 g/mol (penciclovir)

Data from Australian Product Information Sheets or listed articles

[a] calculated from concentration resulting in >50% reduction of maximal viral replication = 2.5 µM

EBV = Epstein-Barr virus

EC50 = half maximal effective concentration

IC50 = half maximal inhibitory concentration

t_{max} = time to maximum concentration

C_{max} = maximum plasma concentration

Spironolactone

Spironolactone is rapidly converted to canrenone which is the primary active metabolite in terms of the mineralocorticoid effect. There are additional metabolites that also have mineralocorticoid effects. In terms of the anti-EBV effects of spironolactone, only spironolactone and canrenone have been studied. These data indicate that spironolactone itself has a slightly more potent effect against EBV than canrenone, although the difference is marginal.[32] Spironolactone causes degradation of xeroderma pigmentosum group B-

complementing protein (XPB) which is a component of human transcription factor TFIIH, in both B lymphocytes and epithelial cells. Depletion of XPB inhibits EBV SM protein. SM protein acts as a transcriptional activator for 15 late lytic genes that are essential for virion production. In vitro studies suggest that spironolactone and canrenone have IC_{50} concentrations of around 1 µg/mL.[32] However, it should be noted that there are technical difficulties with measuring plasma levels of spironolactone.[45] The peak concentrations of spironolactone (0.21 µg/mL) and canrenone (0.43 µg/mL) after a single dose of 100 mg are are approximately 25% and 40% of their respective IC_{50} concentrations.[32, 46] Some increase in peak concentration (around 30%) is typically seen with repeated daily dosing. Peak concentrations with 200 mg are higher. With a half-life of 12 hours for canrenone a similar effect could be expected with twice daily dosing of 50 mg (equivalent daily dosage).

Famciclovir

Famciclovir exerts its anti-EBV effects through the inhibition of EBV DNA synthesis. For famciclovir the EC_{50} and IC_{50} values for the active metabolite penciclovir are in the range of 1.5 – 5.1 µg/mL. The maximum concentration of penciclovir after 500 mg daily (steady state) of famciclovir (3.3 µg/mL) sits in the middle of this range. The half-life of penciclovir is 2.3 hours and a dose of 500 mg BD is likely to results in slightly higher peak concentrations.

Clinical Data

Spironolactone

Spironolactone was used to treat reactivation of EBV in an immunosuppressed person with MS and non-Hodgkin lymphoma (in addition to ganciclovir), but the dose used was not provided.[34]

Spironolactone 12.5 - 25 mg OD was used to treat EBV related fatigue in chronic fatigue syndrome.[48] This dose was not tolerated in 5/21 (24%), but this is a group that may be particularly sensitive to the hypotensive effects of spironolactone.

Doses of 25 – 200 mg per day have been used in young women to treat PCOS for 6-12 months and have been found to be safe.[49]

A dose-doubling analysis of the effects of spironolactone on various measures indicates the following mean changes in serum potassium levels:

25 mg Daily = +0.22 mmol/L

50 mg Daily = +0.35 mmol/L

100 mg Daily = +0.50 mmol/L

Famciclovir

Famciclovir at doses of 125 - 1000 mg per day have been found to be safe and well tolerated for durations up to 12 months.[50-53]

Famciclovir 500 mg tds was used to successfully treat severe IM in an immunocompetent person.

A recent study, which is yet to be peer reviewed, has shown that famciclovir 500 mg bd did not show a statistically significant difference in EBV salivary shedding or EBNA1 antibody titres, but the duration of this study was only 12 weeks.[54] This dose was not tolerated in 6/30 (29%) of cases.

The TGA approved dose for suppression of genital herpes in people with HIV is 500 mg bd and can be taken indefinitely. Several trials have demonstrated the efficacy and safety of famciclovir for the suppression of genital herpes in adult populations at doses ranging from 125 mg bd up to 500 mg bd for prolonged periods of time (up to one year).[1-3] A recent phase II clinical trial of famciclovir at a dose of 500 mg bd for 12 weeks in people with multiple sclerosis showed a reassuring safety profile.[4]

Final Dosage Selection

The proposed dose of spironolactone (50 mg BD) is likely to result in plasma levels that are in the order of 25-50% of the IC_{50} for spironolactone and canrenone. It is likely the effective of these would be added (75% of IC_{50}) and other metabolites may also contribute additional anti-EBV effects. This dose has been well tolerated in other settings outside of the main indications for spironolactone. Therefore 50 mg BD represents the best compromise of potential anti-EBV efficacy and tolerability. The proposed dose of famciclovir (500 mg BD) results in plasma levels that are within the predicted EC_{50}/IC_{50} range for penciclovir and therefore has the potential to be effective against EBV. This dose has been well tolerated in prior studies over long periods (up to 12 months) for current indications, although the same dose was less well tolerated in a recent cohort of people with MS. For both spironolactone and famciclovir we have proposed a protocol of commencing with half-dose therapy and then increasing to the full-dose only if well tolerated and all safety parameters are satisfactory. The inclusion of an option to remain on half-dose, or return to half-dose in the event of tolerability issues, provides some reassurance that tolerability can be optimised. This also reflects would likely be any future real-world use of these agents.

Add-on Study: - A clear message from our Consumer and Community Reference Committee was the need to ensure that the study would be open to as many people with progressive MS as possible and that enrolment should not be precluded by being on any existing MS therapy. The control arm has therefore been designed to ensure that participants will still have access to appropriate therapies currently available for progressive MS; current SOC. Thus, the control arm will be SOC plus placebo and the active arms will be SOC plus spironolactone or famciclovir.

1.9. Regulatory Approval

1.9.1. Trial Registration

The STOP-MS trial has a World Health Organisation, Unique Trial Number (U1111-1293-1787) and has been registered with the Australia and New Zealand Clinical Trial Registry

(ANZCTN12623000849695p). A copy of this protocol is available at: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=386167&isReview=true

The trial has been notified under the Clinical Trial Notification (CTN) scheme with the Therapeutic Goods Administration (CT-2023-CTN-03505-1-v1).

1.9.2. Human Research Ethics Committee Approval

The STOP-MS trial will seek ethics approval from the lead Human Research Ethics Committee (HREC), Gold Coast Health. Additional HREC approval will be sought for any sites not included in the Ethics Research Management (ERM) system.

1.9.3. Governance approval

Site Specific Approval (SSA) will be sought at each participating site either through the ERM system directly or via separate applications.

2. Study Objectives

2.1. Research Question and Aims/Objectives

2.1.1. Primary Aims/Objectives

2.1.1.1. Stage 1

The primary aim of stage 1 of this trial will be to demonstrate that spironolactone or famciclovir plus SOC reduce the frequency of EBV DNA being present in saliva and/or reduce EBNA1 antibody titres in people with progressive MS when compared to placebo plus SOC.

2.1.1.2. Stage 2

The primary aim of stage 2 will be to demonstrate that spironolactone or famciclovir plus SOC reduce the likelihood of 6-month CDP (6mCDP) in people with progressive MS when compared to placebo plus SOC.

2.1.2. Secondary Aims/Objectives

The secondary aims will be to demonstrate that spironolactone or famciclovir plus SOC:

- Are safe when used to treat people with progressive MS.
- Reduce the rate of brain atrophy at 3 years compared to placebo plus SOC.
- Reduce the numbers of new/expanded T2/FLAIR and Gadolinium (Gd)-enhancing lesions on MRI brain compared to placebo plus SOC.
- Reduce the level of whole brain atrophy on MRI brain compared to placebo plus SOC.
- Improve participant-reported outcome measures (PROMs) of disease impact compared to placebo plus SOC.
- Are cost-effective.

2.2. Hypotheses

The hypotheses that the STOP-MS trial aim to address are:

1. Spironolactone or famciclovir plus SOC will reduce the frequency of salivary EBV shedding or EBNA1 antibody titres in people with progressive MS when compared to placebo plus SOC.
2. Spironolactone or famciclovir plus SOC will reduce the likelihood of 6mCDP in people with progressive MS when compared to placebo plus SOC.
3. Spironolactone or famciclovir plus SOC will be associated with a similar rate of adverse events compared to placebo plus SOC when used in people with progressive MS.

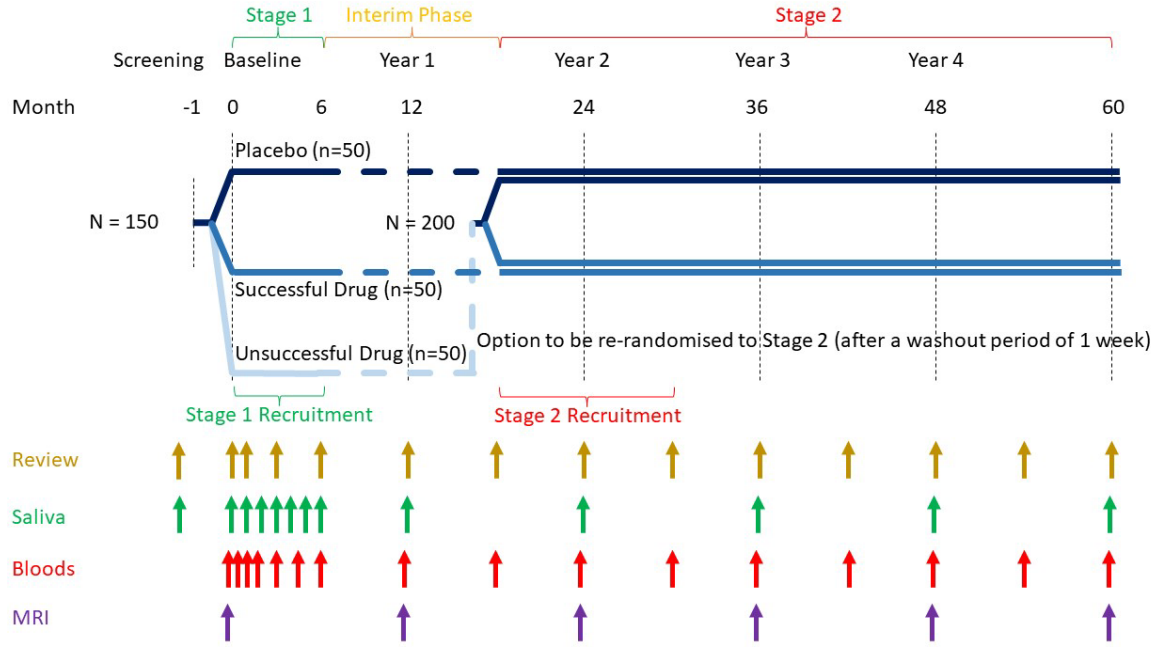
4. Spironolactone or famciclovir plus SOC will reduce the numbers of new/expanded T2/FLAIR and Gd-enhancing lesions on MRI brain when compared to placebo plus SOC (this hypothesis is subject to additional funding).
5. Spironolactone or famciclovir plus SOC will be associated with reduced whole brain atrophy on MRI brain when compared to placebo plus SOC.
6. Spironolactone or famciclovir plus SOC will be associated with improvement in PROMs when compared to placebo plus SOC.
7. Spironolactone or famciclovir will be cost effective in reducing disability accrual in progressive MS.

3. Methods

3.1. Methodological Approach

STOP-MS will be a MAMS trial designed to yield effective therapies for progressive MS as quickly as possible. It will be a phase III clinical trial of spironolactone and famciclovir against placebo in preventing disability progression in MS. The study will be conducted in two stages. In stage 1, which will last 6 months, the co-primary outcome measures will be presence of salivary EBV DNA and serum ENBA1 antibody titres. In stage 2, provided minimum criteria for evidence of anti-EBV efficacy, the best performing agent from stage 1 will be translated into a single treatment arm study against placebo with 6mCDP being the primary outcome measure. A summary of the study design is given pictorially in Figure 1.

Figure 1. Summary of Study Design



3.2. Study Sites/Settings

A total of 22 participating clinical sites across five states of Australia will participate in this study (Gold Coast University Hospital, Royal Brisbane and Women's Hospital, Princess Alexandra Hospital, Mater Hospital Brisbane, Sunshine Coast Hospital, John Hunter Hospital, Royal North Shore Hospital, Brain and Mind Centre, Westmead Hospital, Liverpool Hospital, Concord Hospital (Sydney), Monash Medical Centre, Alfred Hospital, Austin Hospital, Royal Melbourne Hospital, Box Hill Hospital, Royal Adelaide Hospital, Flinders Medical Centre, Royal Hobart Hospital, Launceston General Hospital, Perron Institute). Each site would be expected to recruit 5-10 participants over 6 months in stage 1 and 5-15 participants over 12 months in stage 2. These sites all have extensive experience of investigator-initiated and commercially sponsored clinical trials (this team worked together on the PREVANZ trial [ACTRN12612001160820] – the largest clinical trial of vitamin D in MS). We have chosen a per patient funding model to most efficiently reflect the actual costs for sites and to facilitate recruitment.

3.3. Study Population

Potential participants with progressive MS will be recruited from MS Clinics at the participating sites. The following inclusion and exclusion criteria will be applied:

3.3.1. Inclusion criteria

- Age 25-70 years (inclusive)
- Diagnosed with primary or secondary progressive MS according to McDonald 2017 criteria[55]
- EDSS[27] of 4.0 – 8.0 (inclusive) at the time of randomisation
- Evidence of disability progression over the previous 24 months
- English speaking or non-English speaking but 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

3.3.2. Exclusion criteria

- A clinical relapse within 3 months of randomisation
- A significant co-morbidity that in the opinion of the principal investigator (PI) would negatively affect MS disease outcomes or preclude administration of spironolactone or famciclovir (including renal failure; estimated glomerular filtration rate < 30ml/min)
- Currently taking medication or supplements known to cause hyperkalaemia as listed in Appendix 16
- Hypersensitivity to spironolactone or famciclovir
- Female participants who are pregnant
- Female participants who are breast-feeding
- Women of childbearing potential who are unwilling or unable to use an acceptable method of contraception (see Appendix 17) whilst on trial treatment and for up to 30 days after the last dose of study drug
- Have received treatment with steroids (intravenous and/or oral) for MS relapse/progression within 3 months before randomisation
- Have received any trial therapy within the last 6 months (other than as part of the STOP-MS Stage 1 trial)
- Recent or current history of major depression, bipolar disorder, psychosis or suicidality
- Currently or recently taking any illicit substances (excluding cannabis products used for symptomatic relief)

3.4. Recruitment/Selection

Potential participants will be recruited through the MS or CNS inflammatory disease clinics of the 22 participating hospitals with a cap of 40 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. travel and parking costs).

Potential participants will be identified by neurologists at each site, who will provide an introduction and overview of the study and an invitation to review more detailed information. Those potential participants who are interested will be provided with the Participant Information and Consent Form (PICF) to take away and read, and a further screening visit will be arranged at a later date when the participant will have the opportunity to ask questions and have all queries answered by the investigators, before indicating their understanding and providing written consent.

Participants will be enrolled in the study at the screening visit provided they:

- Have given written informed consent
- Meet all the inclusion criteria
- Meet none of the exclusion criteria

3.4.1. Aboriginal and Torres Strait Islander Peoples

Owing to the rarity of MS in Aboriginal and Torres Strait Islander Peoples, it is considered unlikely that anyone of these populations is likely to be recruited to the STOP-MS trial. However, if this were to eventuate, the following will be implemented to ensure culturally safe practices:

(i) All site staff will be required to be up to date with all locally mandated cultural safety training.
(ii) Local Aboriginal and Torres Strait Islander Health Support Services will be approached for guidance and support.

3.4.2. Randomisation

Stage 1: Participants will be randomised at enrolment to treatment with spironolactone, famciclovir or placebo at a 1:1:1 ratio. Randomisation will be performed using the randomisation module of the REDCap® database based on a random number sequence produced in Excel®, Microsoft (Seattle, CA, USA) stratified by sex and age. This randomisation sheet will be generated by Assoc/Prof Jing Sun (statistician). There will a cap of 30 participants for any single site in Stage 1. The random sequence spreadsheet will be used by the REDCap® database to produce a unique randomisation code number (RCN). A copy of the randomization sequence spreadsheet will be held at each participating pharmacy and used to prescribe study treatment.

Stage 2: Participants will be randomised to either the successful treatment arm from stage 1 (spironolactone or famciclovir) or placebo at a 1:1 ratio. The same randomization process as above will be utilised but using a second randomisation schedule with just two treatment allocation options (treatment or placebo). There will be a cap of 40 participants for any single site in phase 2.

3.4.3. 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 Identification Number (PIDN). The PIDN will consist of the participants three initials (using X for middle initial if no middle name), the site number and a unique number assigned by the REDCap® database. At the baseline/enrolment visit, after eligibility is confirmed and the full consent form has been signed, the PIDNs will be randomly assigned to intervention or placebo groups in REDCap® by the randomiser. Treatment allocation codes will only be visible through REDCap® to site pharmacists, who will make up the active treatment and placebo, and to the randomiser.

To maintain study blinding, all tablets will be over-encapsulated, so they all look the same.

3.4.4. Labelling of IMP

The IMP will be delivered from the manufacturer with both a permanent blinded label indicating the trial details, batch number, dose (half strength or full strength) and expiry date, and a detachable unblinded label indicating the specific IMP and dose (in mg). The on-site pharmacist will take the participants RCN and use this to select the next bottle of appropriate IMP, according to the randomization schedule, and affix a blinded label indicating the participants identifying details (PIDN, name, date of birth, study details) and the possible IMP (e.g. “Spironolactone, Famciclovir or Placebo”). The detachable unblinded label will be removed and attached to the prescription request for documentation purposes.

3.4.5. 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 Coordinating Chief Investigator (or delegate) before breaking the blind. The randomisation code will be stored electronically at the Griffith University by the study statistician. If the blind is broken for a participant, the time, date, participant number and reason for opening must be documented.

3.4.6. On completion of the study

Unblinded study data will only be available once all data collected have been entered into the electronic Care Report Form (eCRF) for every participant and the database has been finalized (locked), except in the case of an emergency, as detailed above.

3.5. Consent

The PICF provides details of the clinical trial and 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 specified that any decision

to participate or not, will in no way affect subsequent MS care at the treating facility for the potential participant.

At the screening visit prior to any study-related activity, the site investigator will review the details of the study, assess the potential participants 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.

3.6. Risk Mitigation Procedures

There are five principal risks of physical harm for participants in the proposed trial:

1. Risk of death from hyperkalaemia - spironolactone can cause hyperkalaemia (see Appendix 18). This principally occurs in three settings:

a) A pre-existing medical condition known to cause hyperkalaemia (chronic kidney disease, uncontrolled diabetes, congestive cardiac failure, hyperaldosteronism, congenital adrenal hyperplasia, Addison's disease, parathyroidectomy).

b) Renal impairment with an eGFR <30 ml/min.

c) Concomitant use of medications/supplements known to cause hyperkalaemia (carenoate potassium, eperenone, dropirenone, captropril, enalapril, lisinopril, candesartan, losartan, ibuprofen, naproxen, diclofenac, meloxicam, enoxaparin, amiloride, triamterene, trimethoprim, pentamidine, propranolol, atenolol, metoprolol, bisoprolol, digoxin, lithium, potassium chloride, epoetin alfa, epoetin beta, alfalfa, dandelion, horsetail, Lily of the Valley, milkweed, nettle, muscle-building supplements, salt-substitutes, diets high in potassium – for a full list of medications and supplements that can cause hyperkalaemia with trade names see Appendix 16).

2. Coincidental development of renal impairment causing:

a) Hyperkalaemia with spironolactone.

b) Confusion and drowsiness with famciclovir.

3. Development of gynaecomastia in males taking spironolactone, the risk of which may be related to dose and duration of therapy (see Appendix 19)

4. Spironolactone is a diuretic and therefore worsen or bring out for the first time symptoms of bladder dysfunction in people with MS. This might include increase frequency, nocturia, urgency and incontinence.

5. Spironolactone is potentially harmful to the foetus in pregnancy (category B3) and to the child during breast feeding. Famciclovir has been shown to be safe in animals during pregnancy and lactation but there is insufficient data in humans.

We propose the following mitigation strategies for these risks:

A. To reduce the risk of hyperkalaemia:

a) All PIs and delegates will be required to undergo training with regards to clinical impacts of hyperkalaemia, this will include (but is not limited to), causes of hyperkalaemia, clinical consequences of hyperkalaemia, ECG changes of hyperkalaemia (see Appendix 20), acute management of hyperkalaemia (see Appendix 21) and monitoring for renal impairment.

In brief the treatment of hyperkalaemia will be as follows:

(i) Serum potassium of 5.0 -6.0 mmol/l – urgent ECG to look for signs of hyperkalaemia – if present treat as for (ii) below; repeat potassium level and check renal function (creatinine and eGFR); check for acidosis; full blood count to exclude haemolysis; remove all agents causing hyperkalaemia; remove potentially cardiotoxic agents (e.g. digoxin, lithium); treat any identified underlying cause of hyperkalaemia; ensure adequate hydration; Resonium A if hyperkalaemia confirmed (oral or PR).

(ii) Confirmed serum potassium >6.0 mmol/l or if ECG changes present – in addition to the above, IV calcium gluconate (stabilises cardiac membranes); IV insulin and glucose; consider nebulised salbutamol, sodium bicarbonate if acidotic, frusemide if fluid overloaded, intravenous calcium chloride (via central line) and dialysis. This level of care should be provided in a suitable environment (e.g. emergency department, high dependency unit or intensive care unit).

b) All the conditions in 1 a) and drugs/supplements in 1 c) above will be exclusion criteria for participation in the trial.

c) Participants will be provided at enrolment with a laminated card indicating the list of drugs and supplements in 1 c) above that should be avoided for the duration of the trial (see Appendix 22).

d) Participants will be advised to indicate their involvement in the trial when being prescribed any new medications and in particular, if being prescribed antibiotics (trimethoprim) or pain medication (NSAIDs), and if purchasing NSAIDs over the counter (see Appendix 23 and Appendix 22).

e) Participants will be recommended to check the list of complimentary medicines and supplements (see Appendix 22) when choosing to start any such products and consult with site staff if in doubt.

f) All participants will undergo an ECG at the initial screening visit and ECG features of hyperkalaemia (peaked T waves, widening/flattening of P waves, PR prolongation, bradyarrhythmia, conduction block, QRS widening) or an increased risk of death from hyperkalaemia (sinus bradycardia, AV block, slow junctional rhythm, slow AF, bundle branch block, fascicular block) are exclusion criteria to participation in the trial.

g) All participants will undergo baseline testing for serum potassium level and a level >5.0 mmol/L is an exclusion criteria for participation in the trial.

h) All participants will undergo baseline testing of eGFR and a level <30 ml/min is an exclusion criteria for participation in the trial.

i) All participants will undergo testing for serum potassium and eGFR at weeks 1, 2, 6 and 12 during treatment initiation and dose escalation phases and every 12 weeks for the duration of the trial, a potassium level >5.0 mmol/L or eGFR <30 ml/min if confirmed to not be a spurious result will be actively managed and will result in withdrawal of the participant from the trial, except where remediable alternative explanation can be identified.

j) A serum potassium level >5.0 mmol/L will be managed with an urgent ECG to check for signs of hyperkalaemia and a repeat serum level. If confirmed, the hyperkalaemia will be managed as per recommended protocols.

k) An eGFR <30 ml/min will result in a repeat level, and if confirmed as <30 ml/min will result in withdrawal from the trial.

l) Persistently rising serum potassium levels (still within normal limits) and falling eGFR (>30 ml/min) will be managed as deemed appropriate by the treating physician through any combination of: removal/avoidance of potential precipitants; more frequent testing; reduction to half-dose treatment; and/or withdrawal from the study.

m) If for medical or other reasons a participant is required to commence on one of the contraindicated medications, one of the following outcomes will be determined by the treating neurologist in consultation with the participant and advice from any relevant treating specialist:

(i) Cease IMP.
(ii) Reduce to half-dose IMP with close monitoring of potassium levels (2, 4, 8 and 12 weeks after introduction of contraindicated medication).
(iii) Continue IMP at full dose with close monitoring of potassium levels (2, 4, 8 and 12 weeks after introduction of contraindicated medication).

If IMP is ceased the participant would be encouraged to continue in the study and undertake the annual outcome assessments, but they will be offered the opportunity to withdraw if they so choose.

B. To reduce the potential impacts of renal impairment.

a) An eGFR <30 ml/min at screening is an exclusion criteria for the trial.

b) A confirmed (confirmed at repeat testing) eGFR <30 ml/min at any time point in the trial will result in withdrawal of the participant.

c) Falling eGFR (>30 ml/min) will be managed as deemed appropriate by the treating physician through any combination of: removal/avoidance of potential precipitants; more frequent testing; reduction to half-dose treatment; and/or withdrawal from the study.

d) Any adverse events of confusion, drowsiness will be investigated with an urgent check of renal function and electrolytes.

C. To reduce the risk of gynaecomastia:

Gynaecomastia is a recognised adverse effect of spironolactone and we are not aware of any strategies to reduce this risk. Our approach has been to consider the risks and review these with our consumer advisory group.

a) Our panel of people with MS were reassured by the fact that this complication of spironolactone therapy is usually reversible on withdrawal of the treatment.

b) The dose of spironolactone chosen is a compromise between the dose anticipated to have an adequate anti-EBV effect (based on animal studies) and dose that has a lower risk of gynaecomastia. An analysis of dose response curves and the effect of duration of therapy from a published meta-analysis[56] suggests a peak incidence rate of around 10% of men at 3 years for the 100 mg per day dose planned for this current trial (see Appendix 24).

c) All PIs will be trained in the clinical assessment of gynaecomastia, with a recommendation for immediate cessation of the trial medication in the case of gynaecomastia being detected.

b) MS predominantly affects women, with a sex ratio of 4:1 (females:males), although this may be closer to 3:1 for progressive forms of MS. In Stage 1 a total of 50 people with MS will be treated with spironolactone. Approximately 10-15 will be male. From the above estimate of risk, we might expect to see 1 case of gynaecomastia in Stage 1. If spironolactone is the drug chosen to move into Stage 2 of the trial 150 people with MS will receive this drug. Therefore the total number of males that may be exposed to spironolactone would be 30-45, meaning that approximately 3-5 cases of gynaecomastia might occur.

D. Participants will be warned of the risks of increased urinary symptoms and asked to report these. Severe symptoms may require an adjustment of dosage or withdrawal from the study.

E. To reduce the potential risks of pregnancy or breast feeding we propose the following:

a) Pregnancy and current breast feeding will be exclusion criteria for participation in the trial.

b) Women of childbearing potential will undergo a pregnancy test (serum βHCG) at screening and will be excluded if this is positive.

c) Women of childbearing potential will be required to agree to using an acceptable form of contraception for the duration of trial and for a period of 30 days after ceasing any IMP. Unwillingness or inability to comply with this will be an exclusion criteria to participation in the trial.

There is no evidence that spironolactone or famciclovir taken by males is associated with any risk to a foetus conceived by them.[57] Therefore, only women of child-bearing age will be required to use contraception during the STOP-MS trial.

A list of currently approved therapies for MS and other commonly prescribed symptomatic therapies, and their potential interactions with spironolactone and famciclovir is given in Table 1 (source MIMS Online). Neither spironolactone or famciclovir have an effect on cytochrome P450 isoenzymes.

Spironolactone may interfere with the elimination of digoxin leading to potentially increased toxicity.[58] Spironolactone may also interfere with digoxin assays making interpretation of serum levels.[58] Digoxin has therefore been included on the list of contraindicated drugs.

In addition to the physical harms listed above, the following ethical risks have been considered:

1. People with progressive MS can be in a state of desperation, having tried available therapies and yet still progressing or not being eligible for existing therapies. In addition, the prospect of a new “clinical trial” might be seen as offering more than it can realistically deliver.

Table 1

MS Therapy – generic (trade names)	Spironolactone	Famciclovir
β-interferon 1B (Betaferon)	–	–
Peginterferon-β 1A (Plegridy)	–	–
Glatiramer acetate (Copaxone)	–	–
Teriflunomide (Aubagio)	–	–
Dimethyl fumarate (Tecfidera)	–	–
Diroximel fumarate (Vumerity)	–	–
Fingolimod (Gilenya)	–	–
Siponimod (Mayzent)	–	–
Ozanimod (Zeposia)	–	–
Cladribine (Mavenclad)	–	–
Natalizumab (Tysabri)	–	–
Ocrelizumab (Ocrevus)	–	–
Ofatumumab (Kesimpta)	–	–
Alemtuzumab (Lemtrada)	–	–
Oxybutynin (Ditropan, Oxytrol)	–	–
Solifenacin (Vesicare)	–	–
Mirabegron (Betmiga)	–	–
Sildenafil (Viagra)	–	–
Amitriptyline (Endep)	–	–
Duloxetine (Dytrex, Duloxecor, Tixol, Cymbolta)	–	–
Citalopram (Celapram, Talam, Cipramil)	–	–
Baclofen (Clofen, Lioresal, Stelax)	–	–
Clonazepam (Rivotril, Paxam)	–	–
Modafinil (Modafin, Modavigil)	–	–
CBD Oil (Sativex)	–	–
Gabapentin (Neurontin, Gabacor, Nupentin)	–	–
Pregabalin (Lyrica, Lypralin, Lyzalon, Neuroccord)	–	–
Carbamazepine (Tegretol	–	–
Famridine (Fampyra)	–	–
4-aminopyridine	–	–

"-" = no interaction

"+" = interaction

In view of this, site PIs will carefully counsel potential participants with regards to the experimental nature of the STOP-MS trial and ensure that their expectations are realistic. It will be important to ascertain that potential participants are not waiving opportunities to access therapies of known potential benefit based on their preference to enter into a clinical trial. Participants already taking any existing proven SOC therapy for MS, will be permitted to participate in the STOP-MS trial. Whilst it would be preferred that this therapy not be changed for the duration of the trial, a change of therapy to any other approved SOC therapy for MS will be permitted.

2. People with progressive MS are, by definition, significantly affected in their activities of daily living to a lesser or greater degree. This may add additional burdens upon participants.

Site PIs will discuss fully with participants the specific issues that they may face through enrolment in the STOP-MS clinical trial (e.g. need to travel for regular visits and assessments).

3. MS rarely causes cognitive impairment to such a degree that capacity to provide informed consent is affected. However, those with progressive MS are most at risk of this.

Site PIs will carefully assess capacity according to recommended criteria. Namely that the potential participant understands the nature of the trial, what alternatives might be available, that they can withdraw at any time, what the potential side effects of the investigational products are, what the practical requirement for participation are, and that they do not have any significant mental health issues.

3.7. Participant Withdrawal Procedures

3.7.1. 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 study drug. 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 eCRF in order to meet the CONSORT publishing requirements,[59] respond to queries from regulatory authorities and to ensure transparent reporting of screen failure participants.

3.7.2. Lost to Follow Up

Investigational staff will go to all reasonable 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 withdraw due to study burden, the trial coordinator and site nurse will make reasonable attempts to reduce trial duties for the participant, with a focus on continued collection of data relating to the primary outcome. If the participant wishes to withdraw for health reasons (such as intolerable side effects), the final assessment visit will be brought forward.

3.7.3. Stopping Rules

Participants may withdraw from the study at any time for any reason with no impact on any future care they may require from the recruitment site or study staff. Participants may also 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 can

remain in the trial and will be followed-up through to the end of the study.In the event of a participant withdrawing from the trial an end of study visit should be organised if possible. The participant should be asked to sign the withdrawal of consent section of the PICF and should be asked to indicate their preferences in terms of data and biobank samples collected to that point as included on the withdrawal of consent form. If they are happy to, participants should also be asked to indicate the reason for withdrawal but they are not obliged to do so.

3.8. Study Procedures

3.8.1. Treatment arms

Stage 1

- Arm 1: Spironolactone + SOC
- Arm 2: Famciclovir + SOC
- Arm 3: Placebo + SOC

Stage 2

- Arm 1: Successful active drug + SOC
- Arm 2: Placebo + SOC

3.8.1.1. Intervention Description, Dosage and Route of Administration

SOC plus Spironolactone: - Spironolactone 50 mg twice daily administered orally.

Spironolactone is approved for the treatment of essential hypertension, congestive heart failure, cirrhotic liver disease and nephrotic syndrome. It is contraindicated in renal insufficiency, hyperkalaemia and pregnancy. Agents that can cause hyperkalaemia should not be used in conjunction with spironolactone. Adverse events include gynaecomastia which is usually reversible on discontinuation, gastrointestinal upset, drowsiness and allergic reactions (see Appendix 18). For these reasons we have chosen a medium dose of spironolactone, screen carefully as outlined above and monitor throughout the study as outlined below. Dose escalation will also be used to minimise potential adverse events (see below).

SOC plus Famciclovir: - Famciclovir 500 mg twice daily administered orally.

Famciclovir is a synthetic nucleoside analogue and is approved for the treatment and prevention of herpes zoster and herpes simplex infections (shingles, genital herpes, herpes labialis and herpes encephalitis). Common adverse effects include headache and nausea. Renal impairment is a contraindication (see Appendix 19). We have chosen the chronic treatment dose to ensure adequate CNS penetrance and participants will be screened for renal failure before proceeding to study randomisation.

SOC plus Placebo: - Matched placebo capsules twice daily administered orally.

3.8.1.2. Blinding

The two investigational products and placebo will be manufactured as over-encapsulated capsules that will be indistinguishable from each other and the placebo.

3.8.1.3. Concurrent MS therapies

Participants will be permitted to continue, start or discontinue any currently approved SOC therapy for their form of MS.

3.8.1.4. Dose escalation

Both active study interventions will be commenced at half dose (spironolactone 25 mg twice daily and famciclovir 250 mg twice daily) for the first 4 weeks. Subject to satisfactory review of adverse events and laboratory investigations, dosage will be increased to the full dose at 4 weeks. In the event of concern regarding adverse events or changes in laboratory parameters there will be options to either, (1) discontinue the study intervention or (2) continue with the half-dose. If all parameters are satisfactory then the dose will be escalated to the full dose for both active treatment arms. Bottles of placebo will be marked as either half-dose or full dose, but will contain identical capsules.

3.8.1.5. Dose reduction

If during the follow up phase of both Stage 1 and Stage 2 of the STOP-MS trial the site PI suspects that a participant may be experiencing clinical or laboratory parameter IMP-related adverse event (AE) then they can elect to:

- Cease the IMP
- Reduce the dose of the IMP to half-dose for the remainder of the study.

In the case of IMP cessation, participants will be encouraged to remain in the study and complete all remaining visits and assessments as per the protocol so that their complete data can be used in the intention-to-treat analysis.

3.8.2. Study Drug Accountability

A nominated pharmacist at each participating site will maintain an inventory of receipt, use, return and collection of study drug.

3.8.2.1. 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 REDCap® database. 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 PI.

3.8.2.2. Return of Supplies

At the conclusion of the trial, the Principal Investigator (or delegate) 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 original version of the product accountability and dispensing logs must be sent to the primary trial coordinator. The Principal Investigator will retain copies of these logs on file.

3.8.2.3. 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 International Conference on Harmonisation – Good Clinical Practice (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.

3.8.3. Study Procedures and Visits

Participants will be required to undergo the following study procedures and visits.

Screening Visit (-28 to -7 days)

- Written informed consent will be provided
- Comprehensive review of medical history including; details of MS history, other past medical and surgical history, , family history, social history and allergies
- Review of past and current medications and health supplements
- Comprehensive medical and neurological examination
- EDSS[27]
- MSReactor computerised cognitive assessment (optional)[30]
- Hospital Anxiety and Depression Scale (HADS)[60] – administered online through REDCap®
- Blood tests – full blood count (FBC), electrolytes, urea creatinine (EUC), eGFR, liver function tests (LFT)
- EBNA1 titres
- Pregnancy test (for women of child-bearing age)
- Collection and storage of DNA sample
- Collection and storage of serum sample

- Saliva collection for EBV DNA polymerase chain reaction (PCR) quantification
- MRI Brain (as routine standard of care – non-study protocol scan – see below for time constraints for MRI)

Enrolment Visit (Day 0)

- Review of neurological symptoms/relapse
- Review of concomitant medications and supplements
- EDSS[27]
- MS Functional Composite (MSFC)[28] - including T25FW, 9-HPT and SDMT
- MSReactor computerised cognitive assessment (optional)[30]PROMs – all PROMs will be administered online through REDCap®
    - Multiple Sclerosis Impact Scale-29 (MSIS-29)[61]
    - Multiple Sclerosis Walking Scale-12 (MSWS-12)[62]
    - Neuropathic Pain Scale (NPS)[63]
    - Fatigue Scale Motor and Cognitive Functions (FSMCF)[64]
    - EuroQol – 5 Domains – 5 Levels (EQ-5D-5L)[65]
- Completion of eligibility criteria checklist
- Randomisation
- Participants will be provided with a Participant ID Card (see Appendix 23)
- Participants will be provided with a list of drugs and supplements that can cause hyperkalaemia that they should avoid taking (see Appendix 22)

Blood Tests (+/- 7 days)

- EUC, eGFR – weeks 1, 3, 6 and 12 weeks and then every 12 weeks
- FBC, LFTs – every 24 weeks
- EBNA1 titres – week 24 (Stage 1 only), week 48, then every 48 weeks
- Collection and storage of serum sample – every 48 weeks

Saliva Collection (+/- 7 days)

- EBV DNA detection – weeks -4, 0, 4, 8, 12, 16, 20 and 24 (Stage 1 only)

Serum Collection (+/- 7 days) – weeks -4, 24 (Stage 1) only, then weeks 48, 96 and 144

- EBNA1 antibody titres
- Serum and plasma storage

Dose Escalation Visit (week 4 +/- 7 days)

- Review of adverse events
- Review of concomitant medications and supplements
- MSReactor computerised cognitive assessment (optional)[30]Review of FBC, LFTs, EUC and eGFR

- Decision regarding dose escalation

Review Visits (Every 24 weeks; +/- 7 days in first 6 months then +/- 14 days)

- Review of relapse history
- Review of adverse events
- Review of intercurrent illness
- Review of concomitant medications and supplements
- Review of current MS SOC therapy
- General physical examination
- EDSS[27]
- MSFC[28]
- MSReactor computerised cognitive assessment (optional)[30]
- Compliance monitoring (remaining pill count)

General examination will include specific examination for gynaecomastia in males.

Review visits may be conducted remotely via telehealth whether for reasons of COVID-lockdowns or remoteness of the participant.

The following PROMs will be repeated at annual review visits (weeks 48, 96, 144 and 192):

- MSIS-29[61]
- MSWS-12[62]
- Neuropathic Pain Scale[63]
- FSMCF[64]
- EQ-5D-5L[65]

Telephone Monitoring (Every 24 weeks; +/- 7 days in first 6 months then +/- 14 days – offset from Review Visits by 12 weeks)

- Review of relapse history
- Review of adverse events
- Review of intercurrent illness
- Review of concomitant medications
- MSReactor computerised cognitive assessment (optional)[30]

MRI (At baseline and every 48 weeks - +/- 90 days)

- MRI Brain (as part of SOC – non-protocol scans)

The full schedule of study events is provided in Appendix 25.

The timeline of participant involvement and total time commitment per patient in the trial is outlined in Table 2.

Table 2. Summary of patient participation as total time in hours*

Procedure	Screen	MRI Brain 1	Enrolment	Dose Escaltion Visit	24 Week Visit	MRI Brain 2	48 Week Visit	72 Week Visit	MRI Brain 3	96 Week Visit	120 Week Visit	MRI Brain 4	144 Week Visit	168 Week Visit	MRI Brain 5	192 Week Visit	Time (Hrs)	Total Time (Hrs)
Time (wks)	-1	-1	0	4	24	48	48	72	96	96	120	144	144	168	192	192		
Screening Visit	1																1	1
Clinical Rev			1		1		1	1		1			1	1		1	0.5	4
EDSS	1		1		1		1	1		1			1	1		1	0.5	4.5
MRI		1				1			1			1			1		1	5
Bloods	1			1	1		1	1		1	1		1	1		1	0.25	2.5
Saliva	1			1	5												0.1	0.7
													Total participant hours					17.7

*Estimate of participant time commitment does not include telephone reviews

Follow up will be for a minimum of 2 years and a maximum of 5 years, with a mean of approximately 3 years. This assumes that the trial progresses to the completion of Stage 2.

3.9. Outcome Measures

3.9.1. Stage 1

The co-primary outcome measures for stage 1 will be:

- Salivary EBV DNA detection
- Serum EBNA1 antibody titres

During stage 1, saliva samples (1-2 ml) will be collected monthly by participants at home which they will then post to QIMR-Berghofer using a pre-paid envelope and kit designed specifically for this purpose. DNA will be extracted using a saliva DNA extraction kit (Qiagen®, Netherlands). EBV DNA will be detected using a TaqMan® assay and EBV shedding considered present if viral count is >5.8 virus copies/µl. A serum sample will be collected at week 24 and separated using a centrifuge and pipette. Serum will be stored locally at -70 °C and shipped to QIMR-Berghofer in batches every 6 months. Enzyme-linked immunosorbent assay kits (Diamedix®, FL, US) with serial dilutions will be used to measure EBNA1 antibody titres with normalisation to the manufacturer's cut-off calibrator standard. These tests will be performed in the laboratory of Chief Investigator (CI) Smith (QIMR-Berghofer, QLD). Standard Operating Procedures (SOPs) will be provided for these processes and should be followed by all participating sites.

3.9.2. Stage 2

The Stage 2 primary outcome measure will be time to 6mCDP using a composite of EDSS,[27] T25FW[28] and 9-HPT.[29] Definitions of progression will be: an increase in EDSS (of 1 point if baseline EDSS was <5.5, or 0.5 points if baseline EDSS was ≥5.5); ≥20% increase in 9-HPT time; or ≥20% increase in T25FW. Any qualifying change must be confirmed at repeat assessment 6 months later. Inclusion of a measure of upper limb function (9-HPT) also addresses consumer interest in assessing arm function and is particularly important for people with higher levels of disability. This composite definition of 6mCDP has been chosen for other recent clinical trials in MS.[66]

3.9.3. Secondary Outcome Measures

The following outcome measures will also be used.

3.9.3.1. Clinical

Time to first relapse, time to 6mCDP based on EDSS alone and mean changes in EDSS, MS Functional Composite (MSFC) Score,[28] utilising the Symbol Digit Modalities Test (SDMT)[67] in place of the Paced Auditory Serial Addition Test, T25FW and 9-HPT.[68]

Cogntive assessment using MSReactor computerised assessment will also be collected. Participants will be requested to complete the MSReactor tasks at the first three trial visits and then once every 3 months. The tasks can be completed within 5 minutes. The recorded reaction times will be uploaded to a server hosted by Monash University in a de-identified format, using the participants MSReactor login details (email and user-defined password). These data will be compared with traditional measures of physical disability (EDSS) and cognitive function (SDMT) to assess if the MSReactor reaction times are any more sensitive in detecting change over time as a purely exploratory outcome measure. Participants may choose to opt-out of this part of the study.

3.9.3.2. MRI

MRI brain will be performed annually as part of SOC. We will leverage existing standard protocols for routine clinical MRI brain which have been implemented at the participating sites as part of the existing MSBase collaboration. These scans include fixed acquisition protocols that include 3D volumetric T1 (with and without Gd) and FLAIR sequences. New and enlarging lesion counts between timepoints, and Gd-enhancing lesion counts will be documented as reported by the local radiologist/neurologist. Subject to additional study funding, we will have the potential to use an artificial intelligence-based software with additional manual quality assurance by trained neuroimaging analysts to detect new/enlarging lesions and measure whole brain atrophy (SIENA technique)[69] through the Sydney Neuroimaging Analysis Centre (SNAC) under CI Barnett.

3.9.3.3. PROMs

The MS Impact Scale-29,[61] which measures physical and psychological wellbeing, the MS Walking Scale-12,[62] Neuropathic Pain Scale[63] and the Fatigue Scale for Motor and Cognitive

Functions[64] will be measured every 12 months. At each visit the number and severity of relapses since the last study visit will be recorded.

Health economics: A treatment that slows disability progression could represent a highly cost-effective use of public health resources given the high costs of progressive MS and the very low cost of repurposed drugs. Cost effectiveness from a health payer and societal perspective will be measured by cost per quality-adjusted life year (QALY) and will be assessed 6-monthly using the EQ-5D-5L.43 For the analysis, results will be reported as the incremental cost per QALY gained as well as the expected net-benefit statistic. We will use societal cost data from our previously published work to attribute avoided societal costs with reduced disability progression.[65]

3.9.3.4. Adverse Events

All AEs will be recorded in the eCRF. Information recorded will include the date of onset of the AE, a description of the AE, severity, relationship to IMP, whether or not the AE is an SAE and date of resolution or if ongoing. MS relapses will also require additional information to be reported in the on-study relapse form. All SAEs must be reported to the Sponsor and the lead HREC within 24 hours of study staff being made aware of the event. All adverse events will be coded according to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE), National Cancer Institute, National Institutes of Health, MD, US. Severity will also be graded according to CTCAE v5.0 definitions.

SAE will be defined as any AE that:

- Results in death
- Is life-threatening – reported medical condition has a known substantial risk of death
- Results in hospitalisation – visits to the emergency department will not be counted as SAE unless other criteria apply
- Results in disability or permanent damage – a substantial change to a person's ability to conduct their usual functions (i.e. significant persistent or permanent change, impairment, damage or disruption to body function/structure, physical activities or quality of life)
- Results in a congenital anomaly or birth defect
- Required significant intervention to avoid injury (e.g. treatment for an allergic reaction or seizures in an emergency department)
- Results in any other serious ongoing change in health (e.g. drug dependence, blood dyscrasias)

De-identified, aggregate AE data will be reviewed by the Data Safety Monitoring Board (DSMB) every 3 months. Once a minimum of 50 AEs have been reported these data will be summarised according to treatment allocation (unblinded) and analysed statistically using Fisher's exact test for comparison between each of the active treatment arms and placebo. Comparisons will be made for all treatment emergent AEs, treatment related AEs (definite or probable), higher grade AEs (grade 2 or 3), SAEs and deaths. SAEs as they occur, will be reported to the sponsor and the lead HREC within 24 hours of site study staff being made aware of them and to local HREC/RGO in accordance with local guidelines. Comparisons will also be made individual AE conditions where more than 5 events have occurred. Event rates will be compared with reported expected rates of these AEs for the two active treatments using 95% confidence intervals. Any rates significantly outside of these expected

limits will be reported to the Sponsor and lead HREC. A data safety report will be provided to the Sponsor and lead HREC annually.

3.9.3.5. Data Collection

Data will be collected in an eCRF created using a specifically created REDCap® database. 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 participating sites and necessary regulatory authorities. Access to the data will be further restricted according to utilisation requirements and for maintaining blinding.

The data to be collected is indicated in the Data Collection Sheet (see Appendix 26).

In addition, data from the MSReactor mobile phone app will also be collected. Participants will be requested to complete the MSReactor tasks on their own mobile phone once every 3 months. The tasks can be completed within a minute or two. The recorded reaction times will be uploaded to a server hosted by Monash University in a de-identified format, using the participants PIN. These data will be compared with traditional measures of physical disability (EDSS) and cognitive function (SDMT) to assess if the MS Reactor reaction times are any more sensitive in detecting change over time as a purely exploratory outcome measure. This component of the study is optional.

3.9.4. Data Storage and Confidentiality

Participants' privacy and confidentiality will be protected through the following:

- The PICF will be held in dedicated files for each participant at individual 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. Integrated electronic medical record system are 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 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 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.
- In accordance with recommendations of the funding body (MRFF) after the full analysis of data has been completed, fully de-identified data from the trial will be posted to an open-access data repository such as Figshare.
- Specific consent for potential future use of bio-banked samples (serum and DNA samples) together with de-identified clinical data has been included in PICF. The precise nature of these studies will depend upon the outcomes of the trial, but the current proposals and general outline of possibilities is included below.

3.9.5. Data Analysis and Statistical Considerations

3.9.5.1. Stage 1

Linear regression analysis with appropriate transformation of data and inclusion of potential confounders (e.g. age, sex, disease modifying therapy (DMT), baseline EDSS) will be used to compare mean frequency of salivary EBV DNA detection and mean EBNA1 titres between treatment arms.

3.9.5.2. Progression to Stage 2

Minimum criteria for consideration of progression to stage 2 will be a 10% reduction in mean salivary EBV DNA detection frequency or mean EBNA1 titre. The agent associated with the greatest change in these parameters will be selected for progression to stage 2.

In the event of both drugs being tested proving to be highly effective in reducing evidence of EBV activity in Stage 1, the CI team will seek additional funding (e.g. MS Australia, National MS Society (US)) to support a third arm in Stage 2. If spironolactone (the cheaper of the two agents) proves to be the more effective agent in Stage 1, then the CI team would seek approval from the Medical Research Future Fund (MRFF) to divert the associated cost savings towards proposed biomarker studies.

If both agents fail to reach nominal levels of significance in Stage 1, in the case of borderline results or presence of a trend in support of effectiveness against EBV then the CIs would seek approval from MRFF to potentially extend participant numbers in Stage 1. Otherwise, the trial would be abandoned.

3.9.5.3. Stage 2

The primary analysis will be a Cox-proportional hazards analysis of cumulative hazard of 6mCDP adjusted for potential residual imbalance at baseline for age, sex, DMT and disability level. Secondary outcome measures will be analysed with Cox (or accelerated

failure time) models and mixed generalised regression models. Analyses will be conducted on an intention to treat basis. Observations will be censored upon leaving the trial for participants without an event, including for those who have withdrawn from the trial, been lost to follow-up, or who have died due to causes other than MS. Death due to MS (EDSS 10.0) will count as 6mCDP. Sensitivity analyses will investigate the per-protocol treatment exposure to assess the impact of non-compliance.

3.9.6. Sample size and power calculations

For stage 1 sample size calculations we have used a relatively relaxed α=0.1 which is suitable for this interim step. Based on the observed rate of salivary samples with EBV DNA in the placebo arms of the teriflunomide clinical trial program of 40% and a rate of 20% in treated arms a sample size of 50 participants in each arm would provide 70% power to detect this level of difference.10 The same sample size would have 80% power to detect a 25% fall in EBNA1 titres towards healthy control levels.

For stage 2, sample size calculations have been based on the observed rate of 6mCDP in the placebo arm of the ORATORIO trial of 40%. A sample size of 132 participants in each arm would have 80% power to detect a 40% reduction in 6mCDP with α=0.05. Allowing for a 14% drop-out rate gives a sample size of 150 in each arm. See Appendix 10 for assumptions made with regards to expected rates of progression.

We would aim to recruit 50 participants to each of the 3 arms in Stage 1 over a period of 6-12 months. Once all Stage 1 participants had reached 6 months follow up (12-18 months) we would analyse the Stage 1 co-primary outcome measures and make a determination on which therapy should proceed to Stage 2. The successful treatment arm and placebo arm would continue into Stage 2. Participants in the unsuccessful treatment arm will be advised (unblinded) as to their treatment allocation and would be discontinued from Stage 1. They would be invited to enrol in Stage 2 after a washout period of 4 weeks if they so wished.

For Stage 2 we would aim to recruit an additional 100 participants to each of the 2 arms over a 12-18 month period. We would plan to conduct final analyses when the last participant enrolled reached the 96 week follow up. Thus the period of follow up for Stage 2 will range from 96 weeks to 240 weeks, with a mean follow up of around 168 weeks (3.5 years).

3.10. Biobanking and Future Studies

A part of Stage 1 the 4-weekly collection of saliva for the detection of EBV DNA from baseline to week 24 and the collection of serum samples at baseline and 24 weeks for EBNA1 antibody titres has been planned as part of primary outcome measures of the STOP-MS trial. In addition, it is proposed that blood for DNA extraction be collected at baseline and blood for serum separation be collected at baseline and every 48 weeks until the end of the trial. At present the research team have not secured funding to support this component of the project, but further applications for funding are planned. Specific consent for collection and storage of these samples will be included on the consent form (“opt-in” check box).

Specific studies to be potentially included are:

1. Whole blood (EDTA tube) will be stored locally at -70 °C and then transferred to a central laboratory (site yet to be determined, but in Australia) for DNA extraction. Genomwide sequencing for allelic variants of single nucleotide polymorphisms would

be performed using chip technology on a standard platform (Affymetrix or Illumina). These data would be used to explore potential genetic markers of a positive clinical response to a particular treatment or correlation with clinical or laboratory markers of disease outcomes (e.g. disability or biomarker levels).

2. Whole blood samples (SST tube) will be separated using a centrifuge at each participating site and serum samples pipetted and aliquoted into 2 x 4 ml tubes and sored locally at -70 °C. These samples will be transferred to central facilities within Australia (sites yet to be determined) for further analyses as outlined below at regular intervals (e.g. annually).
3. Serum samples will be tested for EBNA1 antibody titres (most likely at QIMR/Berghofer Institute, Queensland) and potentially for other antibodies (e.g. VCA IgG).
4. Serum samples will be tested for serum neurofilament light levels as a marker of CNS axonal degeneration using SIMOA technology at one of several potential sites that can provide this service. Other potential markers of CNS degeneration may also be measured (e.g. GFAP, NCAM1).
5. Serum samples might also be used for other exploratory studies of immune cytokines and other soluble markers.

The research team have long established collaborations with the International MS Genetics Consortium who have been successful in identifying over 200 genetic loci associated with MS susceptibility. Current endeavours are focused on potential genetic markers of progressive MS. Since the STOP-MS will be specifically recruiting people with progressive MS we would propose that fully de-identified genomic data (from analyses conducted in Australia on STOP-MS participants who have consent to such use) be combined in meta-analyses with other international cohorts.

Any additional future studies (outside of the scope of those outlined above) using biobanked samples from STOP-MS would require approval from the lead HREC.

Any approach to use de-identified data from STOP-MS by any third party would require a written proposal and approval of the lead HREC.

4. Translation to Changes in Clinical Practice

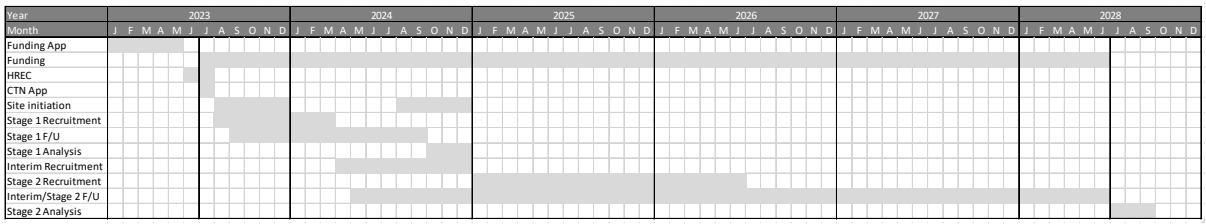
If spironolactone or famciclovir prove to be effective in reducing disability progression in people with MS, the CI team will apply to the Therapeutic Goods Administration for a new indication (progressive MS) for the relevant drug and prepare new treatment guidelines. The results of this study will be reported in a leading neurology journal. This study will be registered with the Australian and New Zealand Clinical Trials Registry and the results will be reported there when finalised. At the completion of the study, a lay summary of the results will be provided to participants (where they have requested this on the PICF) and will also be posted on the Griffith University Facebook page and the MS Australia website.

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 July 2023, with a proposed start date for the trial in December 2023. Recruitment of participants will span the

first 3 years and follow up will continue for a minimum of 96 week (maximum of 240 weeks). Please see Gant chart of proposed timeline in Table 3.

Table 3. Gantt chart of proposed timeline.



6. Funding and Resources

This project is being funded by an MRFF grant of $1,999,362.50 which was awarded in May 2023. Griffith University will be the sponsor of the trial.

These funds will be utilised to cover the costs of a clinical trial manager to be based at Griffith University a clinical trial monitor, manufacture of the investigational medical product, all site related costs related to conduct of the study and all of the proposed monitoring and investigational laboratory tests.

Additional funding for the proposed analysis of biobanked sample will be sought.

6.1. Governance

6.1.1. Independent Steering Committee

The STOP-MS clinical trial will be overseen at a strategic level by the PLatform Adaptive Trial for remYelination and neuroProtection in mUltiple Sclerosis (PLATYPUS) Platform Steering Committee which has been established for the PLATYPUS clinical trial funded by MS Australia. This committee consisting of national and international leaders in neurology, immunology, clinical trial design and statistics will convene twice per year to review trial design and their progress at a strategic level. The members of the committee will have no direct involvement in the conduct of any of the trials that they oversee. The Coordinating Chief Investigators of each trial (PLATYPUS, STOP-MS and a third study which also received MRFF funding, known as Fatigue In Relapsing Multiple Sclerosis – Epstein Barr Virus (FIRMS-EBV)) will report to the committee and seek guidance on specific issues and overall direction of the projects. This will include oversight of trial budgets. Members of the committee will be paid an honorarium for their services. Professor Pam McCombe (MS Neurologist at Royal Brisbane and Women's Hospital) has kindly agreed to chair this committee.

6.1.2. Investigator Meetings

The Coordinating Chief Investigator will convene investigator meetings, initially once per fortnight in the setup phase and then monthly thereafter. All site PI's, AI's, clinical trial

coordinators and pharmacists will be invited to the investigatory meetings. The purpose of these meetings will be to finalise the protocol, ensure consistent practices at all sites, relay any amendments, monitor progress of the trial and troubleshoot any day-to-day matters.

6.1.3. Data Safety Monitoring Board

The data safety monitoring board will convene every 3 months to review summary reports of adverse events every 3 months. These meetings will commence once any adverse events have occurred. When fewer than 50 participants have been recruited reports will remain blinded. Once 50 participants have been recruited data safety reports will be presented in di-identified, aggregate, unblinded format to permit potential trends in safety profile between the investigational medicinal products. In addition to being reported to the relevant HREC within 24 hours of notification, any SAE's will be reported to the Data Safety Monitoring Board out of session for comment and if there are significant concerns an ad hoc meeting will be convened. The members of the Data Safety Monitoring Board will be independent of the STOP-MS trial and will be shared with the PLATYPUS and FIRMS-MS trials. The members will be paid an honorarium for their time.

6.1.4. Sponsor

Griffith University will be the sponsor of the STOP-MS clinical trial. Griffith University is a leading public university in Australia with over 49,000 students, 4,000 academic staff and over $100 million in research funding for 2023. Griffith University holds indemnity insurance covering claims up to $25 million for research activity including clinical trials. The clinical trials unit at Griffith University has the staff and physical resources to provide clinical trial management and monitoring services.

6.1.5. Trial Site Resources

The STOP-MS Clinical Trial sites will be Academic MS Centres located in major public hospitals, private hospitals and universities. The site PIs are all experience MS Neurologists who have considerable experience of both investigator-driven and commercially sponsored clinical trials.

All key site staff (PIs, AIs, clinical trial coordinators and pharmacists) will be required to have current ICH-GCP certification and provide a current CV. Site PIs and AIs will be required to have current EDSS certification (performed as per Neurostatus® guidelines – neurostatus.net). Clinical trial coordinators/nurses will be required to have completed training for the 9-HPT and SDMT.

Trial sites will be required to have the following before trial commencement:

- A nominated PI
- A nominated AI (independent EDSS rater)
- A nominated clinical trial coordinator/nurse
- A nominated pharmacist
- A suitable space for clinical visits (e.g. clinical trials unit, clinic space)
- A paper-based or electronic medical record system with appropriate storage in case of paper-based system

- Secure storage space for relevant study files
- An accredited pharmacy
- An ECG machine and staff trained in performing ECGs
- Access to a centrifuge capable of 2000g (preferably refrigerated)
- Access to a -70 °C freezer
- EDTA and Serum collection tubes
- Measured 25-foot walking area
- Area suitable for assessing walking distances of up to 500m
- 9-HPT kit
- 6 m Snellen chart

The Sponsor will provide to sites the following:

- Templates for data collection
- Salivary collection kits (x8 per participant)
- 9-HPT kit (if required)
- Symbol Digit Modalities Test kit and score sheets

Sites will be responsible for facilitating relevant local HREC and SSA approvals.

Engagement of clinical sites will be through a Clinical Trial Research Agreement between the Sponsor and the host institution.

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