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HEALTHY AGING RESEARCH

Supporting evidence for using Perispinal Etanercept to inhibit TNFα when treating neuropathologies including dementia, chronic stroke, neuropathic pain or traumatic brain injury: Clinical outcomes and role of TNF in neuropathologies other than Alzheimer's Dementia (Part III)

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Abstract

Part III examines the evidence for the involvement of the pro-inflammatory cytokine, Tumour Necrosis Factor-alpha (TNFα) in neuropathologies other than Alzheimer's Dementia (AD), and for using an anti-TNF therapy, Etanercept (ENBREL), to target and treat these health problems, including chronic stroke, neuropathic pain or traumatic brain injury (TBI). All of these can become chronic illnesses and are of major incidence with a grossly unmet need to improve their treatment. The three-part review presents the overwhelming scientific and medical basis as to why research studies and more trials to evaluate the use of the perispinally administered anti-TNFα drug, Etanercept, are justified to allow it to become a front-line standard therapy. Part I established the role of TNFα as a direct regulator of neuronal synaptic activity. It is in this context, as detailed below, that targeting TNF in the brain holds major significance, not only for treating the dementias, but also its great benefits in reducing long term pain during rehabilitation from TBI or chronic stroke. Given the increasing numbers of families afflicted with Alzheimer's disease, chronic stroke, neuropathic pain or TBI, clinical studies are now imperative to improve the treatment of these life-threatening and debilitating illnesses.

Citation: Ralph SJ, Clark I (2015) Supporting evidence for using Perispinal Etanercept to inhibit TNFα when treating neuropathologies including dementia, chronic stroke, neuropathic pain or traumatic brain injury: Clinical outcomes and the role of TNF in neuropathologies other than Alzheimer's Dementia (Part III). Healthy Aging Research 4:17. doi:10.12715/har.2015.4.17.

Received: December 22, 2014; **Accepted:** January 30, 2015; **Published:** February 24, 2015.

Competing interests: The authors have declared that no competing interests exist.

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Evidence from clinical disease states and genetic studies supports a role for TNF not only in dementia but also in depression, migraine and size of the hippocampus

The neurological consequence of excess brain concentrations of TNF (usually defined by cerebrospinal fluid (CSF) concentrations exceeding the upper limit of the homeostatic range required for normal physiological function) is to inhibit neurotransmission. Examples of the normal limits being exceeded include the increased brain and CSF levels of TNF reported in human and experimental meningitis [1, 2], cerebral malaria [3], Alzheimer's Dementia (AD) [4, 5], frontotemporal dementia (FTD) [6] and Parkinson's disease [7]. Where examined, CSF and serum levels of TNF did not correlate, implying distinct production within either of these two compartments.

Several studies have shown that production of TNFα is increased in the brain tissue of patients suffering Late-Onset Alzheimer's Dementia (LOAD) and plays an important role in the pathogenesis of this disease. Epidemiological studies also suggest that patients taking anti-inflammatory drugs have a decreased risk of developing AD [8, 9]. Functional polymorphisms

in the TNFα promoter can also affect immune response, inflammation, tissue injury and likely relate to the susceptibility to migraine and AD [10–12]. Other genetic studies have linked specific mutations within the TNF genes with variations associated with beta-amyloid levels in the CSF [13], as well as predicting the hippocampus volume in healthy individuals [14]. Also, two meta-analyses have shown that inflammatory marker levels such as C-reactive protein (CRP), interleukin (IL)-6 and TNFα were increased in depressed persons compared with non-depressed subjects, and this was further confirmed in elderly depressed patients in a more recent study [15]. Thus, considerable research connects TNF with the pathogenesis of major depression. It is therefore not surprising that there are current clinical trials to determine the link between depression, stress and the onset of Alzheimer's. Increased serum levels of a range of inflammatory cytokines, including TNF, have also already been documented in such patients [16–18] as have serum levels of soluble TNF receptors, which are less labile, and therefore more reliable, markers of enhanced TNF activity [19,20]. Studies showing increased TNF levels in depression have since been extended to samples of various brain regions collected at autopsy, with the same conceptual outcome [21]. Indeed, a recent meta-analysis covering 24 studies has reported significantly higher concentrations of TNF and IL-6 in the state of mental depression compared with control subjects [22].

A further clue to the chronic inflammatory process of the innate immune system in the brain being associated with AD comes from two recent articles and an accompanying editorial [23] identifying mutations in the *TREM2* gene, an innate immune receptor strongly linked to AD, involved in A_{β} clearance.

A very pertinent study by Dr Clive Holmes *et al.* [24] has reported that both acute and chronic systemic inflammation was associated with increased serum levels of TNF and increased cognitive decline in Alzheimer's patients, and they are currently carrying out clinical trials (A phase II, double-blind, placebo-controlled study of the safety and tolerability of Etanercept in patients with Alzheimer's disease. US ClinicalTrials.gov identifier: NCT01068353) using subcutaneous administration of Etanercept rather than the Tobinick perispinally administered patented therapy [25–31] to determine its effects on cognitive function in 40 AD patients (55 years and older). The phase II trial was supported by the University of Southampton, funded by the UK National Health Service, and also supported by the pharmaceutical giant Pfizer. Hence, support and interest does exist for researching Etanercept therapy for Alzheimer's. In the Holmes *et al.* study, Etanercept (50 mg doses) was given as a once weekly subcutaneous injection over a six-monthly period. The results from this study were announced at the Copenhagen Alzheimer's Association International Conference in July 2014, which showed that the course of Etanercept treatment halted the progression of AD in those patients receiving it, whereas the placebo control continued to show a progressive decline in their cognitive functions [32]. In addition, the Etanercept treated group showed marked improvements in their neuropsychiatric behaviour inventory (NPI) scores, indicating more active and engaging/positive attitudes to life. These double blind, randomised clinical trial results are consistent with the observations found for rheumatoid arthritis patients using anti-TNFs [33], and if validated, are of immense importance for humanity [32].

A problem with the UK study was that it utilised abdominal subcutaneous injection of Etanercept, which would greatly reduce drug availability and access into the brain of treated patients. This contrasts with the perispinal route employed by Tobinick, used in a phase I/II trial of Etanercept at Griffith University, Gold Coast, Australia. There is overwhelming evidence supporting the delivery of perispinally administered Etanercept travelling via the Batson's plexus to directly gain entry into the brain based on radiological imaging and/or anatomical studies [29,30, 34].

In every current model for the different neurodegenerative diseases, including Alzheimer's models in animals, substantial evidence exists that anti-TNF therapy ameliorates the disease, blocks TNF in the brain and can even reduce amyloid plaque formation. For example, the use of anti-TNF targeted monoclonal antibody therapy has been shown to protect the brain from ischaemia-induced stroke in animal studies [35]. Marchand *et al.* [36] proposed pharmacological targeting of TNFα as a therapy for spinal cord injury and Chio *et al.* [37] and Chen *et al.*

[38] have shown that Etanercept is an effective treatment reducing tissue damage after traumatic brain injury (TBI), improving locomotor function. Similar results were obtained in models of brain injury involving experimental subarachnoid haemorrhage [39]. The study of Shi *et al.* [40] demonstrated that intracerebroventricular injection of the monoclonal TNFα antibody Infliximab, had positive effects on the pathological features of AD in the mouse model, APP/PS1 double transgenic mice. They found that Infliximab administration reduced the levels of TNFα, amyloid plaques, and tau phosphorylation as early as three days after daily injection of 150 μg Infliximab per day. The authors concluded that this provided strong support for its similar use for Alzheimer's in humans. Tweedie *et al.* [41] from the Drug Design & Development Section of the National Institutes of Health (NIH) in the USA have also proposed TNFα inhibition as a treatment strategy for the neurodegenerative disorders.

Safety of the perispinal procedure for administering Etanercept therapy – evidence from treating neuropathic pain

Doctors working at three clinical branches of the Institute for Neurological Research, based in California and Florida, USA, have been treating patients with lower back pain and have over a decade of clinical experience utilising the Perispinal Etanercept therapy. They have reported successfully treating over 3000 patients with intractable disc-related pain ([27]; reviewed in [30]). In spite of having administered nearly 10,000 injections of Etanercept, via the perispinal delivery route, they have never encountered an adverse reaction or response to the needle injection procedure. This speaks to its safety, as a very benign procedure [30]. Regarding the safety aspect of the treatment, it should also be pointed out that in their desperation caregivers have been travelling from around the globe either to the USA or to Nicaragua, to either obtain therapy or receive medical training to administer this therapy to their loved ones. Although Etanercept administration by non-medically qualified persons is not to be encouraged, it does indicate that the technique is relatively safe and can be administered to good effect by laypersons, much like insulin injections for diabetes.

In several clinical studies establishing Etanercept as a treatment for back pain, it has been delivered by epidural injection directly into the cerebrospinal fluid (CSF) and animal and human safety studies have consistently revealed no behavioural, neurologic, or histologic evidence of drug-related toxicity. Significant improvements in leg and back pain were collectively noted for the Etanercept-treated patients, but not for the saline-treated groups, one month after treatment [42]. Another study, in Australia, using epidural injection of Etanercept to treat back pain has been reported [43], originally presented at the annual meeting of the International Society for the Study of the Lumbar Spine at Spine Week 2012, Amsterdam, Netherlands. In this double-blinded, randomised clinical trial, Freeman *et al.* treated 49 subjects with persistent lumbosacral radicular pain of between six and 26 weeks duration. Although they used lower doses of Etanercept, these were directly injected into the CSF (providing a greater localised dose) and they reported that this was safe, well tolerated, and 63% of the Etanercept group recorded that they were "improved" or "very much improved" [43].

The low doses used in the Cohen *et al.* report [42] and the Freeman *et al.* [43] trial for alleviating back pain involved directly injecting into the CSF in line with the targeting of the spinal cord via epidural injection. However, this approach is a much more invasive procedure, necessitating specialist clinical involvement, whereas the perispinal approach is much less invasive, lower risk and more readily amenable to administering by doctors within the context of their general practice. This should be the potential outcome of any clinical trials in the event that Perispinal Etanercept therapy proves to be successful, making the treatment more widespread and readily available to the public by trained general practitioners. More recently, and for the same reasons outlined above, anti-TNF therapy by similar means has been proposed as an early stage intervention in spinal cord injury [44], pointing to the widening applications of this therapy.

The drug, Etanercept, has generally proved to be very safe and well tolerated and has been used to treat many millions of people worldwide for arthritic-

related autoimmune diseases. Recently, meta-analyses of large scale randomised clinical trials of over 30,000 patients showed that there is no increased risk of cancer from Etanercept treatment [45, 46]. A review of observational research designs has revealed a slightly higher risk of infection within the first few months of initiating anti-TNF treatment [47–49], followed by a progressive reduction. A recent report analysing a large cohort of 16,506 patients treated with anti-TNFα showed around 7% serious infections most of which were pneumonia, skin and soft tissue infections [50]. Overall, Etanercept has a very well publicised safety record and it is continuing to be very widely used, despite its significant purchase price.

Perispinal Etanercept therapy for chronic stroke or TBI-induced impairment and recovery

Brain injury from stroke and traumatic brain injury (TBI) often results in a persistent neuroinflammatory response in the injury penumbra and currently no drug treatment exists that is specifically approved to treat the spectrum of chronic neurological dysfunction that affects the millions of survivors with chronic stroke. Given that stroke is the second highest cause of morbidity and functional disability worldwide and a common cause of death, this represents a major health issue globally. Several recent studies have shown linkage associations of TNF promoter mutations with increased risk of ischaemic stroke [10, 51–53]. The results of stroke include microglial activation and excess levels of TNF that can persist from hours to days and patients with ischaemic stroke classified as cardio-embolic compared to other subtypes showed significantly higher median plasma levels of TNFα, IL-6 and IL-1β [54]. A recent study of focal ischaemia in mice caused by occluding their left middle cerebral artery (MCA), inducing an infarct also showed that systemic anti-TNF therapy significantly improved functional outcomes [55]. Thus, the previous experimental data provide overwhelming evidence that anti-TNF therapy ameliorates microglial activation and can mitigate the adverse synaptic effects of excessive TNF (see Part I and II and preceding sections above).

The most highly supportive study comes from a recent large open label trial that reported the results of a systematic examination of the clinical responses following perispinal administration of Etanercept in a cohort of over 600 patients with chronic neurological dysfunction after stroke or TBI [31, 56]. Highly significant improvements across the board in motor impairment, spasticity, sensory impairment, cognition, psychological/behavioural function, aphasia and pain were noted in the stroke group, with a wide variety of additional clinical improvements noted in individuals, such as reductions in pseudobulbar affect and urinary incontinence. Improvements in multiple domains were typical. Surprisingly, significant improvements continued irrespective of the length of time before the Perispinal Etanercept treatment was initiated. In fact, there was evidence of a strong treatment effect even in the subgroup of patients treated more than ten years after stroke or TBI. In the TBI cohort, motor impairment and spasticity were statistically significantly reduced. These studies provide overwhelming evidence that perispinal injection of Etanercept is safe and offers consistent improvements in the cognitive and neuromuscular function of a very high percentage of patients with chronic stroke or TBI.

Alternative therapies for age-related and other neuropathologies

Given the overwhelming scientific rationale and substantial evidence justifying research trials testing the Perispinal Etanercept (PSE) therapy as outlined above, and the dismal failure of recent drugs like Dimebon [57] and the amyloid targeted antibody-based therapies, other alternative therapies are mostly irrelevant to the proposed Etanercept research trials. As pointed out in a previous review [58], many of the other therapies (e.g. intravenous immunoglobulin; IV-IG), sigma receptor agonists, alpha-7 adrenergic receptor modulators) and more recently, progranulin modulators [59] probably act indirectly by inhibiting TNF and its receptor signalling pathways anyway.

Conclusions

Everyone is facing the prospect of developing Alzheimer's disease or a related dementia and the risk increases so that by the time we reach 80–90 years of age, nearly one in two people will develop it, as the third leading cause of death. Within the next 10 years, it will become the leading health issue and major cause of death, unless research into its treatment can soon produce successful treatments. The overwhelming volume of supportive evidence outlined herein not only identifies that Perispinal Etanercept (PSE) therapy can help to improve the cognitive function of dementia patients, but other brain disorders as well, including neuropathic pain, and that it is very safe, effective and a non-invasive procedure. Worldwide trials should be supported that develop potential breakthrough therapies such as Etanercept for dementia and for rehabilitation of chronic stroke, neuropathic pain or brain trauma victims. It is already proving its worth in treating patients throughout the USA and UK. Many medical practitioners have witnessed firsthand the safe, low risk procedure and the ensuing improvements in the general demeanour of patients who were given Etanercept treatment. Recent discussions in the medical literature are supporting the rights of patients to seek out off-label drug treatments for related illnesses [60, 61]. It is in the public interest to promote greater awareness and support for properly conducted randomised clinical research trials assessing Perispinal Etanercept (PSE) treatment (http://www.pseag.com).

References

1. Møller K, Tofteng F, Qvist T, Sahl C, Sønderkaer S, Pedersen BK. Cerebral output of cytokines in patients with pneumococcal meningitis. Crit Care Med. 2005;33(5):979-83.
2. Barichello T, Dos Santos I, Savi GD, Florentino AF, Silvestre C, Comim CM, et al. Tumor necrosis factor alpha (TNF-alpha) levels in the brain and cerebrospinal fluid after meningitis induced by *Streptococcus pneumoniae*. Neurosci Lett. 2009;467:217-9.
3. John CC, Panoskaltsis Mortari A, Opoka RO, Park GS, Orchard PJ, Jurek AM, et al. Cerebrospinal fluid cytokine levels and cognitive impairment in cerebral malaria. Am J Trop Med Hyg. 2008;78(2):198-205.
4. Tarkowski E, Liljeroth AM, Minthon L, Tarkowski A, Wallin A, Blennow K. Cerebral pattern of pro- and anti-inflammatory cytokines in dementias. Brain Res Bull. 2003;61(3):255-60.
5. Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. J Neurol Neurosurg Psych. 2003;74(9):1200-5.
6. Sjögren M, Folkesson S, Blennow K, Tarkowski E. Increased intrathecal inflammatory activity in frontotemporal dementia: pathophysiological implications. J Neurol Neurosurg Psych. 2004;75(8):1107-11.
7. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from Parkinsonian patients. Neurosci Lett. 1994;165(1-2):208-10.
8. Coté S, Carmichael PH, Verreault R, Lindsay J, Lefebvre J, Laurin D. Nonsteroidal anti-inflammatory drug use and the risk of cognitive impairment and Alzheimer's disease. Alzheimers Dement. 2012;8(3):219-26.
9. Vlad SC, Miller DR, Kowall NW, Felson DT. Protective effects of NSAIDs on the development of Alzheimer disease. Neurology. 2008;70(19):1672-7.
10. Gu L, Yan Y, Long J, Su L, Hu Y, Chen Q, et al. The TNF-alpha-308G/A polymorphism is associated with migraine risk: A meta-analysis. Exp Ther Med. 2012;3(6):1082-6.
11. Ardebili SM, Yeghaneh T, Gharesouran J, Rezazadeh M, Farhoudi M, Ayromlou H, et al. Genetic association of TNF-alpha-308 G/A and -863 C/A polymorphisms with late onset Alzheimer's disease in Azeri Turk population of Iran. J Res Med Sci. 2011;16(8):1006-13. Di Bona D, Candore G, Franceschi C, Licastro F, Colonna-Romano G, Camma C, et al. Systematic review by meta-analyses on the possible role of TNF-alpha polymorphisms in association with Alzheimer's disease. Brain Res Rev. 2009;61(2):60-8.
12. McCusker SM, Curran MD, Dynan KB, McCullagh CD, Urquhart DD, Middleton D, et al. Association between polymorphism in regulatory region of gene encoding tumour necrosis factor alpha and risk of Alzheimer's disease and vascular dementia: a case-control study. Lancet. 2001;357(9254):436-9.
13. Laws SM, Perneczky R, Wagenpfeil S, Muller U, Forstl H, Martins RN, et al. TNF polymorphisms in Alzheimer disease and functional implications on CSF beta-amyloid levels. Hum Mutat. 2005;26(1):29-35.
14. Baune BT, Konrad C, Grotegerd D, Suslow T, Ohrmann P, Bauer J, et al. Tumor necrosis factor gene variation predicts hippocampus volume in healthy individuals. Biol Psychiatry. 2012;72(8):655-62.
15. Vogelzangs N, Duivis HE, Beekman AT, Kluft C, Neuteboom J, Hoogendijk W, et al. Association of

depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry. 2012;2:e79.

16. Hestad KA, Tønseth S, Støen CD, Ueland T, Aukrust P. Raised plasma levels of tumor necrosis factor alpha in patients with depression: normalization during electroconvulsive therapy. J ECT. 2003;19(4):183-8.
17. Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31(5):1044-53.
18. Himmerich H, Fulda S, Linseisen J, Seiler H, Wolfram G, Himmerich S, et al. Depression, comorbidities and the TNF-alpha system. Eur Psychiatry. 2008;23(6):421-9.
19. Grassi-Oliveira R, Brietzke E, Pezzi JC, Lopes RP, Teixeira AL, Bauer ME. Increased soluble tumor necrosis factor-alpha receptors in patients with major depressive disorder. Psychiatry Clin Neurosci. 2009;63(2):202-8.
20. Diniz BS, Teixeira AL, Ojopi EB, Talib LL, Mendonca VA, Gattaz WF, et al. Higher serum sTNFR1 level predicts conversion from mild cognitive impairment to Alzheimer's disease. J Alzheimers Dis. 2010;22(4):1305-11.
21. Dean B, Tawadros N, Scarr E, Gibbons AS. Regionally-specific changes in levels of tumour necrosis factor in the dorsolateral prefrontal cortex obtained postmortem from subjects with major depressive disorder. J Affect Disord. 2010;120(1-3):245-8.
22. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010;67(5):446-57.
23. Neumann H, Daly MJ. Variant TREM2 as Risk Factor for Alzheimer's Disease. N Engl J Med. 2013;368(2):182-4.
24. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, et al. Systemic inflammation and disease progression in Alzheimer disease. Neurology. 2009;73(10):768-74.
25. Tobinick EL, Gross H, Weinberger A, Cohen H. TNF-alpha modulation for treatment of Alzheimer's disease: A 6- month pilot study. MedGenMed. 2006;8(2):25.
26. Tobinick EL. Perispinal etanercept for neuroinflammatory disorders. Drug Discov Today. 2009;14)3–4):168-77.
27. Tobinick EL, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. J Neuroinflamm. 2008;5:2.
28. Tobinick EL, Chen K, Chen X. Rapid intracerebroventricular delivery of Cu-DOTA-etanercept after peripheral administration demonstrated by PET imaging. BMC Res Notes. 2009;2:28.
29. Tobinick E. Tumour necrosis factor modulation for treatment of Alzheimer's disease: rationale and current evidence. CNS Drugs. 2009;23(9):713-25.
30. Tobinick E. Perispinal etanercept: a new therapeutic paradigm in neurology. Expert Rev Neurother. 2010;10(6):985-1002.
31. Tobinick E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. CNS Drugs. 2011;25(2):145-55.
32. Coghlan A. Drug stalls attack of Alzheimer's. New Sci. 2014;223(2978):10-1.
33. Chou RC, Kane, M.A., Ghimire, S., Gautam, S. Tumor Necrosis Factor inhibition reduces the incidence of Alzheimer's disease in rheumatoid arthritis patients. Presented at the American College of Rheumatology Annual Scientific Meeting; 2010.Abstract No:640
34. Griessenauer CJ, Raborn J, Foreman P, Shoja MM, Loukas M, Tubbs RS. Venous drainage of the spine and spinal cord: a comprehensive review of its history, embryology, anatomy, physiology, and pathology. Clin Anat. 2015;28(1):75-87.
35. Sumbria RK, Boado RJ, Pardridge WM. Brain protection from stroke with intravenous TNFalpha decoy receptor-Trojan horse fusion protein. J Cereb Blood Flow Metab. 2012;32(10):1933-8.
36. Marchand F, Tsantoulas C, Singh D, Grist J, Clark AK, Bradbury EJ, et al. Effects of Etanercept and Minocycline in a rat model of spinal cord injury. Eur J Pain. 2009;13(7):673-81.
37. Chio CC, Lin JW, Chang MW, Wang CC, Kuo JR, Yang CZ, et al. Therapeutic evaluation of etanercept in a model of traumatic brain injury. J Neurochem. 2010;115(4):921-9.
38. Chen KB, Uchida K, Nakajima H, Yayama T, Hirai T, Watanabe S, et al. Tumor necrosis factor-alpha antagonist reduces apoptosis of neurons and oligodendroglia in rat spinal cord injury. Spine. 2011;36(17):1350-8.
39. Zhang BF, Song JN, Ma XD, Zhao YL, Liu ZW, Li Y, et al. Etanercept alleviates early brain injury following experimental subarachnoid hemorrhage and the possible role of tumor necrosis factor-alpha and c-Jun N-terminal kinase pathway. Neurochem Res. 2014. Epub 27 Dec 2014.
40. Shi JQ, Shen W, Chen J, Wang BR, Zhong LL, Zhu YW, et al. Anti-TNF-alpha reduces amyloid plaques and tau phosphorylation and induces CD11c-positive dendritic-like cell in the APP/PS1 transgenic mouse brains. Brain Res. 2011;1368:239-47.
41. Tweedie D, Sambamurti K, Greig NH. TNF-alpha inhibition as a treatment strategy for neurodegenerative disorders: new drug candidates and targets. Curr Alzheimer Res. 2007;4(4):378-85.
42. Cohen SP, Bogduk N, Dragovich A, Buckenmaier CC, 3rd, Griffith S, Kurihara C, et al. Randomized, double-blind, placebo-controlled, dose-response, and preclinical safety study of transforaminal epidural etanercept for the treatment of sciatica. Anesthesiology. 2009;110(5):1116-26.

43. Freeman BJ, Ludbrook GL, Hall S, Cousins M, Mitchell B, Jaros M, et al. Randomized, double-blind, placebo-controlled, trial of transforaminal epidural etanercept for the treatment of symptomatic lumbar disc herniation. Spine. 2013;38(23):1986-94.

44. Esposito E, Cuzzocrea S. Anti-TNF therapy in the injured spinal cord. Trends Pharmacol Sci. 2011;32(2):107-15.

45. Moulis G, Sommet A, Bene J, Montastruc F, Sailler L, Montastruc JL, et al. Cancer risk of anti-TNF-alpha at recommended doses in adult rheumatoid arthritis: a meta-analysis with intention to treat and per protocol analyses. PloS One. 2012;7(11):e48991.

46. Lopez-Olivo MA, Tayar JH, Martinez-Lopez JA, Pollono EN, Cueto JP, Gonzales-Crespo MR, et al. Risk of malignancies in patients with rheumatoid arthritis treated with biologic therapy: a meta-analysis. JAMA. 2012;308(9):898-908.

47. Askling J, Baecklund E, Granath F, Geborek P, Fored M, Backlin C, et al. Anti-tumour necrosis factor therapy in rheumatoid arthritis and risk of malignant lymphomas: relative risks and time trends in the Swedish Biologics Register. Ann Rheum Dis. 2009;68(5):648-53.

48. Askling J, Bongartz T. Malignancy and biologic therapy in rheumatoid arthritis. Curr Opin Rheumatol. 2008;20(3):334-9.

49. Askling J, Dixon W. The safety of anti-tumour necrosis factor therapy in rheumatoid arthritis. Curr Opin Rheumatol. 2008;20(2):138-44.

50. Grijalva CG, Chen L, Delzell E, Baddley JW, Beukelman T, Winthrop KL, et al. Initiation of tumor necrosis factor-alpha antagonists and the risk of hospitalization for infection in patients with autoimmune diseases. JAMA. 2011;306(21):2331-9.

51. Cui G, Wang H, Li R, Zhang L, Li Z, Wang Y, et al. Polymorphism of tumor necrosis factor alpha (TNF-alpha) gene promoter, circulating TNF-alpha level, and cardiovascular risk factor for ischemic stroke. J Neuroinflammation. 2012;9(1):235.

52. Gu L, Wu G, Long J, Su L, Yan Y, Chen Q, et al. The role of TNF-alpha 308G>A Polymorphism in the risk for ischemic stroke. Am J Med Sci. 2013;345(3):227-33.

53. Gu L, Su L, Wu G, Chen Q, Yan Y, Xie J, et al. Association between TNF-delta 238G/A polymorphisms and the risk of ischemic stroke. Int J Neurosci. 2013;123(1):1-6.

54. Licata G, Tuttolomondo A, Di Raimondo D, Corrao S, Di Sciacca R, Pinto A. Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke. Thromb Haemost. 2009;101(5):929-37.

55. Lambertsen KL, Østergaard K, Clausen BH, Hansen S, Stenvang J, Thorsen SB, et al. No effect of ablation of surfactant protein-D on acute cerebral infarction in mice. J Neuroinflammation. 2014;11:123.

56. Tobinick E, Kim NM, Reyzin G, Rodriguez-Romanacce H, Depuy V. Selective TNF inhibition for chronic stroke and traumatic brain injury: an observational study involving 629 consecutive patients treated with perispinal etanercept. CNS Drugs. 2012;26(12):1051-70.

57. Bezprozvanny I. The rise and fall of Dimebon. Drug News Perspect. 2010;23(8):518-23.

58. Clark IA, Alleva LM, Vissel B. The roles of TNF in brain dysfunction and disease. Pharmacol Ther. 2010;128:519-48.

59. Tang W, Lu Y, Tian QY, Zhang Y, Guo FJ, Liu GY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science. 2011;332(6028):478-84.

60. Boumil MM. Off-label marketing and the First Amendment. N Engl J Med. 2013;368(2):103-5.

61. Lowenthal J, Hull SC, Pearson SD. The ethics of early evidence--preparing for a possible breakthrough in Alzheimer's disease. N Engl J Med. 2012;367(6):488-90.