LEADING ARTICLE

Perispinal Etanercept for Post-Stroke Neurological and Cognitive Dysfunction: Scientific Rationale and Current Evidence

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Abstract There is increasing recognition of the involvement of the immune signaling molecule, tumor necrosis factor (TNF), in the pathophysiology of stroke and chronic brain dysfunction. TNF plays an important role both in modulating synaptic function and in the pathogenesis of neuropathic pain. Etanercept is a recombinant therapeutic that neutralizes pathologic levels of TNF. Brain imaging has demonstrated chronic intracerebral microglial activation and neuroinflammation following stroke and other forms of acute brain injury. Activated microglia release TNF, which mediates neurotoxicity in the stroke penumbra. Recent observational studies have reported rapid and sustained improvement in chronic post-stroke neurological and cognitive dysfunction following perispinal administration of etanercept. The biological plausibility of these results is supported by independent evidence demonstrating reduction in cognitive dysfunction, neuropathic pain, and microglial activation following the use of etanercept, as well as multiple studies reporting improvement in stroke outcome and cognitive impairment following therapeutic strategies designed to inhibit TNF. The causal association between etanercept treatment and reduction in post-stroke disability satisfy all of the Bradford Hill Criteria: strength of the association; consistency; specificity; temporality; biological gradient; biological plausibility; coherence; experimental evidence; and analogy. Recognition that chronic microglial activation and pathologic TNF concentration are targets that may be therapeutically addressed for years following stroke and other forms of acute brain injury provides an exciting new direction for research and treatment.

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Key Points

Accumulating evidence suggests that chronic post-stroke intracerebral microglial activation and neuroinflammation mediated by pathologic levels of tumor necrosis factor constitute new therapeutic targets that may persist for years after stroke.

Perispinal etanercept for chronic post-stroke neurological and cognitive dysfunction is an emerging treatment modality that may lead to rapid and sustained clinical improvement in this patient population.

1 Introduction

Post-stroke disability represents a major public health problem throughout the world [1, 2]. Current drug

treatments are grossly inadequate [1, 2]. The world stroke research community recognizes the urgent need for improved stroke treatments [3].

In February 2011, rapid improvement in cognition; improvement in chronic neurological dysfunction; and reduction in chronic, intractable post-stroke pain was noted among a series of three patients treated off-label 13, 25, and 36 months after stroke with a single dose of etanercept, administered by perispinal injection [4]. Onset of clinical response was evident within 10 min of the etanercept dose in each patient [4]. Each patient received a second perispinal etanercept dose at 22–26 days after the first, which was followed by additional improvement [4].

In December 2012, an observational study of 629 patients treated off-label with perispinal etanercept was published [5]. The study included 617 consecutive patients treated a mean of 42 months following stroke ('the 617-patient stroke cohort'), and 12 patients following traumatic brain injury (TBI) [5]. Statistically significant improvements in neurological and cognitive function and reduction in pain were noted in the stroke cohort [5]. Perispinal etanercept produced rapid improvement in a variety of chronic post-stroke neurological dysfunctions (Table 1). The 2011 and 2012 etanercept post-stroke studies are designated herein as 'the etanercept stroke studies' [4, 5]. Perispinal etanercept for post-stroke neurological dysfunction was invented and pioneered by the senior author. Perispinal etanercept for this indication has been explored clinically nearly exclusively by the senior author, his colleagues, and a small group of independent physicians who have trained in the perispinal etanercept treatment method. The etanercept stroke studies are previously published studies of the senior author and colleagues.

1.1 Perispinal Administration

Perispinal administration is a novel method of drug delivery. Its use to deliver etanercept for treatment of post-stroke neurological dysfunction is necessitated by the fact that etanercept has difficulty in traversing the blood–brain barrier (BBB) in therapeutically effective concentration when administered systemically, due in large part to its high molecular weight (150,000 Da) [6]. This difficulty in reaching the brain in therapeutic concentrations when administered systemically is consistent with other studies documenting limited (0.1–0.6 %) penetration of large molecules into the brain when administered systemically [7–9]. Perispinal administration of etanercept for treatment of brain disorders involves needle injection overlying the spine superficial (external) to the ligamentum flavum [4, 5, 10, 11]. Perispinal injection of etanercept is designed to facilitate selective delivery of etanercept to the central

nervous system, as drugs administered posterior to the spine are absorbed into the external vertebral venous plexus (Fig. 1) [12, 13]. The external vertebral venous plexus drains into, and is a component of, the cerebrospinal venous system (Fig. 2) [10, 12, 14–18]. The anatomy and physiology of the cerebrospinal venous system, a unique, bi-directional vascular pathway, remains little known in the general medical community, despite recognition in multiple neurosurgical and anatomical publications [18–30]. Trendelenburg positioning may facilitate selective delivery of etanercept into the brain after it reaches the cerebrospinal venous system [10, 31–35]. The cerebrospinal venous system provides a direct vascular pathway to the brain (Figs. 1, 2).

Lack of familiarity with the cerebrospinal venous system and the novelty of etanercept's neurological effects may help explain the skepticism expressed by some and provides a rationale for this article [36]. Do the etanercept stroke studies survive a rigorous analysis with respect to their suggestion of a causal association between post-stroke etanercept treatment and clinical improvement?

2 The Nine Criteria of Hill

To begin such an analysis of the etanercept stroke studies, one may apply the well known criteria laid down by the English epidemiologist and statistician, Sir Austin Bradford Hill [37]. Hill pioneered the randomized clinical trial and was the first to demonstrate the connection between smoking and lung cancer. In his famous Presidential Address to the Royal Society of Medicine, Hill presented nine criteria for determining a causal association that would become the well known 'Bradford Hill Criteria' [37]. Hill's criteria are widely used in the evaluation of causation, have already been applied in the field of neurology, and have been recommended as a useful framework for evaluating healthcare evidence [38–40]. Hill's nine criteria are as follows: strength of the association; consistency; specificity; temporality; biological gradient; biological plausibility; coherence; experimental evidence; and analogy.

2.1 Strength of the Association

The magnitude of the clinical improvements, as reflected by the measures that were quantitated in the 617-patient stroke cohort, including the time to walk 20 m, Montreal Cognitive Assessment, visual analog scale for pain, etc. are consistent with a strong clinical effect. The strength of the association between perispinal etanercept treatment and clinical effect is strong [5].

**Table 1** Rapid improvement in chronic post-stroke neurological dysfunction following perispinal etanercept

Clinical effect	Manifestations	Reference
Statistically significant improvements		
Motor function	Increased strength, improved gait, stronger grip. Improvements in swallowing and dysarthria	[4, 5]
Spasticity	Decreased muscle tone, improved range of motion, decreased shoulder pain	[4, 5]
Sensation	Improved sensation	[4, 5]
Cognition	Improvements in cognitive testing scores and executive function	[4, 5]
Psychological/behavioral function	Improvements in mood, affect, and behavior. Reductions in depression and anxiety	[4, 5]
Aphasia	Improvements in speech and language function	[4, 5]; see also [11]
Pain	Reductions in post-stroke pain, including post-stroke shoulder pain and allodynia	[4, 5]
Case reports		
Urinary incontinence	Regained bladder sensation and control	[5]
Pseudobulbar affect	Reduction in excessive emotionalism	[5]
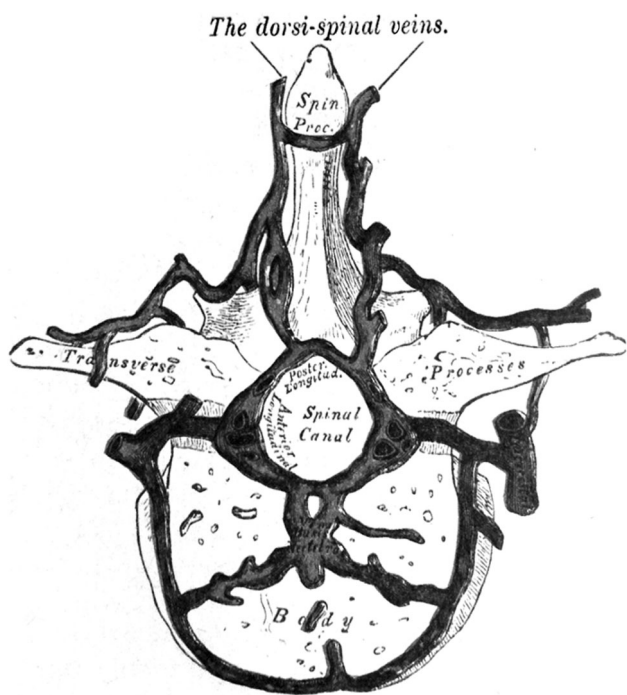
Fig. 1 The vertebral veins. Reproduced from Gray and Holmes [72]

2.2 Consistency

Statistically significant improvements in motor impairment, sensory impairment, cognition, aphasia, pain, and other areas of neurological dysfunction were noted, with p values consistently less than 0.001 in the 617-patient stroke cohort treated with perispinal etanercept [5]. The consistency of the association in the perispinal etanercept stroke studies between treatment and effect is high [4, 5]. Several recent studies using basic science stroke models have documented favorable effects of tumor necrosis factor
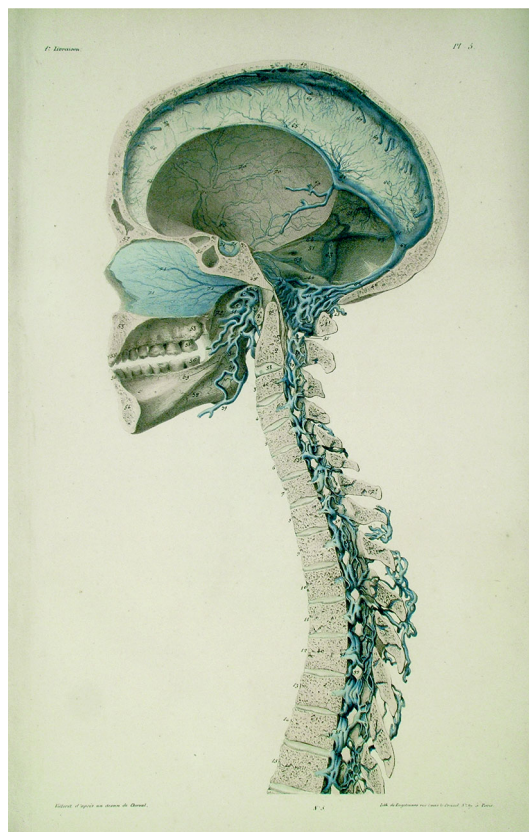
Fig. 2 The cerebrospinal venous system. Reproduced from Breschet [70]

(TNF) inhibition using TNF inhibitors other than etanercept [41–44]. A single study found that etanercept administered *systemically* was ineffective in an acute stroke model, arguing for the necessity of using specialized methods, such as perispinal delivery, to facilitate penetration of etanercept across the blood–cerebrospinal fluid

barrier when treating brain disorders [7, 9, 32–35, 41, 45, 46].

2.3 Specificity

Neither of the etanercept stroke studies utilized a placebo control group, which limits claims of specificity. However, the clinical effects observed in the 617-patient stroke cohort after perispinal etanercept treatment were significant, and many of the results (such as rapid improvement in vision, hearing, and motor function) cannot be explained by any mechanism other than a novel treatment effect, especially considering that patients were treated a mean of 3.5 years after their stroke [5]. The natural history of stroke recovery is well known: the great majority of the neurological recovery occurs in the first 6 months [47–49]. The spectrum of clinical improvement across domains, including improvements in motor function, cognition, sensory function, aphasia, etc., as documented in the etanercept stroke studies (see Case 1 in the 2011 etanercept stroke study, for example) can only be explained by the occurrence of a specific and novel therapeutic effect [4, 5]. The specificity of the association in the etanercept stroke studies between treatment and effect is high.

2.4 Temporality

The temporal relationship between the time of etanercept administration and clinical effect is remarkably strong, since clinical improvement characteristically was observed within minutes of the first dose in both etanercept stroke studies [4, 5].

2.5 Biological Gradient

Hill's biological gradient criteria are meant to examine whether increased exposure to the agent in question is associated with an increased biological effect. "Exposure can be characterized in different ways such as … the duration of exposure … average exposure … or cumulative exposure" [50]. Case reports included within the etanercept stroke studies document enhanced therapeutic responses after additional doses of etanercept in certain patients [4, 5]. Subsequent clinical experience has confirmed additional neurological improvement after additional etanercept doses in multiple patients.

2.6 Biological Plausibility

Biological plausibility is included in the Hill Criteria, with a caveat:

> "It will be helpful if the causation we suspect is biologically plausible. But this is a feature I am

convinced we cannot demand. What is biologically plausible depends upon the biological knowledge of the day … In short, the association we observe may be one new to science or medicine and we must not dismiss it too light-heartedly as just too odd. As Sherlock Holmes advised Dr. Watson, 'when you have eliminated the impossible, whatever remains, however improbable, must be the truth.'"

The evidence supporting biological plausibility is elaborated in detail in Sects. 2.1–2.9.5 and in Table 2. The recent peer-reviewed report of immediate and profound neurological and cognitive improvement following perispinal etanercept injection more than 3 years after acute brain injury provides additional support for the plausibility of rapid neurological improvement following perispinal etanercept for chronic post-stroke neurological and cognitive dysfunction [11].

2.7 Coherence

Reviewing the evidence discussed herein, the published results of perispinal etanercept for post-stroke disability are consistent with the following: (1) known involvement of TNF in the pathophysiology of chronic brain dysfunction in multiple diseases and disorders (review: [32]; 34, 51–62], Table 2); (2) the role of TNF in the pathophysiology of stroke, as discussed herein; (3) the existence of chronic, post-stroke intracerebral glial activation and neuroinflammation, as established by neuroimaging and pathological examination, as discussed herein; and (4) the known ability of etanercept to both rapidly neutralize pathologic TNF and reduce glial activation (Table 2) [45, 63–67].

Additionally, the novel clinical results reported, such as rapid improvement in vision and hearing, etc., may well be attributed to the fact that a potent biologic therapeutic (etanercept) is being administered by a novel route of administration (perispinal). Perispinal administration is designed to deliver etanercept into the cerebrospinal venous system as a method to enhance transport of etanercept across the blood–cerebrospinal fluid barrier [10, 16, 31, 35, 68, 69]. The unique anatomy and physiology of these interconnected venous plexuses is supported by a long series of experimental and pathological investigations recognized by those in the field, particularly in the neurosurgical community [10, 12, 14–19, 27, 31, 35, 68–79].

2.8 Experimental Evidence

Experimental evidence, according to Hill, is where "the strongest support for the causation hypothesis may be revealed" [37]. The experimental evidence supporting the use of perispinal etanercept for post-stroke neurological

Table 2 Evidence supporting the scientific rationale for the use of etanercept for post-stroke neurological and cognitive dysfunction

Pathophysiology and therapeutic rationale	References (exemplary)	Etanercept—effects	References
1. Pathologic TNF is centrally involved in the pathophysiology of stroke	Feuerstein 1994 [80]	Etanercept and other biologic TNF inhibitors improve stroke outcome	Feuerstein 1994 [80]
	Barone 1997 [81]		Barone 1997 [81]
Rationale: Etanercept and other TNF inhibitors reduce pathologic TNF concentration	Nawashiro 1997 [82]		Nawashiro 1997 [82]
	Zaremba 2000 [83], 2001 [85]		Tobinick 2011 [4]
	Kaushal 2008 [86]		Tobinick 2012 [5]
	Tobinick 2011 [4]		Lei 2013 [43]
	Siniscalchi 2014 [87]		King 2013 [42]
			Works 2013 [44]
2. TNF mediates neuropathic pain	Oka 1996 [253]	TNF Ab or TNF siRNA reduces neuropathic pain	Sommer 1998 [156] Ignatowski 1999 [159]
	Sommer 1998 [156]	Etanercept reduces neuropathic pain	Lindenlaub 2000 [157]
Rationale: Etanercept and other TNF inhibitors reduce neuropathic pain	Ignatowski 1999 [159]		Covey 2000 [160]
	Lindenlaub 2000 [157]		Sommer 2001 [158]
	Covey 2000 [160]		Ignatowski 2013 [165]
	Sommer 2001 [166]		Sommer 2001 [166]
	Martuscello 2012 [164]		Tobinick 2003–4 [167–170]
	Ignatowski 2013 [165]		Zanella 2008 [177]
			Cohen 2009 [171]
			Shen 2011 [65]
			Watanabe 2011 [178]
			Tobinick 2011 [4]
			Tobinick 2012 [5]
			Ohtori 2012 [172]
			Freeman 2013 [173]
			Sainoh 2013 [175]
			Kaufman 2013 [174]
			Coelho 2014 [179]

Table 2 continued

Pathophysiology and therapeutic rationale	References (exemplary)	Etanercept—effects	References
3. Excess TNF is centrally involved in the pathophysiology of chronic brain dysfunction in multiple disease states: (a) cerebral malaria; (b) TBI; (c) stroke; (d) Alzheimer's disease; (e) frontotemporal dementia; (f) post-surgery; (g) hepatic encephalopathy Rationale: Etanercept reduces cognitive impairment in disorders associated with excess TNF	Clark 1989, 1991 [51, 181] Goodman 1990 [52] Perry 2001 [53] Tarkowski 2003 [223] Sjogren 2004 [54] Tweedie 2007 [55] Kaushal 2008 [86] John 2008 [56] Clark 2010, 2012 [32, 33] Chio 2010 [45] Terrando 2010 [57] Frankola 2011 [59] Butterworth 2011 [58] Clark 2012 [34] Chastre 2012 [213] Cheong 2013 [60] Chio 2013 [61] Miller 2013 [62]	Etanercept reduces TNF-mediated cognitive impairment in Alzheimer's disease, other dementias, stroke, TBI, rheumatoid arthritis, sarcoidosis, hepatic encephalopathy, post status epilepticus	Tobinick 2006–2012 [10, 35, 68, 69, 146, 216, 218, 219]; Griffin 2008 [217] Shi (infliximab) 2011, 2011 [195, 196] Tobinick 2008 [204, 219] Tobinick 2011–12 [4, 5] Chio 2010 [45] Tobinick 2012 [5] Chen 2010 [206] Efferich 2010 [205] Bassi 2010 [207] Butterworth 2013 [67] Tobinick 2014 [11]
4. Stroke and TBI cause chronic intracerebral glial activation and neuroinflammation Rationale: Etanercept reduces glial activation and pathologic TNF concentration	Dubois 1988 [131] Myers 1991 [132] Pappata 2000 [133] Gentleman 2004 [134] Gerhard 2005 [135] Price 2006 [136] Kaushal 2008 [86] Folkersma 2011 [137] Ramlackhansingh 2011 [138] Johnson 2013 [139]	Etanercept inhibits glial activation and neuroinflammation	Marchand 2009 [64] Chio 2010 [45] Butterworth 2011 [58] Shen 2011 [65] Chastre 2012 [213] Roh 2012 [66] Butterworth 2013 [67]

siRNA small interfering RNA, *TBI* traumatic brain injury, *TNF* tumor necrosis factor

dysfunction is outlined in Table 2. The evidence, as reviewed in the previous and subsequent sections herein, can be separated into the following main categories:

2.8.1 Experimental Evidence in Multiple Models Suggests Pathologic Tumor Necrosis Factor (TNF) is Centrally Involved in the Pathophysiology of Stroke

Experimental evidence implicating TNF in stroke pathophysiology was published in 1994, and has continued through the present [80–89]. A recent study investigated the long-term consequences of subarachnoid hemorrhage (SAH) on behavior, neuroinflammation, and damage to gray and white matter in Wistar rats through day 21 post-insult [90]. Severe SAH induced significant gray- and white-matter damage and changes in multiple cytokines, including increased expression of TNF at 48 h post-insult [90]. Neuroinflammation, including microglial activation, was "very long-lasting and still present at day 21" and accompanied by changes in sensorimotor behavior [90].

2.8.2 Experimental Evidence in Multiple Models Provides Data Demonstrating Improvement in Stroke Outcome Through Inhibition of TNF

TNF was identified as a mediator of post-stroke focal ischemic brain injury 2 decades ago [80–82, 89]. Specific inhibition of TNF, using antibodies or other recombinant TNF inhibitors, was found to reduce neurological damage from stroke, improving stroke outcomes [80–82, 88, 89].

In 2013, inhibition of TNF using three different molecular approaches yielded favorable results in three separate animal models [42–44]. Researchers from Duke summarized the scientific rationale and their results as follows:

> Intracerebral hemorrhage is a devastating stroke sub-type characterized by a prominent neuroinflammatory response. Antagonism of pro-inflammatory cytokines by specific antibodies represents a compelling therapeutic strategy to improve neurological outcome in patients after intracerebral hemorrhage … Post-injury treatment with the TNF-alpha antibody CNTO5048 resulted in less neuroinflammation and improved functional outcomes in a murine model of intracerebral hemorrhage …. TNF-alpha does not serve as a simple "biomarker" of inflammation, but rather plays a central role in mediating and extending neuronal injury after insult … Monoclonal antibodies against TNF-alpha make sense as a therapeutic strategy in intracerebral hemorrhage due to the marked neuroinflammatory effects seen in this disease [43].

Increased peri-hematomal expression of TNF has been functionally associated with neurovascular injury in multiple species and experimental models of intracerebral hemorrhage (ICH) [91–96]. These findings are consistent with clinical reports that found elevated cerebrospinal fluid and plasma concentrations of TNF directly correlated with acute hematoma enlargement, edema development, and poor patient outcomes after ICH [97–102]. In contrast to the early clinical success of biologic inhibitors, which directly bind TNF as a decoy receptor, small molecule inhibitors of TNF signaling pathways remain largely unexplored after ICH. TNF induces biological activity via stimulation of the TNF receptors (TNFR1 and TNFR2) [103, 104]. Post-ICH administration of R-7050, a novel cell-permeable triazoloquinoxaline compound that prevents the association of TNFR with intracellular adaptor molecules [105], reduced vasogenic edema and improved neurological outcomes in a mouse model of ICH [42]. These studies raise the possibility that small molecule inhibitors of TNF-TNFR signaling may possess therapeutic potential after ICH.

A further mechanism to not only mitigate TNF-mediated actions and signaling after ICH but also to aid in defining their roles is to inhibit TNF generation. The controversial sedative, thalidomide, has immunomodulatory actions that are mediated, in large part, by lowering the rate of TNF synthesis [106, 107]. Recent analogs that more effectively achieve this include 3,6′-dithiothalidomide (3,6′-DT) [108], which readily enters the brain [109] and suppresses TNF synthesis post-transcriptionally at the level of translational regulation via the 3′-untranslated region of its messenger RNA (mRNA) [108, 110] as well as through down-regulation of the eukaryotic elongation initiation factor (eIF)-4E [111] to allow its rapid degradation.

In a mouse model of focal ischemic stroke in which brain TNF levels were found to be rapidly elevated within both ipsi- and contralateral brain, 3,6′-DT fully ameliorated this rise and reduced infarct volume, neuronal death, and neurological deficits [112]. This neuroprotection was accompanied by reduced inflammation, with 3,6′-DT lowering the expression of interleukin (IL)-1Beta and inducible nitric oxide synthase, reducing activated microglia/macrophages, astrocyte, and neutrophil numbers, and decreasing the expression of intercellular adhesion molecule (ICAM)-1 within ischemic brain tissue [112]. TNF plays a role in the induction of ICAM-1 expression and also promotes BBB leakage by inducing the expression of matrix metalloproteinase (MMP)-9 [113, 114], which degrades BBB tight junction proteins [115, 116]. Mitigating the rise in TNF by 3,6′-DT treatment suppressed the known TNF-induced activation of MMP-9 [117] and, thereby, decreased stroke-induced BBB disruption by preserving junction proteins [112]. In support of a major role of TNF in processes mediating stroke as well as TNF inhibition as the primary

mechanism for the neuroprotective action of 3,6′-DT, the ability of 3,6′-DT to decrease ischemic brain damage was abolished in mice lacking TNF receptors [112].

The mechanisms underlying the detrimental effects of TNF signaling after ICH remain poorly defined and could provide additional therapeutic targets upon elucidation. Emerging data suggest that TNF induces necroptosis, a novel form of cell death with characteristic features of apoptosis, necrosis, and type 2 autophagic death [118–121]. In an experimental model, hemorrhagic injury increased TNF expression and promoted necroptotic cell death in cultured glial cells [122]. This effect was reversed by inhibition of receptor-interacting serine/threonine-protein kinase (RIPK)-1, a multi-functional protein kinase that interacts with TNFR to activate the pro-inflammatory transcription factor, nuclear factor (NF)-κB [123–125]. In line with this finding, it was observed that necrostatin-1, a pharmacological inhibitor of RIPK [124, 125], similarly limited neurovascular injury and improved outcomes in a pre-clinical model of ICH [126]. This finding is also consistent with reports showing necrostatin-1 is neuroprotective in experimental models of ischemic stroke and TBI [125, 127, 128]. Taken together, these experimental results support the assertion that TNF induces detrimental effects after neurological injury and suggests that directed targeting of TNF and downstream signaling pathways may improve patient outcomes.

Additional research involving multiple animal models of stroke and TBI provides documentation of a favorable therapeutic response to TNF inhibition [42–45, 60, 61, 81, 82, 86, 129]. As an example, brain TNF levels were found to have elevated rapidly (within 1 h) following concussive (weight drop-induced) mild TBI in mice, and were maximal at 12 h [109]. Inhibition of this TBI-induced rise by administration of a single dose of the TNF synthesis inhibitor 3,6′-DT fully ameliorated cognitive impairments evaluated both 7 and 30 days later; supporting both a role for TNF in TBI-induced neuroinflammation/cognitive impairment and its targeting for treatment [109]. Most recently, inhibition of phosphoinositide 3-kinase delta, a molecule that controls intracellular TNF trafficking in macrophages, was shown to reduce TNF secretion and neuroinflammation and confer protection in a mouse cerebral stroke model [130].

2.8.3 Positron Emission Tomographic Brain Imaging and Pathologic Evidence Demonstrate that Chronic Glial Activation and Neuroinflammation May Last for Years after Stroke and Other Forms of Acute Brain Injury

In 1988, researchers used autoradiography to investigate the effects of cerebral infarction induced by unilateral middle cerebral artery occlusion in rats. The radiolabeled ligand PK11195 that binds primarily to activated microglia was used. Seven days after stroke, [3H]PK11195 bound significantly in the cortical and striatal regions surrounding the focus of cerebral infarction with smaller increases in the ventrolateral and posterior thalamic complexes and in the substantia nigra, all ipsilateral to the occlusion [131].

In 1991, increased [3H]PK11195 binding in the thalamus during the second week after experimentally induced stroke in rats was found using ex vivo autoradiography, at a time when [3H]PK11195 binding around the primary lesion was beginning to subside [132].

In 2000, a multi-national European academic collaboration of neurologists, neuroscientists, and nuclear medicine specialists demonstrated that brain inflammation may persist for months or years after stroke in humans [133]. The physicians and scientists investigated the potential of positron emission tomography (PET) using [11C]PK11195 to assess the microglial reaction in secondary thalamic lesions in patients with infarcts in the territory of the middle cerebral artery. All patients studied were found to have increased [11C]PK11195 binding in the ipsilateral thalamus, indicating microglial activation in projection areas remote from the primary lesion [133]. The only patient studied more than 7 months after stroke was a 50-year-old patient with a primary stroke involving the left temporo-parietal region, and he demonstrated bilateral thalamic microglial activation 24 months after stroke [133].

In 2004, an international collaboration of neuroscientists found pathological evidence of a long-term intracerebral inflammatory response after TBI in a series of patients who had sustained blunt head injury. They described microglial hyperplasia and hypertrophy with major histocompatibility complex (MHC) class II upregulation, and inflammatory changes up to 16 years after the injury [134].

In 2005, Gerhard et al. [135], in another international collaboration of academic scientists and physicians, studied a series of patients between 3 and 150 days after onset of ischemic stroke in order to measure the time course of microglial activation. Utilizing (R)-[11C]-PK11195 PET, they found that brain inflammation was long-lasting after stroke, with (R)-[11C]-PK11195 binding involving both the area of the primary lesion and areas distant from the primary lesion site [135]. They described the spread of the glial response beyond the ischemic core as closely resembling the progression of microglial activation in animal experiments, with "early recruitment of microglia in the ischemic border zone and later involvement of the neocortex and thalamus" [135].

In 2006, Price et al. [136], in a multi-center academic collaboration, used (R)-[11C]-PK11195 imaging to study a series of patients after stroke. Using this imaging

methodology, they documented persistent neuroinflammation in the stroke penumbra and elsewhere in the brain in patients following stroke, and recognized that this neuroinflammatory response might represent a therapeutic opportunity that extends beyond time windows traditionally reserved for neuroprotection [136].

In 2011, Folkersma et al. [137], studied microglial activation in patients with moderate and severe TBI using (R)-[^{11}C]-PK11195 brain PET, 6 months after trauma. In both whole-brain and regional analysis, increased (R)-[^{11}C]-PK11195 binding potential was found compared with age- and sex-matched healthy controls. From these series, increased (R)-[^{11}C]-PK11195 binding potential was found not only in the ipsilateral but also in the contralateral hemisphere, indicating prolonged and widespread microglia activation after TBI.

Subsequent studies, using either PET imaging or pathologic examination, have confirmed the existence of chronic intracerebral glial activation that has been documented to last for 17 years after even a single acute brain injury [138, 139].

Microglial imaging using (R)-[^{11}C]-PK11195 brain PET can be of meaningful clinical and diagnostic value in terms of visualization and quantification of active neuroinflammatory and neurodegenerative disease processes and in elucidation of the long-term effects of neuroinflammatory sequelae and its implications for neurological outcome [137]. Taken together, along with additional research showing that pathologic TNF mediates neurotoxicity in the ischemic penumbra, these data suggest that chronic microglial activation and neuroinflammation may be a common pathological response to stroke and other forms of acute brain injury [86, 133–140].

There is a need to understand the long-term relationship between late microgliosis and TNF. Although the PET data discussed in this section do not describe TNF actions or changes, PET imaging before and after therapeutic intervention with TNF inhibitors that can quantify and describe patterns of microglial activation promises to be a fertile area for future investigation. As suggested by Price et al. [136], the accumulating evidence indicates that chronic glial activation after acute brain injury represents a therapeutic target that persists far longer than the time windows traditionally reserved for neuroprotection. This evidence provides a scientific basis for considering pharmacologic therapeutic intervention that targets chronic glial activation months or years after stroke, and supports the plausibility of achieving a therapeutic response in patients with chronic post-stroke neurological dysfunction by targeting pathologic TNF concentration [86, 133–139].

2.8.4 Experimental Evidence Implicates TNF in the Neurotoxicity Produced by Glial Activation in the Stroke Penumbra

In an in vitro model of microglial activation and propagated neuron killing in the stroke penumbra, TNF inhibition using a soluble TNF receptor reduced neurotoxicity [86]. In addition, experimental data suggest that TNF functions as a gliotransmitter that is involved in the mechanisms whereby glia modulate synaptic transmission and neuronal network function [141–155].

2.8.5 Etanercept is Both a Potent TNF Inhibitor and an Inhibitor of Microglial Activation

The plausibility of beneficial effects of etanercept for treatment of chronic post-stroke neurological dysfunction is supported by the fact that, in addition to its known role as a potent biologic inhibitor of TNF, etanercept has also been shown to be capable of reducing glial activation in multiple experimental models [45, 64–67]. The known physiological effects of etanercept on TNF and glial activation make it a well matched candidate to address the chronic glial activation and pathologic TNF that may be a long-lasting consequence of stroke [45, 64–67, 86, 133, 135, 136].

2.9 Analogy

Review of the medical literature provides evidence supporting the plausibility of the results of the etanercept stroke studies by analogy, as discussed below.

2.9.1 Etanercept and Other Biologic TNF Inhibitors Reduce Neuropathic Pain

Statistically significant improvements in pain, including improvements in hyperesthesia, allodynia, pain associated with spasticity, post-stroke shoulder pain, and neuropathic pain were reported in the 617-patient stroke cohort [5]. These results are supported by a long series of experiments documenting the effects of etanercept and other biologic TNF inhibitors in experimental models and in the clinic.

In 1998 and thereafter, Sommer and colleagues [156–158], in a series of basic science experiments, demonstrated the central involvement of TNF in the pathophysiology of neuropathic pain and the favorable effects of anti-TNF antibody treatment in these models. In 1999, a separate group of investigators [159] showed that neuropathic pain was mediated by brain-derived TNF. Subsequent studies provided further supportive evidence [159–165]. In 2001, etanercept was shown to reduce hyperalgesia in experimental painful neuropathy [166]. In 2003 and 2004, the first human evidence of the effectiveness of etanercept

for treating neurological spinal pain was published [167–170]. Many of these early studies were performed by the authors and their colleagues. Subsequently, four randomized controlled clinical trials have provided favorable data supporting the efficacy of etanercept for neurological spinal pain, and TNF inhibition is emerging as a treatment strategy for intractable sciatica and other forms of intervertebral disc-related pain [171–176]. The accumulated evidence is substantial [65, 156–175, 177–179]. This evidence, taken together, suggests by analogy the plausibility of pain improvement following etanercept in patients with chronic post-stroke pain.

2.9.2 TNF is Centrally Involved in the Pathophysiology of Chronic Brain Dysfunction in Multiple Disease States

Statistically significant reduction in cognitive impairment is reported in the 617-patient stroke cohort following perispinal etanercept treatment [5]. The data included improvement in a standardized instrument, the Montreal Cognitive Assessment, with *p*-values less than 0.0001 immediately post-treatment and 1 and 3 weeks later [5]. The cognitive improvement documented in the etanercept stroke studies is supported, by analogy, by substantial scientific evidence that suggests that TNF is centrally involved in the pathophysiology of chronic brain dysfunction.

Beginning in the 1980s, and continuing into the present, TNF has been implicated in the pathophysiology of multiple diseases and disorders associated with chronic brain dysfunction, including cerebral malaria [32, 51, 56, 180–185]; TBI [45, 52, 60, 61, 129, 186, 187]; Alzheimer's disease [32, 34, 53, 55, 59, 149, 188–203], frontotemporal dementia [54]; primary progressive aphasia [62, 204]; sarcoidosis [205]; rheumatoid arthritis [206]; surgery-induced cognitive decline [57]; and a wide variety of additional diseases and disorders [32, 58, 67, 207]. For example, an increasing body of evidence supports a major role for central neuroinflammatory mechanisms in the pathogenesis of hepatic encephalopathy, a neuropsychiatric complication of both acute and chronic liver failure. Microglial activation in liver failure has been attributed to the accumulation of lactate in the brain, and focal accumulation of brain lactate is a common feature of stroke, TBI, and status epilepticus, conditions that are known to result in significant neuroinflammation [67, 208]. Neuroinflammation characterized by microglial activation and increased expression of pro-inflammatory cytokines in the brain has been reported in both human and experimental liver failure of diverse etiology, including viral hepatitis [208] and biliary cirrhosis [209], as well as in acute liver failure resulting from toxic [210] or ischemic [211] liver

injuries. Microglial activation and increased pro-inflammatory cytokine expression are significantly correlated with the grade of encephalopathy in these disorders. Moreover, slowing of hepatic encephalopathy progression has been demonstrated following inhibition of microglial activation by hypothermia [211] or minocycline [212] and following the use of anti-TNF strategies such as etanercept [213]. TNFR gene deletion delays the progression of hepatic encephalopathy in mice with acute liver failure resulting from toxic liver injury [210].

2.9.3 Infusion of Recombinant Human TNF Produced Focal Neurological Dysfunction in Early Human Studies, Supporting a Role of Excess TNF in the Pathogenesis of Such Disorders

Additionally, it is notable that among the 69 patients who participated in the early phase I studies of prolonged (24-h or 5-day) intravenous infusions of recombinant human TNF, three developed transient focal neurological symptoms. One patient developed diplopia, lethargy, and expressive dysphasia after receiving recombinant TNF at 2.0×10^5 U/m^2/d for 2 days, with return to baseline neurologic status within 48 h without sequelae [214]. The second study, involving a 24-h infusion of human recombinant TNF documented two cases of neurological toxicity, as follows:

> Two elderly patients had transient episodes of focal neurological deficits. One patient had an isolated loss of recent memory, while the other had transient expressive aphasia. No abnormalities were noted upon computerized tomography brain scan or cerebrospinal fluid analysis. In each case, the symptoms occurred near the completion of treatment and resolved without sequelae within 6 h. These two toxic events occurred at doses of 182 and 327 µg/m^2 and did not represent dose-limiting toxicity [215].

These early cases of focal neurological toxicity following TNF infusion provide further scientific support for the involvement of excess TNF in the pathophysiology of post-stroke neurological dysfunction and the perispinal etanercept results.

2.9.4 Specific Evidence Suggests that Etanercept has the Potential to Reduce Cognitive Impairment in Multiple Disorders Associated with Chronic Brain Dysfunction

Etanercept has demonstrated favorable effects in neuroinflammatory disorders, both in the clinic and in multiple experimental models [4, 5, 10, 35, 45, 58, 60, 61, 64–69, 146, 166–173, 177–179, 204, 207, 216–221].

TNF levels in the cerebrospinal fluid 25 times higher than in controls have been found in patients with Alzheimer's disease [222]. In patients with mild cognitive impairment (MCI) followed prospectively, "only MCI patients who progressed to Alzheimer's disease at follow up, showed significantly higher CSF levels of TNF-alpha than controls … Indicating that CNS inflammation is a early hallmark in the pathogenesis of AD" [223]. A later study from these investigators supported this conclusion regarding the role of TNF in Alzheimer's disease pathogenesis [224].

In 2006, the clinical results of a prospective, single-center, open-label, pilot clinical trial of perispinal etanercept for Alzheimer's disease was reported by the senior author and colleagues [216]. The authors included two neurologists, a rheumatologist, and an internist, and the study included 15 patients treated with perispinal etanercept weekly over a period of 6 months [216]. The main outcome measures included three standard instruments for measuring cognition: the Mini-Mental State Examination (MMSE), the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog), and the Severe Impairment Battery (SIB). There was significant improvement with treatment, as measured by all of the primary efficacy variables, through 6 months: MMSE increased by 2.13 ± 2.23 ($p < 0.003$), ADAS-Cog improved (decreased) by 5.48 ± 5.08 ($p < 0.006$), and SIB increased by 16.6 ± 14.52 ($p < 0.04$).

In 2008, rapid cognitive improvement in a patient with Alzheimer's disease following treatment with perispinal etanercept was reported by the senior author and a neurologist [218]. Sue Griffin, co-editor of the *Journal of Neuroinflammation*, reported her independent observations after witnessing rapid clinical improvement in additional patients with Alzheimer's disease following treatment with perispinal etanercept [217]. Subsequent publications by the senior author and colleagues documented cognitive improvement in patients with Alzheimer's disease and other forms of dementia following treatment with perispinal etanercept [10, 68, 69, 146, 204, 219].

In a basic science study conducted by the senior author and Stanford scientists and published in 2009, perispinal administration of radiolabeled etanercept followed by head-down positioning was discovered to deliver radiolabeled etanercept into the choroid plexus and cerebrospinal fluid within the cerebral ventricles within minutes of injection, as visualized by PET scan [31].

In 2010, Chio et al. [45] studied etanercept in an experimental model of TBI. They found that etanercept caused attenuation of TBI-induced cerebral ischemia, reduction of motor and cognitive function deficits, and reduction of microglial activation [45].

Chen et al. [206] studied the effects of anti-TNF treatment on cognition in 15 patients with rheumatoid arthritis over a period of 6 months with subcutaneous anti-TNF treatment: eight received etanercept 25 mg twice weekly and seven received adalimumab 40 mg twice monthly. Cognitive function determined by MMSE scores was significantly improved in the patient cohort [206].

Elfferich et al. [205] studied 343 sarcoidosis patients over a period of 6 months, with all patients completing the Cognitive Failure Questionnaire (CFQ) at baseline and at 6 months [206]. Patients were separated into three groups: (1) no immunomodulating drugs; (2) prednisone with or without methotrexate; and (3) anti-TNF drugs. Only patients receiving anti-TNF drugs demonstrated a significant improvement in CFQ score [205].

Chou et al. [225] presented the results of their review of medical and pharmacy claims data from January 2000 to November 2007 for a commercially insured cohort of 8.5 million adults throughout the USA. They derived a sub-cohort of 42,193 patients with a pre-existing diagnosis of rheumatoid arthritis. In this population of adults with rheumatoid arthritis, they found a 55 % decreased incidence in Alzheimer's in those patients treated with TNF inhibitors, but not with other disease-modifying agents used for treatment of rheumatoid arthritis [225]. When they further analyzed the risk according to the individual anti-TNF agent used, they found that only etanercept was significantly ($p = 0.024$) associated with reduced risk [226].

In 2011, Shi et al. [195, 196] reported cognitive improvement in a woman with Alzheimer's disease following intrathecal administration of infliximab, a chimeric TNF monoclonal antibody, following the favorable results of the use of infliximab in an experimental Alzheimer's model [195, 196].

In 2012, Gabbita et al. [227] found that early intervention with a small molecule inhibitor of TNF prevented cognitive deficits and improved the ratio of resting to reactive microglia in the hippocampus in a murine triple transgenic model of Alzheimer's disease. Belarbi et al. [228] found that a TNF protein synthesis inhibitor restored neuronal function and reversed cognitive deficits induced by chronic neuroinflammation. McNaull et al. [197, 229] and Butchart and Holmes [197, 229] discussed the rationale for TNF inhibition as a treatment approach for Alzheimer's disease in their review articles [197, 229].

Bassi and De Filippi [207] reported verbal, cognitive, and behavioral improvement in a patient with long-standing neurological dysfunction, in whom etanercept was used for treatment of psoriasis. The beneficial effect on cognition and social interaction was a surprising side effect of etanercept used to treat the cutaneous psoriasis [207].

In 2013, Cheong et al. [197, 229] studied etanercept in an experimental model of TBI. They found that

neurological and motor deficits, cerebral contusion, and increased brain TNF-alpha contents caused by TBI were attenuated by etanercept [60].

In 2014, Detrait et al. [197, 229] reported favorable effects of etanercept administered systemically in a basic science Alzheimer's model [230]. However, the only dose that was effective across all measures of efficacy was the highest dose, 30 mg/kg given every 2 days (for a total dose of 90 mg/kg given during the first week). This 90-mg/kg weekly dose is more than 100 times the normal human etanercept dose. Etanercept doses of 3 mg/kg every 2 days, about 15 times the usual human dose, were not effective. The lack of efficacy of systemically administered etanercept in this Alzheimer's disease model at doses closer to the usual human therapeutic dose is consistent with a previous Alzheimer's disease clinical trial in which etanercept administered systemically at a dose of 25 mg twice weekly was not found to be effective [231].

The totality of this evidence suggests, by analogy, the plausibility of cognitive improvement following perispinal administration of etanercept in patients with chronic post-stroke cognitive impairment.

2.9.5 Independent Eye-Witness Observations

Finally, rapid neurological improvement following perispinal etanercept has been witnessed first-hand by independent third parties, including several of the authors of this commentary as well as others [11, 35, 216, 217, 232]. A new report has documented that a single dose of perispinal etanercept produced an immediate, profound, and sustained improvement in expressive aphasia, speech apraxia, cognitive dysfunction, and left hemiparesis in a patient with chronic, intractable, debilitating neurological dysfunction present for more than 3 years after acute brain injury [11]. Replication of experimental results with validation by different observers is a time-honored cardinal scientific principle supporting the reliability of a scientific observation [39].

3 Conclusion

In summary, perispinal etanercept for post-stroke neurological and cognitive dysfunction satisfies all of Hill's nine criteria: strength of the association; consistency; specificity; temporality; biological gradient; biological plausibility; coherence; experimental evidence; and analogy.

The Oxford Centre for Evidence-Based Medicine (OCEBM) is widely regarded as an authority in the development of evidence-ranking schemes in medicine [233]. OCEBM documents "a growing recognition that observational studies—even case-series and *anecdotes* can

sometimes provide definitive evidence" and allows for "observational studies with dramatic effects to be 'upgraded'" with respect to level of evidence. The current evidence hierarchy standard promulgated by the OCEBM ranks observational studies that demonstrate dramatic effects as level 2 evidence [233]. The etanercept stroke studies, each of which documents dramatic clinical improvement following perispinal etanercept administration, therefore provide level 2 evidence of the effectiveness of perispinal etanercept for post-stroke neurological dysfunction [233–236]. The weight of the evidence calls for the initiation and funding of the exceedingly costly, large-scale, randomized controlled trials necessary to obtain US FDA approval of perispinal etanercept for these indications. The cost of clinical trials for brain disorders can exceed $US1 billion [237]. Until such trials are completed, the elaborated evidence and unmet medical need provide an ethical mandate that together support this off-label treatment approach [33, 40, 238–246]. With the additional weight of recent basic science studies reporting favorable effects of etanercept in a diverse group of brain disorders, and scientists from several independent academic centers reporting favorable effects of TNF inhibition in other stroke models, now is the time to seriously consider systematic testing of perispinal etanercept for brain injury, especially in stroke. Clinical trials should be directed at early and late post-stroke interventions that can validate the drug for potential future use.

4 Future Directions

On the 40th anniversary of the journal *Stroke*, leading stroke researchers met to devise and prioritize new ways of accelerating progress in reducing the risks, effects, and consequences of stroke [3]. Their consensus recommendations regarding stroke research included the following [3]:

> "[T]here is clearly a need to "do things differently" if there is to be a major advance in the development of new interventions … We need to scan the scientific landscape to embrace new ideas and approaches … Be alert to new models of disease that may vertically integrate basic, clinical, and epidemiological disciplines. For example, could advances in the understanding of infectious disease or inflammation dramatically change our thinking about stroke pathogenesis?" [3]

Scientific communities do not easily embrace new ideas, despite the calls of its leaders to do so [3, 36, 247–252]. As Wolinsky has stated, "the advancement of scientific knowledge is an uphill struggle against 'accepted

wisdom'" [36]. Recognition that chronic microglial activation, synaptic plasticity, and pathologic TNF concentration are therapeutic targets that may be therapeutically addressed for years following stroke and other forms of acute brain injury provides an exciting new direction for research and treatment.

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