PROTOCOL

A Prospective multicentre, phase 2b randomised controlled double-blind trial, to determine the safety and efficacy of perispinal etanercept on quality of life at 28 days post treatment.

PESTO

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Author:
Vincent Thijs

Sponsor/s:
Florey Institute of Neuroscience and Mental Health

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

PRINCIPAL INVESTIGATORS

Role	[Title] [First name] [Surname]	[Department], [School], [Organisation]
Chief Investigator A (Applicant)	Professor Vincent Thijs	Stroke theme, Florey Institute of Neuroscience and Mental Health,
Chief Investigator B	Professor Julie Bernhardt	Stroke theme, Florey Institute of Neuroscience and Mental Health,
Chief Investigator C	Professor Nikola Sprigg	Faculty of Health Sciences and Medicine, University of Nottingham, United Kingdom
Chief Investigator D	Professor Geoffrey Cloud	Stroke Medicine, Alfred Health
Chief Investigator E	Professor Leonid Churilov	University of Melbourne
Chief Investigator F	Ms Brooke Parsons	Stroke consumer, Melbourne
Chief Investigator G	Professor Marion Walker	Faculty of Health Sciences and Medicine, University of Nottingham, United Kingdom
Chief Investigator H	Professor Philip Bath	Faculty of Health Sciences and Medicine, University of Nottingham, United Kingdom

DATA MANAGEMENT

Li Chun Quang – Stroke Theme
The Florey Institute of Neuroscience and Mental Health
245 Burgundy Street
Heidelberg VIC 3084 Australia

BIOSTATISTICIAN

Professor Leonid Churilov
Melbourne University
245 Burgundy St
Heidelberg, VIC 3084, Australia
Phone: 61 9035 7089

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STUDY SYNOPSIS

(please provide a brief information)

Title:	A Prospective multicentre, phase 2b randomised controlled double blind trial, to determine the safety and efficacy of perispinal etanercept on quality of life at 28 days post treatment.
Short Title:	Perispinal etanercept to improve STroke Outcomes (PESTO)
Design:	Phase 2b, multicentre, prospective, randomised, double blind, placebo-controlled clinical trial
Sponsor:	Florey Institute of Neuroscience and Mental Health
Study Centres:	Austin Health (AUS), Alfred Health (AUS), Nottingham (UK)
Study Hypothesis:	Perispinal etanercept (PSE) improves quality of life after chronic stroke in stroke survivors of working age
Study Objectives:	To test the safety and efficacy of administration of 25 mg perispinal etanercept in improving patient reported outcomes at 28 days after treatment.
Primary Objectives:	The *Primary Hypothesis* is that treatment with 25 mg perispinal etanercept improves quality of life in stroke survivors
Secondary Objectives	The *Secondary Hypothesis* is that repeated administration of perispinal etanercept 25mg leads to improved quality of life compared to a single administration.
Inclusion Criteria:	1. Patients with a history of acute ischemic or hemorrhagic stroke confirmed on imaging 2. Age ≥18 years-65 years at time of stroke 3. Moderate-to-severe disability *resulting from stroke* as defined by a modified Rankin scale of 3-5 4. Stroke occurred between 1 and 5 years before enrolment. 5. SF-36 score <95 6. Patient is able to complete the SF-36 questionnaire independently or availability of a relative or carer who is able to complete the SF-36 questionnaire on behalf of the patient 7. Consent can be obtained from the participant or person responsible
Exclusion Criteria:	1. Contra-indication to etanercept (eg previous hypersensitivity, ongoing infection, use of IL-1 antagonists) 2. History of hepatitis B and C, tuberculosis, HIV, SLE, multiple sclerosis, moderate to severe heart failure 3. History of malignancy 4. Other use of immunosuppressant 5. Clinical diagnosis of dementia 6. mRS 0-2 7. Botulinum toxin injection to limbs in the 4 months prior to Screening Visit 8. Pregnancy (women of childbearing potential must be tested) 9. Breastfeeding 10. Participation in any investigational study in the last 30 days

	11. Known terminal illness or planned withdrawal of care or comfort care measures. 12. Any condition that, in the judgment of the investigator could impose hazards to the patient if study therapy is initiated or affect the participation of the patient in the study. 13. Prior exposure to etanercept for stroke
Number of Planned Participants:	168
Investigational product:	etanercept (Enbrel) 25 mg
Safety considerations:	Adverse Events and Serious Adverse events will be collected during the course of the trial.
Statistical Methods:	The primary endpoint, an achievement of at least 5 points improvement on the SF-36 score will be tested using a logistic regression model with the treatment arms as an independent variable and baseline value of SF-36, proxy or self- administration of SF-36 as adjustment covariates.

GLOSSARY OF ABBREVIATIONS & TERMS

Abbreviation	Description (using lay language)
AE	Adverse Event
DSMC	Data Safety Monitoring Committee
eCRF	Electronic Case Report Form
EQ-5D	EuroQol Five Dimensions Questionnaire
FAS	Fatigue Assessment Scale
FIM	Functional Independent Measure
GAD -7	General Anxiety Disorder
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HREC	Human Research Ethics Committee
ICF	Informed Consent Form
ICH GCP	International Conference on Harmonisation-Good Clinical Practice
IP	Investigational Product
ITT	Intention-To-Treat
NIHSS	National Institute of Health Stroke Scale
MoCA	Montreal Cognitive Assessment
mRS	Modified Rankin Scale
PHQ -9	Patient Health Questionnaire
PGICS	Patient Global Improvement of Change Scale
PROMS	Patient reported outcome measures
TBC	tuberculosis
SAE	Serious Adverse Event
SF-36	Short Form 36
VAS	Pain Visual Analogue Scale

1. INTRODUCTION/BACKGROUND INFORMATION

a. LAY SUMMARY

Perispinal administration of etanercept, a drug used to treat conditions affecting the joints and skin, has been associated with improved outcomes in patients with impairments and disability resulting from stroke. It is unclear whether these improved outcomes are due to causal effects of etanercept or other factors, as these results were seen in observational studies. The current randomized, placebo-controlled trial proposes to determine whether a single or two injections of etanercept improves quality of life and is safe.

b. INTRODUCTION

Stroke survivors exhibit lifelong, debilitating neurological impairments which negatively affect every aspect of their daily life. Because current treatment options for post-stroke impairments are limited, patients may try out therapies with unproven efficacy and safety. Often,these therapies often incur significant costs (treatment and travel costs overseas) which adds to the stroke survivor and family burden. Perispinal injection of etanercept (PSE) is such a treatment with case series, media reports and online testimonials suggesting spectacular alleviation of neurological impairments, but no proven biological effect in humans, nor reliable evidence from high quality clinical trials.

etanercept is a Tumor Necrosis Factor-alfa inhibitor that is currently used to treat rheumatological and dermatologic conditions. It does not cross the blood brain barrier when given subcutaneously. Proponents of etanercept for neurological symptoms propose that perispinal administration overcomes this limitation. PESTO is a 30-months, phase 2b, multicentre, prospective, randomised, double blind, placebo-controlled trial testing the safety and efficacy of administration of perispinal etanercept in improving patient reported outcomes at 28 days after treatment. To test this hypothesis, the required sample size is 168. This sample size will be able to establish a clinically important difference in quality of life with a high degree of confidence.

PESTO is a clinical trial that will determine whether perispinal etanercept significantly improves quality of life after stroke, alters impairment and function and whether the enthusiasm for this unproven therapy is warranted. We will scientifically test the safety and efficacy of perispinal etanercept administration in stroke patients of working age.

c. BACKGROUND INFORMATION

A large proportion of the 470,000 patients with stroke will have long-lasting, debilitating neurological impairments. As a consequence, many stroke survivors are unable to live at home, return to work or perform their usual activities or hobbies. The burden of stroke on society is immense.

The management of post-stroke symptoms usually consists of physiotherapy, speech or occupational therapy, psychological support, orthoses, spasticity management and pain control. The efficacy of current medical treatment options in reducing many of the post-stroke impairments is limited. Therefore, patients and families are easily attracted by alternative treatment modalities that promise more effective solutions. These alternative treatments are often not based on adequate preclinical and clinical studies. Their efficacy and safety are not tested in controlled randomized clinical trials. The purported effects of treatment may therefore be related to spontaneous improvements or occur due to placebo effects. Moreover, these treatments may impose additional financial burden on stroke survivors or on the community.

d. PERISPINAL ETANERCEPT

Recently, the beneficial effects of perispinal etanercept administration have caught the attention of the stroke survivor community in Australia, Europe and the United States of America.[1, 2] Reports in the literature have described impressive and immediate improvements in gait, memory, pain, swallowing and muscle strength.[3, 4] These improvements were seen in patients with a variety of neurological conditions such as stroke, traumatic brain injury and Alzheimer's dementia.[5-9] For instance, highly significant improvements in grip strength were reported immediately and up to at least 3 weeks after a single administration of perispinal etanercept. Improvements in the time to walk 20 meters were significantly changed. Ninety percent of the respondents reported favourable changes in motor effects after administration. These effects were maintained over at least several weeks. In this published series, the effects were seen after a single administration. Anecdotal reports have suggested that additional improvements were seen with repeated administration. YouTube videos of patients who have experienced such improvements after treatment offer compelling viewing and encourage other stroke survivors to also undergo treatment.

The medical community has however remained very sceptical of this form of therapy.[10] Although etanercept is primarily used for a variety of rheumatologic conditions, it has not been tested rigorously in stroke. etanercept does not cross the blood barrier due to its large molecular size.[11, 12] Administration of etanercept in the peri-spinal region is argued by the proponents of this treatment modality as a way to overcome this problem. The medical community view the potential for any direct effects on the central nervous system to be low or non-existent because it cannot cross the blood brain barrier.[13, 14] Traditional phase 2 studies have not been performed to date. Regulatory authorities have not given approval for the treatment of neurological conditions with etanercept given inconclusive trial results or the lack of randomized clinical trials.[15] In 2016, the American Academy of Neurology published a practice advisory which concluded that: "Clinicians should counsel patients considering etanercept for treatment of poststroke disability that the evidence is insufficient to determine the treatment's effectiveness and that it may be associated with adverse outcomes and high cost (Level U: insufficient evidence)." The authors additionally noted: "We have very low confidence in the evidence for efficacy of etanercept for poststroke disability because of the high risk of bias of the relevant studies. The biological plausibility of benefit was judged to be low because of the reported immediate onset of benefit and single administration of a transiently acting medication. Explanations other than the effectiveness of the treatment for the observed improvements include observer expectation, performance motivation, regression to the mean, and the placebo effect." Finally, the Practice Advisory recommended that "Research is needed on the effects of etanercept on poststroke disability. To minimize the risk of bias, future studies of etanercept for the treatment for poststroke disability should randomly allocate patients in a masked fashion to active and placebo treatment arms and employ masked, standardized poststroke disability outcome measures."[16]

e. ETANERCEPT

etanercept is a human tumour necrosis factor receptor p75 Fc fusion protein produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian expression system.[17] etanercept is a dimer of a chimeric protein genetically engineered by fusing the extracellular ligand binding domain of human tumour

necrosis factor receptor-2 (TNFR2/p75) to the Fc domain of human IgG1. etanercept contains 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons. The mechanism of action of etanercept is thought to be its competitive inhibition of TNF binding to cell surface TNFR, preventing TNF-mediated cellular responses by rendering TNF biologically inactive. etanercept may also modulate biologic responses controlled by additional downstream molecules (e.g., cytokines, adhesion molecules, or proteinases) that are induced or regulated by TNF. etanercept is commonly used in inflammatory conditions (rheumatoid arthritis) or dermatologic conditions (psoriasis). etanercept is slowly absorbed from the site of subcutaneous injection, reaching maximum concentration approximately 48 hours after a single dose. etanercept is cleared slowly from the body. The half-life is long, approximately 70 hours. Patients should be evaluated for infections before, during, and after treatment with Enbrel (etanercept product name), taking into consideration that the mean elimination half-life of etanercept is approximately 70 hours (range 7 to 300 hours). Allergic reactions are common and have included angioedema and urticaria; serious reactions have occurred. Other risks include reactivation of tuberculosis and hepatitis B, worsening of hepatitis C, immunosuppression, possible increased risks of hematologic malignancy, increased risk of skin cancers and demyelination. It is unknown whether these risks, observed in patients who received repeated administrations, are present in patients who receive one or two injections of etanercept. Careful selection of patients and monitoring of risks of this treatment is warranted. When this precautions are taken, etanercept is very safe.[18]

f. SELECTING OUTCOMES FOR PESTO

etanercept is argued to show benefit across a broad range of impairments in chronic stroke survivors, often many years post stroke. To address the current application of etanercept, ensure generalisability and counter claims of selection bias, this trial needs to test the treatment in a broad sample of stroke survivors. In selecting primary and secondary outcomes we considered the recommendations of an international group of leading experts in stroke recovery.[19, 20] Measuring outcome across a number of ICF domains is warranted in this trial. The modified Rankin scale (a global outcome measure in common use in acute trials) is not an appropriate primary outcome for this chronic population, however it will be used as a secondary outcome.[21].[22] The broad target sample means that impairment-specific scales such as Fugl-Meyer scale for motor recovery, Western Aphasia Battery for language or 6m speed test for walking, apply only to subsets of patients with specific impairments, none can serve as a primary outcome measure. The NIHSS is a global neurological impairment scale that is commonly used in the acute phase and captures more stroke specific deficits but is not validated in the chronic phase and does not measure outcomes that matter to patients such as fatigue, depression or dependency.[23] The NIHSS does not require cooperation of the patient and because we include patients with severe disability or language problems can be used as a clinician reported secondary outcome to capture changes in impairment in this trial.

Quality of life scales are increasingly used in stroke trials to assess cost-efficacy but also to reliably measure stroke survivor's perception of outcome.[23] The Short Form (SF-36) is a 36-item, patient-reported survey of patient health. The SF-36 consists of eight scaled scores, which are the weighted sums of the questions in their section. Each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight. A score of zero is equivalent to maximum disability and a score of 100 is equivalent to no disability. The SF-36, in use since 1991, has both internal and external validity. Higher scores are associated with return to work,

reduced hospitalization and mortality. The SF-36 has been used in randomized trials of a wide range of conditions. In stroke, it avoids ceiling effects seen with disability scales, and captures both physical and mental health issues commonly seen after stroke.[24] Clinically important differences have been defined and, interestingly, are similar across a wide range of conditions.[25] An improvement of 5 points is considered of importance, whereas an improvement of 10 points is considered of major importance. We selected a change in SF-36 of 5 points as the trial primary endpoint. Standard safety reporting of adverse and serious adverse events will be undertaken.

2. STUDY OBJECTIVES

a. HYPOTHESIS

Perispinal etanercept (25 mg) improves quality of life in working age patients who have a history of stroke and moderate to severe disability. The *Primary Hypothesis* is that treatment with peri-spinal etanercept improves quality of life in stroke survivors. The *Secondary Hypothesis* is that repeated administration of peri-spinal administration leads to improved quality of life compared to a single administration.

b. STUDY AIMS

To demonstrate the efficacy and safety of perispinal etanercept (25 mg) on patient reported outcome measures after stroke.

c. OUTCOME MEASURES

Primary Efficacy endpoint:
To estimate the proportion of patients at day 28 with an improvement of 5 points or more on the SF-36 compared to baseline following single injection of etanercept 25 mg or equivalent placebo.

Safety Endpoints*:*
Proportion of serious adverse events at day 28
Proportion of serious adverse events at day 56

Secondary Efficacy Endpoint
To estimate the proportion of patients at day 56 with an improvement of 5 points or more on the SF-36 compared to baseline between the 3 treatment arms (placebo, single administration of etanercept, two administrations of etanercept).

Exploratory endpoints
1. To estimate change in neurological impairment using the NIHSS after injection of placebo or etanercept between baseline and day 56 in patients receiving none, one or two injections of etanercept.
2. To estimate change in neurological impairment using the NIHSS after injection of placebo or etanercept between baseline and day 28 after injection of placebo or etanercept
3. To estimate the change in functional independence using the FIM after injection of placebo or etanercept between baseline and day 28
4. To estimate the change in mRS between baseline and day 28 after injection of placebo or etanercept between baseline and day 28
5. To estimate immediate (30 min after injection) patient reported improvement using the Patient Global Improvement of Change Scale after injection of placebo or etanercept

6. To estimate the change in Fatigue Assessment Scale between baseline and day 28 after injection of placebo or etanercept
7. To estimate the change in the PHQ-9 depression scale between baseline and day 28 after injection of placebo or etanercept
8. To estimate the change in the GAD-7 anxiety scale between baseline and day 28 after injection of placebo or etanercept
9. To estimate the change in Visual Analogue Scale for Pain between baseline and day 28 after injection of placebo or etanercept
10. To estimate the change in MOCA between baseline and day 28 after injection of placebo or etanercept

d. STUDY TYPE & DESIGN & SCHEDULE

This will be a multicentre, prospective, randomised, placebo controlled, double-blind, parallel group phase IIb trial in stroke survivors enrolled between 1 and 5 years after their index event. Participants will be pre-screened using the SF36 and are required to score 95 or less to be considered eligible for the trial, and their modified Rankin Scale should be between 3 and 5. Additional eligibility checks against the study inclusion and exclusion criteria will be performed.

The study uses a two-stage design (Figure 1). In the first phase of the study, patients will be randomized to receive etanercept or placebo and outcome assessments will be performed at day 28. In the second phase, the participants in the placebo arm will be further re-randomized into receiving another dose of placebo or a dose of etanercept at day 28 (based on 2:1 allocation ratio), while patients in the etanercept arm will be further re-randomized into receiving a dose of placebo or the second dose of etanercept at day 28 (based on 1:2 allocation ratio). This will result in three equally sized arms (Placebo n=56, Single Dose etanercept n=56, and Double Dose etanercept n=56) for the secondary dose-response analysis of the efficacy outcome at day 56. (see Figure 2)

The study flow is described in the Figure 1 below.

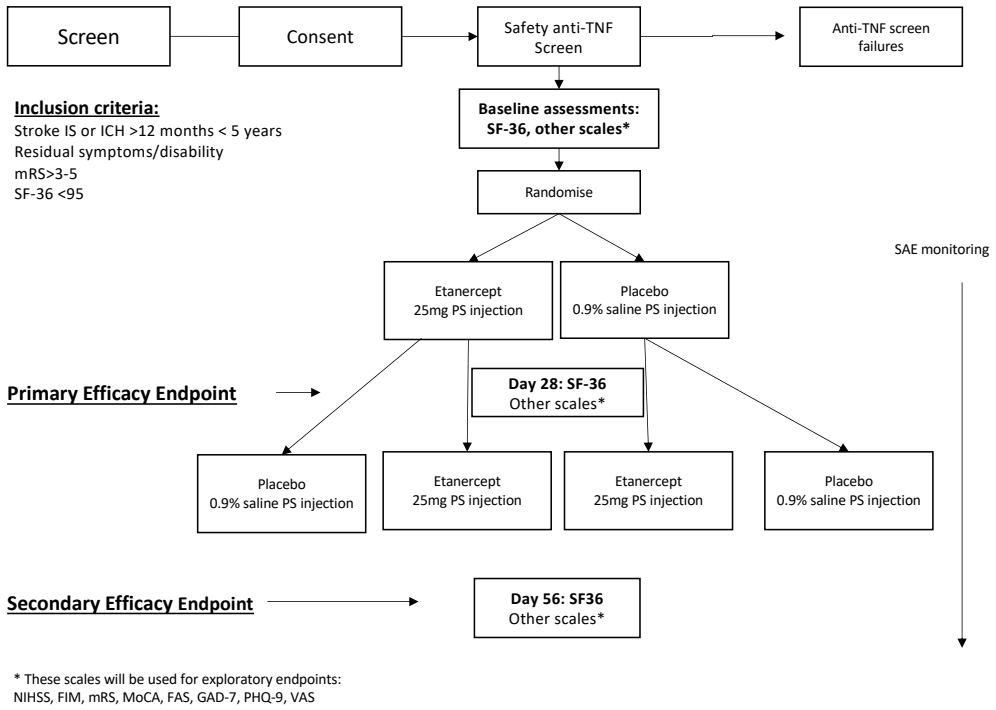
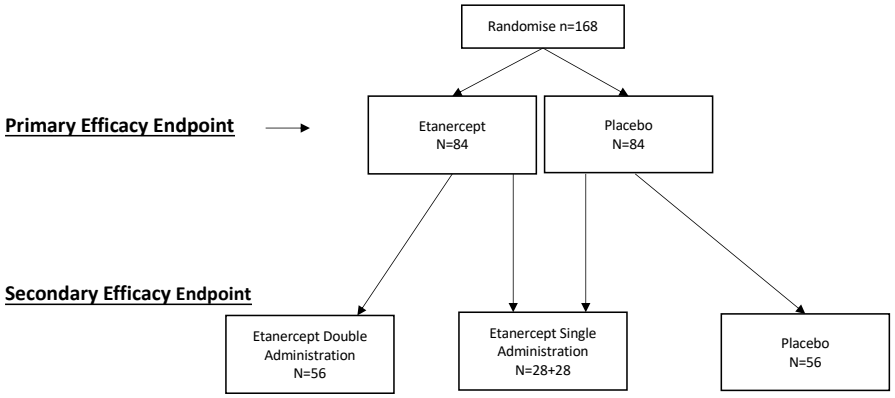


Figure 2 shows the phases and effective sample sizes for each efficacy endpoint.



During the study, participants will be required to attend the clinic/study centre at day 28 (+/- 4 days) and day 56 (+/- 4 days) post study enrolment to complete study outcome assessments. Visits outside of these timeframes will be a protocol deviation.
Following completion of the outcome assessments at day 56, participants will have completed their study involvement.

Participants will attend the clinic for follow up visits, however, home visits may be performed when the participant is unable to attend the clinic/hospital. Peri-spinal etanercept will not be administered at home.

Recruitment for the study will be for 24 months. Analysis will take 2 months.

Data collected will be entered into a secure online database maintained at the Florey Institute of Neuroscience and Mental Health.

e. BLINDING

Patients and outcome assessors will be masked to investigational product allocation. Unblinding will only be done after study completion for the analysis, or in the event of a serious adverse event where the information relating to the drug administered is required for safety reasons.

3. STUDY ASSESSMENTS AND PROCEDURES

a. VISIT 1 SCREENING AND CONSENT (0-14 DAYS PRIOR TO DAY 0)

A brief eligibility screen of interested persons will be performed. The eligibility screen may be performed in person or by telephone.
This will include questions on age, the index stroke (timing since index stroke), current ability to walk and queries about other related stroke impairments (speech, cognition, muscle weakness, coordination problems) and ability to perform activities of daily living. Additionally, we will ask about a history of infectious disease, immunosuppression, heart failure and use of other immunosuppressants. Patients who fulfil entry criteria and exclusion criteria will be provided an information sheet and consent form. Only those who meet the study inclusion criteria will proceed to consent.

b. CONSENT

Interested persons or their medical decision maker will be provided with comprehensive verbal and written information about the study by a member of the research team. They will be given time to consider their inclusion and be given the opportunity to speak to others about their involvement before deciding whether or not to be involved. They will be given the opportunity to ask any further questions regarding their participation before providing written consent via the relevant Participant Information and Consent Forms. Documentation of the process will be made in the patient's clinic notes.

c. BASELINE ASSESSMENT AND SCREENING FOR INFECTION

Following written informed consent, a safety eligibility screen for etanercept will be performed using the QuantiFERON-Gold assay for TBC and screening tests for hepatitis B and C (blood test).

A pregnancy test will be performed in all women of child-bearing potential (urine dipstick).
To further confirm eligibility, certified raters will assess the participants global disability score using the modified Rankin scale (mRS). Participants will also be asked to complete the SF-36 questionnaire independently. If the participant is not able, a relative or carer who is familiar with the patient will be asked to complete the questionnaire.

Participants who do not meet criteria for SF 36 or fail the safety screen, will be considered screening failures at this point and not proceed.

Details of the participants medical history (including stroke history) and concomitant medications will be recorded.

d. Visit 2 Investigational Product Administration (Day 0)

At the beginning of this visit, trained raters will assess neurologic impairment using the NIHSS and global disability using the modified Rankin scale (mRS). GCP-certified assessors will also assess baseline mood using the PHQ-9, fatigue using the Fatigue Assessment Scale (FAS), anxiety using the (GAD-7), pain using the Visual Analogue scale (VAS), cognition using the Montreal Cognitive Assessment (MOCA), functional independence using the Functional Independence Measure (FIM).

e. Randomisation

After confirmation of eligibility, participants will be randomised into the treatment arms using a centralised computer-generated random assignment procedure via the electronic case record form, stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or if not possible by a relative or carer proxy, and baseline modified Rankin Scale (dichotomized as mRS 3 vs mRS4-5). A researcher not otherwise involved in the trial will randomise the participant and allocate to treatment arms, hence allocation concealment will be assured. The treatment allocation will be sent electronically to the study pharmacist who will prepare the investigational product for blinded use. Study pharmacists will be instructed not to disclose treatment allocation.

f. Intervention

An unblinded pharmacist will prepare the investigational product (IP), based on treatment allocation. The IP will be provided to the researchers in a masked fashion to ensure concealment of the allocated treatment.

g. Active treatment

etanercept (Enbrel) is supplied as a single-use dose in a vial as a sterile, lyophilised preservative-free powder which when reconstituted is for subcutaneous injection. etanercept, when dissolved by the addition of sterile water is clear to slightly yellow solution. Each single-use dose vial will contain up to 1.0 mL prepared with a 25 mg lyophilized powdered solution of etanercept. Given the possible slight discoloration of the IP, pharmacy will mask the syringe.

h. Placebo control

The placebo control will be sterile saline (suitable for use as an injection) as a clear colourless solution and prepared in the same type of syringe with the same volume as for the active test drug. Given the possible slight discoloration of the IP and to avoid unblinding, pharmacy will mask the syringe.

i. Drug administration Baseline

Active treatment or placebo, in an identical volume of 1.0 mL, will be administered via a posterior cervical interspinous (between the C6-C7 or C7-T1 interspace) injection subcutaneously in the midline with a 27-gauge half to one inch needle by an experienced neurologist or stroke physician. The participant is then placed on an inversion table in the Trendelenburg position. The body is laid supine, on a 20 degree incline with the feet elevated above the head for four minutes as described by treatment proponents. The injection will be videotaped, such that correct administration can be verified independently. It will not be possible to identify the patient from the videotape. The doctors administering the IP will be trained to correctly administer the injection.

j. POST-INJECTION ASSESSMENT BASELINE

30 minutes after the injection the participant will be asked to complete the Patient Global Improvement of Change Scale (PGICS).

k. VISIT 3 OUTCOME ASSESSMENT AND SECOND INJECTION (DAY 28+/4)

Participants will return to the clinic for the first outcome assessment performed by trained, blinded raters. They will assess neurologic impairment using the NIHSS and global disability using the modified Rankin scale. GCP-certified assessors will also assess cognition (MOCA) and functional independence (FIM).
At this visit, the participant or their carer will fill out questionnaires including quality of life SF-36. In addition, baseline mood (PHQ-9), pain (VAS), fatigue (Fatigue Assessment Scale), anxiety (GAD-7) will be assessed. The same carer should be used for the questionnaires at all visits.
Any Adverse events or serious adverse events that have occurred since the last visit will be recorded. Any changes in medication taken by the participants will be recorded.

l. INJECTION VISIT 3

Participants then proceed to the second stage of the study, where they then receive Active treatment or placebo, in a volume of 1.0 mL, is administered via a posterior cervical interspinous (between the C6-C7 or C7-T1 interspace) injection subcutaneously in the midline with a 27-gauge half to one inch needle by an experienced neurologist or stroke physician. The participant is then placed on an inversion table in the Trendelenburg position. The body is laid supine, on a 20 degree incline with the feet elevated above the head for four minutes as described by treatment proponents. The injection will be videotaped, such that correct administration can be verified independently. It will not be possible to identify the patient from the videotape. The doctors administering the IP will be trained to correctly administer the injection.

m. POST-INJECTION ASSESSMENT VISIT 3

30 minutes after the injection the participant will be asked to complete the Patient Global Improvement of Change Scale (PGICS).

n. VISIT 4 OUTCOME ASSESSMENT (DAY 56+/-4)

Trained, blinded raters will assess neurologic impairment using the NIHSS and global disability using the modified Rankin scale. GCP-certified assessors will also assess cognition (MOCA), functional independence (FIM).

The participant or their carer will fill out questionnaires: quality of life SF-36, mood (PHQ-9), pain (VAS), fatigue (FAS), and anxiety (GAD-7). The same carer should be used for the questionnaires at all visits.

Adverse events and serious adverse events will be recorded. Any changes in medication taken by the participant will be recorded.

The Table below shows the schedule of events.

4. SCHEDULE OF EVENTS

	Visit 1 Screening	Visit 2 Baseline	Visit 3 Follow up 1	Visit 4 Follow up 2
Time period	**D-14-0**	**D0**	**D28+/-4**	**D56+/-4**
Brief eligibility screen	X			
Informed consent	X			
Medical history including allergy	X			
Dipstick (urine test)[1]	X[1]			
Quantiferon gold test	X			
Hepatitis B screening	X			
Hepatitis C screening	X			
Concomitant medication	X	X	X	X
AE and SAE		X	X	X
Randomisation		X		
Administration of IP		X	X	
National Institute of Health Stroke Scale (NIHSS)		X	X	X
modified Rankin scale (mRS)	X	X	X	X
Short Form (36) SF-36	X	X	X	X
Patient Health Questionnaire (PHQ-9)		X	X	X
Fatigue assessment scale (FAS)		X	X	X
General Anxiety Disorder (GAD-7)		X	X	X
Pain visual analogue scale (VAS)		X	X	X
Montreal Cognitive Assessment (MOCA)		X	X	X
Functional Independent Measure (FIM)		X	X	X
Patient Global Impression of Change Scale		X[2]	X[2]	

[1] in females of child-bearing potential only

[2] 30 minutes after IP administration

5. OUTCOME ASSESSMENTS

a. 36-ITEM SHORT FORM HEALTH SURVEY (SF-36)

The Short Form-36 (SF-36) is a 36-item self-administered questionnaire which measures Quality of Life (QoL) across eight domains. The eight domains that the SF-36 measures are as follows: physical functioning; role limitations due to physical health; role limitations due to emotional problems; energy/fatigue; emotional well-being; social functioning; pain; general health. For participants unable to complete themselves, a carer will be asked to complete this on behalf of the participant.

b. MODIFIED RANKIN SCALE (MRS)

The Modified Rankin Scale (mRS) is used to measure the degree of disability in patients who have had a stroke, as follows:

0: No symptoms at all
1: No significant disability despite symptoms; able to carry out all usual duties and activities
2: Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3: Moderate disability; requiring some help, but able to walk without assistance
4: Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5: Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6: Dead

The assessment will be performed by a certified neurologist or stroke physician or health care professional.

c. MONTREAL COGNITIVE ASSESSMENT (MOCA)

Cognition will be evaluated using Montreal Cognitive Assessment, used to assess different cognitive domains including: attention, concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The total score ranges from 0 to 30. The assessment will be performed by a neurologist or stroke physician or trained health care professional.

d. NATIONAL INSTITUTES OF HEALTH STROKE SCALE (NIHSS)

The National Institutes of Health Stroke Scale,/ NIH Stroke Scale (NIHSS) is a tool used by healthcare providers to objectively quantify the impairment caused by a stroke. The NIHSS is composed of 11 items, each of which scores a specific ability between a 0 and 4. For each item, a score of 0 typically indicates normal function in that specific ability, while a higher score is indicative of some level of impairment.[1] The individual scores from each item are summed in order to calculate a patient's total NIHSS score. The maximum possible score is 42, with the minimum score being a 0. The assessment will be performed by a certified neurologist, stroke physician or health care professional.

e. GENERALIZED ANXIETY DISORDER 7 (GAD-7)

This is a self-reported questionnaire for screening and severity measuring of generalized anxiety disorder (GAD). GAD-7 has seven items, which measure severity of various signs of GAD according to reported response categories with assigned points (see below). Assessment is indicated by the total score, which made up by adding together the scores for the scale all seven items.

f. PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

PHQ-9 is a 9-question instrument to screen for the presence and severity of depression. The results of the PHQ-9 may be used to make a depression diagnosis according to DSM-IV criteria and takes less than 3 minutes to complete.

g. FUNCTIONAL INDEPENDENCE MEASURE (FIM)

The FIM's assessment of degree of disability depends on the patient's score in 18 categories, focusing on motor and cognitive function. Each category or item is rated on a 7-point scale (1 = <25% independence; total assistance required, 7 = 100%

independence). As such, FIM scores may be interpreted to indicate level of independence or level of burden of care. The scale is used to assess how well a person can carry out basic activities of daily living and thus how dependent he or she will be on help from others. Other areas assessed include the physical like how well patients move and walk, and the cognitive, how well they interact with others, communicate, and process information.

h. FATIGUE ASSESSMENT SCALE (FAS)

The FAS is a 10-item general fatigue questionnaire to assess fatigue. Five questions reflect physical fatigue and 5 questions (questions 3 and 6-9) mental fatigue.

i. PATIENT GLOBAL IMPRESSION OF CHANGE (PGIC)

The self-report measure Patient Global Impression of Change (PGIC) reflects a patient's belief about the efficacy of treatment. PGIC is a 7-point scale depicting a patient's rating of overall improvement. Patients rate their change as ''very much improved,'' ''much improved,'' ''minimally improved,'' ''no change,'' ''minimally worse,'' ''much worse,'' or ''very much worse.''

j. PAIN VISUAL ANALOGUE SCALE (VAS)

The pain VAS is a continuous scale comprised of a line, 10 centimetres (100 mm) in length, anchored by 2 verbal descriptors and pictures, one for each symptom extreme. For pain intensity, the scale is anchored by "no pain" (score of 0) and "worst imaginable pain". The pain VAS is self-completed by the respondent. The respondent is asked to place a line perpendicular to the VAS line at the point that represents their pain intensity in the past 24 hours.

6. STANDARD CARE AND ADDITIONAL TO STANDARD CARE PROCEDURES

All of the procedures are additional to standard of care.

	Time to complete procedure in minutes
Brief eligibility screen	5
Dipstick (urine test)*	5
Blood test (Quantiferon, hep B, hep C)	10
Concomitant medication collection	10
Randomisation	2
Preparation of IP	30
Administration of IP	5
Patient Global Improvement of Change Scale **	2
National Institute of Health Stroke Scale (NIHSS)	15
modified Rankin scale (mRS)	10
Patient Health Questionnaire (PHQ-9)	3
Fatigue assessment scale (FAS)	5
General Anxiety Disorder (GAD-7)	5

Pain visual analogue scale (VAS)	3
Montreal Cognitive Assessment (MOCA)	20
Functional Independent Measure (FIM)	20
Short Form (36) SF-36	30
AE and SAE assessment	20

7. RANDOMISATION

After confirmation of eligibility, participants will be randomised into either the etanercept group or the control group using a centralised computer-generated random assignment procedure with permuted blocks of various sizes as part of the electronic case record form stored on a secure server based at the Florey. Randomisation will be based on a 1:1 allocation ratio. Randomisation will be stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or if not possible by a relative or carer proxy, and baseline modified Rankin Scale (dichotomized as mRS 3 vs mRS4-5). Randomisation for the primary endpoint will be based on a 1:1 allocation ratio (Placebo vs etanercept). As the second part of the same randomization procedure conducted at the same time point at Day 0, the participants in the placebo arm will be further re-randomized into receiving another dose of placebo or a dose of etanercept at day 28 (based on 2:1 allocation ratio), while patients in the etanercept arm will be further re-randomized into receiving a dose of placebo or the second dose of etanercept at day 28 (based on 1:2 allocation ratio). This will result in three equally sized arms (Placebo n=56, Single Dose etanercept n=56, and Double Dose etanercept n=56) for the secondary dose-response analysis of the efficacy outcome at day 56. (see figure above). Randomisation will be stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or if not possible by a relative or carer proxy. A researcher not otherwise involved in the trial will undertake the randomisation and allocation to treatment arms, hence allocation concealment will be assured.

The treatment allocation will be sent electronically to the study pharmacist who will prepare the investigational product. Study pharmacists will be instructed not to disclose treatment allocation. The investigator administering the investigational product, patients and outcome assessors will therefore be blinded.

8. STUDY POPULATION

a. RECRUITMENT PROCEDURE

Patients will be recruited from multiple outpatient and community sources. Expressions of interest will also be obtained through the Stroke Foundation who have access to people who are interested in being involved in clinical trials. We will additionally advertise through relevant stroke websites (such as the Stroke Foundations EnableMe), and relevant stroke and GP clinics.

b. INCLUSION CRITERIA

The inclusion and exclusion criteria for PESTO either match or exceed those published by the American College of Rheumatology's 2015 guidelines for the use of biologicals in patients with rheumatoid arthritis and the Product Information criteria for use of etanercept in Australia.[17]

1. Patients with a history of acute ischemic or haemorrhagic stroke confirmed on imaging

2. Age ≥18 years-65 years at time of stroke
3. Moderate-to-severe disability resulting from stroke as defined by a modified Rankin scale of 3-5
4. Stroke occurred between 1 and 5 years before enrolment.
5. SF-36 score <95
6. Patient is able to complete the SF-36 questionnaire independently or availability of a relative or carer who is able to complete the SF-36 questionnaire on behalf of the patient
7. Consent can be obtained from the participant or medical decision maker.

c. EXCLUSION CRITERIA

1. Contra-indication to using etanercept (e.g. previous hypersensitivity, patients with, or at risk of sepsis, ongoing infection, use of IL-1 antagonists)
2. History of hepatitis B and C, tuberculosis, HIV, SLE, multiple sclerosis,
3. Moderate to severe heart failure
4. History of malignancy
5. Other current use of immunosuppressant
6. History of alcoholic hepatitis, current ongoing hepatitis
7. History of juvenile idiopathic arthritis
8. History of blood dyscrasia
9. Clinical diagnosis of dementia
10. mRS 0-2
11. Botulinum toxin injection to limbs in the 4 months prior to Screening Visit
12. Pregnancy (women of childbearing potential must be tested)
13. Breastfeeding
14. Participation in any investigational study in the last 30 days
15. Known terminal illness or planned withdrawal of care or comfort care measures.
16. Any condition that, in the judgment of the investigator could impose hazards to the patient if study therapy is initiated or affect the participation of the patient in the study.
17. Prior exposure to etanercept for stroke

d. CONSENT

Informed consent will be obtained from the participant or a medical decision maker. Potentially interested participants or their decision maker will be provided with comprehensive verbal and written information about the study by a member of the research team.

In particular, the researcher will:

i. Present the potential participants or medical decision maker with a written Participant Information Sheet, providing an appropriate and well-informed explanation of the proposed research and the terms of participation

ii. Have sufficient understanding of the research to explain it fully and answer the potential participant's questions

iii. Present the potential participants or medical decision maker with a written Participant Consent Form outlining the terms of research participation (i.e. voluntary, with identifiable personal information, for research purposes only)

iv. Ensure that the obtained participation consent, in the form of a written signed Participant Information and Consent Form, is both well-informed and completely voluntary.

If individuals would like to take more time to think about their participation in the study and to discuss it with family or friends, they will be asked for permission for the

Researcher to contact them by phone after a specified time (e.g., one week) to further discuss the possibility of participation. Individuals who decline to participate will not be contacted again.

9. INVESTIGATIONAL PRODUCT

a. DESCRIPTION OF INVESTIGATIONAL PRODUCT

ENBREL (etanercept) 25 mg powder for injection and water for injections.
Each vial of ENBREL powder for injection contains 25 mg of etanercept. The content of the diluent syringe is 1 mL of sterile water for injections. Following reconstitution with water for injections, ENBREL is a colourless to slightly yellow and clear to slightly opalescent solution, with a pH of 7.1-7.7.
etanercept is a human tumour necrosis factor receptor p75 Fc fusion protein produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian expression system. etanercept is a dimer of a protein genetically engineered by fusing the extracellular ligand-binding domain of human tumour necrosis factor receptor-2 (TNFR2/p75) to the Fc domain of human IgG1. This Fc component contains the hinge, CH2 and CH3 regions but not the CH1 region of IgG1. etanercept contains 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons. etanercept is now manufactured using a serum-free process. The potency is determined by measuring the ability of etanercept to neutralise the TNFα-mediated growth inhibition of A375 cells. The specific activity of etanercept is 1.7 x 106 units/mg.

b. PREPARATION OF IP

ENBREL contains no antibacterial preservative and therefore, solutions prepared with water for injections should be administered as soon as possible and within six hours following reconstitution. In the absence of compatibility studies, ENBREL must not be mixed with other medicinal products.

Reconstitute the ENBREL powder aseptically by injecting 1 mL of sterile water for injections very slowly into the vial with the vial adaptor attached to the syringe. Gently swirl the contents to avoid excessive foaming. Some foaming will occur, this is normal. To avoid excessive foaming, do not shake or vigorously agitate. Dissolution of ENBREL usually takes less than 10 minutes. Visually inspect the solution for particulate matter and discolouration prior to administration. The solution should not be used if discoloured or cloudy, or if particulate matter remains. Withdraw the solution into the empty syringe, removing only the dose to be given from the vial. Some foam or bubbles may remain in the vial. Do not filter reconstituted solution during preparation or administration.

ENBREL should not be used if all the powder in the vial is not dissolved within 10 minutes. In that situation another vial should be. Once the ENBREL solution has been aspirated into the syringe, discard the vial adaptor and replace with a needle from the pack for injection.
Injections should never be given into areas where the skin is tender, bruised, red or hard.

c. PREPARATION OF PLACEBO

The placebo control will be sterile saline (suitable for use as an injection) as a clear colourless solution, with the same appearance as etanercept, and prepared in the same type of syringe with the same volume as for the active test drug.

d. RANDOMISATION

After randomisation (see previous section), treatment allocation is received by the unblinded study pharmacist at each site. The pharmacist will prepare the IP according to the instructions described in 6.2 and label. A study member will pick up the IP and bring it to the clinic where it will be administered. Administration should be performed within 6 hours after preparation of IP.

e. BLINDING/UNBLINDING

This is a double-blind trial. The participant and all those involved in the clinical assessments of the participants will be blinded to treatment allocation. In the case of reported Suspected Unexpected Serious Adverse Reactions (SUSARs), or if treatment identification is necessary for emergency patient management, cases may be unblinded through Redcap.

The Data Safety Monitoring Committee (DSMC) will have access to unblinded grouped data.

f. PRODUCT LABELLING

Details on the labels will include: Protocol name and identification number, name, address and contact number of the sponsor, the participant randomisation number, and the words 'for clinical trial use only'. Labelling of the investigational product will comply with Therapeutic Goods Administration (TGA) code of Good Manufacturing Practice (GMP) for medicinal products, Annex 13, section 17 in Australia, or applicable national and/or local requirements for international sites where applicable.

g. HANDLING AND STORAGE OF INVESTIGATIONAL PRODUCT

Enbrel should be stored at 2ºC to 8°C, refrigerated, but not frozen. The solution should be used immediately after reconstitution. If not used immediately, ENBREL solution must be refrigerated in the vial at 2°C to 8°C after reconstitution and used within 6 hours.
Prior to reconstitution, the powder may be stored at temperatures up to a maximum of 25°C for a single period of up to 4 weeks. ENBREL should be discarded if exposed to high temperatures, or if not used within 4 weeks of initial removal from refrigeration.

h. DOSE JUSTIFICATION

The 25 mg etanercept dose is in common use for treatment of rheumatological and dermatologic use and is used off-label in stroke patients.

i. ADMINISTRATION

Active treatment or placebo, in an identical volume of 1.0 mL, will be administered via a posterior cervical interspinous (between the C6-C7 or C7-T1 interspace) injection subcutaneously in the midline with a 27-gauge half to one inch needle followed by Trendelenburg positioning, with patient supine on an inversion table with the head dependent for four minutes at an incline of 20 degrees, as described by treatment proponents.

2. PARTICIPANT SAFETY AND WITHDRAWAL

a. RISK MANAGEMENT AND SAFETY

Standard treatment will not be delayed for the purpose of this study. Current medications will not be discontinued for the purposes of this study.

Blood sample collection
Insertion of a needle for blood sample collection may be commonly associated with slight pain or bruising at the puncture site and rare chance of infection. There is a rare risk of fainting due to the venous needle insertion.

Perispinal injection
This is a subcutaneous injection of a small volume of liquid. Bruising or pain at the injection site are possible. There is a rare chance of infection at the injection site.

Reported side effects of etanercept
Many of the reported serious events have occurred in patients receiving concomitant medicines including immunosuppressants, or with underlying diseases that, in addition to their rheumatoid arthritis, could predispose them to infections. The side effect profile of etanercept has been derived from patients undergoing chronic treatment. Whether these side effects also occur in patients receiving one or two doses of etanercept is unknown.

(1) Serious infections including sepsis and tuberculosis, have been reported with the use of etanercept. Some of these infections have been fatal. These infections were due to bacteria, mycobacteria, fungi, viruses and parasites (including protozoa). Opportunistic infections have also been reported (including listeriosis, legionellosis and invasive fungal infections) in patients receiving etanercept.

In some cases, fungal and other opportunistic infections are not recognised and this has resulted in delays in appropriate treatment, sometimes resulting in death.

Patients will be screened for tuberculosis, hepatitis B and C prior to administration of study drug. Patients will not be allowed to participate in the trial if any active or latent infection is identified. During the trial participants will be monitored for signs and symptoms of infection. We will inform patients to seek medical advice if signs of infection appear. Any participant who develops a new infection will be monitored closely.

Administration of the study drug will be discontinued if a patient develops a serious infection (e.g. tuberculosis or an atypical mycobacterial infection) or sepsis and appropriate medical care will be administered.

All patients will be evaluated for infections, and the researchers will consider the patient's risk for relevant opportunistic infections (e.g., exposure to endemic mycoses).

Researchers will exercise caution when considering the use of the study drug in patients with a history of recurring or chronic infections, or with underlying conditions,

which may predispose patients to infections such as advanced or poorly controlled diabetes.

(2) Increased infection risk and mortality in alcoholic hepatitis

Risk reduction strategy: Patients with alcoholic hepatitis will not be allowed to participate in the trial.

(3) Hypoglycemia

There have been reports of hypoglycaemia following initiation of etanercept in patients receiving medication for diabetes, necessitating a reduction in anti-diabetic medication in some of these patients.

Risk reduction strategy: Patients with diabetes will be warned about symptoms of hypoglycemia.

(4) Inflammatory bowel disease and uveitis in patients with juvenile idiopathic arthritis.

Risk reduction strategy: Patients with juvenile idiopathic arthritis will not participate in the trial.

(5) Miscellaneous serious side effects in patients who receive other immunosuppressive drugs including infection, hematological problems.

Risk reduction strategy: patients requiring other immunosuppressive drugs will not participate in the trial.

(6) Haematological reactions including aplastic anemia, pancytopenia.

Risk reduction strategy: patients with a history of blood dyscrasia will not participate in the trial

All patients will be advised that if they develop signs and symptoms suggestive of blood dyscrasias or infections (e.g., persistent fever, sore throat, bruising, bleeding, paleness), they should seek immediate medical advice. Such patients should be evaluated urgently, including full blood count; if any blood dyscrasias are confirmed, IP should be discontinued.

(7) Allergic reactions: Allergic reactions associated with etanercept administration have been reported commonly. Allergic reactions have included angioedema and urticaria. Serious reactions have occurred.

Risk reduction strategy: Patients will be monitored for allergic reactions and asked to report any reaction immediately to the study team.

(8) Congestive heart failure

Worsening heart failure and rare reports of new onset heart failure in patients without known preexisting cardiovascular disease, even in patients under 50 years have been reported.

Risk reduction strategy: Patients with known congestive heart failure will not be recruited. Patients with new onset dyspnea and/or other signs of heart failure will be assessed for possible heart failure.

(9) Other neurological disorders

Treatment with ENBREL and other agents that inhibit TNF have been associated with rare cases of new onset or exacerbation of central nervous system demyelinating disorders, some presenting with mental status changes and some associated with permanent disability.

Risk reduction strategy: Patients with known inflammatory neurological disorders will not be recruited. Patients with new onset neurological symptoms following etanercept administration will receive neurological assessments using the NIHSS as part of the clinical trial.

(10) Malignancies

A possible risk for the development of lymphomas, leukemia, melanoma and non-melanoma skin cancer or other malignancies in patients treated with a TNF-antagonist cannot be excluded. Rates were low and were observed in patients with conditions associated with development of malignancies.

Risk reduction strategy: Patients with ongoing malignancy will not be included in the study. Periodic skin examination will be recommended for all patients who are at increased risk for skin cancer, especially in patients with psoriasis, who may be at increased risk.

(11) Live vaccines

No safety data are available on the effects of live vaccine when used in combination with ENBREL. Live vaccines should therefore not be given concurrently with ENBREL.

Risk reduction strategy: Patients will be recommended to undergo vaccination with live vaccines during the course of the trial.

(12) Pregnancy

The safe use of ENBREL during pregnancy has not been established.

Risk reduction strategy:
Women of childbearing potential should be advised to use appropriate contraception to avoid becoming pregnant during ENBREL therapy and for three weeks after discontinuation of therapy.

(13) Lactation

The safe use of ENBREL during lactation has not been established. etanercept has been reported to be excreted in human breast milk following subcutaneous administration.

Risk reduction strategy:

Nursing mothers will not be included in the study.

b. Handling of Withdrawals

Participants may voluntarily withdraw from the study at any time. Data obtained prior to withdrawal of consent will be retained, unless otherwise requested by the withdrawing participant.

c. Replacements

Dropouts from the study will not be replaced. The sample size calculations allow for up to 10 participants withdrawing from the study.

3. Adverse Events (AEs) and Serious Adverse Events (SAEs)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE) as provided in this protocol.

During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

a. Definition of an Adverse Event (AE)

An Adverse Event (AE) is any untoward medical occurrence in a participant temporarily associated with the use of an investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the product, whether or not considered related to the product. For marketed products, this also includes failure to produce benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition. New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication.

Examples of an AE do not include:

- Medical or surgical procedure (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to hospital).
- The disease being studied, or signs/symptoms associated with the disease unless more severe than expected for the participant's condition. In this study, AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of participants' previous therapeutic regimen).

b. Definition of a Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence that, at any dose:
a) Results in death
b) Is life threatening
c) Requires hospitalisation or prolongation of an existing hospitalisation.

Note: In general, hospitalisation signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious. Hospitalisation for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in disability/incapacity

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.

e) Is a congenital abnormality / birth defect, or
f) Is a significant medical event

Note: Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardise the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious.

c. Time Period, Frequency, and Method of Detecting AEs

All adverse events will be recorded between the time of consent and each study visit. Each participant will be monitored regularly by the investigator and study personnel for adverse events and all SAEs will be reported during study participation. SAEs will be reported to the Human Research Ethics Committee as soon as practicable and to the Therapeutic Goods Administration (TGA), within the specified reporting time lines.

d. Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the site investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostic reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in to the eCRF. Supporting documentation for SAEs will be collected. In this instance, all participant identifiers will be blinded on the copies of the medical records prior to submission to sponsor.

For each adverse event, start and stop dates, action taken, outcome, intensity and relationship to study product (causality) will be documented.

If an AE changes in frequency or intensity during a study, a new entry of the event must be made in the eCRF.

The investigator will establish a diagnosis of the event based on signs, symptoms,

and/or other clinical information. In the absence of a diagnosis, the individual signs/symptoms will be documented. All details of any treatments initiated due to the adverse event should be recorded in the participant's notes and the eCRF.

e. Prompt Reporting of SAEs

Serious Adverse Events require immediate action. Once an investigator becomes aware that an SAE has occurred, they will immediately notify the Sponsor. The SAE form must be completed on the eCRF as thoroughly as possible with all available details of the event and signed by the investigator (or appropriately qualified designee and sent to the study monitor within 24 hours of first becoming aware of the event. If the investigator does not have all information regarding an SAE, they will not wait to receive additional information before notifying the study monitor of the event and completing the form. The form will be updated when additional information is received. The investigator will provide an assessment of causality at the time of the initial report. The SAE form may be appropriately amended if required by the investigator as more information comes to hand and resubmitted to the Sponsor.
In accordance with local ethics requirements, the investigator must also notify their Ethics Committee of SAEs according to local guidelines.
The investigator and others responsible for participant care, should institute any investigations of serious adverse events based on their clinical judgement of the likely causative factors. This may include seeking further opinion from a specialist in the field of the event. If a participant dies, a cause of death and any post-mortem findings, including histopathology will be collected if available.

f. Assessment of intensity of AEs and SAEs

The investigator will assess intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgement. The intensity of each AE and SAE recorded in the CRF should be assigned to one of the following categories:

Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday
activities.

Severe: An event which is incapacitating and prevents normal everyday activities.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilised for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

g. Assessment of Causality for AEs and SAEs

The investigator will assess the relationship between study drug and the occurrence of each AE/SAE using clinical judgment.
Alternative causes, such as natural history of the underlying disease, concomitant therapy,
other risk factors, and the temporal relationship of the event to the investigational product will

be considered and investigated. The investigator will also consult the product information in the determination of their assessment.
The causal relationship to the study product should be assessed using the following classifications:
Not Related: In the Investigator's opinion, there is no causal relationship between the study product and the adverse event.
Related: The adverse event follows a reasonable temporal sequence from the time of study product administration or reappears when study product is reintroduced.

There may be situations when an SAE has occurred, and the investigator has minimal information to include in the initial report. It is very important that the investigator always makes an assessment of causality for every event prior to sending the SAE form. The investigator may change their opinion of causality in light of follow-up information, amending the SAE form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

h. Assessment of Expectedness for SAEs

The assessment of the expectedness of the event will also be made by the investigator and reported on the SAE form.

Expected An adverse reaction, the nature or severity of which is consistent with the applicable product information
Unexpected An adverse reaction, the nature or severity of which is not consistent with the Product Information.

i. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to actively follow each participant and provide further information to the Sponsor on the participant's condition. All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits or contacts. All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the participant is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, or consultation with other health care professionals. New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the investigator. The updated SAE form should be re-sent to the sponsor after any updates.

4. Trial Governance and Conduct

a. Management Committee

The trial Chief Investigators, central coordinator, trial statistician and finance manager will form the Management Committee and maintain oversight of regular operations of the trial.

b. Steering Committee

Principal investigators from all Australian and international sites (when added) will form the trial steering committee, co-chaired by the trial Chief Investigators.

c. Data Safety Monitoring Committee

An independent Data Safety Monitoring Committee (DSMB) will meet regularly and review data from the study after each 50 patients are recruited. They will provide a blinded report to the management and Steering Committees according to a DSMC charter specifically outlining requirements. Committee discussions will remain confidential to the DSMC unless there are

safety issues or a reason to amend the protocol, in which required information will be provided to the Management and Steering Committee.

d. STUDY TERMINATION

The study may be terminated prematurely by the principal investigator or his/her designee if: The number and/or severity of adverse events justify discontinuation of the study based on the recommendation of the Data Safety Monitoring Committe. In addition, The Florey reserves the right to discontinue the study prior to inclusion of the intended number of participants but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must send written notification to the Ethics Committee. The study may also be terminated at any time at the request of any regulatory authority, with proper and timely notification of all parties concerned. The local or lead HREC will be informed promptly and the investigator will supply reason(s) for the termination or suspension. Otherwise, the study will be considered terminated upon completion of all patient interventions, outcome evaluations, and submission of final reports.

5. STATISTICAL METHODS

a. SAMPLE SIZE ESTIMATION & JUSTIFICATION

Earlier work in chronic stroke populations have shown that 11% of patients report a 5-point improvement in SF-36 when given placebo. Based on the description of spectacular improvement with etanercept in up to 90% of patients, we conservatively anticipate that at day 28, 30% of the patients will have a 5-point improvement on SF-36. Recruiting 160 patients (equally distributed between two arms) would yield 0.8 power to observe the absolute risk difference of 19%, assuming two-tailed Alpha of 0.05 and 11% of patients achieving a 5-point improvement in SF-36 when given placebo. To allow for potential dropouts, the proposed total sample size for this study is 168 patients, equally distributed between two arms.

b. POWER CALCULATIONS

Power calculations were performed in STATA IC15.0 (StataCorp, College Station, TX, USA)

c. STATISTICAL METHODS TO BE UNDERTAKEN

The analysis will be conducted following intention-to-treat principles. All outcomes and analyses are prospectively categorized as primary or secondary or exploratory. Differences in all endpoints between the 2 arms of the study will be tested independently at the two-tailed 0.05 level of significance. All estimates of treatment effects will be presented with 95% confidence intervals. No formal adjustments will be undertaken to constrain the overall type I error associated with the secondary and exploratory analyses. Their purpose is to supplement evidence from the primary analysis to more fully characterise the treatment effect. Results from the secondary analyses will be interpreted in this context. Descriptive statistics will be generated for each of the measures used in study.

The primary endpoint will be tested using a logistic regression model with achievement of at least 5 points improvement on the SF-36 compared to baseline as the dependent variable and with the treatment arm as an independent variable and baseline value of SF-36, proxy or self-administration of SF-36 as adjustment covariates. The secondary efficacy endpoint will be tested using a logistic regression model with achieving at least 5 points improvement from the baseline value on the SF-36 scale as the outcome, the treatment arms (placebo, single dose, and double dose) as an independent variable, and baseline value of SF-36, proxy or self-administration of SF-36 as adjustment covariates.

Between arm differences in safety outcomes will be analyzed identically to the efficacy outcomes.

Between arms differences in secondary and exploratory outcomes will be assessed using the generalised linear mixed models (GLMM) approach, using a link function as appropriate to the response outcome distribution.
The details of the statistical analysis will be summarized in a separate Statistical Analysis Plan prior to the lock of the trial data.

6. DATA SECURITY & HANDLING

a. ELECTRONIC CASE REPORT FORM (ECRF)

An electronic Case Report Form (eCRF) will be completed for each study participant summarising all clinical screening and study data. Participants will only be referred to in the eCRF by their participant number and initials in order to retain participant confidentiality.
The completed original eCRF data is to be submitted via REDCap® online database as soon as practical after completion. Source data relating to the participants involvement in the trial is to be retained by the investigator for 15 years.

b. DETAILS OF WHERE RECORDS WILL BE KEPT & HOW LONG WILL THEY BE STORED

For the duration of the study, any paper-based information will be kept in a locked office at the Florey Melbourne Brain Centre (Austin campus). Electronic recording of case report forms will be managed using REDCap software for secure web-based data recording. All imaging data will be managed using best practice principles, with all identifiable data stored and backed up on secure servers. After conclusion of the study, the data will be securely archived for at least 15 years. Only with the participants approval, deidentified data collected as part of the study may also be used by other members of the research team for related research projects and/or transmitted to an online database for use by external researchers.

c. CONFIDENTIALITY AND SECURITY

Only the researchers responsible for this project will have access to identifiable participant data. After conclusion of the study; the data will be deidentified and securely archived for at least 15 years. The data will be stored at the Florey Institute of Neuroscience and Mental Health.

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APPENDIX A - FATIGUE ASSESSMENT SCALE (FAS)

This assessment cannot be completed by proxy

The following 10 statements refer to how you usually feel. For each statement you can choose one out of five answer categories, varying from *Never* to *Always*. 1 = *Never* ; 2 = *Sometimes* ; 3 = *Regularly* ; 4 = *Often* ; 5 = *Always*. Please circle the answer to each question that is applicable to you. Please give an answer to each question, even if you do not have any complaints at the moment.

	Never	Sometimes	Regularly	Often	Always
1. I am bothered by fatigue (WHOQOL)	1	2	3	4	5
2. I get tired very quickly (CIS)	1	2	3	4	5
3. I don't do much during the day (CIS)	1	2	3	4	5
4. I have enough energy for everyday life (WHOQOL)	1	2	3	4	5
5. Physically, I feel exhausted (CIS)	1	2	3	4	5
6. I have problems starting things (FS)	1	2	3	4	5
7. I have problems thinking clearly (FS)	1	2	3	4	5
8. I feel no desire to do anything (CIS)	1	2	3	4	5
9. Mentally, I feel exhausted	1	2	3	4	5
10. When I am doing something, I can concentrate quite well (CIS)	1	2	3	4	5

APPENDIX B – SHORT FORM 36 (SF-36)

SF-36 Questionnaire

This questionnaire asks for your views about your health. For ALL questions, please tick, cross or colour the circle that most closely matches your response. There are no right or wrong answers. Please answer ALL questions.

1. In general, would you say your health is:	Poor ◯	Fair ◯	Good ◯	Very good ◯	Excellent ◯
2. Compared to one year ago, how would you rate your health general in now?	Much worse now than one year ago ◯	Somewhat worse than one year ago ◯	About the same as one year ago ◯	Somewhat better than one year ago ◯	Much better than one year ago ◯

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	No, not limited at all	**Yes, limited a little**	**Yes, limited a lot**
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	◯	◯	◯
b. Moderate activities, such as moving a table pushing a vacuum cleaner, bowling, or playing	◯	◯	◯
c. Lifting or carrying groceries	◯	◯	◯
d. Climbing <u>several</u> flights of stairs	◯	◯	◯
e. Climbing <u>one</u> flight of stairs	◯	◯	◯
f. Bending, kneeling or stooping	◯	◯	◯
g. Walking more than a mile	◯	◯	◯
h. Walking several blocks	◯	◯	◯
i. Walking one block	◯	◯	◯
j. Bathing or dressing yourself	◯	◯	◯

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other daily activities as a result of your physical health?

	None of the time	A little of the time	Some of the time	Most of the time	All of the time
a. Cut down on the amount of time you spent on work or other activities	◯	◯	◯	◯	◯
b. Accomplished less than you would like	◯	◯	◯	◯	◯
c. Were limited in the kind of work or other activities	◯	◯	◯	◯	◯
d. Had difficulty performing the work or other activities (e.g. it took extra effort)	◯	◯	◯	◯	◯

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	None of the time	A little of the time	Some of the time	Most of the time	All of the time
a. Cut down on the amount of time you spent on work or other activities	◯	◯	◯	◯	◯
b. Accomplished less than you would like	◯	◯	◯	◯	◯
c. Did work or other activities less carefully than usual	◯	◯	◯	◯	◯

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a bit	All of the time

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?					
	Not at all	A liitle bit	Moderately	Quite a bit	Extremely
9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...					
	None of the time	A little of the time	Some of the time	Most of the time	All of the time
a. did you feel full of life?	○	○	○	○	○
b. have you been very nervous?	○	○	○	○	○
c. have you felt so down in the dumps that nothing could cheer you up?	○	○	○	○	○
d. have you felt calm and peaceful?	○	○	○	○	○
e. did you have a lot of energy?	○	○	○	○	○
f. have you felt downhearted and depressed?	○	○	○	○	○
g. did you feel worn out?	○	○	○	○	○
h. have you been happy?	○	○	○	○	○
i. did you feel tired?	○	○	○	○	○
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?					
	None of the time	A little of the time	Some of the time	Most of the time	All of the time
11. How TRUE or FALSE is each of the following statements for you?					
	Defintely false	Mostly false	Don't know	Mostly true	Definitely true
a. I seem to get sick a little easier than other people	○	○	○	○	○

b. I am as healthy as anybody I know	◯	◯	◯	◯	◯
c. I expect my health to get worse	◯	◯	◯	◯	◯
d. My health is excellent	◯	◯	◯	◯	◯

APPENDIX C - MONTREAL COGNITIVE ASSESSMENT SCALE

1-3 VISUOSPATIAL / EXECUTIVE	**Refer to specific instructions for administration in the Blinded Assessor manual. Ask the patient to complete the patient worksheet attached.**					**POINTS**
Trail Making ok? ☐ Yes ☐ No	**The Cube ok?** ☐ Yes ☐ No	**The Clock:** Contour ok? ☐ Yes ☐ No; Numbers ok? ☐ Yes ☐ No; Hands ok? ☐ Yes ☐ No				/5
4. NAMING	**Refer to specific instructions for administration in the Blinded Assessor manual. Ask the patient to identify the three animals on the patient worksheet attached.**					
Lion identified? ☐ Yes ☐ No	**Rhinoceros identified?** ☐ Yes ☐ No	**Camel /Dromedary identified?** ☐ Yes ☐ No				/3
5. MEMORY	**Read list of words. Patient must repeat them. Do 2 trials. Recall later in test.**					
	FACE	**VELVET**	**CHURCH**	**DAISY**	**RED**	
1st Trial						**NO POINTS**
2nd Trial						
6. ATTENTION	**Read list of digits (1 digit/sec.)**					
	Patient has to repeat the following numbers in the forward order: 2 1 8 5 4 ☐ Yes ☐ No **Patient has to repeat the following numbers in the backward order: 7 4 2** ☐ Yes ☐ No					/2
Tell the patient: 'I am going to read a sequence of letters. Every time I say the letter 'A', tap your hand once. (No point if ≥ 2 errors).						
F B A C M N A A J K L B A F A K D E A A A J A M O F A A B						/1
Ask the patient to subtract 7 from 100 and continue to subtract 7 from his/her answer (down to 65). ☐ **4 or 5 correct (3 points)** ☐ **1 correct (1 point)** ☐ **2 or 3 correct (2 points)** ☐ **0 correct (0 points)**						
93 ☐ 86 ☐ 79 ☐ 72 ☐ 65 ☐						/3
7-8. LANGUAGE	**Read each sentence once and then ask the patient to repeat it.**					
	I only know that John is the one to help today. **Correct? Yes ☐ No ☐** **The cat always hid under the couch when dogs were around.** **Correct? Yes ☐ No ☐**					/2
Name maximum number of words in one minute that begin with the letter 'F'. (1 point if ≥ 11 words).						
Patient generated 11 words or more? Yes ☐ No ☐						/1
9. ABSTRACTION	**Ask the patient: 'Tell me how an orange and banana are alike'. Answer: Fruit. Next, tell the patient: 'Now tell me how these items are alike'.**					
Train and bicycle **Correct? Yes ☐ No ☐** **Watch and ruler** **Correct? Yes ☐ No ☐**						/2
10. DELAYED RECALL	**Ask the patient to recall the words with no cueing. (Points for uncued recall only – 1 (FACE, VELVET, CHURCH, DAISY, RED).**					
Points for uncued recall only – 1 point per correct word.						/5
11. ORIENTATION	**Ask the patient: 'Tell me the date today' (DATE, MONTH, YEAR, DAY OF THE WEEK), then the exact location (PLACE, CITY).**					
Day ☐ Month ☐ Year ☐ Day ☐ Place ☐ City ☐						/6
Add 1 point if patient has had ≤ 12 years of education.						/1
(Normal ≥ 26/30)					**TOTAL**	/30

APPENDIX D - MODIFIED RANKIN SCALE (MRS)

Note: The modified Rankin Scale is to be assessed based on the participant's symptoms **for the current stroke event. This score must be ASSESSED by a person Certified for the modified Rankin Scale.**

		Tick ONE box only
0 -	**No symptoms at all** The participant should be unaware of any new limitations caused by the stroke, however minor.	☐
1 -	**No significant disability despite symptoms; able to carry out all usual duties and activities** The participant has some symptoms as a result of the stroke, whether physical, cognitive (e.g. affecting speech, reading, writing; or physical movement; or sensation; or vision; or swallowing; or mood) - but can continue to take part in all previous work, social and leisure activities. The crucial question to distinguish grade 1 from grade 2 (below) may be 'Is there anything that you can no longer do that you used to do until you had the stroke?' As a guide, an activity that was undertaken more frequently than monthly could be regarded as a 'usual' activity.	☐
2 -	**Slight disturbance; unable to carry out all previous activities, but able to look after own affairs without assistance** The participant will be unable to undertake some activity that was possible before the stroke (e.g. driving a car, dancing, reading, or working) but is still able to look after him/herself without the help from others on a day to day basis. Thus, the participant can manage dressing, moving around, feeding, toileting, preparing simple meals, shopping, and travelling locally without needing assistance from anyone else. Supervision may not be necessary. This grade assumed that the participant could be left alone at home for periods of a week or more without concern.	☐
3 -	**Moderate disability; requiring some help, but able to walk without assistance** At this grade, the participant is independently mobile (using a walking aid or frame if necessary) and can manage dressing, toileting, feeding etc. but needs help from someone else with more complex tasks (e.g. someone else may need to undertake the shopping, cooking, cleaning, and will need to visit the participant more often than weekly to ensure that these activities are completed). The assistance can be advisory rather than physical e.g. a participant who needs supervision or encouragement to cope with financial affairs would be in this grade.	☐
4 -	**Moderately severe disability; unable to walk without assistance, unable to attend to own bodily needs without assistance** The participant requires someone else to help with some daily tasks, whether walking, dressing, toileting, or eating. This participant will be visited at least once and usually twice or more times daily or must live in proximity to a carer. To distinguish grade 4 from grade 5 (below), consider whether the participant can regularly be left alone for moderate periods during the day.	☐
5 -	**Severe disability; (usually) bedridden, incontinent, and requiring constant nursing care and attention** Someone else will always need to be available during the day and at times during the night, though not necessarily a trained nurse.	☐

APPENDIX E – NATIONAL INSTITUTE OF HEALTH STROKE SCALE (NIHSS)

Category	Score/Definition
1a. Level of consciousness	☐ 0 = Alert, keenly responsive ☐ 1 = Not alert, but arousable with minimal stimulation ☐ 2 = Not alert, requires repeated stimulation or obtunded ☐ 3 = Responds only with reflex motor or automatic effects or totally unresponsive (see item 5)
1b. LOC questions (ask month and age)	☐ 0 = Answers both correctly ☐ 1 = Answers one correctly ☐ 2 = Both incorrect
1c. LOC commands (ask to open /close eyes and make a fist)	☐ 0 = Obeys both correctly ☐ 1 = Obeys one correctly ☐ 2 = Both incorrect
2. Best Gaze (horizontal eye movement)	☐ 0 = Normal ☐ 1 = Partial gaze palsy, gaze abnormal in 1 or both eyes, but forced deviation or total gaze paresis not present ☐ 2 = Forced deviation or total gaze paresis not overcome with oculocephalic manoeuvre
3. Visual fields testing	☐ 0 = No visual field loss ☐ 1 = Partial hemianopia ☐ 2 = Complete hemianopia, if participant has severely impaired consciousness (Q1=3), score 2 here ☐ 3 = Bilateral hemianopia, blind, including cortical blindness
4. Facial Palsy (ask subject to show teeth and raise eyebrows, and close eyes tightly)	☐ 0 = Normal symmetrical movement ☐ 1 = Minor paralysis or “mild” noted ☐ 2 = Partial paralysis (total or near total paralysis of lower face) ☐ 3 = Complete absence of movement in upper and lower face
5a. Motor Function Right arm	☐ 0 = Normal (extends arm 0 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
5b. Motor Function Left arm	☐ 0 = Normal (extends arm 90 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
6a. Motor Function Right leg	☐ 0 = Normal (extends leg 90 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
6b. Motor Function Left Leg	☐ 0 = Normal (extends leg 90 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
7. Limb ataxia	☐ 0 = Absent, score 0 if unrecorded, participant unconscious, uncooperative or limb too weak to perform task ☐ 1 = Present in one limb ☐ 2 = Present in two limbs (UL and LL)
8. Sensory (use pinprick to test arms, legs, trunk and face – compare sides)	☐ 0 = Normal ☐ 1 = Mild to moderate sensory loss, participant feels pin prick is less sharp or dull on affected side ☐ 2 = Severe/total sensory loss, participant is unaware of being touched. Brainstem stroke with bilateral sensory loss

Category	Score/Definition
9. Best language (describe picture, name items, read sentences)	☐ 0 = Normal ☐ 1 = Mild to moderate aphasia ☐ 2 = Severe aphasia ☐ 3 = Mute
10. Dysarthria (read several words)	☐ 0 = Normal articulation ☐ 1 = Mild to moderate slurring of words ☐ 2 = Near unintelligible or unable to speak ☐ 9 = Intubated or other physical barrier
11. Extinction/ inattention	☐ 0 = No abnormality ☐ 1 = Visual tactile auditory or personal inattention or extinction to bilateral simultaneous in 1 modality ☐ 2 = Profound hemi-inattention or inattention in >1 sensory modality. Does not recognise own hand or orientates to one side of space
Total Score ☐☐	(do not include items scores of '9' in total)

APPENDIX F - GENERALISED ANXIETY DISORDER ASSESSMENT – 7 (GAD-7)

Over the last 2 weeks how often have you been bothered by any of the following problems?

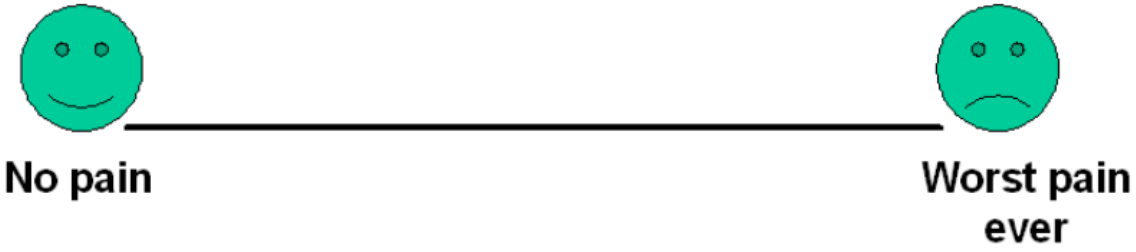
	Not at all	Several Days	More than half the days	Nearly every day
Feeling nervous or on edge?	☐	☐	☐	☐
Not being able to stop or control worrying?	☐	☐	☐	☐
Worrying too much about different things?	☐	☐	☐	☐
Trouble relaxing?	☐	☐	☐	☐
Being so restless it is hard to sit still?	☐	☐	☐	☐
Becoming easily annoyed or irritable?	☐	☐	☐	☐
Feeling afraid as if something awful might happen?	☐	☐	☐	☐

APPENDIX G - PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

Over the last 2 weeks how often have you been bothered by any of the following problems?

	Not at all	Several Days	More than half the days	Nearly every day
Little interest or pleasure in doing things	☐	☐	☐	☐
Feeling down, depressed or hopeless	☐	☐	☐	☐
Trouble falling asleep, staying asleep or sleeping too much	☐	☐	☐	☐
Feeling tired or having little energy	☐	☐	☐	☐
Poor appetite or overeating	☐	☐	☐	☐
Feeling bad about yourself or that you are a failure or have let your family down	☐	☐	☐	☐
Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
Moving or speaking so slowly that other people could have noticed. Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐
Thoughts that you would be better off dead or of hurting yourself in some way	☐	☐	☐	☐

APPENDIX H - VISUAL ANALOGUE SCALE (VAS)



APPENDIX I - PATIENTS GLOBAL IMPRESSION OF CHANGE (PGIC) SCALE

Since beginning treatment at this clinic, how would you describe the change (if any) in ACTIVITY LIMITATIONS, SYMPTOMS, EMOTIONS and OVERALL QUALITY OF LIFE, related to your painful condition? (tick ONE box).

No change (or condition has got worse)	☐	1
Almost the same, hardly any change at all	☐	2
A little better, but no noticeable change	☐	3
Somewhat better, but the change has not made any real difference	☐	4
Moderately better, and a slight but noticeable change	☐	5
Better, and a definite improvement that has made a real and worthwhile difference	☐	6
A great deal better, and a considerable improvement that has made all the difference	☐	7

In a similar way, please circle the number below, that matches your degree of change since beginning care at this clinic:

Much Better — **No Change** — **Much Worse**

0 1 2 3 4 5 6 7 8 9 10

Permissions to be obtained- https://eprovide.mapi-trust.org/instruments/patient-global-impression-of-change#contact_and_conditions_of_use

Reference: Hurst H, Bolton J. Assessing the clinical significance of change scores recorded on subjective outcome measures. J Manipulative Physiol Ther 2004;27:26-3

APPENDIX J - FUNCTIONAL INDEPENDENCE MEASURE (FIM)

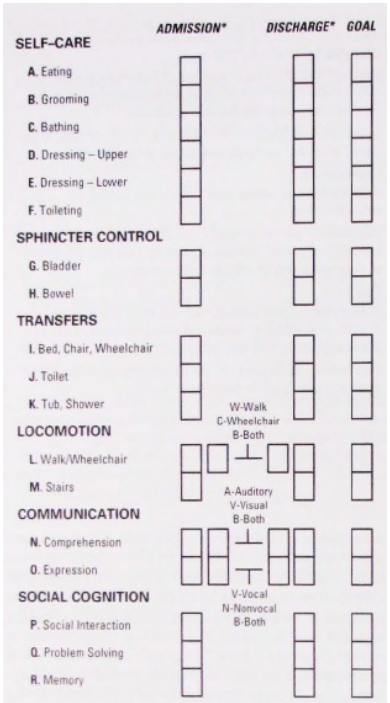
The Functional Independence Measure (FIM) is an 18-item of physical, psychological and social function. The tool is used to assess a patient's level of disability as well as change in patient status in response to rehabilitation or medical intervention.

The FIM is used by health care practitioners to assess and grade the functional status of a person based on the level of assistance he or she requires. Grading categories range from total independence to total assistance. Irrespective of the use of any assistive device, the person is considered complete independence.

Tasks that are evaluated using the FIM include bowel and bladder control, transfers, locomotion, communication, social cognition as well as the following six self-care activities:

- Feeding
- Grooming
- Bathing
- Upper Body Dressing
- Lower Body Dressing
- Toileting

The FIM measures what an individual can perform and not what that person could do under certain circumstances.



Permissions to be obtained. http;//www.udsmr.org

References: Linacre JM, Heinemann JW, Wright BD, Granger CV, Hamilton BB. The structure and stability of the functional independence measure. Arch Phys Med Rehabil. 1994. 75: 127-132. Heinemann AW, Linacre JM, Wright BD, Hamilton BB, Granger C. Relationships between impairment and physical disability as measured by the functional independence measure. Arch Phys Med Rehabil. 1993. 74: 566-573.

PROTOCOL

A Prospective multicentre, phase 2b randomised controlled double-blind trial, to determine the safety and efficacy of perispinal etanercept on quality of life at 28 days post treatment.

PESTO

Protocol Number 2
Version: 2.1
Date: 31 May 2022

Author:
Vincent Thijs

Sponsor/s:
Florey Institute of Neuroscience and Mental Health

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

Version 2 Changes

Background: Language changes, clarification of phase of trial (phase 2b),

Methods:
-Added missing information on timing of exploratory endpoint (FIM) measurement
-Added stratification variable to randomization (1-5 years after index stroke or 6-15 years after index stroke)
-Changes to inclusion criteria
    -Stroke occurred between 1 and 15 years before enrolment (previously 1-5 years).
    -Added: At time of enrolment patient is <70 years old

Whole protocol:
-changed etanercept to Etanercept when appearing at beginning of sentence
-header changes

Version 2.1 Changes
Site Nottingham never started so was removed from protocol
Site Christchurch was not mentioned in prior protocol, now added
Change in inclusion criteria: age increase, inclusion of patients with stroke at age 16 or 17 that are now 18 or older, inclusion of patients with milder disability (mRS=2) but decrease in quality of life similar to dependent patients, as operationalised with an SF-36 score of <80

PRINCIPAL INVESTIGATORS

Role	[Title] [First name] [Surname]	[Department], [School], [Organisation]
Chief Investigator A (Applicant)	Professor Vincent Thijs	Stroke theme, Florey Institute of Neuroscience and Mental Health,
Chief Investigator B	Professor Julie Bernhardt	Stroke theme, Florey Institute of Neuroscience and Mental Health,
Chief Investigator C	Professor Nikola Sprigg	Faculty of Health Sciences and Medicine, University of Nottingham, United Kingdom
Chief Investigator D	Professor Geoffrey Cloud	Stroke Medicine, Alfred Health
Chief Investigator E	Professor Leonid Churilov	University of Melbourne
Chief Investigator F	Ms Brooke Parsons	Stroke consumer, Melbourne
Chief Investigator G	Professor Marion Walker	Faculty of Health Sciences and Medicine, University of Nottingham, United Kingdom
Chief Investigator H	Professor Philip Bath	Faculty of Health Sciences and Medicine, University of Nottingham, United Kingdom

DATA MANAGEMENT

Li Chun Quang – Stroke Theme
The Florey Institute of Neuroscience and Mental Health
245 Burgundy Street
Heidelberg VIC 3084 Australia

BIOSTATISTICIAN

Professor Leonid Churilov
Melbourne University
245 Burgundy St
Heidelberg, VIC 3084, Australia
Phone: 61 9035 7089

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STUDY SYNOPSIS

(please provide a brief information)

Title:	A Prospective multicentre, phase 2b randomised controlled double blind trial, to determine the safety and efficacy of perispinal etanercept on quality of life at 28 days post treatment.
Short Title:	Perispinal etanercept to improve STroke Outcomes (PESTO)
Design:	Phase 2b, multicentre, prospective, randomised, double blind, placebo-controlled clinical trial
Sponsor:	Florey Institute of Neuroscience and Mental Health
Study Centres:	Austin Health (AUS), Alfred Health (AUS), Christchurch (New Zealand)
Study Hypothesis:	Perispinal etanercept (PSE) improves quality of life after chronic stroke in stroke survivors of working age
Study Objectives:	To test the safety and efficacy of administration of 25 mg perispinal etanercept in improving patient reported outcomes at 28 days after treatment.
Primary Objectives:	The *Primary Hypothesis* is that treatment with 25 mg perispinal etanercept improves quality of life in stroke survivors
Secondary Objectives	The *Secondary Hypothesis* is that repeated administration of perispinal etanercept 25mg leads to improved quality of life compared to a single administration.
Inclusion Criteria:	1. Patients with a history of acute ischemic or hemorrhagic stroke confirmed on imaging 2. Age ≥18 years -70 years at time of stroke or stroke occurred at age 16 or 17 and participant is now 18 or more 3. Moderate-to-severe disability *resulting from stroke* as defined by a modified Rankin scale of 3-5 or modified Rankin scale 2 with SF-36 score <80 4. Stroke occurred between 1 and 15 years before enrolment. 5. At time of enrolment, the patient is <= 70 years 5. SF-36 score <95 6. Patient is able to complete the SF-36 questionnaire independently or availability of a relative or carer who is able to complete the SF-36 questionnaire on behalf of the patient 7. Consent can be obtained from the participant or person responsible
Exclusion Criteria:	1. Contra-indication to etanercept (eg previous hypersensitivity, ongoing infection, use of IL-1 antagonists) 2. History of hepatitis B and C, tuberculosis, HIV, SLE, multiple sclerosis, moderate to severe heart failure 3. History of malignancy 4. Other use of immunosuppressant 5. Clinical diagnosis of dementia 6. mRS 0-1 OR if mRS=2, SF-36 score is 80 or more 7. Botulinum toxin injection to limbs in the 4 months prior to Screening Visit 8. Pregnancy (women of childbearing potential must be tested)

	9. Breastfeeding 10. Participation in any investigational study in the last 30 days 11. Known terminal illness or planned withdrawal of care or comfort care measures. 12. Any condition that, in the judgment of the investigator could impose hazards to the patient if study therapy is initiated or affect the participation of the patient in the study. 13. Prior exposure to etanercept for stroke
Number of Planned Participants:	168
Investigational product:	etanercept (Enbrel) 25 mg
Safety considerations:	Adverse Events and Serious Adverse events will be collected during the course of the trial.
Statistical Methods:	The primary endpoint, an achievement of at least 5 points improvement on the SF-36 score will be tested using a logistic regression model with the treatment arms as an independent variable and baseline value of SF-36, proxy or self- administration of SF-36 as adjustment covariates.

GLOSSARY OF ABBREVIATIONS & TERMS

Abbreviation	Description (using lay language)
AE	Adverse Event
DSMC	Data Safety Monitoring Committee
eCRF	Electronic Case Report Form
EQ-5D	EuroQol Five Dimensions Questionnaire
FAS	Fatigue Assessment Scale
FIM	Functional Independent Measure
GAD -7	General Anxiety Disorder
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HREC	Human Research Ethics Committee
ICF	Informed Consent Form
ICH GCP	International Conference on Harmonisation-Good Clinical Practice
IP	Investigational Product
ITT	Intention-To-Treat
NIHSS	National Institute of Health Stroke Scale
MoCA	Montreal Cognitive Assessment
mRS	Modified Rankin Scale
PHQ -9	Patient Health Questionnaire
PGICS	Patient Global Improvement of Change Scale
PROMS	Patient reported outcome measures
TBC	tuberculosis
SAE	Serious Adverse Event
SF-36	Short Form 36
VAS	Pain Visual Analogue Scale

1. INTRODUCTION/BACKGROUND INFORMATION

a. LAY SUMMARY

Perispinal administration of etanercept, a drug used to treat conditions affecting the joints and skin, has been associated with improved outcomes in patients with impairments and disability resulting from stroke. It is unclear whether these improved outcomes are due to causal effects of etanercept or other factors, as these results were seen in observational studies. The current randomized, placebo-controlled trial proposes to determine whether a single or two injections of etanercept improves quality of life and is safe.

b. INTRODUCTION

Stroke survivors exhibit lifelong, debilitating neurological impairments which negatively affect every aspect of their daily life. Because current treatment options for post-stroke impairments are limited, patients may try out therapies with unproven efficacy and safety. Often, these therapies incur significant costs (treatment and travel costs overseas) which adds to the stroke survivor and family burden. Perispinal injection of etanercept (PSE) is such a treatment with case series, media reports and online testimonials suggesting spectacular alleviation of neurological impairments, but no proven biological effect in humans, nor reliable evidence from high quality clinical trials.

Etanercept is a Tumor Necrosis Factor-alfa inhibitor that is currently used to treat rheumatological and dermatologic conditions. It does not cross the blood brain barrier when given subcutaneously. Proponents of etanercept for neurological symptoms propose that perispinal administration overcomes this limitation. PESTO is a 30-months, phase 2b, multicentre, prospective, randomised, double blind, placebo-controlled trial testing the safety and early efficacy of administration of perispinal etanercept in improving patient reported outcomes at 28 days after treatment. To test this hypothesis, the required sample size is 168. This sample size will be able to establish a clinically important difference in quality of life.

PESTO is a phase 2b clinical trial that will determine whether perispinal etanercept significantly improves quality of life after stroke, alters impairment and function and whether the enthusiasm for this unproven therapy is warranted. We will scientifically test the safety and efficacy of perispinal etanercept administration in stroke patients of working age. Outcomes from this (and other) trials may inform further definitive phase 3 clinical trials of etanercept in ischemic stroke.

c. BACKGROUND INFORMATION

A large proportion of the 470,000 patients with stroke will have long-lasting, debilitating neurological impairments. As a consequence, many stroke survivors are unable to live at home, return to work or perform their usual activities or hobbies. The burden of stroke on society is immense.

The management of post-stroke symptoms usually consists of physiotherapy, speech or occupational therapy, psychological support, orthoses, spasticity management and pain control. The efficacy of current medical treatment options in reducing many of the post-stroke impairments is limited. Therefore, patients and families are easily attracted by alternative treatment modalities that promise more effective solutions. These alternative treatments are often not based on adequate preclinical and clinical studies. Their efficacy and safety are not tested in controlled randomized clinical trials. The purported effects of treatment may therefore be related to spontaneous

improvements or occur due to placebo effects. Moreover, these treatments may impose additional financial burden on stroke survivors or on the community.

d. PERISPINAL ETANERCEPT

Recently, the beneficial effects of perispinal etanercept administration have caught the attention of the stroke survivor community in Australia, Europe and the United States of America.[1, 2] Reports in the literature have described impressive and immediate improvements in gait, memory, pain, swallowing and muscle strength.[3, 4] These improvements were seen in patients with a variety of neurological conditions such as stroke, traumatic brain injury and Alzheimer's dementia.[5-9] For instance, highly significant improvements in grip strength were reported immediately and up to at least 3 weeks after a single administration of perispinal etanercept. Improvements in the time to walk 20 meters were significantly changed. Ninety percent of the respondents reported favourable changes in motor effects after administration. These effects were maintained over at least several weeks. In this published series, the effects were seen after a single administration. Anecdotal reports have suggested that additional improvements were seen with repeated administration. YouTube videos of patients who have experienced such improvements after treatment offer compelling viewing and encourage other stroke survivors to also undergo treatment.

The medical community has however remained very sceptical of this form of therapy.[10] Although etanercept is primarily used for a variety of rheumatologic conditions, it has not been tested rigorously in stroke. Etanercept does not cross the blood barrier due to its large molecular size.[11, 12] Administration of etanercept in the peri-spinal region is argued by the proponents of this treatment modality as a way to overcome this problem. The medical community view the potential for any direct effects on the central nervous system to be low or non-existent because it cannot cross the blood brain barrier.[13, 14] Traditional phase 2 studies have not been performed to date. Regulatory authorities have not given approval for the treatment of neurological conditions with etanercept given inconclusive trial results or the lack of randomized clinical trials.[15] In 2016, the American Academy of Neurology published a practice advisory which concluded that: "Clinicians should counsel patients considering etanercept for treatment of poststroke disability that the evidence is insufficient to determine the treatment's effectiveness and that it may be associated with adverse outcomes and high cost (Level U: insufficient evidence)." The authors additionally noted: "We have very low confidence in the evidence for efficacy of etanercept for poststroke disability because of the high risk of bias of the relevant studies. The biological plausibility of benefit was judged to be low because of the reported immediate onset of benefit and single administration of a transiently acting medication. Explanations other than the effectiveness of the treatment for the observed improvements include observer expectation, performance motivation, regression to the mean, and the placebo effect." Finally, the Practice Advisory recommended that "Research is needed on the effects of etanercept on poststroke disability. To minimize the risk of bias, future studies of etanercept for the treatment for poststroke disability should randomly allocate patients in a masked fashion to active and placebo treatment arms and employ masked, standardized poststroke disability outcome measures."[16]

e. ETANERCEPT

Etanercept is a human tumour necrosis factor receptor p75 Fc fusion protein produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian

expression system.[17] Etanercept is a dimer of a chimeric protein genetically engineered by fusing the extracellular ligand binding domain of human tumour necrosis factor receptor-2 (TNFR2/p75) to the Fc domain of human IgG1. Etanercept contains 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons. The mechanism of action of etanercept is thought to be its competitive inhibition of TNF binding to cell surface TNFR, preventing TNF-mediated cellular responses by rendering TNF biologically inactive. etanercept may also modulate biologic responses controlled by additional downstream molecules (e.g., cytokines, adhesion molecules, or proteinases) that are induced or regulated by TNF. Etanercept is commonly used in inflammatory conditions (rheumatoid arthritis) or dermatologic conditions (psoriasis). Etanercept is slowly absorbed from the site of subcutaneous injection, reaching maximum concentration approximately 48 hours after a single dose. etanercept is cleared slowly from the body. The half-life is long, approximately 70 hours. Patients should be evaluated for infections before, during, and after treatment with Enbrel (etanercept product name), taking into consideration that the mean elimination half-life of etanercept is approximately 70 hours (range 7 to 300 hours). Allergic reactions are common and have included angioedema and urticaria; serious reactions have occurred. Other risks include reactivation of tuberculosis and hepatitis B, worsening of hepatitis C, immunosuppression, possible increased risks of hematologic malignancy, increased risk of skin cancers and demyelination. It is unknown whether these risks, observed in patients who received repeated administrations, are present in patients who receive one or two injections of etanercept. Careful selection of patients and monitoring of risks of this treatment is warranted. When this precautions are taken, etanercept is very safe.[18]

f. SELECTING OUTCOMES FOR PESTO

Etanercept is argued to show benefit across a broad range of impairments in chronic stroke survivors, often many years post stroke. To address the current application of etanercept, ensure generalisability and counter claims of selection bias, this trial needs to test the treatment in a broad sample of stroke survivors. In selecting primary and secondary outcomes we considered the recommendations of an international group of leading experts in stroke recovery.[19, 20] Measuring outcome across a number of ICF domains is warranted in this trial. The modified Rankin scale (a global outcome measure in common use in acute trials) is not an appropriate primary outcome for this chronic population, however it will be used as a secondary outcome.[21].[22] The broad target sample means that impairment-specific scales such as Fugl-Meyer scale for motor recovery, Western Aphasia Battery for language or 6m speed test for walking, apply only to subsets of patients with specific impairments, none can serve as a primary outcome measure. The NIHSS is a global neurological impairment scale that is commonly used in the acute phase and captures more stroke specific deficits but is not validated in the chronic phase and does not measure outcomes that matter to patients such as fatigue, depression or dependency.[23] The NIHSS does not require cooperation of the patient and because we include patients with severe disability or language problems can be used as a clinician reported secondary outcome to capture changes in impairment in this trial.

Quality of life scales are increasingly used in stroke trials to assess cost-efficacy but also to reliably measure stroke survivor's perception of outcome.[23] The Short Form (SF-36) is a 36-item, patient-reported survey of patient health. The SF-36 consists of eight scaled scores, which are the weighted sums of the questions in their section. Each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight. A score of zero is equivalent to maximum disability and

a score of 100 is equivalent to no disability. The SF-36, in use since 1991, has both internal and external validity. Higher scores are associated with return to work, reduced hospitalization and mortality. The SF-36 has been used in randomized trials of a wide range of conditions. In stroke, it avoids ceiling effects seen with disability scales, and captures both physical and mental health issues commonly seen after stroke.[24] Clinically important differences have been defined and, interestingly, are similar across a wide range of conditions.[25] An improvement of 5 points is considered of importance, whereas an improvement of 10 points is considered of major importance. We selected a change in SF-36 of 5 points as the trial primary endpoint. Standard safety reporting of adverse and serious adverse events will be undertaken.

2. STUDY OBJECTIVES

a. HYPOTHESIS

Perispinal etanercept (25 mg) improves quality of life in working age patients who have a history of stroke and moderate to severe disability. The *Primary Hypothesis* is that treatment with peri-spinal etanercept improves quality of life in stroke survivors. The *Secondary Hypothesis* is that repeated administration of peri-spinal administration leads to improved quality of life compared to a single administration.

b. STUDY AIMS

To demonstrate the efficacy and safety of perispinal etanercept (25 mg) on patient reported outcome measures after stroke.

c. OUTCOME MEASURES

Primary Efficacy endpoint:
To estimate the proportion of patients at day 28 with an improvement of 5 points or more on the SF-36 compared to baseline following single injection of etanercept 25 mg or equivalent placebo.

Safety Endpoints*:*
Proportion of serious adverse events at day 28
Proportion of serious adverse events at day 56

Secondary Efficacy Endpoint
To estimate the proportion of patients at day 56 with an improvement of 5 points or more on the SF-36 compared to baseline between the 3 treatment arms (placebo, single administration of etanercept, two administrations of etanercept).

Exploratory endpoints
1. To estimate change in neurological impairment using the NIHSS after injection of placebo or etanercept between baseline and day 56 in patients receiving none, one or two injections of etanercept.
2. To estimate change in neurological impairment using the NIHSS after injection of placebo or etanercept between baseline and day 28 after injection of placebo or etanercept
3. To estimate the change in functional independence using the FIM after injection of placebo or etanercept between baseline and day 28

4. To estimate the change in mRS between baseline and day 28 after injection of placebo or etanercept between baseline and day 28

5. To estimate immediate (30 min after injection) patient reported improvement using the Patient Global Improvement of Change Scale after injection of placebo or etanercept
6. To estimate the change in Fatigue Assessment Scale between baseline and day 28 after injection of placebo or etanercept
7. To estimate the change in the PHQ-9 depression scale between baseline and day 28 after injection of placebo or etanercept
8. To estimate the change in the GAD-7 anxiety scale between baseline and day 28 after injection of placebo or etanercept
9. To estimate the change in Visual Analogue Scale for Pain between baseline and day 28 after injection of placebo or etanercept
10. To estimate the change in MOCA between baseline and day 28 after injection of placebo or etanercept

d. Study Type & Design & Schedule

This will be a multicentre, prospective, randomised, placebo controlled, double-blind, parallel group phase IIb trial in stroke survivors enrolled between 1 and 5 years after their index event. Participants will be pre-screened using the SF36 and are required to score 95 or less to be considered eligible for the trial, and their modified Rankin Scale should be between 3 and 5 or if 2 the SF36 should be <80. Additional eligibility checks against the study inclusion and exclusion criteria will be performed.

The study uses a two-stage design (Figure 1). In the first phase of the study, patients will be randomized to receive etanercept or placebo and outcome assessments will be performed at day 28. In the second phase, the participants in the placebo arm will be further re-randomized into receiving another dose of placebo or a dose of etanercept at day 28 (based on 2:1 allocation ratio), while patients in the etanercept arm will be further re-randomized into receiving a dose of placebo or the second dose of etanercept at day 28 (based on 1:2 allocation ratio). This will result in three equally sized arms (Placebo n=56, Single Dose etanercept n=56, and Double Dose etanercept n=56) for the secondary dose-response analysis of the efficacy outcome at day 56. (see Figure 2)

The study flow is described in the Figure 1 below.

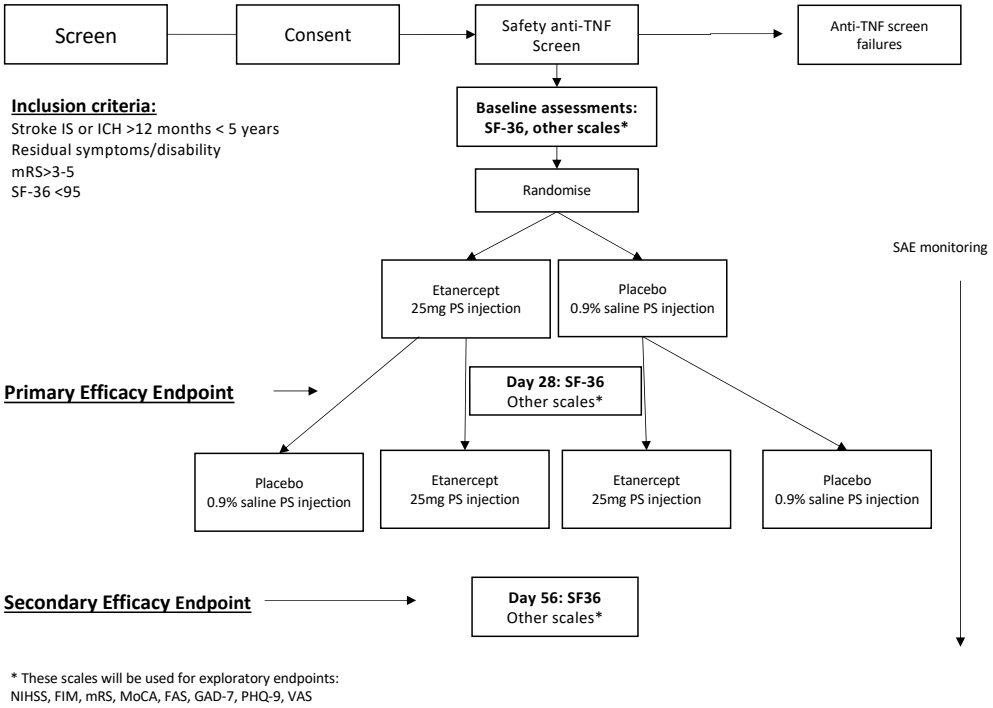
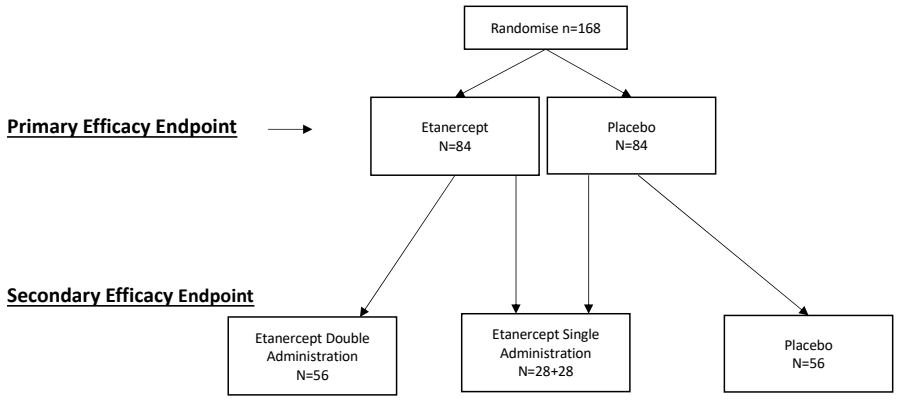


Figure 2 shows the phases and effective sample sizes for each efficacy endpoint.



During the study, participants will be required to attend the clinic/study centre at day 28 (+/- 4 days) and day 56 (+/- 4 days) post study enrolment to complete study outcome assessments by a blinded outcome assessor. Visits outside of these timeframes will be a protocol deviation.
Following completion of the outcome assessments at day 56, participants will have completed their study involvement.

Participants will attend the clinic for follow up visits, however, home visits may be performed when the participant is unable to attend the clinic/hospital. Peri-spinal etanercept will not be administered at home.

Recruitment for the study will be for 24 months. Analysis will take 2 months.

Data collected will be entered into a secure online database maintained at the Florey Institute of Neuroscience and Mental Health.

e. Blinding

Patients and outcome assessors will be masked to investigational product allocation. Unblinding will only be done after study completion for the analysis, or in the event of a serious adverse event where the information relating to the drug administered is required for safety reasons.

3. Study Assessments and Procedures

a. Visit 1 Screening and consent (0-14 days prior to Day 0)

Potential patients will self refer or will be referred by their medical practitioner to the researchers. A flyer will be used to advertise the study. A brief eligibility screen of interested persons will be performed by the study personnel. The eligibility screen may be performed in person or by telephone.

This will include questions on age, the index stroke (timing since index stroke), current ability to walk and queries about other related stroke impairments (speech, cognition, muscle weakness, coordination problems) and ability to perform activities of daily living. Additionally, we will ask about a history of infectious disease, immunosuppression, heart failure and use of other immunosuppressants. Patients who fulfil entry criteria and exclusion criteria will be provided an information sheet and consent form. Only those who meet the study inclusion criteria will proceed to consent.

b. Consent

Interested persons or their medical decision maker will be provided with comprehensive verbal and written information about the study by a member of the research team. They will be given time to consider their inclusion and be given the opportunity to speak to others about their involvement before deciding whether or not to be involved. They will be given the opportunity to ask any further questions regarding their participation before providing written consent via the relevant Participant Information and Consent Forms. Documentation of the process will be made in the patient's clinic notes.

c. Baseline Assessment and Screening for Infection

Following written informed consent, a safety eligibility screen for etanercept will be performed using the QuantiFERON-Gold assay for TBC and screening tests for hepatitis B and C (blood test).

A pregnancy test will be performed in all women of child-bearing potential (urine dipstick).

To further confirm eligibility, certified raters will assess the participants global disability score using the modified Rankin scale (mRS). Participants will also be asked to complete the SF-36 questionnaire independently. If the participant is not able, a

relative or carer who is familiar with the patient will be asked to complete the questionnaire.
Participants who do not meet criteria for SF 36 or fail the safety screen, will be considered screening failures at this point and not proceed.

Details of the participants medical history (including stroke history) and concomitant medications will be recorded.

d. Visit 2 Investigational Product Administration (Day 0)

At the beginning of this visit, trained raters will assess neurologic impairment using the NIHSS and global disability using the modified Rankin scale (mRS). GCP-certified assessors will also assess baseline mood using the PHQ-9, fatigue using the Fatigue Assessment Scale (FAS), anxiety using the (GAD-7), pain using the Visual Analogue scale (VAS), cognition using the Montreal Cognitive Assessment (MOCA), functional independence using the Functional Independence Measure (FIM).

e. Randomisation

After confirmation of eligibility, participants will be randomised into the treatment arms using a centralised computer-generated random assignment procedure via the electronic case record form, stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or if not possible by a relative or carer proxy, and baseline modified Rankin Scale (dichotomized as mRS 3 vs mRS4-5), and whether the index stroke occurred between 1 and 5 years or between 6 and 15 years before enrolment. A researcher not otherwise involved in the trial will randomise the participant and allocate to treatment arms, hence allocation concealment will be assured. The treatment allocation will be sent electronically to the study pharmacist who will prepare the investigational product for blinded use. Study pharmacists will be instructed not to disclose treatment allocation.

f. Intervention

An unblinded pharmacist will prepare the investigational product (IP), based on treatment allocation. The IP will be provided to the researchers in a masked fashion to ensure concealment of the allocated treatment.

g. Active treatment

Etanercept (Enbrel) is supplied as a single-use dose in a vial as a sterile, lyophilised preservative-free powder which when reconstituted is for subcutaneous injection. etanercept, when dissolved by the addition of sterile water is clear to slightly yellow solution. Each single-use dose vial will contain up to 1.0 mL prepared with a 25 mg lyophilized powdered solution of etanercept. Given the possible slight discoloration of the IP, pharmacy will mask the syringe.

h. Placebo control

The placebo control will be sterile saline (suitable for use as an injection) as a clear colourless solution and prepared in the same type of syringe with the same volume as for the active test drug. Given the possible slight discoloration of the IP and to avoid unblinding, pharmacy will mask the syringe.

i. Drug administration Baseline

Active treatment or placebo, in an identical volume of 1.0 mL, will be administered via a posterior cervical interspinous (between the C6-C7 or C7-T1 interspace) injection subcutaneously in the midline with a 27-gauge half to one inch needle by an experienced neurologist or stroke physician. The participant is then placed on an inversion table in the Trendelenburg position. The body is laid supine, on a 20 degree incline with the feet elevated above the head for four minutes as described by treatment proponents. The injection will be videotaped, such that correct administration can be verified independently. It will not be possible to identify the patient from the videotape. The doctors administering the IP will be trained to correctly administer the injection.

j. Post-injection assessment Baseline

30 minutes after the injection the participant will be asked to complete the Patient Global Improvement of Change Scale (PGICS).

k. Visit 3 Outcome Assessment and Second Injection (Day 28+/4)

Participants will return to the clinic for the first outcome assessment performed by trained, blinded raters. They will assess neurologic impairment using the NIHSS and global disability using the modified Rankin scale. GCP-certified assessors will also assess cognition (MOCA) and functional independence (FIM).
At this visit, the participant or their carer will fill out questionnaires including quality of life SF-36. In addition, baseline mood (PHQ-9), pain (VAS), fatigue (Fatigue Assessment Scale), anxiety (GAD-7) will be assessed. The same carer should be used for the questionnaires at all visits.
Any Adverse events or serious adverse events that have occurred since the last visit will be recorded. Any changes in medication taken by the participants will be recorded.

l. Injection Visit 3

Participants then proceed to the second stage of the study, where they then receive Active treatment or placebo, in a volume of 1.0 mL, is administered via a posterior cervical interspinous (between the C6-C7 or C7-T1 interspace) injection subcutaneously in the midline with a 27-gauge half to one inch needle by an experienced neurologist or stroke physician. The participant is then placed on an inversion table in the Trendelenburg position. The body is laid supine, on a 20 degree incline with the feet elevated above the head for four minutes as described by treatment proponents. The injection will be videotaped, such that correct administration can be verified independently. It will not be possible to identify the patient from the videotape. The doctors administering the IP will be trained to correctly administer the injection.

m. Post-injection assessment Visit 3

30 minutes after the injection the participant will be asked to complete the Patient Global Improvement of Change Scale (PGICS).

n. VISIT 4 OUTCOME ASSESSMENT (DAY 56+/-4)

Trained, blinded raters will assess neurologic impairment using the NIHSS and global disability using the modified Rankin scale. GCP-certified assessors will also assess cognition (MOCA), functional independence (FIM).

The participant or their carer will fill out questionnaires: quality of life SF-36, mood (PHQ-9), pain (VAS), fatigue (FAS), and anxiety (GAD-7). The same carer should be used for the questionnaires at all visits.

Adverse events and serious adverse events will be recorded. Any changes in medication taken by the participant will be recorded.

The Table below shows the schedule of events.

4. SCHEDULE OF EVENTS

	Visit 1 Screening	Visit 2 Baseline	Visit 3 Follow up 1	Visit 4 Follow up 2
Time period	**D-14-0**	**D0**	**D28+/-4**	**D56+/-4**
Brief eligibility screen	X			
Informed consent	X			
Medical history including allergy	X			
Dipstick (urine test)[1]	X[1]			
Quantiferon gold test	X			
Hepatitis B screening	X			
Hepatitis C screening	X			
Concomitant medication	X	X	X	X
AE and SAE		X	X	X
Randomisation		X		
Administration of IP		X	X	
National Institute of Health Stroke Scale (NIHSS)		X	X	X
modified Rankin scale (mRS)	X	X	X	X
Short Form (36) SF-36	X	X	X	X
Patient Health Questionnaire (PHQ-9)		X	X	X
Fatigue assessment scale (FAS)		X	X	X
General Anxiety Disorder (GAD-7)		X	X	X
Pain visual analogue scale (VAS)		X	X	X
Montreal Cognitive Assessment (MOCA)		X	X	X
Functional Independent Measure (FIM)		X	X	X
Patient Global Impression of Change Scale		X[2]	X[2]	

[1] in females of child-bearing potential only

[2] 30 minutes after IP administration

5. OUTCOME ASSESSMENTS

a. 36-ITEM SHORT FORM HEALTH SURVEY (SF-36)

The Short Form-36 (SF-36) is a 36-item self-administered questionnaire which measures Quality of Life (QoL) across eight domains. The eight domains that the SF-36 measures are as follows: physical functioning; role limitations due to physical health; role limitations due to emotional problems; energy/fatigue; emotional well-being; social functioning; pain; general health. For participants unable to complete themselves, a carer will be asked to complete this on behalf of the participant.

b. MODIFIED RANKIN SCALE (MRS)

The Modified Rankin Scale (mRS) is used to measure the degree of disability in patients who have had a stroke, as follows:

0: No symptoms at all
1: No significant disability despite symptoms; able to carry out all usual duties and activities
2: Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3: Moderate disability; requiring some help, but able to walk without assistance
4: Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5: Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6: Dead

The assessment will be performed by a certified neurologist or stroke physician or health care professional.

c. MONTREAL COGNITIVE ASSESSMENT (MOCA)

Cognition will be evaluated using Montreal Cognitive Assessment, used to assess different cognitive domains including: attention, concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The total score ranges from 0 to 30. The assessment will be performed by a neurologist or stroke physician or trained health care professional.

d. NATIONAL INSTITUTES OF HEALTH STROKE SCALE (NIHSS)

The National Institutes of Health Stroke Scale,/ NIH Stroke Scale (NIHSS) is a tool used by healthcare providers to objectively quantify the impairment caused by a stroke. The NIHSS is composed of 11 items, each of which scores a specific ability between a 0 and 4. For each item, a score of 0 typically indicates normal function in that specific ability, while a higher score is indicative of some level of impairment.[1] The individual scores from each item are summed in order to calculate a patient's total NIHSS score. The maximum possible score is 42, with the minimum score being a 0. The assessment will be performed by a certified neurologist, stroke physician or health care professional.

e. GENERALIZED ANXIETY DISORDER 7 (GAD-7)

This is a self-reported questionnaire for screening and severity measuring of generalized anxiety disorder (GAD). GAD-7 has seven items, which measure severity of various signs of GAD according to reported response categories with assigned points (see below). Assessment is indicated by the total score, which made up by adding together the scores for the scale all seven items.

f. PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

PHQ-9 is a 9-question instrument to screen for the presence and severity of depression. The results of the PHQ-9 may be used to make a depression diagnosis according to DSM-IV criteria and takes less than 3 minutes to complete.

g. FUNCTIONAL INDEPENDENCE MEASURE (FIM)

The FIM's assessment of degree of disability depends on the patient's score in 18 categories, focusing on motor and cognitive function. Each category or item is rated on a 7-point scale (1 = <25% independence; total assistance required, 7 = 100%

independence). As such, FIM scores may be interpreted to indicate level of independence or level of burden of care. The scale is used to assess how well a person can carry out basic activities of daily living and thus how dependent he or she will be on help from others. Other areas assessed include the physical like how well patients move and walk, and the cognitive, how well they interact with others, communicate, and process information.

h. Fatigue Assessment Scale (FAS)

The FAS is a 10-item general fatigue questionnaire to assess fatigue. Five questions reflect physical fatigue and 5 questions (questions 3 and 6-9) mental fatigue.

i. Patient Global Impression of Change (PGIC)

The self-report measure Patient Global Impression of Change (PGIC) reflects a patient's belief about the efficacy of treatment. PGIC is a 7-point scale depicting a patient's rating of overall improvement. Patients rate their change as ''very much improved,'' ''much improved,'' ''minimally improved,'' ''no change,'' ''minimally worse,'' ''much worse,'' or ''very much worse.''

j. Pain Visual Analogue Scale (VAS)

The pain VAS is a continuous scale comprised of a line, 10 centimetres (100 mm) in length, anchored by 2 verbal descriptors and pictures, one for each symptom extreme. For pain intensity, the scale is anchored by "no pain" (score of 0) and "worst imaginable pain". The pain VAS is self-completed by the respondent. The respondent is asked to place a line perpendicular to the VAS line at the point that represents their pain intensity in the past 24 hours.

6. Standard Care and Additional to Standard Care Procedures

All of the procedures are additional to standard of care.

	Time to complete procedure in minutes
Brief eligibility screen	5
Dipstick (urine test)*	5
Blood test (Quantiferon, hep B, hep C)	10
Concomitant medication collection	10
Randomisation	2
Preparation of IP	30
Administration of IP	5
Patient Global Improvement of Change Scale **	2
National Institute of Health Stroke Scale (NIHSS)	15
modified Rankin scale (mRS)	10
Patient Health Questionnaire (PHQ-9)	3
Fatigue assessment scale (FAS)	5
General Anxiety Disorder (GAD-7)	5

Pain visual analogue scale (VAS)	3
Montreal Cognitive Assessment (MOCA)	20
Functional Independent Measure (FIM)	20
Short Form (36) SF-36	30
AE and SAE assessment	20

7. RANDOMISATION

After confirmation of eligibility, participants will be randomised into either the etanercept group or the control group using a centralised computer-generated random assignment procedure with permuted blocks of various sizes as part of the electronic case record form stored on a secure server based at the Florey. Randomisation will be based on a 1:1 allocation ratio. Randomisation will be stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or if not possible by a relative or carer proxy, and baseline modified Rankin Scale (dichotomized as mRS 3 vs mRS4-5). Randomisation for the primary endpoint will be based on a 1:1 allocation ratio (Placebo vs etanercept). As the second part of the same randomization procedure conducted at the same time point at Day 0, the participants in the placebo arm will be further re-randomized into receiving another dose of placebo or a dose of etanercept at day 28 (based on 2:1 allocation ratio), while patients in the etanercept arm will be further re-randomized into receiving a dose of placebo or the second dose of etanercept at day 28 (based on 1:2 allocation ratio). This will result in three equally sized arms (Placebo n=56, Single Dose etanercept n=56, and Double Dose etanercept n=56) for the secondary dose-response analysis of the efficacy outcome at day 56. (see figure above). Randomisation will be stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or if not possible by a relative or carer proxy. A researcher not otherwise involved in the trial will undertake the randomisation and allocation to treatment arms, hence allocation concealment will be assured.

The treatment allocation will be sent electronically to the study pharmacist who will prepare the investigational product. Study pharmacists will be instructed not to disclose treatment allocation. The investigator administering the investigational product, patients and outcome assessors will therefore be blinded.

8. STUDY POPULATION

a. RECRUITMENT PROCEDURE

Patients will be recruited from multiple outpatient and community sources. Expressions of interest will also be obtained through the Stroke Foundation who have access to people who are interested in being involved in clinical trials. We will additionally advertise through relevant stroke websites (such as the Stroke Foundations EnableMe), and relevant stroke and GP clinics.

b. INCLUSION CRITERIA

The inclusion and exclusion criteria for PESTO either match or exceed those published by the American College of Rheumatology's 2015 guidelines for the use of biologicals in patients with rheumatoid arthritis and the Product Information criteria for use of etanercept in Australia.[17]

1. Patients with a history of acute ischemic or haemorrhagic stroke confirmed on imaging

2. Age ≥18 years-70 years at time of stroke or stroke occurred at age 16 or 17 and patients is now 18 or more

3. Moderate-to-severe disability resulting from stroke as defined by a modified Rankin scale of 3-5 or modified Rankin scale 2 with SF-36 score <80

4. Stroke occurred between 1 and 15 years before enrolment.
5. At time of enrolment patient is <=70 years old
6. SF-36 score <95 (<80 if mRS is 2)
7. Patient is able to complete the SF-36 questionnaire independently or availability of a relative or carer who is able to complete the SF-36 questionnaire on behalf of the patient
8. Consent can be obtained from the participant or medical decision maker.

c. EXCLUSION CRITERIA

1. Contra-indication to using etanercept (e.g. previous hypersensitivity, patients with, or at risk of sepsis, ongoing infection, use of IL-1 antagonists)
2. History of hepatitis B and C, tuberculosis, HIV, SLE, multiple sclerosis,
3. Moderate to severe heart failure
4. History of malignancy
5. Other current use of immunosuppressant
6. History of alcoholic hepatitis, current ongoing hepatitis
7. History of juvenile idiopathic arthritis
8. History of blood dyscrasia
9. Clinical diagnosis of dementia
10. mRS 0-1 OR if mRS=2, SF-36 score is 80 or more

11. Botulinum toxin injection to limbs in the 4 months prior to Screening Visit
12. Pregnancy (women of childbearing potential must be tested)
13. Breastfeeding
14. Participation in any investigational study in the last 30 days
15. Known terminal illness or planned withdrawal of care or comfort care measures.
16. Any condition that, in the judgment of the investigator could impose hazards to the patient if study therapy is initiated or affect the participation of the patient in the study.
17. Prior exposure to etanercept for stroke

d. CONSENT

Informed consent will be obtained from the participant or a medical decision maker. Potentially interested participants or their decision maker will be provided with comprehensive verbal and written information about the study by a member of the research team.

In particular, the researcher will:

i. Present the potential participants or medical decision maker with a written Participant Information Sheet, providing an appropriate and well-informed explanation of the proposed research and the terms of participation

ii. Have sufficient understanding of the research to explain it fully and answer the potential participant's questions

iii. Present the potential participants or medical decision maker with a written Participant Consent Form outlining the terms of research participation (i.e. voluntary, with identifiable personal information, for research purposes only)

iv. Ensure that the obtained participation consent, in the form of a written signed Participant Information and Consent Form, is both well-informed and completely voluntary.
If individuals would like to take more time to think about their participation in the study and to discuss it with family or friends, they will be asked for permission for the Researcher to contact them by phone after a specified time (e.g., one week) to further discuss the possibility of participation. Individuals who decline to participate will not be contacted again.

9. INVESTIGATIONAL PRODUCT

a. DESCRIPTION OF INVESTIGATIONAL PRODUCT

ENBREL (etanercept) 25 mg powder for injection and water for injections.
Each vial of ENBREL powder for injection contains 25 mg of etanercept. The content of the diluent syringe is 1 mL of sterile water for injections. Following reconstitution with water for injections, ENBREL is a colourless to slightly yellow and clear to slightly opalescent solution, with a pH of 7.1-7.7.
Etanercept is a human tumour necrosis factor receptor p75 Fc fusion protein produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian expression system. etanercept is a dimer of a protein genetically engineered by fusing the extracellular ligand-binding domain of human tumour necrosis factor receptor-2 (TNFR2/p75) to the Fc domain of human IgG1. This Fc component contains the hinge, CH2 and CH3 regions but not the CH1 region of IgG1. etanercept contains 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons. etanercept is now manufactured using a serum-free process. The potency is determined by measuring the ability of etanercept to neutralise the TNFα-mediated growth inhibition of A375 cells. The specific activity of etanercept is 1.7 x 106 units/mg.

b. PREPARATION OF IP

ENBREL contains no antibacterial preservative and therefore, solutions prepared with water for injections should be administered as soon as possible and within six hours following reconstitution. In the absence of compatibility studies, ENBREL must not be mixed with other medicinal products.

Reconstitute the ENBREL powder aseptically by injecting 1 mL of sterile water for injections very slowly into the vial with the vial adaptor attached to the syringe. Gently swirl the contents to avoid excessive foaming. Some foaming will occur, this is normal. To avoid excessive foaming, do not shake or vigorously agitate. Dissolution of ENBREL usually takes less than 10 minutes. Visually inspect the solution for particulate matter and discolouration prior to administration. The solution should not be used if discoloured or cloudy, or if particulate matter remains. Withdraw the solution into the empty syringe, removing only the dose to be given from the vial. Some foam or bubbles may remain in the vial. Do not filter reconstituted solution during preparation or administration.

ENBREL should not be used if all the powder in the vial is not dissolved within 10 minutes. In that situation another vial should be. Once the ENBREL solution has been aspirated into the syringe, discard the vial adaptor and replace with a needle from the pack for injection.

Injections should never be given into areas where the skin is tender, bruised, red or hard.

c. PREPARATION OF PLACEBO

The placebo control will be sterile saline (suitable for use as an injection) as a clear colourless solution, with the same appearance as etanercept, and prepared in the same type of syringe with the same volume as for the active test drug.

d. RANDOMISATION

After randomisation (see previous section), treatment allocation is received by the unblinded study pharmacist at each site. The pharmacist will prepare the IP according to the instructions described in 6.2 and label. A study member will pick up the IP and bring it to the clinic where it will be administered. Administration should be performed within 6 hours after preparation of IP.

e. BLINDING/UNBLINDING

This is a double-blind trial. The participant and all those involved in the clinical assessments of the participants will be blinded to treatment allocation. In the case of reported Suspected Unexpected Serious Adverse Reactions (SUSARs), or if treatment identification is necessary for emergency patient management, cases may be unblinded through Redcap.

The Data Safety Monitoring Committee (DSMC) will have access to unblinded grouped data.

f. PRODUCT LABELLING

Details on the labels will include: Protocol name and identification number, name, address and contact number of the sponsor, the participant randomisation number, and the words 'for clinical trial use only'. Labelling of the investigational product will comply with Therapeutic Goods Administration (TGA) code of Good Manufacturing Practice (GMP) for medicinal products, Annex 13, section 17 in Australia, or applicable national and/or local requirements for international sites where applicable.

g. HANDLING AND STORAGE OF INVESTIGATIONAL PRODUCT

Enbrel should be stored at 2ºC to 8°C, refrigerated, but not frozen. The solution should be used immediately after reconstitution. If not used immediately, ENBREL solution must be refrigerated in the vial at 2°C to 8°C after reconstitution and used within 6 hours.
Prior to reconstitution, the powder may be stored at temperatures up to a maximum of 25°C for a single period of up to 4 weeks. ENBREL should be discarded if exposed to high temperatures, or if not used within 4 weeks of initial removal from refrigeration.

h. DOSE JUSTIFICATION

The 25 mg etanercept dose is in common use for treatment of rheumatological and dermatologic use and is used off-label in stroke patients.

i. ADMINISTRATION

Active treatment or placebo, in an identical volume of 1.0 mL, will be administered via a posterior cervical interspinous (between the C6-C7 or C7-T1 interspace) injection subcutaneously in the midline with a 27-gauge half to one inch needle followed by Trendelenburg positioning, with patient supine on an inversion table with the head dependent for four minutes at an incline of 20 degrees, as described by treatment proponents.

2. PARTICIPANT SAFETY AND WITHDRAWAL

a. RISK MANAGEMENT AND SAFETY

Standard treatment will not be delayed for the purpose of this study. Current medications will not be discontinued for the purposes of this study.

Blood sample collection
Insertion of a needle for blood sample collection may be commonly associated with slight pain or bruising at the puncture site and rare chance of infection. There is a rare risk of fainting due to the venous needle insertion.

Perispinal injection
This is a subcutaneous injection of a small volume of liquid. Bruising or pain at the injection site are possible. There is a rare chance of infection at the injection site.

Reported side effects of etanercept
Many of the reported serious events have occurred in patients receiving concomitant medicines including immunosuppressants, or with underlying diseases that, in addition to their rheumatoid arthritis, could predispose them to infections. The side effect profile of etanercept has been derived from patients undergoing chronic treatment. Whether these side effects also occur in patients receiving one or two doses of etanercept is unknown.

(1) Serious infections including sepsis and tuberculosis, have been reported with the use of etanercept. Some of these infections have been fatal. These infections were due to bacteria, mycobacteria, fungi, viruses and parasites (including protozoa). Opportunistic infections have also been reported (including listeriosis, legionellosis and invasive fungal infections) in patients receiving etanercept.

In some cases, fungal and other opportunistic infections are not recognised and this has resulted in delays in appropriate treatment, sometimes resulting in death.

Patients will be screened for tuberculosis, hepatitis B and C prior to administration of study drug. Patients will not be allowed to participate in the trial if any active or latent infection is identified. During the trial participants will be monitored for signs and symptoms of infection. We will inform patients to seek medical advice if signs of infection appear. Any participant who develops a new infection will be monitored closely.

Administration of the study drug will be discontinued if a patient develops a serious infection (e.g. tuberculosis or an atypical mycobacterial infection) or sepsis and appropriate medical care will be administered.

All patients will be evaluated for infections, and the researchers will consider the patient's risk for relevant opportunistic infections (e.g., exposure to endemic mycoses).

Researchers will exercise caution when considering the use of the study drug in patients with a history of recurring or chronic infections, or with underlying conditions, which may predispose patients to infections such as advanced or poorly controlled diabetes.

(2) Increased infection risk and mortality in alcoholic hepatitis

Risk reduction strategy: Patients with alcoholic hepatitis will not be allowed to participate in the trial.

(3) Hypoglycemia

There have been reports of hypoglycaemia following initiation of etanercept in patients receiving medication for diabetes, necessitating a reduction in anti-diabetic medication in some of these patients.

Risk reduction strategy: Patients with diabetes will be warned about symptoms of hypoglycemia.

(4) Inflammatory bowel disease and uveitis in patients with juvenile idiopathic arthritis.

Risk reduction strategy: Patients with juvenile idiopathic arthritis will not participate in the trial.

(5) Miscellaneous serious side effects in patients who receive other immunosuppressive drugs including infection, hematological problems.

Risk reduction strategy: patients requiring other immunosuppressive drugs will not participate in the trial.

(6) Haematological reactions including aplastic anemia, pancytopenia.

Risk reduction strategy: patients with a history of blood dyscrasia will not participate in the trial

All patients will be advised that if they develop signs and symptoms suggestive of blood dyscrasias or infections (e.g., persistent fever, sore throat, bruising, bleeding, paleness), they should seek immediate medical advice. Such patients should be evaluated urgently, including full blood count; if any blood dyscrasias are confirmed, IP should be discontinued.

(7) Allergic reactions: Allergic reactions associated with etanercept administration have been reported commonly. Allergic reactions have included angioedema and urticaria. Serious reactions have occurred.

Risk reduction strategy: Patients will be monitored for allergic reactions and asked to report any reaction immediately to the study team.

(8) Congestive heart failure

Worsening heart failure and rare reports of new onset heart failure in patients without known preexisting cardiovascular disease, even in patients under 50 years have been reported.

Risk reduction strategy: Patients with known congestive heart failure will not be recruited. Patients with new onset dyspnea and/or other signs of heart failure will be assessed for possible heart failure.

(9) Other neurological disorders

Treatment with ENBREL and other agents that inhibit TNF have been associated with rare cases of new onset or exacerbation of central nervous system demyelinating disorders, some presenting with mental status changes and some associated with permanent disability.

Risk reduction strategy: Patients with known inflammatory neurological disorders will not be recruited. Patients with new onset neurological symptoms following etanercept administration will receive neurological assessments using the NIHSS as part of the clinical trial.

(10) Malignancies

A possible risk for the development of lymphomas, leukemia, melanoma and non-melanoma skin cancer or other malignancies in patients treated with a TNF-antagonist cannot be excluded. Rates were low and were observed in patients with conditions associated with development of malignancies.

Risk reduction strategy: Patients with ongoing malignancy will not be included in the study. Periodic skin examination will be recommended for all patients who are at increased risk for skin cancer, especially in patients with psoriasis, who may be at increased risk.

(11) Live vaccines

No safety data are available on the effects of live vaccine when used in combination with ENBREL. Live vaccines should therefore not be given concurrently with ENBREL.

Risk reduction strategy: Patients will be recommended to undergo vaccination with live vaccines during the course of the trial.

(12) Pregnancy

The safe use of ENBREL during pregnancy has not been established.

Risk reduction strategy:
Women of childbearing potential should be advised to use appropriate contraception to avoid becoming pregnant during ENBREL therapy and for three weeks after discontinuation of therapy.

(13) Lactation

The safe use of ENBREL during lactation has not been established. etanercept has been reported to be excreted in human breast milk following subcutaneous administration.

Risk reduction strategy:
Nursing mothers will not be included in the study.

b. Handling of Withdrawals

Participants may voluntarily withdraw from the study at any time. Data obtained prior to withdrawal of consent will be retained, unless otherwise requested by the withdrawing participant.

c. Replacements

Dropouts from the study will not be replaced. The sample size calculations allow for up to 10 participants withdrawing from the study.

3. Adverse Events (AEs) and Serious Adverse Events (SAEs)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE) as provided in this protocol.

During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

a. Definition of an Adverse Event (AE)

An Adverse Event (AE) is any untoward medical occurrence in a participant temporarily associated with the use of an investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the product, whether or not considered related to the product. For marketed products, this also includes failure to produce benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition. New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication.

Examples of an AE do not include:

- Medical or surgical procedure (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to hospital).
- The disease being studied, or signs/symptoms associated with the disease unless more severe than expected for the participant's condition. In this study,

AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of participants' previous therapeutic regimen).

b. Definition of a Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence that, at any dose:

a) Results in death

b) Is life threatening

c) Requires hospitalisation or prolongation of an existing hospitalisation.

Note: In general, hospitalisation signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious. Hospitalisation for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in disability/incapacity

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.

e) Is a congenital abnormality / birth defect, or

f) Is a significant medical event

Note: Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardise the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious.

c. Time Period, Frequency, and Method of Detecting AEs

All adverse events will be recorded between the time of consent and each study visit. Each participant will be monitored regularly by the investigator and study personnel for adverse events and all SAEs will be reported during study participation. SAEs will be reported to the Human Research Ethics Committee as soon as practicable and to the Therapeutic Goods Administration (TGA), within the specified reporting time lines.

d. Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the site investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostic reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in to the eCRF. Supporting documentation for SAEs will be collected. In this instance, all participant identifiers will be blinded on the copies of the medical records prior to submission to sponsor.

For each adverse event, start and stop dates, action taken, outcome, intensity and relationship to study product (causality) will be documented.

If an AE changes in frequency or intensity during a study, a new entry of the event must be made in the eCRF.
The investigator will establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In the absence of a diagnosis, the individual signs/symptoms will be documented. All details of any treatments initiated due to the adverse event should be recorded in the participant's notes and the eCRF.

e. Prompt Reporting of SAEs

Serious Adverse Events require immediate action. Once an investigator becomes aware that an SAE has occurred, they will immediately notify the Sponsor. The SAE form must be completed on the eCRF as thoroughly as possible with all available details of the event and signed by the investigator (or appropriately qualified designee and sent to the study monitor within 24 hours of first becoming aware of the event. If the investigator does not have all information regarding an SAE, they will not wait to receive additional information before notifying the study monitor of the event and completing the form. The form will be updated when additional information is received. The investigator will provide an assessment of causality at the time of the initial report. The SAE form may be appropriately amended if required by the investigator as more information comes to hand and resubmitted to the Sponsor.
In accordance with local ethics requirements, the investigator must also notify their Ethics Committee of SAEs according to local guidelines.
The investigator and others responsible for participant care, should institute any investigations of serious adverse events based on their clinical judgement of the likely causative factors. This may include seeking further opinion from a specialist in the field of the event. If a participant dies, a cause of death and any post-mortem findings, including histopathology will be collected if available.

f. Assessment of intensity of AEs and SAEs

The investigator will assess intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgement. The intensity of each AE and SAE recorded in the CRF should be assigned to one of the following categories:

Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday
activities.

Severe: An event which is incapacitating and prevents normal everyday activities.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilised for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

g. Assessment of Causality for AEs and SAEs

The investigator will assess the relationship between study drug and the occurrence of each AE/SAE using clinical judgment.

Alternative causes, such as natural history of the underlying disease, concomitant therapy,
other risk factors, and the temporal relationship of the event to the investigational product will
be considered and investigated. The investigator will also consult the product information in the determination of their assessment.
The causal relationship to the study product should be assessed using the following classifications:
Not Related: In the Investigator's opinion, there is no causal relationship between the study product and the adverse event.
Related: The adverse event follows a reasonable temporal sequence from the time of study product administration or reappears when study product is reintroduced.

There may be situations when an SAE has occurred, and the investigator has minimal information to include in the initial report. It is very important that the investigator always makes an assessment of causality for every event prior to sending the SAE form. The investigator may change their opinion of causality in light of follow-up information, amending the SAE form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

h. Assessment of Expectedness for SAEs

The assessment of the expectedness of the event will also be made by the investigator and reported on the SAE form.

Expected An adverse reaction, the nature or severity of which is consistent with the applicable product information
Unexpected An adverse reaction, the nature or severity of which is not consistent with the Product Information.

i. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to actively follow each participant and provide further information to the Sponsor on the participant's condition. All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits or contacts. All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the participant is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, or consultation with other health care professionals. New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the investigator. The updated SAE form should be re-sent to the sponsor after any updates.

4. Trial Governance and Conduct

a. Management Committee

The trial Chief Investigators, central coordinator, trial statistician and finance manager will form the Management Committee and maintain oversight of regular operations of the trial.

b. Steering Committee

Principal investigators from all Australian and international sites (when added) will form the trial steering committee, co-chaired by the trial Chief Investigators.

c. Data Safety Monitoring Committee

An independent Data Safety Monitoring Committee (DSMB) will be confirmed and will include a statistician and two clinicians working in the area of stroke. All members will be independent of the study and will have experience in data monitoring and oversight of clinical trials. The committee will meet regularly and review data from the study after each 50 patients are recruited. They will provide a blinded report to the management and Steering Committees according to a DSMC charter specifically outlining requirements. Committee discussions will remain confidential to the DSMC unless there are safety issues or a reason to amend the protocol, in which required information will be provided to the Management and Steering Committee.

d. Study termination

The study may be terminated prematurely by the principal investigator or his/her designee if: The number and/or severity of adverse events justify discontinuation of the study based on the recommendation of the Data Safety Monitoring Committee. In addition, The Florey reserves the right to discontinue the study prior to inclusion of the intended number of participants but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must send written notification to the Ethics Committee. The study may also be terminated at any time at the request of any regulatory authority, with proper and timely notification of all parties concerned. The local or lead HREC will be informed promptly and the investigator will supply reason(s) for the termination or suspension. Otherwise, the study will be considered terminated upon completion of all patient interventions, outcome evaluations, and submission of final reports.

5. Statistical Methods

a. Sample Size Estimation & Justification

Earlier work in chronic stroke populations have shown that 11% of patients report a 5-point improvement in SF-36 when given placebo. Based on the description of spectacular improvement with etanercept in up to 90% of patients, we conservatively anticipate that at day 28, 30% of the patients will have a 5-point improvement on SF-36. Recruiting 160 patients (equally distributed between two arms) would yield 0.8 power to observe the absolute risk difference of 19%, assuming two-tailed Alpha of 0.05 and 11% of patients achieving a 5-point improvement in SF-36 when given placebo. To allow for potential dropouts, the proposed total sample size for this study is 168 patients, equally distributed between two arms.

b. Power Calculations

Power calculations were performed in STATA IC15.0 (StataCorp, College Station, TX, USA)

c. Statistical Methods To Be Undertaken

The analysis will be conducted following intention-to-treat principles. All outcomes and analyses are prospectively categorized as primary or secondary or exploratory. Differences in all endpoints between the 2 arms of the study will be tested independently at the two-tailed 0.05 level of significance. All estimates of treatment effects will be presented with 95% confidence intervals. No formal adjustments will be undertaken to constrain the overall type I error associated with the secondary and exploratory analyses. Their purpose is to supplement evidence from the primary analysis to more fully characterise the treatment effect. Results from the secondary analyses will be interpreted in this context. Descriptive statistics will be generated for each of the measures used in study.

The primary endpoint will be tested using a logistic regression model with achievement of at least 5 points improvement on the SF-36 compared to baseline as the dependent variable and with the treatment arm as an independent variable and baseline value of SF-36, proxy or self-

administration of SF-36 as adjustment covariates. The secondary efficacy endpoint will be tested using a logistic regression model with achieving at least 5 points improvement from the baseline value on the SF-36 scale as the outcome, the treatment arms (placebo, single dose, and double dose) as an independent variable, and baseline value of SF-36, proxy or self-administration of SF-36 as adjustment covariates.
Between arm differences in safety outcomes will be analyzed identically to the efficacy outcomes.
Between arms differences in secondary and exploratory outcomes will be assessed using the generalised linear mixed models (GLMM) approach, using a link function as appropriate to the response outcome distribution.
The details of the statistical analysis will be summarized in a separate Statistical Analysis Plan prior to the lock of the trial data.

6. DATA SECURITY & HANDLING

a. ELECTRONIC CASE REPORT FORM (ECRF)

An electronic Case Report Form (eCRF) will be completed for each study participant summarising all clinical screening and study data. Participants will only be referred to in the eCRF by their participant number and initials in order to retain participant confidentiality.
The completed original eCRF data is to be submitted via REDCap® online database as soon as practical after completion. Source data relating to the participants involvement in the trial is to be retained by the investigator for 15 years.

b. DETAILS OF WHERE RECORDS WILL BE KEPT & HOW LONG WILL THEY BE STORED

For the duration of the study, any paper-based information will be kept in a locked office at the Florey Melbourne Brain Centre (Austin campus). Electronic recording of case report forms will be managed using REDCap software for secure web-based data recording. All imaging data will be managed using best practice principles, with all identifiable data stored and backed up on secure servers. After conclusion of the study, the data will be securely archived for at least 15 years. Only with the participants approval, deidentified data collected as part of the study may also be used by other members of the research team for related research projects and/or transmitted to an online database for use by external researchers.

c. CONFIDENTIALITY AND SECURITY

Only the researchers responsible for this project will have access to identifiable participant data. After conclusion of the study; the data will be deidentified and securely archived for at least 15 years. The data will be stored at the Florey Institute of Neuroscience and Mental Health.

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APPENDIX A - FATIGUE ASSESSMENT SCALE (FAS)

This assessment cannot be completed by proxy

The following 10 statements refer to how you usually feel. For each statement you can choose one out of five answer categories, varying from *Never* to *Always*. 1 = *Never* ; 2 = *Sometimes* ; 3 = *Regularly* ; 4 = *Often* ; 5 = *Always*. Please circle the answer to each question that is applicable to you. Please give an answer to each question, even if you do not have any complaints at the moment.

	Never	Sometimes	Regularly	Often	Always
1. I am bothered by fatigue (WHOQOL)	1	2	3	4	5
2. I get tired very quickly (CIS)	1	2	3	4	5
3. I don't do much during the day (CIS)	1	2	3	4	5
4. I have enough energy for everyday life (WHOQOL)	1	2	3	4	5
5. Physically, I feel exhausted (CIS)	1	2	3	4	5
6. I have problems starting things (FS)	1	2	3	4	5
7. I have problems thinking clearly (FS)	1	2	3	4	5
8. I feel no desire to do anything (CIS)	1	2	3	4	5
9. Mentally, I feel exhausted	1	2	3	4	5
10. When I am doing something, I can concentrate quite well (CIS)	1	2	3	4	5

APPENDIX B – SHORT FORM 36 (SF-36)

SF-36 Questionnaire

This questionnaire asks for your views about your health. For ALL questions, please tick, cross or colour the circle that most closely matches your response. There are no right or wrong answers. Please answer ALL questions.

1. In general, would you say your health is:	Poor ◯	Fair ◯	Good ◯	Very good ◯	Excellent ◯
2. Compared to one year ago, how would you rate your health general in now?	Much worse now than one year ago ◯	Somewhat worse than one year ago ◯	About the same as one year ago ◯	Somewhat better than one year ago ◯	Much better than one year ago ◯

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	No, not limited at all	**Yes, limited a little**	**Yes, limited a lot**
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	◯	◯	◯
b. Moderate activities, such as moving a table pushing a vacuum cleaner, bowling, or playing	◯	◯	◯
c. Lifting or carrying groceries	◯	◯	◯
d. Climbing several flights of stairs	◯	◯	◯
e. Climbing one flight of stairs	◯	◯	◯
f. Bending, kneeling or stooping	◯	◯	◯
g. Walking more than a mile	◯	◯	◯
h. Walking several blocks	◯	◯	◯
i. Walking one block	◯	◯	◯
j. Bathing or dressing yourself	◯	◯	◯

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other daily activities as a result of your physical health?

	None of the time	A little of the time	Some of the time	Most of the time	All of the time
a. Cut down on the amount of time you spent on work or other activities	◯	◯	◯	◯	◯
b. Accomplished less than you would like	◯	◯	◯	◯	◯
c. Were limited in the kind of work or other activities	◯	◯	◯	◯	◯
d. Had difficulty performing the work or other activities (e.g. it took extra effort)	◯	◯	◯	◯	◯

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	None of the time	A little of the time	Some of the time	Most of the time	All of the time
a. Cut down on the amount of time you spent on work or other activities	◯	◯	◯	◯	◯
b. Accomplished less than you would like	◯	◯	◯	◯	◯
c. Did work or other activities less carefully than usual	◯	◯	◯	◯	◯

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a bit	All of the time

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?					
	Not at all	A liitle bit	Moderately	Quite a bit	Extremely
9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...					
	None of the time	A little of the time	Some of the time	Most of the time	All of the time
a. did you feel full of life?	○	○	○	○	○
b. have you been very nervous?	○	○	○	○	○
c. have you felt so down in the dumps that nothing could cheer you up?	○	○	○	○	○
d. have you felt calm and peaceful?	○	○	○	○	○
e. did you have a lot of energy?	○	○	○	○	○
f. have you felt downhearted and depressed?	○	○	○	○	○
g. did you feel worn out?	○	○	○	○	○
h. have you been happy?	○	○	○	○	○
i. did you feel tired?	○	○	○	○	○
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?					
	None of the time	A little of the time	Some of the time	Most of the time	All of the time
11. How TRUE or FALSE is each of the following statements for you?					
	Defintely false	Mostly false	Don't know	Mostly true	Definitely true
a. I seem to get sick a little easier than other people	○	○	○	○	○

b. I am as healthy as anybody I know	○	○	○	○	○
c. I expect my health to get worse	○	○	○	○	○
d. My health is excellent	○	○	○	○	○

APPENDIX C - MONTREAL COGNITIVE ASSESSMENT SCALE

1-3VISUOSPATIAL / EXECUTIVE	**Refer to specific instructions for administration in the Blinded Assessor manual. Ask the patient to complete the patient worksheet attached.**					**POINTS**
Trail Making ok? ☐ Yes ☐ No	**The Cube ok?** ☐ Yes ☐ No	**The Clock:** Contour ok? ☐ Yes ☐ No; Numbers ok? ☐ Yes ☐ No; Hands ok? ☐ Yes ☐ No				/5
4. NAMING	**Refer to specific instructions for administration in the Blinded Assessor manual Ask the patient to identify the three animals on the patient worksheet attached.**					
Lion identified? ☐ Yes ☐ No	**Rhinoceros identified?** ☐ Yes ☐ No	**Camel /Dromedary identified?** ☐ Yes ☐ No				/3
5. MEMORY	**Read list of words. Patient must repeat them. Do 2 trials. Recall later in test.**					
	FACE	**VELVET**	**CHURCH**	**DAISY**	**RED**	
1st Trial						**NO POINTS**
2nd Trial						
6. ATTENTION	**Read list of digits (1 digit/sec.)**					
	Patient has to repeat the following numbers in the forward order: 2 1 8 5 4 ☐ Yes ☐ No **Patient has to repeat the following numbers in the backward order: 7 4 2** ☐ Yes ☐ No					/2
Tell the patient: 'I am going to read a sequence of letters. Every time I say the letter 'A', tap your hand once. (No point if ≥ 2 errors).						
F B A C M N A A J K L B A F A K D E A A A J A M O F A A B						/1
Ask the patient to subtract 7 from 100 and continue to subtract 7 from his/her answer (down to 65). ☐ 4 or 5 correct (3 points) ☐ 1 correct (1 point) ☐ 2 or 3 correct (2 points) ☐ 0 correct (0 points)						
93 ☐ 86 ☐ 79 ☐ 72 ☐ 65 ☐						/3
7-8. LANGUAGE	**Read each sentence once and then ask the patient to repeat it.**					
	I only know that John is the one to help today. Correct? ☐ Yes ☐ No **The cat always hid under the couch when dogs were around.** Correct? ☐ Yes ☐ No					/2
Name maximum number of words in one minute that begin with the letter 'F'. (1 point if ≥ 11 words).						
Patient generated 11 words or more? ☐ Yes ☐ No						/1
9. ABSTRACTION	**Ask the patient: 'Tell me how an orange and banana are alike'. Answer: Fruit. Next, tell the patient: 'Now tell me how these items are alike'.**					
Train and bicycle Correct? ☐ Yes ☐ No **Watch and ruler** Correct? ☐ Yes ☐ No						/2
10. DELAYED RECALL	**Ask the patient to recall the words with no cueing. (Points for uncued recall only – 1 (FACE, VELVET, CHURCH, DAISY, RED).**					
Points for uncued recall only – 1 point per correct word.						/5
11. ORIENTATION	**Ask the patient: 'Tell me the date today' (DATE, MONTH, YEAR, DAY OF THE WEEK), then the exact location (PLACE, CITY).**					
Day ☐ Month ☐ Year ☐ Day ☐ Place ☐ City ☐						/6
Add 1 point if patient has had ≤ 12 years of education. (Normal ≥ 26/30)						/1
					TOTAL	/30

APPENDIX D - MODIFIED RANKIN SCALE (MRS)

Note: The modified Rankin Scale is to be assessed based on the participant's symptoms **for the current stroke event. This score must be ASSESSED by a person Certified for the modified Rankin Scale.**

		Tick ONE box only
0 -	**No symptoms at all** The participant should be unaware of any new limitations caused by the stroke, however minor.	☐
1 -	**No significant disability despite symptoms; able to carry out all usual duties and activities** The participant has some symptoms as a result of the stroke, whether physical, cognitive (e.g. affecting speech, reading, writing; or physical movement; or sensation; or vision; or swallowing; or mood) - but can continue to take part in all previous work, social and leisure activities. The crucial question to distinguish grade 1 from grade 2 (below) may be 'Is there anything that you can no longer do that you used to do until you had the stroke?' As a guide, an activity that was undertaken more frequently than monthly could be regarded as a 'usual' activity.	☐
2 -	**Slight disturbance; unable to carry out all previous activities, but able to look after own affairs without assistance** The participant will be unable to undertake some activity that was possible before the stroke (e.g. driving a car, dancing, reading, or working) but is still able to look after him/herself without the help from others on a day to day basis. Thus, the participant can manage dressing, moving around, feeding, toileting, preparing simple meals, shopping, and travelling locally without needing assistance from anyone else. Supervision may not be necessary. This grade assumed that the participant could be left alone at home for periods of a week or more without concern.	☐
3 -	**Moderate disability; requiring some help, but able to walk without assistance** At this grade, the participant is independently mobile (using a walking aid or frame if necessary) and can manage dressing, toileting, feeding etc. but needs help from someone else with more complex tasks (e.g. someone else may need to undertake the shopping, cooking, cleaning, and will need to visit the participant more often than weekly to ensure that these activities are completed). The assistance can be advisory rather than physical e.g. a participant who needs supervision or encouragement to cope with financial affairs would be in this grade.	☐
4 -	**Moderately severe disability; unable to walk without assistance, unable to attend to own bodily needs without assistance** The participant requires someone else to help with some daily tasks, whether walking, dressing, toileting, or eating. This participant will be visited at least once and usually twice or more times daily or must live in proximity to a carer. To distinguish grade 4 from grade 5 (below), consider whether the participant can regularly be left alone for moderate periods during the day.	☐
5 -	**Severe disability; (usually) bedridden, incontinent, and requiring constant nursing care and attention** Someone else will always need to be available during the day and at times during the night, though not necessarily a trained nurse.	☐

APPENDIX E – NATIONAL INSTITUTE OF HEALTH STROKE SCALE (NIHSS)

Category	Score/Definition
1a. Level of consciousness	☐ 0 = Alert, keenly responsive ☐ 1 = Not alert, but arousable with minimal stimulation ☐ 2 = Not alert, requires repeated stimulation or obtunded ☐ 3 = Responds only with reflex motor or automatic effects or totally unresponsive (see item 5)
1b. LOC questions (ask month and age)	☐ 0 = Answers both correctly ☐ 1 = Answers one correctly ☐ 2 = Both incorrect
1c. LOC commands (ask to open /close eyes and make a fist)	☐ 0 = Obeys both correctly ☐ 1 = Obeys one correctly ☐ 2 = Both incorrect
2. Best Gaze (horizontal eye movement)	☐ 0 = Normal ☐ 1 = Partial gaze palsy, gaze abnormal in 1 or both eyes, but forced deviation or total gaze paresis not present ☐ 2 = Forced deviation or total gaze paresis not overcome with oculocephalic manoeuvre
3. Visual fields testing	☐ 0 = No visual field loss ☐ 1 = Partial hemianopia ☐ 2 = Complete hemianopia, if participant has severely impaired consciousness (Q1=3), score 2 here ☐ 3 = Bilateral hemianopia, blind, including cortical blindness
4. Facial Palsy (ask subject to show teeth and raise eyebrows, and close eyes tightly)	☐ 0 = Normal symmetrical movement ☐ 1 = Minor paralysis or “mild” noted ☐ 2 = Partial paralysis (total or near total paralysis of lower face) ☐ 3 = Complete absence of movement in upper and lower face
5a. Motor Function Right arm	☐ 0 = Normal (extends arm 0 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
5b. Motor Function Left arm	☐ 0 = Normal (extends arm 90 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
6a. Motor Function Right leg	☐ 0 = Normal (extends leg 90 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
6b. Motor Function Left Leg	☐ 0 = Normal (extends leg 90 or 45 degrees for 10 seconds without drift) ☐ 1 = Drift ☐ 2 = Some effort against gravity ☐ 3 = No effort against gravity ☐ 4 = No movement ☐ 9 = Untestable
7. Limb ataxia	☐ 0 = Absent, score 0 if unrecorded, participant unconscious, uncooperative or limb too weak to perform task ☐ 1 = Present in one limb ☐ 2 = Present in two limbs (UL and LL)
8. Sensory (use pinprick to test arms, legs, trunk and face – compare sides)	☐ 0 = Normal ☐ 1 = Mild to moderate sensory loss, participant feels pin prick is less sharp or dull on affected side ☐ 2 = Severe/total sensory loss, participant is unaware of being touched. Brainstem stroke with bilateral sensory loss

Category	Score/Definition
9. Best language (describe picture, name items, read sentences)	☐ 0 = Normal ☐ 1 = Mild to moderate aphasia ☐ 2 = Severe aphasia ☐ 3 = Mute
10. Dysarthria (read several words)	☐ 0 = Normal articulation ☐ 1 = Mild to moderate slurring of words ☐ 2 = Near unintelligible or unable to speak ☐ 9 = Intubated or other physical barrier
11. Extinction/ inattention	☐ 0 = No abnormality ☐ 1 = Visual tactile auditory or personal inattention or extinction to bilateral simultaneous in 1 modality ☐ 2 = Profound hemi-inattention or inattention in >1 sensory modality. Does not recognise own hand or orientates to one side of space
Total Score ☐☐	(do not include items scores of '9' in total)

APPENDIX F - GENERALISED ANXIETY DISORDER ASSESSMENT – 7 (GAD-7)

Over the last 2 weeks how often have you been bothered by any of the following problems?

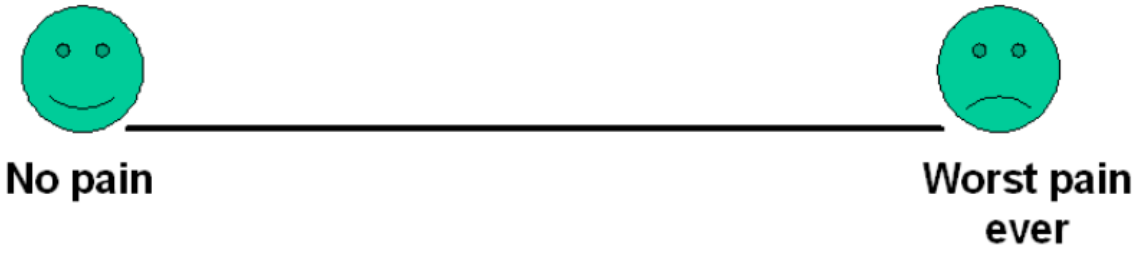
	Not at all	Several Days	More than half the days	Nearly every day
Feeling nervous or on edge?	☐	☐	☐	☐
Not being able to stop or control worrying?	☐	☐	☐	☐
Worrying too much about different things?	☐	☐	☐	☐
Trouble relaxing?	☐	☐	☐	☐
Being so restless it is hard to sit still?	☐	☐	☐	☐
Becoming easily annoyed or irritable?	☐	☐	☐	☐
Feeling afraid as if something awful might happen?	☐	☐	☐	☐

APPENDIX G - PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

Over the last 2 weeks how often have you been bothered by any of the following problems?

	Not at all	Several Days	More than half the days	Nearly every day
Little interest or pleasure in doing things	☐	☐	☐	☐
Feeling down, depressed or hopeless	☐	☐	☐	☐
Trouble falling asleep, staying asleep or sleeping too much	☐	☐	☐	☐
Feeling tired or having little energy	☐	☐	☐	☐
Poor appetite or overeating	☐	☐	☐	☐
Feeling bad about yourself or that you are a failure or have let your family down	☐	☐	☐	☐
Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
Moving or speaking so slowly that other people could have noticed. Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐
Thoughts that you would be better off dead or of hurting yourself in some way	☐	☐	☐	☐

APPENDIX H - VISUAL ANALOGUE SCALE (VAS)



APPENDIX I - PATIENTS GLOBAL IMPRESSION OF CHANGE (PGIC) SCALE

Since beginning treatment at this clinic, how would you describe the change (if any) in ACTIVITY LIMITATIONS, SYMPTOMS, EMOTIONS and OVERALL QUALITY OF LIFE, related to your painful condition? (tick ONE box).

No change (or condition has got worse)	☐	1
Almost the same, hardly any change at all	☐	2
A little better, but no noticeable change	☐	3
Somewhat better, but the change has not made any real difference	☐	4
Moderately better, and a slight but noticeable change	☐	5
Better, and a definite improvement that has made a real and worthwhile difference	☐	6
A great deal better, and a considerable improvement that has made all the difference	☐	7

In a similar way, please circle the number below, that matches your degree of change since beginning care at this clinic:

Much Better					No Change					Much Worse
0	1	2	3	4	5	6	7	8	9	10

Permissions to be obtained- https://eprovide.mapi-trust.org/instruments/patient-global-impression-of-change#contact_and_conditions_of_use

Reference: Hurst H, Bolton J. Assessing the clinical significance of change scores recorded on subjective outcome measures. J Manipulative Physiol Ther 2004;27:26-3

APPENDIX J - FUNCTIONAL INDEPENDENCE MEASURE (FIM)

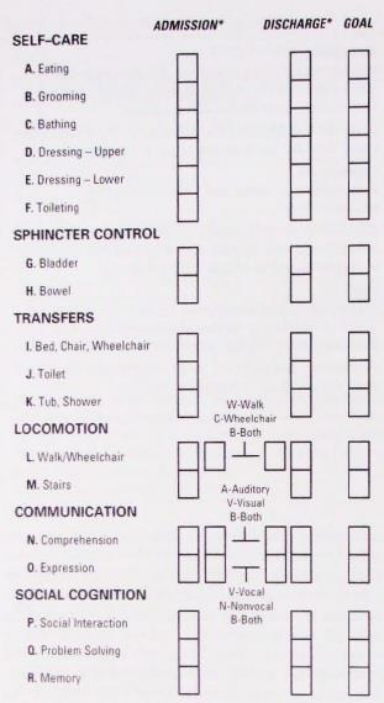
The Functional Independence Measure (FIM) is an 18-item of physical, psychological and social function. The tool is used to assess a patient's level of disability as well as change in patient status in response to rehabilitation or medical intervention.

The FIM is used by health care practitioners to assess and grade the functional status of a person based on the level of assistance he or she requires. Grading categories range from total independence to total assistance. Irrespective of the use of any assistive device, the person is considered complete independence.

Tasks that are evaluated using the FIM include bowel and bladder control, transfers, locomotion, communication, social cognition as well as the following six self-care activities:

- Feeding
- Grooming
- Bathing
- Upper Body Dressing
- Lower Body Dressing
- Toileting

The FIM measures what an individual can perform and not what that person could do under certain circumstances.



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The Perispinal Etanercept to Improve Stroke Outcomes (PESTO)

A prospective multicentre, phase 2b randomised controlled double-blind trial, to determine the safety and efficacy of perispinal etanercept on quality of life at 28 days post treatment in stroke survivors

Statistical Analysis Plan

Version 1; Dated 18 December 2023

Protocol Number: 2

Protocol Version: 2.1; Dated 31 May 2022

Leonid Churilov, Vincent Thijs

for the PESTO Investigators

Author for correspondence:

Prof Leonid Churilov, PhD
Department of Medicine & Melbourne Brain Centre at Royal Melbourne Hospital
The University of Melbourne
T: +61 3 9035 7089 E: leonidc@unimelb.edu.au

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1 Abbreviations

Abbreviation	Description (using lay language)
AE	Adverse Event
DSMC	Data Safety Monitoring Committee
eCRF	Electronic Case Report Form
FAS	Fatigue Assessment Scale
FIM	Functional Independent Measure
GAD -7	General Anxiety Disorder
GCP	Good Clinical Practice
HREC	Human Research Ethics Committee
ICF	Informed Consent Form
ICH GCP	International Conference on Harmonisation-Good Clinical Practice
IP	Investigational Product
ITT	Intention-To-Treat
NIHSS	National Institute of Health Stroke Scale
MoCA	Montreal Cognitive Assessment
mRS	Modified Rankin Scale
PHQ -9	Patient Health Questionnaire
PGICS	Patient Global Improvement of Change Scale
PROMS	Patient reported outcome measures
TBC	tuberculosis
SAE	Serious Adverse Event
SF-36	Short Form 36
VAS	Pain Visual Analogue Scale

2 Administrative Information

Protocol: 2; Version 2.1; 31 Month 2022
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2.1 Document Version History

Version Date	Version	Author	Signature	Change Description	Reason/Comment
18 Dec 2023	1.0	Leonid Churilov		Initial release	Not applicable

2.2 Approvals

The undersigned have reviewed this plan and approve it as final. They find it to be consistent with the requirements of the protocol as it applies to their respective areas. They also find it to be compliant with ICH-E9 principles and confirm that this analysis plan was developed in a completely blinded manner (i.e., without knowledge of the effect of the intervention being assessed)

Name	Role on Study	Affiliation	Signature	Date
Vincent Thijs	PI	The Florey, Melbourne, Australia		14 April 2024
Leonid Churilov	Biostatistics Lead	University of Melbourne, Australia		14 April 2024

3 Study design

3.1 Overview

PESTO is a phase 2b, multicentre, international (Australia and Aotearoa New Zealand), prospective, randomised, placebo controlled, double-blind, parallel group trial with concealed allocation, blinded measurement and intention-to-treat analysis in adult stroke survivors aged up to 70 years between 1 and 15 years after their index ischemic or hemorrhagic event. Participants are pre-screened using the 36-Item Short Form Health Survey and are required to score a total of 95 or less out of a total of 100 to be considered eligible for the trial, and their modified Rankin Scale (mRS) score should be between 3 and 5. Participants with mRS score of 2 (stroke leading to slight disability with inability to carry out all previous tasks) can also be included provided their SF-36 score is below 80. Participants are randomised in 1:1 ratio to either receive a single dose of the etanercept (etanercept arm) or placebo (the control arm). Participants randomized into placebo arm, are subsequently randomised into receiving the second dose of placebo or a single dose of etanercept at day 28 after the first injection (based on 2:1 allocation ratio), while participants in the etanercept arm are subsequently randomised into receiving a single dose of placebo or the second dose of etanercept at day 28 (based on 1:2 allocation ratio), resulting in three equally sized arms for the secondary dose-response analysis of the efficacy outcome at day 56.

3.2 Aims and hypotheses

The primary aim of PESTO is to test the hypothesis that, compared to placebo, perispinal subcutaneous injection of etanercept improves quality of life and is safe in participants with chronic, disabling, effects of stroke

3.3 Patient population

Inclusion criteria

1. Participants with a history of acute ischemic or haemorrhagic stroke confirmed on imaging
2. Age ≥18 years-70 years at the time of stroke or if stroke occurred at the age of 16 or 17 and participant is now 18 or more
3. Moderate-to-severe disability resulting from stroke as defined by a modified Rankin scale of 3-5 or modified Rankin scale 2 with SF-36 score <80
4. Stroke occurred between 1 and 15 years before enrolment.
5. At the time of enrolment patient is <=70 years old
6. SF-36 score <95 (<80 if mRS is 2)
7. Participant can complete the SF-36 questionnaire independently or availability of a relative or carer who is able to complete the SF-36 questionnaire on behalf of the patient
8. Consent can be obtained from the participant or medical decision maker.

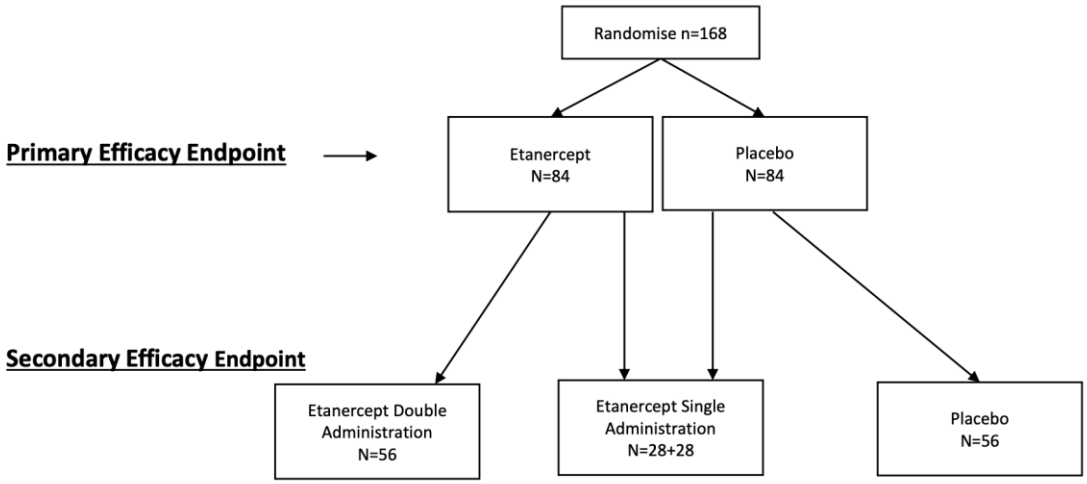
Exclusion criteria

1. Contra-indication to using etanercept (e.g., previous hypersensitivity, participants with, or at risk of sepsis, ongoing infection, use of IL-1 antagonists)
2. History of hepatitis B and C, tuberculosis, HIV, SLE, multiple sclerosis
3. Moderate to severe heart failure
4. History of malignancy
5. Other current use of immunosuppressant
6. History of alcoholic hepatitis, current ongoing hepatitis
7. History of juvenile idiopathic arthritis
8. History of blood dyscrasia
9. Clinical diagnosis of dementia
10. mRS 0-1 OR if mRS=2, SF-36 score is 80 or more
11. Botulinum toxin injection to limbs in the 4 months prior to screening Visit
12. Pregnancy (women of childbearing potential must be tested)
13. Breastfeeding
14. Participation in any investigational study in the last 30 days
15. Known terminal illness or planned withdrawal of care or comfort care measures.
16. Any condition that, in the judgment of the investigator could impose hazards to the patient if study therapy is initiated or affect the participation of the patient in the study.
17. Prior exposure to etanercept for stroke

3.4 Randomization

Participants may be randomized to the trial if they meet the above criteria. Participants are first randomised in 1:1 ratio to either receive a single dose of the etanercept (etanercept arm) or placebo (the control arm) using a centralized computer-generated random assignment procedure with permuted blocks of various sizes as part of the REDCap electronic case record form stored on a secure server based at the Florey. Randomisation is stratified by whether the pre-randomisation SF-36 questionnaire was completed by the patient, or, if not possible, by a relative or carer proxy, baseline modified Rankin Scale (dichotomised as mRS 2-3 vs mRS 4-5), and by time since stroke (1-5 years vs >5 years after stroke onset).

At the second step of the randomisation procedure conducted at the same time point, the participants in the placebo arm are subsequently randomised into receiving either the second dose of placebo or a single dose of etanercept at day 28 after the first injection (based on 2:1 allocation ratio), while participants in the etanercept arm are subsequently randomised into receiving either a single dose of placebo or the second dose of etanercept at day 28 (based on 1:2 allocation ratio). This results in three equally sized arms (Placebo n=56, Single Dose etanercept n=56, and Double Dose etanercept n=56) for the secondary dose-response analysis of the efficacy outcome at day 56. (see Figure below).



To ensure the covariate balance across all arms, operationally this was achieved through stratified randomization into four arms defined by the combination of treatments administered at first/second injection as follows: placebo/placebo n=56; placebo/etanercept n=28; etanercept/placebo=28; etanercept/etanercept =56. For the primary analyses and the analyses of the outcomes at 28 days post first injection administration, placebo/placebo and placebo/etanercept arms were treated as the single placebo arm, while etanercept/placebo and etanercept/etanercept were treated as the single etanercept arm. For the analyses of analyses of the outcomes at 56 days post first injection administration, placebo/placebo arm was treated as the placebo arm, placebo/etanercept arm and etanercept/placebo arm were treated as the single dose etanercept arm, and etanercept/etanercept arm was treated as were treated as the two doses etanercept arm.

A researcher not otherwise involved in the trial undertakes the randomisation and allocation to treatment arms, thereby ensuring allocation concealment. The treatment allocation is sent

electronically to the study pharmacist who prepares the investigational product. Study pharmacists are instructed not to disclose treatment allocation. The investigator administering the investigational product, participants and outcome assessors are therefore blinded to treatment allocation.

3.5 Baseline and follow-up assessments

All responsible investigators receive training in the systems for data collection and data entry and training in Good Clinical Practice (GCP). All investigators responsible for patient assessment participate in training in critical assessment items (NIHSS, mRS, FIM).

The assessment schedule for this trial is found in the protocol. Briefly, once eligibility is confirmed and informed consent is obtained, the responsible investigator is able to randomize the participant through the secure web-based system. The baseline assessments include NIHSS, pre-morbid mRS, SF-36v2, PHQ-9, FAS, GAD-7, Pain VAS, MOCA, FIM. Demographic data, past medical history, stroke history and concomitant medication as well as a safety screening lab are recorded at the screening visit.

All participants are followed up on days 28 and 56 unless death occurs. The assessments are conducted in person at baseline, and at day 28 by an assessor trained in study procedures who is blind to treatment allocation. The day 56 assessment can be done in person or via telehealth.

The trial management team are responsible for ensuring that all data are completed in a timely manner. Participants do not receive payment for participation.

3.6 Sample size considerations

Earlier work has shown that 11% of patients report a 5-point improvement in SF-36 when given placebo. [1] Based on the previous description of markedly significant improvement with etanercept in up to 90% of patients [2], for the primary efficacy outcome we anticipate that at day 28, 30% of the patients will have a 5-point improvement on SF-36. Recruiting 160 participants (equally distributed between two arms) would yield 80% power to observe the absolute risk difference of 19%, assuming a two-tailed alpha of 0.05 and 11% of participants achieving a 5-point improvement in SF-36 when given placebo. To allow for potential dropouts, the estimated total sample size for this study is 168 patients, equally distributed between two arms.

Due to the study recruitment being affected by COVID-19 over multiple years and funding constraints, PESTO steering committee in consultation with the trial DSMB have made the decision to stop the recruiting for PESTO. The last participant was recruited on 01/09/2023, resulting in the total achieved sample size of 126 participants.

3.7 Unblinding

Only the Data and Safety Monitoring Board (DSMB) have access to interim data and results. The DSMB review unblinded data in accordance with the DSMB Charter (Version 1, 07 Oct, 2019). Treatment allocations are securely stored and separated from investigators. Statisticians not involved in the DSMB remain blinded and work on dummy datasets until the computer codes for statistical analysis are validated.

3.8 Definitions of the outcomes

Primary Efficacy Outcome

Proportion of participants at day 28 with an improvement of 5 points or more on the SF-36 compared to baseline following single injection of etanercept 25 mg or equivalent placebo. The change in SF-36 is defined as the sum of changes in the Physical Component Summary score or the Mental Component Summary or both of the SF-36 between baseline and day 28.

Primary Safety Outcome

Proportion of participants with serious adverse events following single injection of etanercept 25 mg or equivalent placebo over 28 days

Secondary outcomes

- Efficacy: Proportion of participants at day 56 with an improvement of 5 points or more on the SF-36 compared to baseline between the 3 treatment arms (placebo, single administration of etanercept, two administrations of etanercept)
- Safety: Proportion of participants with serious adverse events over 56 days in the 3 treatment arms (placebo, single administration of etanercept, two administrations of etanercept)

Exploratory outcomes (some or all of these outcomes may be reported in publications subsequent to primary paper)

Measured at days 28 (compared between participants receiving placebo or single dose of etanercept) and 56 (between participants receiving none, one or two doses of etanercept):

- The National Institute of Health Stroke Scale (NIHSS)
- Functional Independent Measurement (FIM)
- modified Rankin Scale (mRS)
- Fatigue Assessment Scale (FAS)
- General Anxiety Disorder-7 (GAD-7)
- Patient Health Questionnaire (PHQ-9)
- Visual Analogue Scale (VAS) for pain
- Montreal Cognitive Assessment (MOCA)

4 Funding

This study was funded by the Australian Medical Research Future Fund, an Australian Government organization, under a project grant (Return to life, return to work PSE1901). No external parties had input into the study design, data collection, data analysis, data interpretation or writing of the protocol and statistical analysis plan.

5 Statistical analysis

5.1 Analysis principles and general considerations

- All outcomes and analyses are prospectively categorized as primary, secondary, or exploratory
- Differences in all other endpoints between the arms of the study will be tested independently at the two-tailed 0.05 level of significance. Unless specifically stated otherwise, all estimates of treatment effects will be presented with 95% confidence intervals (95%CIs)
- No formal adjustments will be undertaken to constrain the overall type I error associated with the secondary, tertiary, and exploratory analyses. Their purpose is to supplement evidence from the primary analysis to more fully characterize the treatment effect. Results from the secondary and tertiary analyses will be interpreted in this context.
- The analysis for primary outcome and all secondary efficacy and safety outcomes analyses will be conducted on an intention-to-treat (ITT) basis so that all patients will be analyzed in the group to which they were randomized irrespective of whether or not they received the allocated treatment.
- The ITT analysis strategy [3] for PESTO is defined as follows:
    - Is based on an ITT design that aims to collect all outcome data on all randomized subjects;
    - Includes a main analysis that keeps subjects in their randomized groups, analyzes all available outcome data, and is valid under a named plausible assumption about the missing data;
    - Includes sensitivity analyses that consider a range of plausible alternative assumptions that contradict the main assumption about the missing data;
    - All individuals are included in sensitivity analyses
- In addition to the ITT analysis, an "as treated" analysis will also be conducted.
- Subgroup analyses could be carried out irrespective of whether there is a significant treatment effect on the outcome. Their purpose is to supplement evidence from the primary analysis to help to fully characterize the treatment effect. Results from subgroup analyses will be interpreted in this context. The following subgroups are prespecified as being of potential interest to be attempted for the primary analysis subject to sufficient size of the resulting subgroups:

    - Age at time of stroke (young: 16-45y.o. versus older >45y.o.)
    - Sex
    - Number of years elapsed since stroke (1 to 5 years inclusive vs >5 years)
    - Stroke type (ischemic stroke versus other)
    - Baseline mRS (mild to moderate (2-3) versus severe (4-5))
    - SF-36 completed by participant versus proxy
    - Hypertension (present vs absent)
    - Diabetes (Type 1 and Type 2 versus no diabetic history)
    - Atrial fibrillation (present vs absent)

- Analyses will be conducted using Stata and R statistical software.

5.2 Interim analyses

The Independent DSMB reviewed unblinded data. A formal interim safety analysis was performed after 83 participants were enrolled. A recommendation of early termination due to safety reasons were to be considered by the Independent Data Safety Monitoring Board. The Haybittle-Peto procedure for generating early stopping boundaries was chosen as a guideline for deciding to recommend early termination of the trial due to safety reasons for deaths and for SAEs other than serious infections. A risk of serious infection >5% would be of clinical concern and the DSMB would consider a recommendation to stop the trial if the lower limit of the 90% confidence interval for the observed risk of infection in either group exceeds 5% (e.g. if 10 patients in the first 100 in either group have serious infection the lower limit of the 90% confidence interval would be 5.1% and trigger the stopping rule).

No formal interim analyses for efficacy or futility were planned or conducted.

5.3 Trial profile

Flow of patients through the study will be displayed in a standard CONSORT diagram. The report will include the number of patients included, withdrawn, lost to follow up, the number who received the allocated treatment and were analysed.

5.4 Patients' characteristics and baseline comparisons

In order to assess balance, description of the specified baseline characteristics will be presented for the placebo and single dose of etanercept arms (Table 1 – see Appendix) and for the participants receiving none, one or two doses of etanercept arms (Table S1 – see Appendix). Discrete variables will be summarized as frequencies and percentages. Unless otherwise indicated in the tables, percentages will be calculated according to the number of patients for whom data are available. If there are more than 5% missing values, the denominator will be added in a footnote in the corresponding summary table. Continuous variables will be summarized by use of either mean and standard deviation (SD) or median and interquartile range (IQR). Durations and time intervals will be summarized by medians and IQRs.

5.5 Primary efficacy outcome: Difference in proportion of participants at day 28 with an improvement of 5 points or more on the SF-36 compared to baseline following single injection of etanercept 25 mg or equivalent placebo.

Outcome measure

This measure of outcome will occur at 28 days post administration of the first injection. The outcome is measured using the SF-36v2 scale, analysed with the following software: QualityMetric Pro CoRE Smart Measurement System Version 2.3 (Build #2.3.8697.31648) [4]. The change in SF-36 is defined as the sum of changes in the Physical Component Summary score or the Mental Component Summary or both of the SF-36 between baseline and day 28.

Statistical Hypotheses

The set of statistical hypotheses is p(placebo) equal top(etanercept) versus p(placebo) is not equal to p(etanercept), where p(placebo) is the proportion of participants experiencing the defined change in

the placebo arm and p(etanercept) is the proportion of participants experiencing the defined change in the etanercept arm.

Treatment of missing values

Based on blinded monitoring of the amount of missing primary outcome data in the PESTO trial, it is anticipated that there will be minimal missing primary outcome data. Nevertheless, a small amount of missing data may be inevitable. Since both ITT and as treated analysis are pre-specified and all randomized subjects are to be included in the primary ITT outcome analysis, there is a need for a consistent ITT-based strategy of dealing with any missing primary outcome values.

Following clinical input from, and discussions with, the PESTO Management Committee, the primary outcome is assumed to be missing-at-random (MAR), i.e. it is assumed that the probability of data being missing may depend on the values of the observed data, but not on the values of the missing data. Important explanatory and auxiliary variables were collected and will be examined to assess the plausibility of the MAR assumption.

For the primary analysis, the complete case analysis followed by the sensitivity analyses that consider a range of plausible alternative assumptions about missing primary outcome data will be conducted.

Analysis method

The hypothesis is tested using the binary logistic regression model with treatment arm as an independent variable and the improvement of 5 points or more on the SF-36 at day 28 compared to baseline as the dependent variable, including baseline SF-36 score and time since stroke in years as treatment covariates for adjustment purposes. Standardized risk difference will be derived as per FDA Guidance for Industry "Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products" [5]. The treatment effect will be presented as adjusted odds ratio (aOR) and standardized risk difference with the corresponding 95% CIs.

5.6 Primary safety outcome: Difference in proportions of participants over 28 days with Serious Adverse Events following single injection of etanercept 25 mg or equivalent placebo.

Outcome measure

This measure of outcome will occur over 28 days post administration of the first injection.

Statistical Hypotheses

The set of statistical hypotheses is p(placebo) equal to p(etanercept) versus p(placebo) is not equal to p(etanercept), where p(placebo) is the proportion of participants experiencing at least one Serious Adverse Event in the placebo arm and p(etanercept) is the proportion of participants experiencing at least one Serious Adverse Event in the etanercept arm.

Treatment of missing values

As for the Primary Efficacy Analysis.

Analysis method

The hypothesis is tested using the Firth logistic regression model with treatment arm as an independent variable and the presence or absence of SAEs over 28 days as the dependent variable. The treatment effect will be presented as odds ratio (OR) and absolute risk difference with the corresponding 95% CIs.

5.7 Secondary efficacy outcome: differences in proportions at day 56 of participants with an improvement of 5 points or more on the SF-36 compared to baseline between the 3 treatment arms (placebo, single administration of etanercept, two administrations of etanercept)

Outcome measure

This secondary measure of outcome will occur at 56 days post administration of the first injection. The outcome is measured using the SF-36v2 scale, analysed with the following software: QualityMetric Pro CoRE Smart Measurement System Version 2.3 (Build #2.3.8697.31648) [4]. The change in SF-36 is defined as the sum of changes in the Physical Component Summary score or the Mental Component Summary or both of the SF-36 between baseline and day 28.

Statistical Hypothesis

The set of statistical hypotheses is that p(placebo), p(single dose etanercept) and p(two doses etanercept) are equal versus there is a difference in the above proportions, where p(placebo) is the proportion of participants experiencing the defined change in the placebo arm, p(single dose etanercept) is the proportion of participants experiencing the defined change in the arm with single administration of etanercept, and p(two doses etanercept) is the proportion of participants experiencing the defined change in the arm with two doses of etanercept administered.

Treatment of missing values

Complete case analysis with subsequent sensitivity analysis will be undertaken.

Analysis method

The hypothesis is tested using the binary logistic regression model with treatment arms as an independent variable and the improvement of 5 points or more on the SF-36 at day 56 compared to baseline as the dependent variable, including baseline SF-36 score and time since stroke in years as treatment covariates for adjustment purposes. The treatment effects will be presented as adjusted odds ratios (aORs) and standardized risk differences with the corresponding 95% CIs using the placebo arm as the reference.

5.8 Secondary safety outcome: differences in proportions of participants with Serious Adverse Events over day 56 days between the 3 treatment arms (placebo, single administration of etanercept, two administrations of etanercept)

Outcome measure

This measure of outcome will occur over 56 days post administration of the first injection.

Statistical Hypothesis

The set of statistical hypotheses is that p(placebo), p(single dose etanercept) and p(two doses etanercept) are equal versus there is a difference in the above proportions, where p(placebo) is the proportion of participants experiencing serious adverse events in the placebo arm, p(single dose etanercept) is the proportion of participants experiencing serious adverse events in the arm with single administration of etanercept, and p(two doses etanercept) is the proportion of participants experiencing serious adverse events in the arm with two doses of etanercept administered.

Treatment of missing values

Complete case analysis with subsequent sensitivity analysis will be undertaken.

Analysis method

The hypothesis is tested using the Firth regression model with treatment arms as an independent variable and the presence or absence of SAEs over 56 days as the dependent variable. The treatment effect will be presented as odds ratio (OR) and absolute risk difference with the corresponding 95% CIs.

5.9 Exploratory outcomes (some or all of these outcomes may be reported in publications subsequent to primary paper)

- Differences in mRS outcomes at 28 days post first injection between the placebo arm and the etanercept arm will be investigated using Generalized Odds Ratios (GenOR) stratified by baseline mRS with tied observations split between the arms. The effect sizes will be reported as GenOR with 95%CIs.
- Differences in mRS outcomes at 56 days post first injection between the placebo arm, single administration of etanercept arm, and arm with two doses of etanercept administered will be investigated using Generalized Odds Ratios (GenOR) for trend [6] stratified by baseline mRS with tied observations split between the arms. The effect sizes will be reported as GenOR with 95%CIs.
- Differences in all other exploratory outcomes at 28 days post first injection between the placebo arm and the etanercept arm will be investigated using quantile regression with the respective outcome measure at 28 days as the dependent variable, treatment arm as the independent variable, and baseline value of the outcome variable and time since stroke in years as adjustment covariates. The models will be run for the 25^{th}, 50^{th} (median), and 75^{th} percentile of the outcome distributions. The effect sizes will be reported as difference in respective quantiles with 95%CIs.
- Differences in all other exploratory outcomes at 56 days post first injection between the placebo arm, single administration of etanercept arm, and arm with two doses of etanercept administered, will be investigated using quantile regression with the respective outcome measure at 56 days as the dependent variable, treatment arms (placebo vs single administration of etanercept vs two doses of etanercept administered) as the independent variable, and baseline value of the outcome variable and time since stroke in years as adjustment covariates. The models will be run for the 25^{th}, 50^{th} (median), and 75^{th} percentile of the outcome distributions. The effect sizes will be reported as difference in respective quantiles with 95%CIs using placebo arm as the reference.
- Longitudinal changes in exploratory outcomes other than mRS over 56 days post first injection between the treatment sequences (placebo/placebo; placebo/etanercept; etanercept/placebo; and etanercept/etanercept) will be investigated over two periods (0-27 days post first injection and 28-56 days post first injection) using clustered quantile regression with the respective outcome measure at the end of a given period as the dependent variable, treatments for a given period (placebo vs etanercept) and two periods as independent variables, and time since stroke in years as adjustment covariate, and individual participants as clusters. The models will be run for the 25^{th}, 50^{th} (median), and 75^{th}

percentile of the outcome distributions. The effect sizes will be reported as difference in respective quantiles with 95%CIs.

5.10 Sensitivity analyses

In the presence of missing data for the SF-36 outcome, sensitivity analysis will be conducted under a range of assumptions about the missing data. As the main analysis is planned under an assumption of missing at random, the sensitivity of the results to plausible departures from MAR will be explored as a part of an intention-to-treat analysis strategy [3].

According to this approach, the data and missingness are modelled jointly using a pattern-mixture model (modelling the differences between missing and observed data). Assumptions about the missing data are expressed via a parameter delta which measures the degree of departure from missing at random. Results can be investigated over a range of assumptions (a range of values of delta).

5.11 Tables and figures for the main paper

The proposed tables and figures for the main results are presented in Appendix 1. Tables 1 and S1 will report key baseline characteristics of participants by placebo and Etanercept for ITT and as treated populations respectively. Tables 2 and S2 will report key baseline characteristics of participants by placebo, single dose of Etanercept, and two doses of Etanercept for ITT and as treated populations respectively. Tables 3 and S3 will report the primary and some exploratory outcomes for day 28 for ITT and as treated populations respectively. Tables 4 and S4 will report the primary and some exploratory outcomes by placebo, single dose of Etanercept, and two doses of Etanercept for ITT and as treated populations respectively. Supplementary tables will report summary data about safety. Figure 1 will be the CONSORT diagram. Figures 2ab and S2ab will present box plots for the change on the SF-36 compared to baseline at 28 days (for placebo and Etanercept) and 56 days (placebo, single dose of Etanercept, and two doses of Etanercept) for ITT and as treated populations respectively. Figures 3ab and S3ab will present forest plots for subgroup analyses for placebo and Etanercept (a) and placebo, single dose of Etanercept, and two doses of Etanercept (b) pre-specified in Analysis Principles and General Considerations section of this SAP for ITT and as treated populations respectively.

6 References

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7 PESTO Primary Trial Tables (Main Manuscript and Supplement)

Tables 1 and S1. Baseline Characteristics Intention to Treat and As Treated Populations respectively (n=___)			
	Total (n=_)	**Placebo (n=_)**	**Etanercept (n=_)**
Age at enrollment (years), median (IQR)			
Age at time of last stroke, (years), median (IQR)			
Number of years from last stroke (years), median (IQR)			
Stroke Type Ischemic stroke, n (%)			
Stroke Type Intracerebral hemorrhage, n (%)			
Stroke Type subarachnoid hemorrhage, n (%)			
Stroke Type Subtype Unknown, n (%)			
Gender, Female, n (%)			
Baseline NIHSS Score, median (IQR)			
Hypertension, n (%)			
Hypercholesterolemia, n (%)			
Atrial fibrillation, n (%)			
Diabetes IDDM, n (%)			
Diabetes NIDDM, n (%)			
Current Smoker, n (%)			
Past smoker (Ceased			

within last 2 years combined with ceased more than 2 years ago), n (%)			
Coronary artery disease, n (%)			
Modified Rankin Scale at baseline, n (%)			
SF-36 at screening measured **by Subject** Total score, median (IQR)			
SF-36 at screening measured **by Proxy** Total score, median (IQR)			
SF-36 at baseline by Subject Physical Component Summary, median (IQR)			
SF-36 at baseline by Subject Mental Component Summary, median (IQR)			
SF-36 at baseline by Proxy Physical Component Summary, median (IQR)			
SF-36 at baseline by Proxy Mental Component Summary, median (IQR)			
PHQ-9 Total Score, median (IQR)			
FAS Total Score, median (IQR)			
GAD-7, median (IQR)			

VAS, median (IQR)			
MOCA Score, median (IQR)			
FIM V2, median (IQR)			

Tables 2 and S2. Baseline Characteristics Intention to Treat and As Treated Populations respectively (n=___)			
	Placebo (n=_)	**Single Dose Etanercept (n=___)**	**Two Doses Etanercept (n=___)**
Age at enrollment (years), median (IQR)			
Age at time of last stroke, (years), median (IQR)			
Number of years from last stroke (years), median (IQR)			
Stroke Type Ischemic stroke, n (%)			
Stroke Type Intracerebral hemorrhage, n (%)			
Stroke Type subarachnoid hemorrhage, n (%)			
Stroke Type Subtype Unknown, n (%)			
Gender, Female, n (%)			
Baseline NIHSS Score, median (IQR)			
Hypertension, n (%)			
Hypercholesterolemia, n (%)			
Atrial fibrillation, n			

(%)			
Diabetes IDDM, n (%)			
Diabetes NIDDM, n (%)			
Current Smoker, n (%)			
Past smoker (Ceased within last 2 years combined with ceased more than 2 years ago), n (%)			
Coronary artery disease, n (%)			
Modified Rankin Scale at baseline, n (%)			
Modified Rankin Scale at baseline, n (%)			
Modified Rankin Scale at baseline, n (%)			
Modified Rankin Scale at baseline, n (%)			
SF-36 at screening measured **by Subject** Total score, median (IQR)			
SF-36 at screening measured **by Proxy** Total score, median (IQR)			
SF-36 at baseline by Subject Physical Component Summary, median (IQR)			
SF-36 at baseline by Subject Mental Component Summary, median (IQR)			
SF-36 at baseline by Proxy			

Physical Component Summary, median (IQR)			
SF-36 at baseline by Proxy Mental Component Summary, median (IQR)			
PHQ-9 Total Score, median (IQR)			
FAS Total Score, median (IQR)			
GAD-7, median (IQR)			
VAS, median (IQR)			
MOCA Score, median (IQR)			
FIM V2, median (IQR)			

Tables 3 and S3. Primary and Explratory Outcomes at Day 28 (Intention to Treat and As Treated Populations)				
	Placebo (n=_)	**Etanercept (n=___)**	**Effect Size (95% CI)**	**P-value**
Primary Efficacy Outcome (Intention to Treat Population; n=___)				
Participants at day 28 with an improvement of 5 points or more on the SF-36 compared to baseline			Adjusted Odds ratio: Standardized Risk Difference:	
Primary Safety Outcome (Intention to Treat Population; n=___)				
Participants with Serious Adverse Events over 28 days			Adjusted Odds ratio: Standardized Risk Difference:	
Exploratory Outcomes (Intention to Treat Population; n=___)				
The National Institute of Health Stroke Scale (NIHSS), median (IQR)			25th percentile: Median: 75th percentile:	
Functional Independent Measurement (FIM), median (IQR)			25th percentile: Median: 75th percentile:	
modified Rankin Scale (mRS), median (IQR)			25th percentile: Median: 75th percentile:	
Fatigue Assessment Scale (FAS), median (IQR)			25th percentile: Median: 75th percentile:	
General Anxiety Disorder-7 (GAD-7), median (IQR)			25th percentile: Median: 75th percentile:	

Patient Health Questionnaire (PHQ-9), median (IQR) Visual Analogue Scale (VAS) for pain, median (IQR)			25th percentile: Median: 75th percentile:	
Montreal Cognitive Assessment (MOCA), median (IQR)			25th percentile: Median: 75th percentile:	
Data are n (%), n/N (%) or median (interquartile range)				

	Tables 4 and S4. Secondary and Exploratory Outcomes at Day 56 (Intention to Treat and As Treated Populations)					
	Placebo (n=_)	**Single Dose Etanercept (n=___)**	**Two Doses Etanercept (n=___)**	**Effect Size Single Dose vs Placebo (95% CI)**	**Effect Size Two Doses vs Placebo (95% CI)**	
Secondary Efficacy Outcome (Intention to Treat Population; n=___)						
Participants at day 56 with an improvement of 5 points or more on the SF-36 compared to baseline				Adjusted Odds ratio: Standardized Risk Difference:	Adjusted Odds ratio: Standardized Risk Difference:	
Secondary Safety Outcome (Intention to Treat Population; n=___)						
Participants with Serious Adverse Events over 56 days				Adjusted Odds ratio: Standardized Risk Difference:	Adjusted Odds ratio: Standardized Risk Difference:	
Exploratory Outcomes (Intention to Treat Population; n=___)						
The National Institute of Health Stroke Scale (NIHSS), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	

Functional Independent Measurement (FIM), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
modified Rankin Scale (mRS), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
Fatigue Assessment Scale (FAS), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
General Anxiety Disorder-7 (GAD-7), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
Patient Health Questionnaire (PHQ-9), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
Visual Analogue Scale (VAS) for pain, median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
Montreal Cognitive Assessment (MOCA), median (IQR)				25th percentile: Median: 75th percentile:	25th percentile: Median: 75th percentile:	
Data are n (%), n/N (%) or median (interquartile range)						

Supplementary Table __. Number of patients enrolled by site in the intention-to-treat population	
Site Name	**Total**
Australia and New Zealand	
Austin Health	
Alfred Health	
CGM Christchurch	

Supplementary Table __. Type and Incidence of Serious Adverse Events and other important adverse events observed during the conduct of the trial		
Complications	**Placebo (n=___)**	**Etanercept (n=___)**
…		
…		
…		
…		
…		
…		
…		