Trials

STUDY PROTOCOL

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A phase II trial examining the safety and preliminary efficacy of repetitive transcranial magnetic stimulation (rTMS) for people living with multiple sclerosis

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Abstract

Background Multiple sclerosis (MS) is a chronic neurological condition and the leading cause of non-traumatic disability in young adults. MS pathogenesis leads to the death of oligodendrocytes, demyelination, and progressive central nervous system neurodegeneration. Endogenous remyelination occurs in people with MS (PwMS) but is insufficient to repair the damage. Our preclinical studies in mice indicate that endogenous remyelination can be supported by the delivery of repetitive transcranial magnetic stimulation (rTMS). Our phase I trial concluded that 20 sessions of rTMS, delivered over 5 weeks, are safe and feasible for PwMS. This phase II trial aims to investigate the safety and preliminary efficacy of rTMS for PwMS.

Methods Participants must be aged 18–65 years, diagnosed with MS by a neurologist, stable and relapse free for 6 months, have an Extended Disability Status Scale (EDSS) between 1.5 and 6 (inclusive), willing to travel to a study site every weekday for 4 consecutive weeks, and able to provide informed consent and access the internet. Participants from multiple centres will be randomised 2:1 (rTMS to sham) stratified by sex.

The intervention will be delivered with a Magstim Rapid2 stimulator device and circular 90-mm coil or MagVenture MagPro stimulator device with C100 circular coil, positioned to stimulate a broad area including frontal and parietal cortices. For the rTMS group, pulse intensity will be set at 18% (MagVenture) or 25% (Magstim) of maximum stimulator output (MSO), and rTMS applied as intermittent theta burst stimulation (iTBS) (~ 3 min per side; 600 pulses). For the sham group, the procedure will be the same, but the intensity is set at 0%. Each participant will attend 20 intervention sessions over a maximum of 5 weeks.

Outcome measures include MS Functional Composite Score (primary), Fatigue Severity Scale, Hospital Anxiety and Depression Scale, Quality of Life, and Pittsburgh Sleep Quality Index/Numeric Rating Scale and adverse events

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(secondary) and advanced MRI metrics (tertiary). Outcomes will be measured at baseline and after completing the intervention.

Discussion This study will determine if rTMS can improve functional outcomes or other MS symptoms and determine whether rTMS has the potential to promote remyelination in PwMS.

Trial registration Registered with Australian New Zealand Clinical Trials Registry, 20 January 2022; ACTRN12622000064707.

Keywords Multiple sclerosis, Transcranial magnetic stimulation, rTMS, Remyelination, MRI, PROM

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

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Role of sponsor {5c}	The study sponsor and funders were not directly or indirectly involved in designing this study. They will also not be involved in the collection or analysis of the data, or the decision to publish the manuscript. However, the study sponsor may determine the continuation or discontinuation of this study if the choice suits the best interest of the participants. Archiving of study records and participants' data for a minimum of 15 years is the responsibility of the study sponsor.

Introduction

Background and rationale {6a}

Multiple sclerosis (MS) affects a growing number of people, increasing from 2.3 to 2.9 million worldwide over the last decade [1]. MS begins in early adulthood and leads to chronic demyelination, progressive neurodegeneration, and increasing disability [2, 3], costing the Australian economy $2.5 billion each year [1]. Preclinical studies have shown that remyelination can lessen disability progression [4], and whilst people with MS (PwMS) experience endogenous remyelination, it is limited and insufficient to repair the demyelinated brain lesions [5].

There are currently no MS medications that support remyelination, and remyelination therapies were identified as the number one research priority for PwMS in Australia [6].

Preclinical studies in adult mice indicate that low-intensity repetitive transcranial magnetic stimulation (rTMS), delivered as 600 pulses of intermittent theta-burst stimulation (iTBS), daily for 4 weeks, increased survival of newborn oligodendrocytes [7] and enhanced remyelination of surviving and newborn oligodendrocytes [8]. These observations were the basis of our phase I clinical trial, TAURUS [9].

The primary objective of TAURUS was to evaluate the safety and tolerability of 20 sessions of rTMS for PwMS, delivered as 600 pulses of iTBS using 25% maximum stimulator output (MSO), over a maximum of 5 consecutive weeks. The TAURUS trial also evaluated protocol feasibility and collected exploratory data on the effect of rTMS on MS symptoms and advanced magnetic resonance imaging (MRI) metrics [9].

rTMS is generally well tolerated and has been used extensively in clinical trials and research [10]. The known side effects of rTMS include mild scalp discomfort or tingling sensations during stimulation, transient headaches, and dizziness [11, 12]. On rare occasions, rTMS has resulted in serious side effects including hearing loss and seizures [11, 12]. However, rTMS is typically delivered at intensities ranging from ~ 30 to 65% of MSO [13]. The risk of adverse reactions appears to increase with high intensity and higher frequencies [11, 12]. This risk is further reduced by excluding participants who are prone to seizures, migraines, and other contraindications [11, 12]. Diffuse stimulation affects a wide region of the brain, consistent with myelin loss across multiple brain regions. Studies using different forms of rTMS indicate potential benefits for treating MS symptoms including fatigue, depression, working memory, manual dexterity, and lower limb spasticity [14–16].

The TAURUS trial determined that it was safe and feasible to deliver 20 sessions of rTMS (iTBS, 25% MSO) to PwMS over a 4–5-week period [17]. This has informed the design of this phase II clinical trial, which aims to determine the safety and preliminary efficacy of this specific rTMS protocol in PwMS.

Objectives {7}

We hypothesise that 20 sessions of low-intensity rTMS, as iTBS, will improve functional outcomes and symptoms of MS and result in detectable changes to MRI metrics that are consistent with increased myelin in the brains of PwMS.

Primary objective:

- To determine if 20 sessions of rTMS improves the MS functional composite score (MSFC) for PwMS.

Secondary objectives:
To determine if 20 sessions of rTMS.

- Is safe, tolerable, and acceptable for PwMS.
- Improves the quality of life of PwMS.
- Reduces anxiety and depression in PwMS.
- Reduces fatigue in PwMS.
- Improves sleep quality in PwMS.

Tertiary objectives:
To determine.

- Preliminary evidence for efficacy for rTMS to promote myelin addition in the brains of PwMS (measured using MRI measurements).
- Whether myelin addition correlates with treatment effect on functional and patient-reported outcomes.
- If protocol compliance and adherence to treatment schedule is equivalent for PwMS in the rTMS and sham groups.
- If our sham protocol ensures that participants are blind to the treatment group.

Trial design {8}

This is a phase II, multi-centre, randomised (2:1), sham-controlled, parallel, blind, safety, and preliminary efficacy trial.

A schematic of the trial design is shown in Fig. 1.

Methods: participants, interventions, and outcomes

Study setting {9}

This trial will be conducted at six academic hospitals or medical research institutes in Australia: (1) John Hunter Hospital, Hunter New England Health Lookout Rd, New Lambton Heights, New South Wales; (2) Alfred Health, 55 Commercial Rd, Melbourne, Victoria; (3) Perron Institute for Neurological and Translational Science, RR Block, QE II Medical Centre, 8 Verdun St, Nedlands, Western Australia; (4) Launceston General Hospital, 274–280 Charles St, Launceston, Tasmania; (5) Mater Misericordiae Ltd, Level 3, Aubigny Place, Raymond Terrace, South Brisbane, Queensland; (6) Menzies Institute for Medical Research, 17 Liverpool Street, Hobart, Tasmania. Menzies Institute for Medical Research, University of Tasmania (UTAS), Tasmania, Australia, will be the coordinating site.

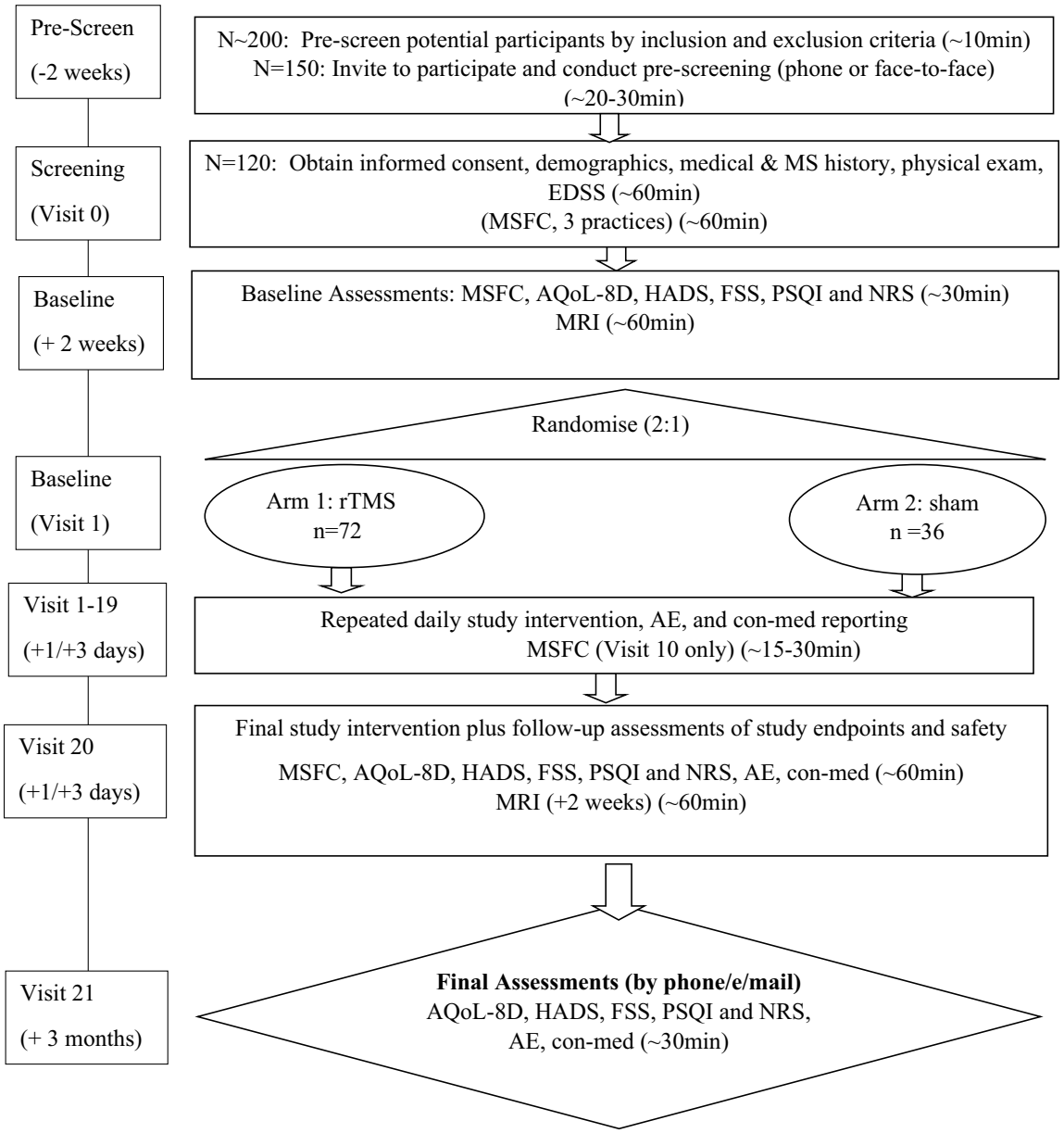

Fig. 1 Study schema (CONSORT)

Eligibility criteria {10}

Inclusion criteria:

(i) Age 18–65 years (inclusive).
(ii) Diagnosed with MS by an MS neurologist (McDonald criteria, 2018).
(iii) Stable and relapse free for 6 months (either on or off MS treatment).
(iv) Expanded Disability Status Scale (EDSS) between 1.5 and 6 (inclusive).
(v) Willing to travel to a trial site every weekday for 4 consecutive weeks.
(vi) Capacity to provide consent.
(vii) Access to the Internet for follow-up assessments.

Exclusion criteria:

(i) Have metal anywhere inside their head (dental brace is not a contraindication). People with MRI compatible metal in their body, outside their head, can participate (e.g. cardiac pacemaker).
(ii) Are pregnant or intend to become pregnant.

(iii) Have a history of seizures, epilepsy, stroke, brain surgery, bipolar, mania, claustrophobia, serious head trauma*, substance abuse*, or migraines*.
(iv) Currently taking tricyclic antidepressants, neuroleptics, antipsychotics, or antiseizure medication for the treatment of any indications listed in iii above.
(v) Have an EDSS < 1.5 and > 6.
(vi) Previously received any form of TMS (to maintain blinding).
(vii) English illiterate** (to enable completion of follow-up questionnaires).
(viii) Currently involved in another interventional clinical trial.

The eligibility criteria have been carefully selected to reduce the risks associated with rTMS and exclude people with any contraindication to either rTMS or MRI [18]. We include participants with an EDSS of 1.5–6 as they are most likely to benefit from any remyelination therapy, and this level of disability would not, in itself, preclude attending daily visits. Pregnancy is an exclusion criterion as pregnancy hormones themselves affect myelination [19].

*Note: participants with minor/moderate head trauma, previous substance abuse, and/or well controlled or rare migraines may be included on PI discretionary basis, e.g. these conditions are prevalent in the community and may not be clinically significant. **English illiterate participants who can be provided with a suitable translator/interpreter for the duration of the study (including phone calls, questionnaires, and visits) may be included.

Who will take informed consent? {26a}

Informed consent will be obtained by a trained, qualified, and delegated researcher in person at the screening visit. The consent process will not involve the participant's treating neurologist to reduce the risk that an unequal relationship affects their decision to participate.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Participants will have the option to consent to the future use of their data in currently unforeseen, ethically approved MS research studies. Participants will consent to their general practitioner and treating neurologist being informed of their involvement in the trial, to allow for notification of clinically relevant outcomes or serious adverse events.

Interventions

Explanation for the choice of comparators {6b}

This study will compare change from baseline for PwMS randomised to either rTMS or sham groups. Both groups will continue concomitant MS treatments. A sham comparator has been chosen to account for placebo effects in our assessments of rTMS efficacy [20].

Intervention description {11a}

Participants will receive 20 sessions of rTMS or sham (Monday–Friday) over a period of 4 to 5 weeks. A maximum of 3 days between sessions is tolerated. A Magstim Rapid2 device (Magstim Ltd, Whitland, UK) and 90-mm circular coil or MagVenture MagPro device (MagVenture, Farum, Denmark) with C100 circular coil will be used to deliver rTMS and sham interventions. The Magstim Rapid2 will be set at a stimulation intensity of 25% MSO and the MagVenture MagPro set at an equivalent intensity of 18% MSO. Device parameters will be set to deliver an iTBS pattern: bursts of 3 pulses at 50 Hz, repeated at 5 Hz for a 2-s period (10 bursts), followed by an 8-s gap (~ 10 s cycle time), repeated for 20 cycles (600 pulses, ~ 3 min) [19]. For the sham group, both the Magstim Rapid2 device and the MagVenture MagPro device will be set to 0% MSO, and an Olympus VN-541PC sound recorder will be used to recreate the rTMS (iTBS, 25% MSO) clicking sounds to maintain blinding.

To deliver the rTMS or sham intervention, participants will be seated upright in a comfortable chair, positioned so that they cannot see the device interface screen or sound recorder. Coil target position will be determined relative to the vertex (top centre point of scalp, where sagittal and coronal planes intersect), found using a flexible tape measure and marking the mid-point between the nasion and inion, and pre-auricular (ear landmark) on each side, respectively [9]. From the vertex, the target coil position will be marked with a whiteboard marker at 2 cm lateral and aligned with the vertex in the anterior–posterior axis on both the left and right sides of the scalp when using the Magstim Rapid2 stimulator with 90-mm Standard Coil [9]. When using the MagVenture MagPro stimulator with C100 circular coil, marks will be made at 2.5-cm lateral and aligned with the vertex in the anterior–posterior axis on both the left and right sides of the scalp. The coil will be oriented so that the plane of the coil is tangential to the scalp and the central hole over the target mark. The coil handle will be pointing backwards, at 45° to the scalp midline. By positioning the coil in this way, we will stimulate a broad cortical area including frontal and parietal regions, consistent with our preclinical and phase I protocols [9].

Stimulation of the left or right hemisphere first will be counter-balanced between participants, but for individual participants, it will be consistent across sessions. After completing the 3 min of iTBS to the first hemisphere, the coil is then flipped and positioned to stimulate the second hemisphere for a further 3 min [9]. Flipping the coil between hemispheres ensures that

both hemispheres of the brain receive anteroposterior-induced current on the lateral portion of the coil for the second phase component of the biphasic pulse [9]. When using Magstim Rapid2, 90-mm circular coil, side A of the coil should be up (label 'A' visible) whilst stimulating the left hemisphere and side B should be up (label 'B' visible) whilst stimulating the right hemisphere. When using the MagVenture MagPro, C100 circular coil, the 'therapy' labelled side, arrows point anticlockwise whilst stimulating the left hemisphere. This is flipped when stimulating the right hemisphere.

Criteria for discontinuing or modifying allocated interventions {11b}

Participation is voluntary; participants are free to withdraw at any time. They may elect to withdraw from treatment only or both treatment and follow-up. However, it will be made clear that any data collected up to the point of withdrawal will still be used in the study analysis as per intention to treat principles. Withdrawal of consent after the study is completed and/or archived will not be possible.

Participants will be withdrawn from the trial intervention if they.

- Develop seizures
- Receive a metal implant that will prevent them from having an MRI
- Become pregnant
- Begin taking tricyclics, neuroleptics, or antiseizure or antipsychotic medications
- Are no longer fit to participate (e.g. MS relapse or change in disease modifying therapy or prescribed high dose steroids) at PI discretion
- Need to be withdrawn as an urgent safety measure

If it is in the best interest of the participants or the trial, the study sponsor (UTAS) or sponsor representative may discontinue the trial. The notification of discontinuation and reason must be communicated in writing to the CI. Reasons for this decision may include futility, serious safety concerns, acts of fraud or serious breaches, critical findings, or persistent non-compliance that negatively impacts participant safety or data integrity. In the case of early termination, arrangements will be made to ensure the appropriate follow-up and care of the participants.

Strategies to improve adherence to interventions {11c}

Participants must attend a study site to receive the intervention. Adherence to the intervention will be monitored through the recording of missed visits and protocol deviations. Appointment times will be flexible between 8 am and 6 pm on weekdays, depending on availability of the participant, clinic room, and staff. A maximum gap of + 3 days is tolerated between intervention sessions to allow for long weekends, illness, and other unexpected factors that may prevent scheduled attendance. Participants will be reimbursed for all reasonable travel expenses for each visit, and a $50 voucher is provided at the end of the intervention period. On-site parking will be provided.

Relevant concomitant care permitted or prohibited during the trial {11d}

During the trial, participants will be allowed to continue with their concomitant care including continuing with physical rehabilitation treatment and disease-modifying therapies. Investigators will keep a record of participants' current treatment and medications, noting changes from baseline to the final intervention session.

Provisions for post-trial care {30}

Participants who complete or discontinue the study will receive post-trial care from their neurologists and return to the care of their general practitioner (GP). The trial sponsor is providing insurance and indemnity which covers participants who may suffer harm as a result of their participation in the trial. UTAS insurance does not cover any claims related to SARS-COV-2.

Outcomes {12}

Primary outcome

MSFC score will be collected at baseline (before the first intervention session) and visit 20 (after the last intervention session, 4–5 weeks from baseline) and will be used to compare change over time between rTMS and sham groups' mean *z*-scores.

Secondary outcomes

The incidence of treatment emergent adverse events (AE) and serious adverse events (SAE) will be recorded at each visit during the intervention phase. Proportions of participants recorded as experiencing at least one adverse reaction (AR) over the trial period (incidence proportion) will be compared between the rTMS and sham groups. ARs leading to withdrawal from the study intervention and suspected unexpected serious adverse reactions (SUSAR) will be presented either in a table or a listing. A clinically acceptable difference in treatment emergent events between the rTMS and sham groups is 10% for AR and 0% for SUSARs [21]. Symptom and

quality of life assessments will be collected at baseline (before the first intervention session), visit 20 (after the last intervention session), and again at visit 21 (4 months after randomisation) and will be used to calculate change over time for the rTMS and sham groups' mean or median scores (as appropriate for distribution) in the assessment of.

- Anxiety and depression, using the Hospital Anxiety and Depression Scale (HADS) [22, 23].
- Overall quality of life, using the AQoL-8D's health state utilities and dimensional scores [24].
- Fatigue, using the Fatigue Severity Scale (FSS) [23, 25].
- Sleep quality, using Pittsburgh sleep quality index (PSQI) and numeric rating scale (NRS).

Tertiary outcomes

MRI data will be extracted from scans collected at baseline (up to 2 weeks prior to the first intervention visit) and post-intervention (within 2 weeks of the last intervention visit). We will compare the mean or median, as appropriate for distribution, for the rTMS and sham groups at each time point, and the change between baseline MRI and post-intervention MRI metrics of sham and rTMS intervention groups, including metrics for whole brain, lobe-specific, and lesion locations.

- Lesion analysis: number of new lesions and number of enlarging T2 hyperintense lesions, combined volume of lesion(s) within each lobe.
- Atrophy analysis: normalised percentage brain volume change from baseline to post-intervention MRI timepoints will be estimated using Structural Image Evaluation using Normalization, of Atrophy (SIENA) [26], part of FMRIB Software Library (FSL) [27]. For substructures, including white matter, grey matter, peripheral (cortical) grey matter, and ventricular cerebrospinal fluid, Structural Image Evaluation using Normalization of Atrophy Cross-sectional (SIENAX) [26], part of FMRIB's Integrated Registration and Segmentation Tool (FSL-FIRST) [27], will be used to determine normalised tissue volumes at each timepoint.
- Diffusion tensor imaging (DTI) metrics: mean diffusivity, fractional anisotropy, axial diffusivity, and radial diffusivity. Considered together, these metrics are indicative of axonal and myelin integrity [28].
- Mean magnetisation transfer ratio (MTR) and quantitative T1 mapping (qT1, mean relaxation time) will be used to measure relatively subtle changes in myelin content [29].

DTI, MTR, and qT1 metrics will be obtained for tissue types combined and for each tissue type segmented into white matter, grey matter, peripheral (cortical) grey matter, and T2 lesional tissue.

- Pearson's correlation co-efficient between MRI measures and MSFC, in both rTMS and sham groups, from baseline to 4 weeks post randomisation.
- Adherence to the treatment schedule and protocol compliance comparison will be conducted between the rTMS and sham groups. This will involve assessing the proportion of participants who adhere to the protocol and those who do not, both overall and separately for each intervention group.
- Blinding success will involve participants indicating which intervention they think they received (rTMS, sham, or uncertain) after the initial intervention, following their 10th session, and after the last intervention. A visual analogue scale will gauge the level of certainty participants have regarding their chosen intervention group. A table will display the number and percentage of participants' choices, categorized by intervention group.

Participant timeline {13}

The participant timeline and visit schedule is described in Fig. 2.

Pre-screening visit (phone option) (~ 30 min)

After verbal consent is obtained, a screening questionnaire on eligibility will be administered. Participant information sheets will be sent to potential participants, who will then be followed up to confirm interest.

Screening visit (~ 120 min)

Informed consent will be obtained. Demographic data and medical and MS history will be collected. Participants will undergo a physical examination and EDSS assessment. Medication history will be checked against the clinical records of each participant and eligibility confirmed by the principal investigator (PI).

The screening visit will require participants to complete three practice sessions of the MSFC score which includes the symbol digit modality test (SDMT), 9-hole peg test (9HPT), and timed 25-foot walk (T-25FW). Each test will be performed following standard operating procedures, adapted from the MSFC manual [30]. The screening MSFC will not be included in the data analysis and is collected to minimise learning effect.

	STUDY PERIOD								
	Screening/ Enrolment		**Baseline***	**Intervention** (20 sessions over 4-5 weeks) +1/+3 days					**Remote follow-up** (4 months post-allocation)
TIMEPOINT**	$-v_2$	$v-_1$	v_0	v_1	v_{2-9}	v_{10}	v_{11-19}	v_{20}	v_{21}
ENROLMENT:									
Pre-screen (phone)	X								
Eligibility assessment	X	X							
Informed consent		X							
Demographics, medical & MS history, physical exam, EDSS		X							
Allocation (randomised)				X					
INTERVENTION: *rTMS* or *sham*				◆—	—	—	—	—◆	
ASSESSMENTS:									
MRI* (may require additional visit)			X					X	
MSFC		X	X			X		X	
HADS			X					X	X
AQoL-8D			X					X	X
FSS			X					X	X
PSQI & NRS			X					X	X
Blinding survey				X		X		X	
Report any adverse events after 1st intervention				X	X	X	X	X	X
Report concomitant medications		X	X	X	X	X	X	X	X

Fig. 2 Schedule of enrolment, interventions, and assessments. Note: An additional 2-week window is tolerated for baseline MRI pre-visit 1 (v1) and post-visit 20 as it may not be possible to schedule scans on the same day. Baseline questionnaires will be emailed with instructions to complete before visit 1 but if not already completed remotely may be completed prior to the first intervention at visit 1

Participants will have their baseline MRI scheduled, for completion prior to, and within 2 weeks of, the baseline visit.

Baseline MRI (~ 60 min)

The acquisition sequence is as follows: 3D FLAIR, 3DT1, MT on/off, qT1 map, and DTI (minimum 32dir).

Baseline visit and first intervention (~ 60 min)

Only after the baseline MRI has passed a SNAC quality assessment will participants proceed with the baseline visit. A series of patient-reported outcome measures (PROM) including the HADS, FSS, AQoL-8D, PSQI, and NRS questionnaires will be collected using the REDCap survey tool. The REDCap survey distribution will be scheduled at screening, for completion of PROMS prior to the baseline visit. Participants will complete the baseline assessments for the MSFC (SDMT, 9-HPT, T-25FW) prior to randomisation and first intervention.

Randomisation

Participants will be randomly assigned 2:1 to the rTMS or sham groups. Randomisation will be performed by REDCap and stratified by sex.

Group 1 ($n=72$): rTMS + usual care.
Group 2 ($n=36$): sham + usual care.

Intervention phase (visits 1–20) (~ 15–30 min each day)

Participants will be asked to confirm details such as their name and date of birth and indicate whether they are experiencing any new or worsening symptoms.

A suitably qualified, unblinded, and well-trained member of the team will perform the intervention, following the SOP. The group allocation will be confirmed in REDCap prior to delivery of the intervention. The intervention will be commenced on the same hemisphere (right or left) for individual participants in all the visits and repeated for 3 min on each hemisphere. Daily intervention visits will be conducted for a period of 4 to 5 consecutive weeks (excluding weekends) until the 20 sessions are completed. If a participant has more than 3 days between visits, they will be considered protocol noncompliant, but they will be allowed to continue in the study until the end of the intervention phase for efficacy and outcome assessments on intention to treat principles. All new AEs and concomitant medications will be recorded, reviewed, and followed up at each visit.

Visits 1, 10, and 20 only

Participants will be asked which intervention group they think they are in, based on their experience of the sessions. A Likert VAS will be used to measure certainty of intervention group allocation: rTMS or sham.

Visit 10 only (allow an additional 30 min)

Participants will undertake the MSFC, including SDMT, 9HPT, and T25-FW.

Visit 20 only (~ 60 min)

The follow-up (post-intervention) MRI will be arranged to coincide with visit 20 (+2 week window). The acquisition sequence will be the same as for the baseline MRI. Participants will undertake the MSFC, including the SDMT, 9HPT, and T25-FW. The PROM questionnaires (AQoL-8D, HADS, FSS, PSQI, and NRS) will be administered via the REDCap survey tool.

Post-intervention follow-up (visit 21) (~ 30 min)

Three months after the last intervention (or four months post randomisation), participants will be asked to complete the final set of PROM questionnaires via the REDCap survey tool. AEs and concomitant medications will also be followed up over the phone.

Sample size {14}

The sample size is based on the primary effect of interest, which is the difference between the rTMS and sham groups in change (pre- and post-intervention) in MSFC score. We have based our target effect size on findings that a change in each component of the MSFC of $\geq 20\%$ corresponds to a change in disability perceivable by the individual, as an increase/decrease in the level of help they require from others [31], and is greater than day-to-day intrapersonal variability [32]. Converting meaningful changes in individual scores to a change in average overall *z*-score requires data on means and standard deviations (SD) for each component. We made assumptions based on Cohen et al. (2001) [33] to convert the changes to an overall *z*-score of 0.57 and then adjusted this downwards to a *z*-score of 0.2 to allow for uncertainty in assumptions and ensure adequate power.

Using the observed effect size of 0.2 in *z*-score in the rTMS group over and above the sham group (that is, 0.2 greater change taking into account any practice effects—for example if the sham group improved by 0.05, then the rTMS group would have to improve by 0.25) and a standard deviation of change of 0.35 (based on data of between-session changes in this measure [33]), we will

have 80% power to detect this effect at $\alpha = 0.10$ with 58 participants in the rTMS group and 29 participants in the sham group. We are employing a 2:1 group size ratio to aid recruitment. We then allow for 20% attrition, resulting in 105 participants total, and round this up to 108 to be evenly divisible by the block size of 6 multiplied by two strata [34].

Recruitment {15}

We will enrol 108 participants from 6 Australian sites. During the phase I trial, the coordinating site recruited 2 to 3 participants per month, indicating that it is feasible to recruit at least 24 participants per site in a year. Participating sites were selected based on their ability to achieve a similar level of recruitment following a site feasibility assessment and review of past MS clinical trial performance.

Participants will be recruited by sites using the following methods:

1. Direct recruitment in MS clinics. The research staff will make an initial approach during MS clinic appointments. These patients will be asked if they would like to hear more about the study and undergo pre-screening.
2. Referral by MS clinic neurologists to the study staff who will make first contact by phone and conduct phone pre-screening.
3. Where applicable, using a local register of participants that have previously participated in research and consented to be contacted about future related research. These participants will be contacted and asked if they would like to hear more about the study and undergo pre-screening.
4. To achieve our local and national recruitment targets, we will promote the trial via the Menzies Institute for Medical Research, MS Research Flagship newsletter, webpage, and social media pages, by placing posters/flyers in the MS clinics and by the distribution of flyers or trial information by patient advocacy groups, such as MS Australia and MS Plus, and at public events. The trial is registered on the ANZCTR and MS Australia trial registries. Promotion and public facing information about the study may result in participants directly contacting study staff requesting more information about the study and to undergo pre-screening.

Sites will pre-screen potential participants using an eligibility questionnaire (either in person or on the phone) based on the eligibility criteria. Verbal consent will be confirmed using the following script.

'Hello, my name is < insert >, and I would like to speak to you about a clinical trial called TAURUS.2. This trial is for people with MS, and we will assess whether magnetic brain stimulation is safe and able to reduce MS symptoms. Would you be interested in finding out more about this study?' Yes/No.
If yes, 'Would you mind if I ask you a few questions about your medical history first to confirm if you are eligible for the study? Answering these questions does NOT obligate you to participate in the trial and you can change your mind or ask me to stop at any time.' Yes/No.
If no, 'OK, thank you for your time, have a good day.'
If yes, ask the pre-screening questionnaire and explain next steps.

Ineligible participants will be recorded on the screening log and informed they are not eligible to participate. If applicable, the referring clinician/neurologist will be informed but no further follow-up will take place. Those who are eligible will be invited to participate and sent the participant information sheet for consideration. The participant information sheet will be provided by email or in person where practical. A follow-up phone call will be made at a mutually agreeable time. Any questions regarding the study will be answered, and participant agreement to take part was confirmed. The participant will then be invited to attend a full screening visit.

Assignment of interventions: allocation

Sequence generation {16a}

The allocation sequence will be generated by the REDCap randomisation module after PI confirmation of eligibility. A group allocation ratio of 2:1 will be used, including a permuted block randomisation with a block size of 6, stratified by sex due to sex differences in presentation of MS [35].

Concealment mechanism {16b}

The REDCap software will be used to perform the randomisation. Only unblinded researchers will have access to the randomisation module in REDCap. The randomisation will be done during the baseline visit before the administration of the intervention. Permutated block sizes of 6 will be used to assist with allocation concealment prior to randomisation.

Implementation {16c}

Participants will be enrolled by study investigators, whilst REDCap will do the group allocation or randomisation.

Assignment of interventions: blinding

Who will be blinded {17a}

Participants will be blind to group allocation. The researcher delivering the intervention and the data manager will not be blind to the group allocation. Researchers performing the statistical analyses will be unblinded due to the uneven group allocation ratio (2:1), but they will not be involved in participant recruitment, allocation, or delivery of the intervention. Unblinded researchers will collect the AE data, but the AE will be evaluated by blinded researchers including the principal and chief investigators (PI/CI). Blinded researchers will collect the MSFC, PROM, and MRI outcome data. The primary outcome will be collected by a blinded researcher not involved in delivery of the intervention to minimise observer bias.

Procedure for unblinding if needed {17b}

It should not be necessary to break the treatment allocation code to unblind the participants during the active study period. If necessary, a request for un-blinding will be sent by the CI of the study, and if permission is granted, the allocation revealed by the researcher delivering the intervention or the data manager. Only at the end of the study will participants be informed of their treatment group, after data analysis is complete.

Data collection and management

Plans for assessment and collection of outcomes {18a}

The MSFC measures will be used to determine whether rTMS improves functional outcomes for PwMS. MSFC will be derived from three components: (1) SDMT scores, (2) 9-HPT scores, and (3) T-25FW. Three practice session of the MSFC will be conducted during the screening phase for the trial. This will reduce any learning effects and will not be used in the analysis [30]. MSFC data for analysis will be collected at baseline, at visit 10 (about 2 weeks post-randomisation) and at visit 20 (4–5 weeks post-randomisation).

Symbol digit modality test (SDMT)

This cognitive performance test is remarkably sensitive for detecting changes in cognitive function. Participants will be instructed to match as many symbols with numbers as they can in 90 s, using the legend which assigns a symbol to each number between 0 and 9. Use of the SDMT, as a component of the MSFC, instead of the traditional paced auditory serial addition test (PASAT), has been well validated [36]. SDMT is a reliable measure with good test–retest reliability (intraclass correlation (ICC) of 0.85) with better construct validity than PASAT [37] and is relatively easy to administer. SDMT is available online for a small fee [36, 38].

Timed 25-foot walk (T25-FW)

This is a quantitative performance-based timed walk. Participants will be instructed to walk as quickly and safely as possible on a twenty five-foot walking track, stop, and walk back. The T25-FW will be administered in person by a trained examiner. The examiner need not be a physician or nurse. The score for the T25-FW is the average of two completed trials. The scores will be analysed individually and used as part of the MSFC. The T25-FW has high inter-rater and test–retest reliability (ICC—0.86, $p \leq 0.001$) and good concurrent validity with other gait measures [39]. Administration and scoring manual can be found online [30, 33].

Nine-hole peg test (9-HPT)

This is a brief quantitative test of upper extremity function. The participant will be seated at a table with a small shallow container holding nine pegs and a wooden or plastic block with nine empty holes. A verbal start command will be issued when a stopwatch is started, and participants will pick up each peg, one at a time, and fit them into the corresponding holes, as quickly as possible. Participants will then remove them as quickly as possible, one at a time, replacing them in the container. The total time to complete the task is recorded. Two consecutive trials with the dominant hand are immediately followed by two consecutive trials with the non-dominant hand. The two trials for each hand are averaged, converted to the reciprocals of the mean times for each hand, and then the two reciprocals are averaged. Administration and scoring manual can be found online [30]. The 9-HPT has high inter-rater and intra-rater reliability (ICC—0.98) and high test–retest reliability (Spearman rho (r) of 0.98) and concurrent and convergent validity [40] and is capable of detecting minor impairments of hand function. As performance on the 9-HPT may be sensitive to practice effects, participants will be required to complete 3 practice sessions prior to baseline [30].

Adverse events

To determine if rTMS is safe and tolerable for PwMS, AEs will be recorded at each intervention visit (from baseline to visit 20). The AE log for each participant will be recorded directly in the REDCap database. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The AE start date, stop date, severity, relationship to study intervention, expectedness, outcome, and duration will be recorded and assessed by the blinded PI. AE tables will be presented by System Organ Class (SOC) and preferred term groupings. A concomitant medication log will collect data on each participant's current medications and any changes throughout the

study. This data will be collected daily from baseline to visit 20 and will aid in the interpretation of both the AE and other outcome data analysis.

PROM—questionnaires

To determine whether rTMS reduces anxiety, depression, or fatigue or improves quality of life or sleep quality over time, participants will complete PROM questionnaires at baseline (visit1), at visit 20 (last intervention visit), and visit 21 (4 months post-randomisation). HADS, AQoL-8D, FSS, and PSQI and NRS will be distributed to participants using the REDCap survey tool with direct data entry by participants. This reduces any data entry errors and improves data completeness and quality.

HADS

This is a self-assessment scale comprising 14 items: seven items each for anxiety and depression. HADS has been used extensively in research, with reliability shown to be high (test–retest intraclass correlation coefficient (ICC) = 0.83; Cronbach's alpha > 0.8) and good criterion validity [22, 41] that correlates well with other measures of anxiety and depression. The use of this scale has been licensed by GL Assessment, MAPI Research Trust, and is available from GL Assessment [42].

AQoL-8D

The AQoL-8D assesses physical and psychosocial aspects of quality of life. The questionnaire consists of 35 items across 8 dimensions: independent living, senses, pain, mental health, happiness, self-worth, coping, and relationships [43]. It is a reliable scale with good convergent and predictive validity, as assessed by a multi-instrument comparison study, and has been validated in PwMS [44]. AQoL-8D data collection instruments are available online [45].

FSS

The FSS is a self-reported nine-item questionnaire (scored from 1 to 7) that measures physical and cognitive aspects of fatigue and fatigue severity [46]. FSS has excellent reliability (ICC = 0.76, over 6 months) and validity metrics in the MS population [47]. Forms for the collection of FSS data are available online [48].

PSQI

The PSQI is an instrument used to measure sleep quality over the past month. The global PSQI is calculated by totalling the seven component scores to give an overall score ranging from 0 to 21, with higher scores indicating worse sleep quality. PSQI has high test reliability and validity metrics, and forms and scoring are available online [49].

Protocol compliance

The number of intervention sessions completed will be summed for each participant, and the proportion of rTMS vs sham participants to complete all 20 intervention sessions within a 5-week period, with no more than 3 days between sessions, will be calculated.

Blinding success

Participants will be asked by the researcher administering the intervention whether they think they are in the rTMS or sham group or if they are unsure. We will also ask participants why they think they are in this group or why they are unsure, and responses will be entered by the researcher into the electronic case report form (eCRF). If participants select either the rTMS or sham option, they will also rate their level of certainty of this guess along a VAS by using a slider on a touchscreen from 'very uncertain' to 'very certain', which is then numerically scored. Successful blinding is defined as a high proportion of 'unsure' responses and an approximately equal proportion of responses across participant guesses; however, participant awareness of the allocation ratio could bias guess choice [50]. Blinding will be considered unsuccessful if more than 60% of participants in the sham arm correctly identify their group and rate their certainty of selection in the top 25% of the scale, in line with recommendations from assessments of bias under various (un)blinding scenarios [51, 52]. Blinding success will be primarily assessed after the first intervention visit as this is less likely to be confounded by any perceived efficacy (or inefficacy) of the intervention [53], in accordance with CONSORT 2010 guidelines, item 11 [54].

MRI data collection and quality control

Participants will complete an MRI brain scan within 2 weeks before the first intervention and within 2 weeks after the last intervention. MRI will be performed at an accredited facility, by fully trained and qualified personnel. MRI will be conducted according to the MRI technologist manual for the study. Approximately 20 scans will be performed at each MRI visit, with the following acquisition sequence: 3D FLAIR, 3DT1, MT on/off, qT1 map, and DTI (minimum 32dir). To ensure sufficient quality for analysis and no movement artefacts, baseline MRI images will be checked by MRI analysts prior to commencing the first intervention and repeated if necessary. The same quality evaluation will be completed on post-intervention MRI scans. At all stages of the manual,

semi-automated, and automated analyses of MRI scans, output will be examined by an expert observer with supervision from Sydney Neuroimaging Analysis Centre (SNAC) directors/image analysis specialists. Where consensus cannot be reached for discrepancies found between analysts, the SNAC director will make the final determination.

Pre-processing of MRI image prior to analysis

Before performing the MRI volumetric analysis, the 3D T1-weighted images will undergo pre-processing using in-house brain-extraction and lesion-filling tools with manual quality assurance and correction where needed. Estimates of brain tissue volumes (whole brain, white matter, grey matter, cortical grey matter, and ventricular cerebrospinal fluid) normalised for individual head sizes will be estimated at each time point using SIENAX [26], a component of FSL-FIRST [26] methods.

Lesion number and volume will be determined from manually drawn lesion masks on baseline 3D-FLAIR images. Lesion masks will be transformed to follow-up 3D T1-weighted image space and adjusted for lesion activity (see the 'Lesion assessment' section) by trained analysts.

Lesion assessment

A T2-weighted lesion will be identified as a rounded or oval area of hyper-intensity, according to the guidelines by Filippi et al. [55]. A lesion will only be counted once per scan. If a lesion extends across more than one slice, it is only counted once, rather than counting the number of lesions per slice. A lesion will be considered 'new' if it was present at the post-intervention MRI and not at baseline. Lesions that are adjacent to a pre-existing lesion but connected to it by a relatively low signal area will also be considered new. Lesions > 5 mm will be classified as 'enlarged' if the lesion size has either increased by at least 100% or the size has increased in at least two consecutive slices. Lesions < 5 mm in size must satisfy both criteria to be classified as enlarged.

Assessments using advanced MRI metrics

The diffusion tensor model is fitted to the pre-processed data using FSL-DTIFIT (diffusion tensor imaging fit). Magnetisation transfer ratio maps are generated as magnetisation transfer-On image (MTon), co-registered to magnetisation transfer-Off image (MToff) by linear registration. Magnetisation transfer ratio is calculated in voxel-wise fashion as MToff subtract MTon, divided by MToff. Quantitative T1 mapping will be obtained by processing the variable flip-angle spoiled gradient recalled echo data with QUIT open-source software [56]. A map of the longitudinal relaxation time (T1) is generated by linear least-squares fitting of the signal intensity curve as a function of flip angle at constant transfer ratio.

Plans to promote participant retention and complete follow-up {18b}

To improve adherence, appointment times will be flexible between 8 am and 6 pm each weekday (room and staff availability permitting), can be at a different time each day, and will not be required on weekends. To allow flexibility for long weekends, illness, or other factors that may prevent attendance, a window of + 3 days is tolerated between intervention sessions, with up to 1 additional week (maximum 5 weeks) tolerated to complete the 20 sessions. A 2-week window is tolerated between screening and baseline for the baseline MRI to be completed. An additional 2-week window is tolerated after the last intervention to allow for the follow-up MRI to be completed. A 1-week window is tolerated for the final follow-up visit. Non-compliance with the visit schedule will not result in withdrawal, and primary and secondary outcome data will continue to be collected. Significant deviations from the intervention schedule may result in withdrawal from follow-up MRI (tertiary outcome data). Withdrawal from treatment does not necessitate withdrawal from primary or secondary outcome data collection. An email with links to trial surveys will be sent to participants at least one day before their scheduled visit, with automatic reminders generated in REDCap. Survey completion will be checked during the visit to avoid missing data. Participants will be encouraged to contact us by phone or email if they are not able to attend their visit. If they do not attend the agreed visit, they will be contacted to identify the reason, and the next visit will be confirmed. Participants will be reimbursed for all reasonable travel expenses for each visit, and a $50 voucher will be provided at the end of the intervention period to promote retention. On-site parking will be provided. Before a participant is considered 'lost to follow-up', they will be contacted three times by two different methods. Contact attempts will be recorded in the case notes.

Data management {19}

Data will be collected, handled, and stored in accordance with the Good Clinical Practice guidelines [57], The Privacy Act (1988) [58], and the Australian Code for the Responsible Conduct of Research, 2018 [59]. A data management plan outlining the collection, handling, storage, security, access controls, and archiving of study data has been developed in accordance with the sponsor's Management of Research Data Procedure. The data management plan can be found in the trial master file and investigator site files.

REDCap will be used to manage our research data. A laptop or tablet will be provided to each site for accessing the REDCap database. REDCap is an open source, free, mature, and secure web application for building and managing online surveys and databases. It is a robust system and provides many useful functions for managing research data (e.g. participant tracking, randomisation, web-based questionnaires, separation of identifying and clinical data and tracking/auditing of data changes and user access controls). REDCap is fully compliant with GCP and international standards for clinical trial data management. The data collected will be stored within a secured database server. This server is housed within a secure computer room equipped with dedicated uninterrupted power supply and air-conditioning system.

Digital medical record source verification of demographics, general medical history, MS diagnosis, MS type and symptoms, MS history, MS relapses, MS treatments, EDSS score, and concomitant medications will be performed at screening.

MSFC and PROMs will be collected directly to eCRF in REDCap, minimising transcription errors and missing data. Built-in validation and range checks will be employed to improve data quality across all eCRF. The trial coordinating centre will conduct data quality checks on a quarterly basis and resolve data queries with sites on an ongoing basis, performing final data cleaning and database lock prior to analysis. If participants request a paper questionnaire, this will be printed from REDCap and then entered by study personnel. Quality control will be performed on all data that is not directly entered.

The primary and secondary outcome data will be analysed by the Menzies Institute for Medical Research, Hobart. The MRI data will be analysed by the Sydney Neuroimaging Assessment Centre (SNAC) at the Brain and Mind Centre, Sydney, and at The Menzies Institute for Medical Research, Hobart. All MRI scans will be codified and transmitted to the Sydney Neuroimaging Assessment Centre (SNAC) via a secure web portal for central analysis.

Confidentiality {27}

Identifiable data (name, address, phone number, email, emergency contact and emergency phone number, neurologist name and neurologist phone number) collected during screening will not leave the study sites. Each participant will be given a unique participant identifier (PID) that will be used on all eCRF and stored separately from their identifiable data. All eCRFs will be pseudonymised. The PID will be assigned sequentially in the following format: site code and participant number: 000–00. Each participating site will only have access to their participants' data. The coordinating site will have access to all pseudonymised data collected during the trial.

Trial data will be made available to suitably qualified members of the study team, monitors, and auditors, HREC, and the Therapeutic Goods Administration (TGA) as far as required by law.

It is a requirement of the sponsor and the National Statement on Ethical Conduct in Research to archive the study records (trial master files and participant data) for a minimum of 15 years. Each site will be responsible for archiving trial documents locally for a minimum of 15 years following study completion. A final review of the trial master file and site files may be conducted by the coordinating site to ensure study closure and archiving is completed correctly.

Participants may choose for their trial data to be retained indefinitely and made available for future ethically approved research. Consent for future use of data will be obtained and noted in the REDCap dataset. Consent will also indicate permission to share data securely and confidentially with named external collaborators.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

N/A.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The methods of analysis will be detailed in a statistical analysis plan (SAP) and signed off by the CI and statistician prior to data lock and data analysis taking place. STATA v18SE (license: 401,809,320,755, UTAS) will be utilised for computing descriptive summaries, and the GEE model will be fitted using the GENMOD procedure in SAS v10 (license: 10,000,108, UTAS).

Primary outcome

MSFC will be derived from three components: (1) SDMT scores, (2) 9HPT scores, and (3) T25-FW. The MSFC score is calculated as the mean of the *z*-scores of the three components, with the means and SDs that are required to derive the *z*-scores for the components taken from a suitable reference population.

Prentice generalised estimating equations (GEE) [60] will be used to compare changes in MSFC *z*-scores between the rTMS and sham group over trial period. We will include in the GEE model fixed effects of time, treatment, and a time-treatment interaction term and adjust for baseline factors including age, sex, and pre-treatment MSFC *z*-scores. Since the time interval between baseline and post-treatment is the same for every participant in our TAURUS 2 trial, a marginal multivariate normal model with a stationary first-order autoregressive type structure [AR(1)] will be considered. The dependence of the post-treatment *z*-score on the baseline *z*-score will be modelled as the following:

$$Y_{ij} = \beta_0 + \beta_1(Trt_{ij}) + \beta_2(Time_{ij}) + \beta_3(Trt_{ij} * Time_{ij}) + \beta_4(Age_i) + \beta_5(Sex_i) + \phi Y_{i,j-1} + \varepsilon_{ij}$$

where Y_{ij} is the 'raw' MSFC *z*-score value for the *i*th participant measured at the *j*th visit, and $Y_{i,j-1}$ is the pre-treatment (baseline) MSFC *z*-score. The parameter β_0 is the bias term (intercept), $\beta_1, \ldots, \beta_5$ are fixed effects regression parameters, and ϕ is the effect of the baseline MSFC *z*-score on the post-treatment *z*-score; ε_{ij} are random measurement errors, where $\varepsilon_{i1} \sim N(0, \sigma^2)$ and $\varepsilon_{ij} \sim N(0, \sigma^2(1-\alpha^2)))$. Here, $\text{var}(Y_{ij}) = \sigma^2$, and $\text{cov}(Y_{ij}, Y_{ij'}) = \phi^{|j'-j|}\sigma^2$. We will perform a sensitivity analysis to check for possible influential outliers, conduct a battery of GEE diagnostic tests, and/or consider other flexible distributions for the *Z*-scores in case the normality assumption is violated. We will further check that the AR(1) type covariance structure is appropriate. For the latter, statistical significance of the correlation parameter ϕ will be assessed at $\alpha = 10\%$. The marginal average benefit of rTMS over sham (effect size) will be captured in the time-treatment interaction term (β_3). The statistical significance will be kept at $\alpha = 10\%$. If the *p*-value for the difference in mean change in *z*-score ($\widehat{\beta_3}$) is < 10%, we will conclude that rTMS is statistically significantly better than sham. All estimates will be reported alongside the (1- α) = 90% confidence intervals. Furthermore, if β_3 is greater than or equal to 0.2, we will conclude a clinically meaningful difference in the MSFC responses to rTMS compared to sham [61].

Secondary outcomes

Adverse events

A Fisher's exact test will be used to compare the proportion of people in the sham group compared to the rTMS group who experience any treatment-emergent AEs. If different categories of treatment-emergent AEs are reported, we will run additional tests to compare proportions of AEs of each severity level (treatment emergent AEs, high intensity AEs, SAEs, and SUSARs).

PROM questionnaires

Continuous outcomes (AQoL-8D, HADS, FSS, PSQI, and NRS) will be compared between the rTMS and sham groups with GEE models. The treatment-time interaction term will be used to estimate the effect of treatment on change in outcomes, as described for the primary outcome. The family-wise error rate (FWER) for each endpoint will be kept at FWER = 5%.

Tertiary outcomes

MRI

Continuous MRI measures will also be analysed with GEE models. Separate measures for different tissue type and brain regions will be analysed depending on distributions of overall measures. The proportion of participants with new (incident) lesions or enlarging lesions at the post-intervention time point will be compared between sham and rTMS using a Fisher's exact test.

We will conduct a canonical discriminant analysis to examine rTMS/sham group separation on linear combinations of measures of myelin.

The association between any change in myelin and change in patient outcomes will be reported, with due consideration of any bias due to missing data.

Compliance

We will use a Fisher's exact test to compare sham and rTMS proportions of participants that are compliant (complete all 20 sessions within the time frame) vs. non-compliant (fail to complete all 20 sessions within the time frame).

Blinding

We will present summary descriptive statistics, frequency (*n*) and percentages per guess category, and median certainty scores (i.e. VAS), cross-tabulated by intervention group, at each time point.

Demographics

Summary descriptive statistics of the participants' characteristics including frequency (*n*) and percentages cross-tabulated by intervention groups, rTMS and sham group,

will be performed. Participants' characteristics that will be reported will include sex, age, EDSS, and duration in years since MS diagnosis.

Interim analyses {21b}

There is no interim analysis planned for this study.

Methods for additional analyses {20b}

There are no additional analyses planned for this study.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

All analyses will be conducted according to intention-to-treat principles. The assessments at baseline occur immediately before randomisation, so by design we will have a baseline value for the primary outcome for everyone. Participants with only baseline measurements will have their baseline data included in the GEE models, though their data cannot contribute to the estimate of change. If there is substantial missingness in a follow-up measurement (greater than 10%), covariates that are predictive of missingness will be included in the model. Intention-to-treat handling of AEs and treatment compliance is straightforward; any missing data on blinding success will be presented as number and proportion of participants who declined blinding assessment and for which group (rTMS or sham).

Plans to give access to the full protocol, participant-level data, and statistical code {31c}

The University of Tasmania's Research Data Portal will host the published data. The RIF-CS metadata will be consistent with FAIR data principles (Findable, Accessible, Interoperable and Reusable) with a Creative Commons license attributing the data owners. This approach is consistent with sponsor policy and the Australian Code for the Responsible Conduct of Research. The clinical trial data will be made available for future ethically approved research by us and external collaborators with appropriate consent, confidentiality, and data sharing agreements in place. The protocol will be published and the statistical code available.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

The local PIs will be responsible for overseeing the trial at their site, ensuring all staff are appropriately delegated, trained, and qualified to conduct the trial, have current GCP training, and follow the trial protocol and SOPs. They will be conversant with the protocol and abreast of protocol amendments and follow the regulatory guidelines.

PIs will manage recruitment and eligibility, data collection and quality, participant withdrawals, and assessment and notification of AEs and SAEs. They will ensure data privacy and confidentiality is maintained and respond to monitoring and auditing requirements. They will also ensure continuity of trial through proper staff management.

The chief investigator (CI) will be responsible for all reporting to the sponsor, HREC, TGA, steering committee (SC), data safety monitoring board (DSMB), and funders. The CI and coordinating team will ensure each site has received ethics and governance approval, site initiation and training, and the complete document set required for their investigator site files (and subsequent amendments) and provide access to the eCRF and trial database via REDCap. The Trial Management Group (TMG) will be managed by the coordinating centre and will include the coordinating CI, PI, clinical research fellow, trial coordinator, data manager, and program manager, with an invitation extended to all site PIs and trial coordinators to attend. The TMG will meet monthly and ensure the smooth day to day running of the trial across all sites. The coordinating site's CI, program manager, statistician, and clinical research fellow will report quarterly to both SC ($n=6$) and DSMB ($n=4$), made up of independent clinical, scientific, consumer, and statistical experts.

Remote data monitoring of the participating sites will be conducted by the coordinating site. On-site monitoring will be performed if triggered by persistent non-compliance or concerns identified during remote data monitoring. The coordinating site and DSMB will review blinded data extracts on a quarterly basis and review recruitment and retention rates, protocol treatment compliance, data quality, and completion rates for all primary and secondary outcomes. In addition, the DSMB will monitor the unblinded randomisation allocation sequence and trends towards safety and efficacy.

The TMG will consider new information of relevance, consider and act on recommendations made by the sponsor, DSMB, HREC, or TGA, and review reports and papers for publication. The CI will resolve authorship disputes.

Consumer and community involvement (CCI)

The Menzies MS Research Flagship, Consumer and Community Reference Committee (C&CRC) will provide ongoing support for the study. The committee is 100% independent and comprises 12–16 diverse members, including people living with MS, and their carers. The C&CRC is independently chaired by A/Prof Des Graham (2019–2023) and Chris Gumley (2023-current). The

C&CRC will meet quarterly and provide lived experience input into.

- Participant information sheets and consent forms
- Advertising material
- Outcome measures
- Study design, feasibility, and management
- Dissemination of results (co-presenting at conferences and plain English summaries)

The consumer and community involvement manager will ensure consumer involvement is conducted according to the NHMRC statement on consumer and community involvement in health and medical research [62] and the Menzies CCI policy.

Composition of the data monitoring committee, its role and reporting structure {21a}

Safety oversight will be conducted by an independent DSMB consisting of independent neurologists (3) and a statistician (1). They will meet quarterly to review unblinded study data and make recommendations to the TMG and sponsor as required. They will follow a defined Damocles charter (TAURUS.2 DSMB Damocles charter_v0.2_Jun2022) [63] and the NHMRC national statement.

Adverse event reporting and harms {22}

Adverse events (AE) and adverse reactions (AR)

Any new or worsening clinical sign, symptom, or disease, in a participant to whom the trial intervention has been administered (even if unrelated) is regarded as an AE. At each visit, AEs will be recorded, and the PI will be required to assess the severity and causal relationship of the AE to the study intervention. An AE is classified as related if the AE is known to occur with the study intervention and there is a reasonable possibility of causation or temporal relationship between the intervention and the event. AEs are unrelated if there is no reasonable possibility that the intervention caused the event, there is no temporal relationship, or an alternative aetiology has been established. Related AEs are termed adverse reactions (AR).

Assessment of AE severity

Grade 1—Mild AEs requiring no specific medical intervention including asymptomatic or mild symptoms, clinical or diagnostic findings.
Grade 2—Moderate AEs where minimal, local, or non-invasive interventions are indicated. Limiting age-appropriate instrumental activity of daily living (ADL).
Grade 3—Severe AEs that are medically significant but not immediately life threatening, requires hospitalisation or prolongation of hospitalisation, disabling, limiting self-care ADL.
Grade 4—A life-threatening AE, emergency or urgent intervention required.
Grade 5—Fatal AEs that results in death.

Serious adverse event (SAE) is defined as any occurrence that.

a) Results in death
b) Is life threatening
c) Requires hospitalisation or prolongation of existing hospitalisation
d) Results in persistence of significant disability or incapacity
e) Is a congenital abnormality or birth defect
f) Is considered medically significant by the investigator

The expected AEs in this clinical trial related to rTMS are scalp discomfort, headache, dizziness, and tingling of fascial muscles. They are expected to be mild and stop shortly after the administration of rTMS. Additional expected SAEs which are unrelated to rTMS are MS disability progression, elective surgery admission, and relapse of MS.

Reporting

Unexpected SAE will be reported to the CI and sponsor within 24 h of investigators becoming aware of the event. Expected SAEs do not require immediate reporting.

Suspected unexpected serious adverse reactions (SUSAR) will be reported by the CI to the sponsor and HREC within 24 h. CI will notify Menzies Director within 72 h. The sponsor will notify the Deputy Vice Chancellor of Research (DVCR) and UTAS authority within 72 h.

SUSARs will also be reported to the TGA. If the event is fatal or life threatening, this will be reported within 7 days; all other SUSARS will be reported to the TGA within 15 days.

PIs will be responsible for local reporting of SAEs according to local procedures. PIs may take urgent safety measures to ensure the safety and protection of the clinical trial participants from any immediate hazard to their health and safety. In this instance, the approval of HREC prior to implementing is not required. The CI must inform the sponsor, HREC, and TGA within 24–72 h. If the trial is temporarily suspended or terminated early due to safety reasons, this will be reported within 15 calendar days.

The CI will send an annual progress and safety report to the HREC on the anniversary date of the approval of

the clinical trial. TGA will receive a Development Safety Update Report when requested.

Frequency and plans for auditing trial conduct {23}

The UTAS clinical trial governance team (independent of investigators) will conduct an annual audit of the trial master file. Data will be made available to representatives of the study sponsor, HRECs, or funders as required by law for auditing purposes.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Hunter New England Human Research Ethics Committees (HNE HREC) is the lead HREC under the National Mutual Acceptance (NMA) scheme. All amendments will be submitted to HNE HREC for review and approval and passed through UTAS HREC (which is not part of the NMA scheme) for approval. Minor administrative amendments will be implemented immediately and notified. All substantial amendments will require HREC review and approval prior to implementation. The CI will be responsible for submitting amendments to both HRECs. Each site PI will be required to notify their local governance team of amendments, sign the protocol amendment acknowledgement and signature page, and ensure their local team is appraised of the amendment. Each site will notify their trial participants of any relevant approved changes and ensure participants voluntarily sign a new consent form based on the changes if they still want to continue with the study. Any relevant amendments will be notified to the TGA under the clinical trial notification (CTN) scheme as may be necessary. The ANZCTR and MS Australia trial registries will also be kept up to date with trial amendments.

Dissemination plans {31a}

Participants will receive the grouped results of this study in plain English and not their individual results. The results of this trial will also be disseminated through Menzies Institute for Medical Research, MS Research Flagship website, e-newsletters, and social media channels and via participating sites and partner organisation (MS Australia and MS Plus) newsletters and social media channels. The clinical trial findings will also be presented at seminars, scientific meetings, and conferences and published in open access journals no later than 12 months after study completion. A final study report will be submitted to the major funding bodies.

Discussion

Ethics approvals

The major practical or operational issue faced by this trial was obtaining ethics approval to perform the trial in multiple sites across Australia. The NMA scheme in Australia does not apply to all sites, including the University of Tasmania. We initially sought approval locally with UTAS HREC to start the trial whilst we obtained ethics and governance approval for our other sites, ordered equipment and resources for them, and conducted site initiation and training. We anticipated this process was going to take time and had intended to initiate recruitment locally whilst undertaking this process. However, HNE HREC twice rejected our application due to (1) choice of MRI markers of remyelination as primary outcomes and (2) choice of RCT design over an open-label single-arm trial. We immediately halted initiation of the trial at Menzies (no participants had been recruited) whilst we adapted our trial design to have a validated clinical outcome as our primary outcome measure and further justified the necessary RCT design. We then sought approval from HNE HREC, followed by UTAS HREC. This not only significantly delayed our trial start up and recruitment time frames but also shifted the emphasis of success of rTMS to a clinical/functional outcome, rather than a radiological marker of remyelination (now our tertiary outcome). MSFC is commonly used in MS trials and is more sensitive to change than EDSS, requiring a shorter assessment time and treatment duration to detect an effect [64]. Therefore, the decision was made to select MSFC as our primary clinical outcome measure. Remyelination on MRI will still be assessed as part of this clinical trial and may substantiate any improvements in MSFC or other PROMs.

Standard operating procedures (SOPs)

Standard operating procedures (SOPs) for rTMS administration using Magstim and MagVenture devices and associated coils, MSFC, informed consent, data management, safety reporting, physical examination, and EDSS were prepared for this trial and will properly guide the participating sites to deliver the protocol and ensure uniformity across sites. This will help to reduce any variability or between centre effects.

Standardisation of MRI and rTMS procedures using healthy volunteers

Healthy volunteers will be recruited to enable standardisation of equipment and protocols and for staff training and competency assessments to take place. Prior to the recruitment of participants to the main RCT, we will advertise for healthy volunteers from the local community. Eligible volunteers will be invited to provide informed consent. Volunteers who decline to participate or decide to withdraw their consent will not suffer any negative consequences.

We will recruit up to 10 healthy volunteers at each participating site to test and standardise the MRI and

rTMS protocols and ensure that the staff at each site have the capabilities to carry out the procedures according to protocol. The rTMS and MRI procedures used in this study are not standard procedures, so specific training and quality evaluation is required.

A healthy volunteer will undertake 1–2 MRI sequences or a single session of rTMS or sham. No additional study visits or questionnaires will be required. The risk of a healthy volunteer experiencing an AE resulting from a single MRI sequence or a single session of rTMS or sham stimulation is extremely low. Data collected from healthy volunteers will be used to inform and confirm the main study protocol and will not be analysed with the main study data.

There will be no direct benefit for the healthy volunteers. The benefit will be derived for the researchers only. The healthy volunteer tests are essential to improve the researcher's skill in delivering the rTMS and sham intervention, ensuring they can maintain participant blinding during each session and ensuring the MRI protocol implemented at each site produces high-quality scans for analysis, prior to commencing the main RCT. Each healthy volunteer will be provided with a unique code to maintain confidentiality of data produced.

Standardisation of intervention across sites

Each site will receive training in our rTMS standard operating procedure (SOP) from experienced rTMS administrators from the co-ordinating study site to ensure a consistency of the intervention across sites. Training will be performed on healthy volunteers, excluding anyone with a contraindication to rTMS, particularly those that are prone to migraines or seizures will be excluded at screening.

Five of the study sites will use Magstim Rapid2 and 90-mm circular coil, whilst one site will use MagVenture MagPro stimulator with C100 circular coil (11 cm outer diameter). To ensure equivalent stimulation intensity across devices, the magnetic field (mT) was assessed at the coil surface for each MSO percentage increment, using a GM08 Gaussmeter (Hirst Magnetic Instruments) and Transverse Hall sensor and Rigol DS1052E oscilloscope. iTBS timing parameters and pulse waveform characteristics were also confirmed to be within acceptable limits of timing variation. Coils have similar geometry, and published data and specifications provided by the manufacturer confirmed that the field distribution across the coil profile is also comparable [65]. We are therefore confident that comparable rTMS and sham will be delivered across all sites in the study.

Standardisation MRI

The Sydney Neuroimaging Analysis Centre (SNAC) will conduct pre-trial scans on healthy volunteers and quality assessments with each site using the standardised technologist manual and assessment frameworks. This is a critical step for all studies where MRI outcomes are used, as MRI scanners differ significantly between clinical sites. Running test scans is the only way to ensure that the MRI protocol has been operationalised at each site and to confirm the site can produce scans that are of suitable quality before commencing the RCT. Generally, only 1–2 MRI scans are required to successfully set the test parameters; however, sometimes repeat scans are required if they fail quality checks.

Blinding

Whilst we have demonstrated successful blinding of the treatment allocation in our phase I trial, we are using a slightly modified version of the sham procedure in phase II. We may reasonably anticipate that this method will also be successful in maintaining participant blinding and reducing bias; however, that is to be determined during the course of this trial. We will reduce observer bias by ensuring each site has a dedicated blinded assessor to administer the MSFC and collect PROMS.

Trial status

This trial is currently recruiting participants after initial ethics approval on 20 July 2022. The current protocol is version 8 dated 12 December 2023. The anticipated recruitment period is June 2022 to December 2023. The actual recruitment period commenced in November 2022 to May 2024. Trial completion is defined as the last patient last visit (LPLV).

Abbreviations

ADL	Activity of daily living
AE	Adverse event
ANZCTR	Australia and New Zealand Clinical Trial Registry
AQoL-8D	Assessment of Quality of Life 8 Dimensions
AR	Adverse reaction
CCI	Consumer and community involvement
C&CRC	Consumer and Community Reference Committee
CI	Chief investigator
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case report form
CTN	Clinical trial notification
DSMB	Data safety monitoring board
DTI	Diffusion tensor imaging
eCRF	Electronic case report form
EDSS	Expanded Disability Status Scale
FSS	Fatigue Severity Score
FWER	Family-wise error rate
GCP	Good Clinical Practice
GEE	Generalised estimating equations
GP	General practitioner
HADS	Hospital Anxiety and Depression Scale

HNE	Hunter New England
9HPT	9-Hole Peg Test
HREC	Human Research Ethics Committee
ICC	Intraclass correlation
ICH	International Conference on Harmonisation
ISF	Investigator site file
iTBS	Intermittent theta-burst stimulation
ITT	Intention-to-treat
LPLV	Last patient last visit
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSO	Maximum stimulator output
MTR	Magnetisation transfer ratio
NAWM	Normal appearing white matter
NHMRC	National Health and Medical Research Council
NRS	Numeric Rating Scale (Sleep quality)
OPC	Oligodendrocyte progenitor cells
PASAT	Paced Auditory Serial Addition Test
PI	Principal investigator
PID	Participant identifier
PROM	Patient reported outcome measure
PSQI	Pittsburgh Sleep Quality Index
qT1	Quantitative measurement of longitudinal relaxation time
r	Spearman's rho
rTMS	Repetitive transcranial magnetic stimulation
SAE	Serious adverse event
SAP	Statistical analysis plan
SDMT	Symbol Digit Modalities Test
SNAC	Sydney Neuroimaging Analysis Centre
SOC	System Organ Class
SOP	Standard operating procedure
SUSARs	Suspected unexpected serious adverse reaction
T25-FW	Timed 25-foot walk
TGA	Therapeutic Goods Administration
TMF	Trial Master File
UTAS	University of Tasmania
VAS	Visual analogue scale

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Authors' contributions {31b}
NS is the program manager and contributed to the study design, protocol development, database development and management, quality assurance, ethics applications, governance, and registration processes; trained researchers in consent processes; managed the oversight committees; developed the DSMB charter; managed CCI; and contributed to writing the final protocol manuscript. CE contributed to the protocol development and implementation, coordinated the writing of the protocol manuscript for publication, contributed to the governance and registration processes and testing the database platform, managed trial ethics approvals, delivered rTMS, and coordinated site activities including site initiation visits and activation. VFN contributed to the statistical analysis plan, data cleaning, and statistical analysis of the trial results. KM contributed to the study design, protocol development and implementation, analysis plan, and writing of the protocol manuscript; performed device assessment; and trained researchers to deliver rTMS. AZ contributed to the study design and the development and implementation of the protocol and trained researchers in participating sites to deliver rTMS. PTN contributed to the study design and the development and implementation of the protocol. LLL contributed to the development of the protocol and statistical analysis of the trial results and edited the final manuscript. MD is a person with lived experience and contributed to the development of the participant information sheets, recruitment and promotional materials, and all public facing materials. They contributed to the trial feasibility and accessibility and edited the final manuscript. MHB contributed to the study design and the development of MRI outcome measures and methodology. KS recruited study participants and collected primary and secondary outcome data and coordinated activities with the sites. JS recruited study participants and collected primary and secondary outcome data and coordinated activities with the sites. MRH contributed to the study design and protocol development and implementation and training researchers to deliver rTMS. CLC contributed to the study design, secured funding for protocol development, and collected primary data. MB contributed to the study design, protocol development, sample size calculation, and statistical analysis section. KMY is the research principal investigator. She conceived the study design, contributed to the protocol development, secured funding for the protocol development, and edited the final manuscript. BVT is the chief investigator and clinical principal investigator. He contributed to the protocol development and implementation, secured funding for protocol development, and edited the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials {29}
There are no contractual agreements that will limit the researchers from accessing the data. The data will be made available to researchers that will perform the statistical analysis at the end of the trial. This will be in de-identified form.

Declarations

Ethics approval and consent to participate {24}
Ethics approval for this clinical trial was provided by HNE HREC (ref: 2022/ETH01012) and UTAS HREC (ref: H0026359). Written, informed consent to participate will be obtained from all participants.

Consent for publication {32}
We can provide a model of our consent form on request.

Competing interests {28}
The authors declare no competing interests.

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