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REVIEW

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Perispinal etanercept stroke trial design: PESTO and beyond

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ABSTRACT

Introduction: Perispinal etanercept (PSE) is an innovative treatment designed to improve stroke recovery by addressing chronic post-stroke neuroinflammation. Basic science evidence, randomized clinical trial (RCT) evidence and 14 years of favorable clinical experience support the use of PSE to treat chronic stroke. This article provides guidance for the design of future PSE RCTs in accordance with current FDA recommendations.

Areas covered: Scientific background and essential elements of PSE RCT design.

Expert opinion: Intimate familiarity with PSE, its novel method of drug delivery, and the characteristics of ideal enriched study populations are necessary for those designing future PSE stroke trials. The design elements needed to enable a PSE RCT to generate valid results include a suitable research question; a homogeneous study population selected using a prospective enrichment strategy; a primary outcome measure responsive to the neurological improvements that result from PSE; trialists with expertise in perispinal delivery; optimal etanercept dosing; and steps taken to minimize the number of placebo responders. RCTs failing to incorporate these elements, such as the PESTO trial, are incapable of reaching reliable conclusions regarding PSE efficacy. SF-36 has not been validated in PSE trials and is unsuitable for use as a primary outcome measure in PSE RCTs.

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KEYWORDS
Etanercept; perispinal; RCT; PESTO; stroke; clinical trial design; enrichment

1. Introduction

TNF (tumor necrosis factor) is an immune signaling molecule that plays a pivotal role in the modulation of synaptic mechanisms in the brain [1–5]. Stroke results in chronic microglial activation and elevation of TNF in the brain, a disturbance of homeostasis that may self-perpetuate through an autocrine feed-forward loop [6–12]. Clinical and imaging evidence suggests that microglial activation is widespread in the brain and persists long after the acute stroke [10–16]. The 18-kDA translocator protein (TSPO), previously named the peripheral benzodiazepine receptor (PBR), is a mitochondrial protein that is markedly increased at sites of brain injury and is considered a marker of microglial activation [17,18]. Radioligands targeting TSPO have been used with positron emission tomography (TSPO PET) to image microglial activation in the brain after stroke, in humans and in basic science models [10,11,15,17,18].

Perispinal etanercept (PSE) is an innovative biological treatment designed to improve recovery from stroke by neutralizing excess TNF and reducing microglial activation [13]. PSE has been used to improve neurological recovery after a stroke since 2010 [13,14,19]. Real-world evidence, derived from favorable clinical experience involving thousands of patients with chronic stroke treated with PSE over the course of 14 years; basic science evidence; expert opinion; imaging studies and a double-blind, placebo-controlled RCT published in 2020 all support the use of PSE to reduce chronic post-stroke neurological dysfunction and improve recovery from stroke [8,12–16,19–28]. However, formidable barriers to widespread acceptance exist, particularly for novel treatments that challenge existing dogma [27,29–35]. It is well known that an incorrect design of an early phase RCT could result in the failure of an effective treatment to reach those who could benefit [36–40]. Such a false-negative result would be considered a Type II error, i.e. the failure to detect the true efficacy of an effective drug.

A primary consideration in the design of chronic stroke RCTs is the fact that the population of individuals with chronic post-stroke neurological dysfunction is inherently heterogeneous [39]. Neurological outcome and the magnitude of response to treatment in chronic stroke is influenced not only by the size and location of the lesion, but also by the type and subtype of stroke – ischemic (thrombotic, embolic, lacunar) or hemorrhagic (intraparenchymal hemorrhage, intraventricular hemorrhage, subarachnoid hemorrhage, hemorrhagic transformation, etc.), as well as by external factors, such as the duration and severity of hypoxia, hypotension, or hypoperfusion. This heterogeneity can present a particular challenge for small or even medium-size RCTs in which heterogeneity of the RCT study population could reduce the trial's ability to detect a beneficial treatment effect. Generic outcome measures meant to quantitate response to treatment for heterogeneous, non-stroke populations, such as the SF-36 quality-of-life measure, may have significant issues regarding validity when used as primary outcome measures for stroke trials [41]. Fortunately, there are available strategies, including the use of a prospectively enriched study population, which

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Article highlights

- **Stroke causes, and etanercept reduces, neuroinflammation**: Stroke results in chronic and widespread neuroinflammation in the brain. Etanercept attenuates neuroinflammation by neutralizing excess TNF and reducing microglial activation.
- **Perispinal etanercept for stroke recovery**: Perispinal etanercept (PSE) has been successfully used in the clinic for 14 years to improve recovery from stroke in thousands of patients. PSE is supported by clinical, basic science and randomized clinical trial (RCT) evidence, imaging studies, and expert opinion.
- **FDA guidance on RCT enrichment strategies**: Current FDA guidance for RCTs recommends study of homogeneous populations selected using a prospective enrichment strategy to improve their ability to accurately detect drug efficacy.
- **Essential design elements for PSE RCTs**: Necessary elements to enable PSE trials to generate valid results include: a homogeneous, prospectively enriched study population; a primary outcome measure responsive to the neurological improvements that result from PSE; trialists with expertise in perispinal delivery; optimal dosing; and steps taken to minimize the number of placebo responders.
- **SF-36 is unsuitable as a primary outcome measure in PSE RCTs**: SF-36 and other generic quality of life measures that have not been validated in PSE trials are unsuitable for use as primary outcome measures in clinical trials testing the efficacy of PSE for treating chronic stroke.
- **PESTO trial is incapable of reaching reliable efficacy conclusions**: PSE RCTs, such as PESTO, that fail to incorporate the design elements necessary for validity, are incapable of reaching reliable conclusions regarding PSE efficacy.
- **Future TSPO brain imaging trials**: Clinical trials utilizing TSPO PET brain imaging may enable visualization of changes in microglial activation following PSE and aid in participant selection in future PSE chronic stroke trials.

can improve the validity and the reliability of the efficacy conclusions of chronic stroke RCTs [39,42]. Trial enrichment is defined as '[*the*] *prospective use of any patient characteristic to select a study population in which detection of a drug effect (if one in fact is present) is more likely than it would be in an unselected population*' [42].

To add historical perspective, both infliximab and etanercept failed in early indications, such as sepsis, when they were first studied, more than 20 years ago [43,44]. Both drugs were repurposed and successfully used for the new indications for which they are now FDA-approved, including rheumatoid arthritis, psoriasis, and inflammatory bowel disease. From these early failures, it appears that, at least for some of these disorders, such as sepsis, the time point within the natural history of the disease when the TNF antagonist is administered may be critical. For sepsis, the inflammatory cascade initiated by gram-negative bacteremia may be so rapid that by the time the patient is in septic shock it is too late to intervene with a TNF antagonist. For chronic stroke, clinical experience indicates that time to intervention is not critical, since patients respond favorably weeks, months, years or even decades after stroke or brain injury [13,14,19,22,45]. Part of the difference is that in the brain TNF plays a pivotal role in the modulation of synaptic function [1–5,25,46,47]. TNF's key role in the regulation of brain function is distinct from its role as the master regulator and initiator of peripheral inflammatory cascades, which if unchecked, may eventuate in a cytokine storm [5,9,24,25,27,47–62]. This distinction is certainly part of the explanation why the required timing of TNF inhibition for these different categories of diseases is not identical.

The PSE chronic stroke RCT guidelines detailed herein are formulated based upon clinical experience using PSE for more than 5,000 chronic stroke patients. Although this review will focus on PSE RCT design, the basic concepts may assist those designing clinical trials for other neurological indications.

2. Perispinal etanercept for stroke recovery: scientific background

The scientific background supporting the clinical use of PSE for brain disorders has been reviewed in detail in previous publications [13,14,21–25,27,45–47,51,55,59,63–72]. The following is a current synopsis of selected aspects of the scientific background.

2.1. TNF

TNF is an immune signaling molecule and a neuromodulator that plays essential roles in immune and inflammatory responses throughout the body [55,73]. In the periphery, TNF is the master regulator of the inflammatory response [24]. In the brain, TNF is a neuromodulator that regulates synaptic and brain network function, including synaptic transmission, synaptic strength, synaptic scaling, and synaptic plasticity [1–3,5,21]. Elevated brain levels of TNF are thought to be centrally involved in the pathogenesis of the chronic brain dysfunction that accompanies stroke, traumatic brain injury, Alzheimer's disease, and other brain disorders by mediating synaptic dysfunction and perpetuating microglial activation [9,13–15,19,21,22,24,25,27,45–47,55,69,74–77].

2.2. Etanercept

Etanercept is a dimeric fully human soluble TNF receptor Fc fusion protein manufactured using recombinant DNA biotechnology. It is a large molecule composed of 934 amino acids with a molecular weight (mw) of 150 kilodaltons (kDa). It consists of the extracellular ligand-binding portion of the human 75 kDa (p75) TNF receptor linked to the Fc portion of human IgG1. Etanercept reversibly binds to circulating TNF, acting as a decoy receptor, reducing the biologic effects of TNF, including TNF's deleterious effects when present at levels in excess of its normal physiologic range. Etanercept's first Food and Drug Administration (FDA) approval was in November 1998 for treatment of rheumatoid arthritis. Etanercept was subsequently approved for chronic use by once or twice weekly subcutaneous injections for multiple additional indications, including ankylosing spondylitis, psoriasis, psoriatic arthritis and juvenile arthritis in children as young as age 2. It has also been used off-label for multiple inflammatory conditions, including several neurological disorders. Since its introduction more than two decades ago, it has maintained a favorable safety profile [78,79].

2.3. Stroke may result in widespread, sustained microglial activation in the brain

A growing body of evidence suggests that post-stroke microglial activation may persist in the stroke penumbra and in regions remote from the region of acute necrosis after both ischemic and hemorrhagic stroke [8,12,16,80,81]. This evidence includes the results of a 2021 positron emission tomographic imaging study (TSPO PET) of individuals with chronic middle cerebral artery (MCA) stroke that was conducted using a radioligand to the 18 kDa translocator protein (TSPO) [12]. TSPO is a mitochrondrial protein upregulated in activated glia and considered a marker of neuroinflammation [12]. The study found evidence of glial activation in multiple non-infarcted brain regions, both ipsilateral and contralateral, outside the MCA infarct zone, consistent with previous TSPO PET clinical imaging studies [10–12].

2.4. Etanercept reduces neuroinflammation by reducing microglial activation and neutralizing excess TNF

Autocrine activation of microglia by TNF creates a positive feed-forward loop that may perpetuate neuroinflammation and have neurotoxic consequences [7,9,13,82,83]. Etanercept reduces neuroinflammation in two ways:

(1) By attenuation of microglial activation through a direct effect on microglia and by interruption of the known microglia/TNF autocrine feed-forward loop [7,9,13,57,60,84–96]; and
(2) By binding to soluble TNF and thereby reducing its biological activity [97,98].

In addition, neutralization of TNF by etanercept would be expected to reduce the concentration of other non-TNF inflammatory cytokines that could activate microglia because TNF sits on top of the inflammatory cascade as the master regulator of the inflammatory response [9,52–55,99–101]. The inflammatory cytokines IL-1, IL-6, etc. are downstream in the inflammatory cascade from TNF [9,52–55,99–101].

2.5. Known physiological mechanisms are consistent with the rapid and sustained neurological improvement observed after PSE administration

Etanercept has been found to reduce microglial activation in at least 17 animal models, including those studying stroke, as previously reviewed [14,57,60,72,84–96]. Interruption of the autocrine microglia-TNF feed-forward loop may explain the sustained and sometimes progressive neurological improvement that has been observed in chronic stroke patients after a single dose of PSE [13,14,19]. The nearly instantaneous attenuation of the biological activity of TNF in the presence of etanercept, and TNF's known pivotal role in the modulation of synaptic mechanisms, provide mechanisms to explain the rapid neurological improvements seen in stroke and other neuroinflammatory conditions following PSE [1–5,14,19,21,22,45,72,75–77].

2.6. The cerebrospinal venous system

The veins, venous sinuses, and venous plexuses of the brain and the spine together comprise the cerebrospinal venous system (CSVS), a unique, large capacity, interconnected, venous system that carries venous blood back and forth between its cerebral and spinal components [102–105]. The first of the two main divisions of this system, the intracranial veins, includes the cortical veins, the dural sinuses, the cavernous sinuses, and the ophthalmic veins. The second main division, the vertebral venous system, includes the vertebral venous plexuses, which course longitudinally up and down the entire length of the spine. The intracranial veins richly anastomose with the internal vertebral venous plexus (IVVP) in the suboccipital region. The external vertebral venous plexus (EVVP), the only division of the CSVS that contains valves, drains the anatomic region posterior to the spine and directs blood flow internally into the IVVP [106]. Venous blood flow within the IVVP can be bidirectional, due to the IVVP's lack of venous valves [102–105]. Drugs or contrast delivered into areas that drain into the EVVP may flow cranially into the cerebral venous system via the IVVP [103–105]. The CSVS plays an important role in the regulation of intracranial pressure by facilitating venous flow into and out of the brain with changes in posture. The CSVS provides a direct vascular route enabling the rapid delivery of etanercept to the brain after perispinal injection [13,14,19–22,45–47,51,69,72,75,76,102,105,107].

2.7. Perispinal injection enables rapid delivery of drugs to the brain via the CSVS

The scientific rationale underlying the perispinal delivery method is supported by basic science experiments [20,26]; clinical investigations [21,105,108]; clinical trials [28,51]; and cadaver studies dating back more than 200 years, with further anatomic evidence developed in the 1940s and in the last decade [102–105,109–112]; as well as 25 years of clinical experience [13,14,19,22,28,45,51,63–66,72,75,76,105]. The proper performance and implementation of this technique requires expertise and hands-on training with an expert [47,113].

The supporting data includes evidence developed in animal models following perispinal delivery of biologics, including *in vivo* brain imaging [20,26,107]. In 2007, an animal positron emission tomography (PET) study performed at Stanford University demonstrated rapid delivery of radiolabeled etanercept (mw 150 kDa) into the cerebral ventricles, the choroid plexus and the CSF within the brain minutes after perispinal injection [20]. In 2015, a second animal study used single-proton emission computed tomography (SPECT) to study the biodistribution of several radiolabeled large molecules after perispinal injection in 5 rats [107].

Figure 1(a) is a SPECT image showing the biodistribution of radiolabeled cetuximab (mw 146 kDa) in Rat 1 following perispinal injection overlying the cervical spine at the C6-C7 level followed by placement of the rat in the head-down position for 3 minutes [107]. Five minutes after the perispinal injection SPECT scanning was performed, which took 20 minutes. The SPECT image reveals rapid delivery of radiolabeled cetuximab

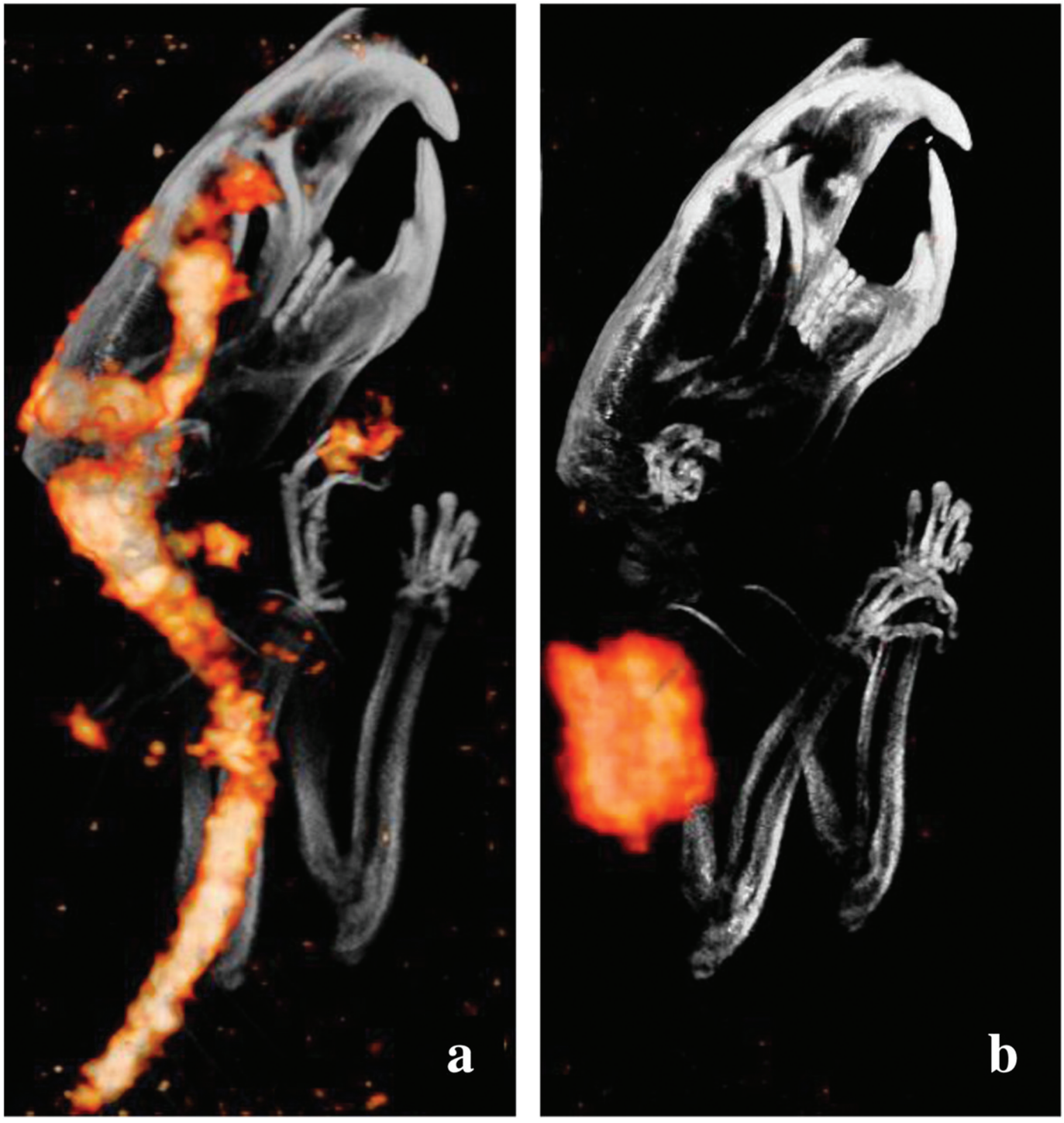

Figure 1. Single-photon emission computed tomography (SPECT) images showing the biodistribution of radiolabeled molecules five minutes after perispinal injection and head-down tilt in rats. (a) Rapid delivery of radiolabeled cetuximab into the brain and the cerebrospinal venous system extending into the ophthalmic venous plexus after perispinal injection in rat 1. (b) Tracer confined to the perispinal area after perispinal injection in another rat. Figure reproduced from [107], © 2015 roerink et al. Licensed with CC by 4.0 (https://creativecommons.org/licenses/by/4.0/). Image unmodified; title and caption modified from the original.

into the brain and the cerebrospinal venous system (CSVS) extending into the ophthalmic venous plexus after the perispinal injection.

The authors of the 2015 study speculated that the injection in Rat 1 was inadvertently intrathecal (i.e. into the CSF) [107]. Close examination of the imaging evidence, however, disproves their hypothesis. Extension of the tracer signal to the orbital venous plexus, as shown in Figure 1(a), would not be expected after an intrathecal injection, because the CSF has no anatomic connection to the orbital venous plexus [114,115]. In fact, the most intense tracer signal is in the veins comprising the CSVS, not in the CSF. This is not the pattern one would see if the injection were intrathecal; in that case the most intense signal would be in the CSF. Instead, the SPECT image clearly

shows that the most intense signal is intravenous and it extends through the spinal and intracranial components of the CSVS all the way to the ophthalmic (orbital) venous plexus, which itself is a component of the CSVS. The biodistribution of the labeled drug into the ophthalmic venous plexus is unmistakable here due to the distinctive appearance of this venous plexus at the back of the eye [114,115] (Figure 1(a)).

Rapid delivery into the brain in Rat 1 after perispinal injection was confirmed postmortem, with 4.05% of the injected dose/gram found in the brain, twice the concentration found in the blood, and 67.5 times the concentration found in the perispinal region, confirming brain delivery after perispinal injection [107]. The rapid delivery to the ophthalmic venous plexus after perispinal injection and head-down tilt documented here is supporting evidence that carriage of etanercept through the CSVS after perispinal injection is the anatomical basis for the nearly immediate improvement in visual perception that is commonly observed in the clinic after PSE [14,19,22,45].

The apparent failure to deliver the radiolabeled drug into the CSVS in the other four rats, as suggested by the image in Figure 1(b), results in accumulation of the drug superficial to the spine. This is not unexpected, because perispinal delivery into the CSVS in rats is technically difficult to accomplish due to the small caliber of the veins comprising their external vertebral venous plexus. Larger animals, such as dogs, pigs, primates, or humans are more suitable for perispinal delivery experiments. The development of expertise in perispinal delivery, in both rats and humans, requires expert training [47].

2.8. Clinical effects of perispinal etanercept in chronic stroke

A spectrum of rapid and sustained neurological improvements in chronic stroke and chronic brain injury following treatment with PSE has been previously described [13,14,19,22,45]. In the clinic the most common neurological effects include improvements in cognition, central post-stroke pain (CPSP), post-stroke shoulder pain, gait, balance, visual perception, swallowing, spasticity, dysarthria, language abilities, fatigue, strength, attention, range of motion and sensation [13,14,19,22,45]; see also the extensive online videos documenting the clinical response to PSE in chronic stroke [116].

3. Essential elements of PSE RCT design

Intimate familiarity with a drug, its precise method of delivery and its clinical effects is indispensable when designing an RCT evaluating the drug's efficacy [36,39,40,113,117–120]. Without such familiarity, the optimal selection of a research question, dosing regimen, outcome measure(s), and study population is not possible [36,38–40,113,117–121]. RCT design is made more difficult when the treatment method is innovative and its clinical effects novel [24,27,47]. The following recommendations, are, in general, applicable to the design of RCTs examining the efficacy of novel drug therapies for the treatment of conditions whose populations are heterogeneous, but are specifically meant to assist in the design of future trials of PSE for chronic stroke and related indications. To help in illustration of these concepts, the differences in the design of the two PSE chronic stroke RCTs that have been completed to date are compared in this section. These RCTs are the PSE RCT conducted on the Gold Coast of Australia that published in 2020 [28] (the 'Gold Coast RCT') and the Perispinal Etanercept to improve Stroke Outcomes ('PESTO') RCT, whose protocol published on 27 April 2024 [122].

3.1. Compose a suitable research question

Appropriate formulation of the research question is the most important first step in clinical trial design [40]. Until the efficacy of an innovative treatment for a disease with a heterogeneous population has been firmly established, the most suitable research questions are those directed to examining efficacy in narrowly defined subpopulations thought most likely to benefit. This guiding RCT design principle conforms with explicit FDA guidance [42].

PSE results in a spectrum of neurological improvements across different domains (cognition, motor function, central pain, spasticity, etc.) that can vary according to the stroke type, location, etc. For this reason, significant clinical experience using PSE and observing its neurological effects in different stroke subpopulations is necessary in order to formulate a research question and a matching trial design that will minimize Type II error. The fundamental differences between the successful Gold Coast RCT and the failed PESTO trial were apparent even before the trials began. The Gold Coast RCT set out to answer a question directed to a narrowly defined stroke subpopulation, those with intractable central post-stroke pain. In contrast, PESTO's research question, concerning quality of life, was directed to a heterogeneous, essentially unselected population of stroke survivors with a wide range of disability. PESTO's choice of research question precluded the use of prospective enrichment in selection of its study population.

3.2. Optimize participant selection through prospective enrichment

> Clinical trial enrichment represents a powerful strategy for selecting a subset of the general population in which the effect of the drug can be more efficiently demonstrated. This approach has the potential to result in smaller studies, increased study power, and/or shortened development times … . Of critical importance, our data suggest that use of any type of enrichment approach is more likely to lead to clinical trial and development program success compared to not using enrichment as part of clinical trial design and execution. [123]

> Smaller trials in clearly defined and homogeneous stroke pathologies can yield answers that may be obscured by dilution in larger groups of patients who are not as well characterized. [39]

Enrichment designs are used to increase the efficiency of drug development and improve the chance of detecting the efficacy of a new drug. It is widely recognized that, with heterogeneous clinical syndromes, optimization of participant selection is central to the likelihood of the RCT's success [39,40,42]. Trial enrichment can greatly increase the chance of reaching a correct conclusion, without compromising the trial's validity or the meaningfulness of the conclusions

reached [42,123,124]. RCT population enrichment as an RCT design strategy is intended both to decrease the time and expense involved in drug development and ' … support precision medicine, i.e. tailoring treatments to those patients who will benefit based on clinical, laboratory, genomic and proteomic factors [42]. '

The Gold Coast RCT utilized prospective population enrichment to select a narrowly defined study population. This homogeneous, enriched population consisted of selected individuals with chronic and intractable central post-stroke pain (CPSP): 'All participants initially demonstrated significant intractable and constant daily CPSP with pain scores at baseline entry between 50 and 80 inclusive on the 0–100 point vNPRS-FPS, with their pain refractory to analgesic medications (including oxycodone or pregabalin [28]. ' Such patients were known through clinical experience to constitute a narrow subset of stroke survivors in which the favorable neurological effects of PSE could be efficiently demonstrated [13,14]. Using this design, the Gold Coast RCT was able to successfully demonstrate that PSE had efficacy for treating CPSP.

In contrast, rather than incorporate enrichment strategies in selection of its trial population, PESTO was deliberately designed to study a heterogeneous population. This is readily apparent from examination of PESTO's inclusion and exclusion criteria. The inclusion criteria were very broad and the exclusion criteria were narrow. For example, PESTO, by design, specifically allowed individuals with a degree of disability ranging from modified Rankin scale (mRS) class 2 to mRS class 5 to enter the trial. By definition, this could include trial participants with only slight disability who are able to look after their own affairs without assistance (mRS 2), all the way to individuals with severe disability who require constant nursing care and attention and are bedridden and incontinent (mRS 5). Patients with aphasia were not excluded from the trial, even if they required a proxy to complete their SF-36 questionnaire. This was not a narrowly defined, homogeneous study population; it was exactly the opposite. PESTO failed; its decision not to study an enriched study population was a critical design error.

Enrichment of clinical study populations is carried out in order to select trial participants in whom a drug effect, if present, is more likely to be demonstrable [124]. Predictive enrichment attempts to identify individuals with an increased likelihood of favorable response to the drug being trialed for a mechanistic reason, such as possession of a suitable receptor, or, in the case of chronic stroke, possession of a well-defined clinical characteristic that reflects underlying pathophysiology likely to respond to the drug [124]. This was exactly the case in the Gold Coast RCT, in which predictive enrichment was used to prospectively select its study population, all of whom had moderate-to-severe CPSP. Trial enrichment has the fundamentally important advantage of enabling the use of an outcome measure that is matched to the study population and in that way increase study power and decrease Type II error. None of this is possible when studying a heterogeneous population, as done in the PESTO trial, that was 'designed to include a representative sample of the patients that undergo off-label treatment with etanercept [122],' a population of patients that is inherently unenriched.

3.3. Use a primary outcome measure sensitive to the drug's effects

> Selecting the best outcome measure requires consideration of issues far beyond the usual questions about reliability and validity. The ultimate value of a clinical trial or outcome study will be directly tied to how well the selected outcome measure matches the researcher's understanding of what he or she expects to change, to what degree it is expected to change, over what period of time this change will happen, and how that change can best be identified. [118]

Selecting an appropriate primary outcome measure is a critical step in designing scientifically sound, ethical clinical trials [36,38,40,117–119]. Selection needs to consider features of outcome measures that may affect their validity and utility for the study's purpose [118,125]. Experience with the drug's effects in the selected study population is necessary to determine beforehand if the outcome measure is likely to be sensitive to the degree of change expected from the drug and thus appropriate for use in any given RCT [36,117–119]. Without the requisite experience, the ability of an outcome instrument to reliably measure the neurological changes produced by a novel drug in a chronic stroke population cannot be certain. This is particularly true for subjective, generic outcome measures, such as SF-36, that are not stroke specific.

The limitations of SF-36 as an outcome measure for stroke trials are illustrated by a real-world example: Botulinum toxin. Botulinum toxin (Botox®) is known to be efficacious for the treatment of post-stroke spasticity. On that basis, one might think that botulinum toxin would increase the quality of life in an individual living with chronic spasticity after stroke. It might well do so, but a clinical trial of botulinum toxin for stroke spasticity showed no change in quality of life, as measured by SF-36 [126]. The take-away is not that botulinum toxin is ineffective as a treatment for spasticity. Rather, it would appear that SF-36 is not responsive to the neurological improvement (reduction in spasticity) produced by botulinum toxin injection.

Other limitations with respect to outcome selection in stroke RCTs require consideration. Patient-reported outcome measures (PROMs), as a class, have less validity and reliability than objective measures, because they are subjective [127,128]. Common sequelae of stroke, including cognitive impairment or difficulties with language comprehension or expression, are likely to confound subjective outcome measures [127]. Such stroke sequelae may make it difficult or impossible for the individual to access and grade on an ordinate scale internal experiences, such as pain, fatigue, or quality of life [127]. Individuals with severe aphasia or severe cognitive dysfunction are unable to reliably self-complete PROM questionnaires [127]. In such cases, proxy completion of written questionnaires, such as SF-36, would be necessary. However, proxy completion can reduce the validity of the data reported [129]. Discrepancies between patient and proxy reporting may be large enough to impact the outcome assessment in stroke clinical trials [129].

Both the Gold Coast RCT and PESTO used PROMs as their primary outcome measure. The Gold Coast RCT used the

vertical Numerical Pain Rating Scale supplemented with a Faces Pain Scale (vNPRS-FPS), a measure that is stroke-specific, has been validated in a chronic stroke population, and was developed specifically for quantitating pain after stroke [130]. The vNPRS-FPS is well suited for a trial evaluating PSE for CPSP. Validated pain scales, such as vNPRS, were known from previous clinical experience to be responsive to the reduction in stroke pain caused by PSE [14]. By design vNPRS does not require proxy completion [13,14]. In contrast, PESTO used SF-36, a generic subjective measure that was not stroke-specific [41]; and that was not matched to any specific symptom in PESTO's heterogeneous study population. PESTO, by design, and as specified in its inclusion criteria, also explicitly allowed proxies to complete the SF-36 questionnaire, its primary outcome measure, for the patient. SF-36 had not been used in any PSE RCT, so PESTO's designers could not be certain that the SF-36 measure would be responsive to PSE's neurological effects. In sum, selection of SF-36 as PESTO's primary outcome measure was another critical design error.

3.4. Select and study an adequate number of trial participants

> [A] study with a sample that is too small will be unable to detect clinically important effects. Such a study may thus be scientifically useless, and hence unethical in its use of subjects and other resources. [121]

One of the requirements for an RCT to be ethical is that it have a sample size large enough to adequately test the research question that is posed, particularly in the absence of explicit plans for definitive studies in the future [38,119]. The greater the heterogeneity of the study population, the more difficult it is to detect a treatment effect [39,40,42,131]. For an RCT to reliably determine whether a new therapy shows at least some promise of benefit, it must be properly powered [38,113]. Thorough familiarity with a novel treatment is necessary to perform power calculations adequate to determine the sample size needed to reliably detect a treatment effect. RCTs evaluating drug efficacy for brain disorders may require a sample size greater than 1,000 to reach valid scientific conclusions if the study population is heterogeneous [117]. Enrichment strategies can enable scientifically reliable results to be obtained with smaller trials, as a comparison between the Gold Coast RCT and PESTO illustrates [39,40,42,131]. The Gold Coast RCT was able to demonstrate efficacy using an enriched trial population consisting of 22 participants. PESTO was unable to demonstrate efficacy using a larger, unenriched, heterogeneous trial population consisting of 126 participants, which fell short of its pre-specified sample size target of 168. This shortfall would tend to increase Type II error.

3.5. Use an optimal dosing regimen

> Insufficient dosing of a new intervention can lead to premature conclusions about the ineffectiveness of a general intervention approach if a different (but untested) dose would have been effective. [120]

Selection of dosing for an RCT includes the determination of the amount of each dose, the number of doses to be studied, the dose interval, and the method of administration [40,117,120,132]. From a large pharmaceutical company perspective, 'the greatest challenge in clinical research is finding the therapeutic dose [133]. ' Clinical experience and RCT evidence (based upon the Gold Coast RCTs published results) suggest optimal dosing for a PSE chronic stroke RCT is as follows:

(1) A 25 mg etanercept dose on day 0 and a second 25 mg etanercept dose on day 14.
(2) Outcome measurement 14 days after the second 25 mg dose.

The above schedule was the dosing used in the successful Gold Coast RCT. PESTO used different dosing:

(1) A 25 mg etanercept dose on day 0.
(2) Outcome measurement 28 days later.

PESTO employed only 50% of the etanercept dose used in the Gold Coast RCT prior to measurement of the primary efficacy outcome, and measured the outcome at an interval twice as long after the last dose (28 days vs. 14 days). PESTO employed a sub-optimal dosing regimen, another flaw in its design that increased its chance of making a Type II error, i.e. failing to detect the true efficacy of PSE.

3.6. Minimize the number of placebo responders

> Placebo responses are especially problematic in studies that rely on subjective patient-reported outcomes, and can have severe detrimental effects on assay sensitivity, contributing to the failure of trials of efficacious therapies. *[134]*

The higher the placebo responder rate, the more difficult it is for the active drug to achieve superiority and the trial to succeed [134,135]. For example, a systematic review of clinical trials of drugs meant to prevent migraines showed a statistically significant difference in trial success rate (60%) between RCTs with ≤20% placebo responders and studies with >30% placebo responders [135].

When PESTO's partial results were presented at the 2024 European Stroke Conference, the 58% placebo responder rate was an immediate red flag. This rate was more than 5½ times larger than the 11% rate expected, as stated in PESTO's published protocol [122]. It is likely that several factors falsely inflated this rate:

(1) A subjective primary outcome measure, SF-36, which was unsuitable. SF-36 is a generic, subjective PROM; has not been shown to be responsive to PSE; and has not been validated as an outcome measure in PSE trials or reports [134];
(2) Heightened expectations of a favorable outcome due to widespread favorable media coverage in Australia and New Zealand, the sites of the RCT [136,137];

(3) PESTO's [inadvertent] use of a painful acidic solution as its placebo control (Sterile saline solution (0.9% sodium chloride solution for injection), used in PESTO as its placebo control, is acidic (pH 5.3) and painful when injected). A fix for this issue could have been as simple as using a buffered saline control solution) [138–141]. In contrast, the etanercept solution reconstituted from lyophylized powder that was used in PESTO is not painful when given by perispinal injection, because its pH (7.4) is physiological, not acidic [97]. These circumstances would tend to lead to higher expectations of a favorable response in those participants randomized to placebo, as they would tend to mistakenly believe that a painful injection meant they had received the active drug, thereby increasing the placebo response.

To minimize the number of placebo responders, it is recommended that future PSE RCTs utilize buffered saline control solutions to eliminate painful perispinal injections and incorporate prospective measures known to reduce the placebo responder rate, such as interventions to reduce staff and subject expectations and improve the ability of subjects to accurately report symptom severity [134].

3.7. An exemplary PSE chronic stroke trial design

Based upon more than a decade of clinical experience, as well as the results of the Gold Coast RCT, the design of an exemplary PSE chronic stroke RCT would include the following elements:

(1) A homogeneous trial population consisting of individuals with chronic, intractable central post-stroke pain (CPSP) and corresponding sensory changes. The pain must be constant, present all day, every day, present at rest and always of moderate-to-severe intensity, ranging from a minimum of 40 mm to a maximum of 95 mm on a 100 mm visual analog scale (VAS). Individuals with CPSP but without corresponding sensory changes will be excluded from the trials. Individuals without significant constant CPSP will be excluded.
(2) Individuals with aphasia or cognitive impairment to a degree that would prevent them from reliably quantitating their pain on a VAS scale will be excluded.
(3) The primary efficacy outcome will be the change in pain intensity as measured using a VAS pain scale from Day 0 to Day 28.
(4) In patients with at least 30 degrees of active shoulder range of motion (ROM) at baseline, the change in their active ROM will be a secondary outcome measure.
(5) The amount of analgesic medication used by the study participant during the study compared with the amount utilized before treatment will be another secondary outcome measure.
(6) Change in the distribution, extent, or intensity of microglial activation as quantitated by TSPO PET brain imaging before and after PSE could be an experimental outcome measure in the future. Incorporation of this element into the trial design will, however, first require collaboration with an imaging group with expertise in TSPO PET brain imaging and the completion of pilot trials.
(7) Trialists must be intimately familiar with the anatomy and physiology of the cerebrospinal venous system; must complete expert, hands-on training in the optimal technique of perispinal injection; and must have experience observing the clinical effects of perispinal etanercept.
(8) For a two-armed trial, there must be a minimum of 168 subjects, ideally at a single center. A second trial center will be added using the same protocol upon successful completion of the first trial.
(9) The etanercept dosing regimen for subjects randomized to the active arm will be cervical perispinal injection of 25 mg of etanercept solution prepared from lyophylized powder, followed by placement in the Trendelenburg position for 7 minutes. Subjects in the active arm will receive perispinal etanercept on Day 0 and Day 14.
(10) Subjects randomized to the control arm will receive perispinal injections of sterile saline buffered with sodium bicarbonate to a pH of 7.4 to minimize inflating the placebo response rate by the use of painful control injections.
(11) To further minimize placebo response the trialists will have taken efforts to reduce placebo response prior to administration of study medications, such as interventions to reduce staff and subject expectations and improve the ability of subjects to accurately report symptom severity [134].
(12) In a second iteration, a similar trial could be designed using subcutaneous injections of etanercept 25 mg in the abdomen as an active control.

4. Conclusions

The elements of future RCTs seeking to confirm PSE's efficacy for specific chronic stroke indications that are necessary to ensure valid results include:

(1) A homogeneous study population selected using a prospective enrichment strategy;
(2) A primary outcome measure responsive to the neurological improvements that result from PSE;
(3) Trialists with experience and expertise in perispinal delivery;
(4) An adequate number of trial participants;
(5) An optimal dosing schedule;
(6) Steps taken to minimize the number of placebo responders.

PESTO failed to incorporate *any* of these elements, making it unable to generate valid conclusions regarding the efficacy of PSE for improving recovery after stroke.

5. Expert opinion

Intimate familiarity with PSE, its novel method of drug delivery and the characteristics of ideal enriched study populations is necessary for those designing future PSE stroke trials. Hands-on training with experts is necessary for trialists to enable them to accomplish effective perispinal delivery of etanercept to the brain through carriage in the CSVS. Careful attention to the design of future PSE RCTs will help ensure that such trials reach valid conclusions regarding efficacy. SF-36 and other generic quality of life measures that have not been validated in PSE trials are unsuitable for use as primary outcome measures in clinical trials to support determination of effectiveness of PSE.

There were multiple critical flaws in the design of the placebo control arm of the PESTO trial, including the use of a painful placebo control injection and the use of a subjective, patient-reported primary outcome measure not well-suited for PSE stroke trials, all of which would tend to falsely inflate the placebo response rate, particularly considering the elevated patient expectations that would be engendered by the widespread favorable media attention that PSE had received. Moreover, there is no indication in the protocol that any measures were taken to mitigate the multiple design flaws in the PESTO trial, all of which would tend to falsely amplify the placebo response.

The preliminary report of some of PESTO results indicated that 53% of the trial participants successfully reached the trial's pre-determined efficacy threshold after a single PSE dose, a rate 76% higher than the 30% success rate predicted in the sample size calculation published in the PESTO protocol [122,142]. However, even this higher than expected efficacy result was unable to overcome the hurdle presented by PESTO's excessive placebo response rate. The flaws in the design of the placebo control arm of the PESTO trial, in combination with the selection of SF-36 as the primary outcome measure, were critical errors that contributed to PESTO's inability to generate valid efficacy conclusions.

If future trials are designed in accordance with the recommended guidelines, PSE for a specific chronic stroke indication, such as severe central post-stroke pain, could receive regulatory approval and become widely available within 10 years. Such approval would open the door to further studies of PSE in populations with other chronic stroke symptoms, such as severe post-stroke cognitive dysfunction, post-stroke shoulder pain, post-stroke spasticity, gait, and balance disturbances, etc., as well as open the door to further study of PSE for additional brain indications.

The ability of PSE to reduce chronic neurological dysfunction after stroke naturally leads to another question: Does PSE have potential as an acute stroke treatment? A definitive answer is not known at this time, because to reach the answer would require large, multi-center, RCTs performed in hospital settings. What has been known for more than two decades is that elevated levels of TNF occur in acute stroke and, at least in animal models, neutralization of TNF reduces focal ischemic brain injury. The evidence suggests that this approach merits immediate investigation.

Basic research increasingly supports the pivotal role of TNF in the regulation of synaptic mechanisms in the brain. In addition to its clinical utility, perispinal administration as a method to enhance delivery of large molecules to the brain has promise as a basic science and clinical research tool. Additional basic science studies of the biodistribution of radiolabeled large molecules given by perispinal injection should be initiated without delay. These biodistribution studies should be performed in large animals because of the difficulty of achieving reliable delivery of drugs into the CSVS after perispinal injection in rodents.

In humans, TSPO PET brain imaging has the potential to contribute valuable information regarding the pathophysiology of chronic stroke and its response to PSE. Several studies have now documented that acute stroke results in chronic, widespread neuroinflammation in the brain. At present, the standard methods of brain imaging, computed tomography and magnetic resonance imaging, are unable to visualize or quantitate neuroinflammation. TSPO PET brain imaging does enable visualization of the pattern and extent of microglial activation after stroke. It is as yet unknown if TSPO PET will be sensitive enough to detect the effects of PSE on microglial activation. It is an exciting prospect. If such changes were visualized, they could be used as an objective measure of biological response and an outcome measure for PSE clinical trials. Additionally, TSPO PET imaging might also have the ability to aid in participant selection in future PSE chronic stroke trials, particularly if one could correlate patterns of microglial activation in patients with their response to PSE treatment.

Favorable basic science and clinical data using etanercept has given a clue to the wide spectrum of brain disorders that could benefit from PSE, including, but not limited to, ischemic and hemorrhagic stroke; Alzheimer's disease and other neurodegenerative disorders; brain injury due to trauma, hypoxia, or cardiac arrest; and long COVID. All of these disorders share in common sustained microglial activation, a pathology that is perpetuated through a positive feed-forward physiological loop created by autocrine activation of microglia by TNF, and all, therefore, may potentially benefit from PSE.

Many of the early discoveries regarding the role of cytokines in regulating synaptic and neuronal network function had yet to be made when PSE first emerged as a potential treatment for neuroinflammatory disorders 25 years ago. Only now is the pivotal role of TNF in modulating these mechanisms, in health and disease, becoming more widely recognized in the brain research community. We look forward to the future, when a new generation of neuroscientists will arise, for whom the central role of cytokines in brain physiology will be basic knowledge, and the promise of perispinal delivery of large molecules to address brain disorders is fully realized.

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Declaration of interest

E Tobinick is the inventor of the perispinal etanercept treatment method, holds US, European, Canadian, and Australian patents covering its use, receives royalties generated by those patents, and was an unpaid design consultant for the Gold Coast RCT. E Tobinick is the founder and director of the Institute of Neurological Recovery, a private medical practice that

utilizes perispinal etanercept for treatment of its chronic stroke patients. D Ucci, K Bermudo and S Asseraf are employees of the Institute of Neurological Recovery. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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