Title
Modulating neuroinflammation in Alzheimer's Disease and Multiple Sclerosis with TREM2 agonist antibodies

Article
Etxeberria A et al. (2024) Neutral or Detrimental Effects of TREM2 Agonist Antibodies in Preclinical Models of Alzheimer's Disease and Multiple Sclerosis. J Neurosci 44:e2347232024

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Alzheimer's Disease (AD) and multiple sclerosis (MS) are neurodegenerative diseases, which feature aberrant inflammatory processes and accumulation of age-related disability. Amyloid-β plaques and neurofibrillary tangles in AD and demyelinated lesions in MS are paradigmatic features in these conditions with activated resident macrophages, known as microglia, being implicated in both. In fact, several lines of evidence point to microglia as central mediators of inflammation in AD and MS through a variety of mechanisms, including release of pro-inflammatory cytokines and clearance of myelin debris (Sen et al., 2022; Zhang et al., 2023). Consequently, microglia have emerged as promising therapeutic targets for these diseases.

Triggering receptor expressed by myeloid cells 2 (TREM2) is a trans-membrane protein and a key regulator of microglia (Ulland and Colonna, 2018; Zhang et al., 2023). TREM2 enables microglia to respond to pathological insults by recognising damage-associated lipids, initiating phagocytosis, increasing energy metabolism, and promoting proliferation (Schlepckow et al., 2023; Zhang et al., 2023). Cleavage of TREM2 attenuates this response, releasing soluble TREM2 (sTREM2), which can, in turn, promote the survival and migration of nearby microglia (Ulland and Colonna, 2018).

Previous studies using models of AD and MS have shown that TREM2 is required for microglia to transition from a resting surveillance state to disease-associated microglia (DAM), which actively engage with core features of disease pathology. In AD models, TREM2 enables DAM to form protective barriers around amyloid-β plaques (Schlepckow et al., 2023). In demyelination models, used to study MS, TREM2 influences microglial recruitment to demyelination sites, myelin debris clearance, and remyelination

(Cignarella et al., 2020). In this way, TREM2 is thought to regulate the balance between the protective and harmful effects of microglia forming the basis of research aimed at modulating TREM2 expression.

In *Trem2*-knockout Alzheimer's disease (AD) models, microglia remain in a surveillance state, resulting in increased expression of pro-inflammatory mediators, greater amyloid-β and tau pathology, and more severe neurodegeneration (Zhang et al., 2023). These findings supported the hypothesis that TREM2 overexpression slows AD progression, but results have been mixed. Some studies showed improved amyloid-β clearance and reduced tau pathology, while others showed no benefit (Zhang et al., 2023; Etxeberria et al., 2024). Similarly, TREM2 agonist antibodies had varied outcomes, from increased amyloid-β clearance to heightened tau pathology and neuritic dystrophy (Schlepckow et al., 2023). In demyelination, TREM2 loss hinders microglial recruitment and clearance of myelin debris, while overexpression promotes debris clearance (Sen et al., 2022). TREM2 agonist antibodies promote remyelination by increasing oligodendrocyte precursor cell density in demyelinated areas (Cignarella et al., 2020). However, stabilising TREM2, by preventing its cleavage into soluble TREM2 (sTREM2) has yielded both positive and negative effects on demyelination (Etxeberria et al., 2024).

The study by Etxeberria *et al.* addresses these discordant findings by investigating the effects of TREM2 agonist antibodies in AD and demyelination models (Etxeberria et al., 2024). As earlier studies focused on the mouse *Trem2* gene and used antibodies with varying structures and off-target effects, Etxeberria et al. employed human cell cultures

and transgenic mice expressing human TREM2 (hTREM2), along with an agonist antibody (Para.09) that binds specifically to a unique hTREM2 epitope. This approach is more precise and relevant for evaluating therapeutic potential in humans.

The authors identified the optimal Para.09 dose by measuring reduced sTREM2 levels in mouse brain and plasma as a proxy for TREM2 activity. In amyloidosis mice, Para.09 increased microglial proliferation, measured using RNA-seq and quantitative fluorescent Iba1 labelling (Etxeberria et al., Fig. 4B,C). High-resolution 3D co-labelling indicated that interactions between microglia and amyloid-β plaques were strengthened after this exposure (Etxeberria et al., Fig. 4D). Prolonged Para.09 treatment (weekly administration for three months) showed no significant benefits on tau or amyloid-β pathologies. Despite evidence of microglial activation, no effect on microglia-plaque interactions or associated neuritic dystrophy (Etxeberria et al., Fig. 7) was observed.

The authors proposed that Para.09 might reduce tau pathology better than amyloid-β, as *Trem2* deletion worsens tau pathology and TREM2 is upregulated in advanced AD. However, despite evidence of TREM2 engagement, after four months of treatment in tauopathy and amyloidosis mice, Para.09 showed no impact on microglia-plaque interactions, neuritic dystrophy, or microglial activation (Etxeberria et al., Fig. 9). Pathological, biochemical, radiological, and behavioural assessments confirmed that prolonged TREM2 agonism with Para.09 offered no measurable benefit in slowing AD progression (Etxeberria et al., Fig. 10).

Similar results were found using a mouse-specific agonist (mAb5) in tauopathy/amyloidosis mice expressing wild-type *Trem2* to address the possibility that the lack of observed effects was not due to partial hTREM2 function in transgenic mice (Etxeberria et al., Fig 12). Overall, these findings strongly suggest that TREM2 agonist antibodies do not notably slow disease progression in AD mouse models.

Etxeberria et al. studied TREM2 agonism in two demyelination models: lysolecithin injections, which induce focal demyelination, and cuprizone feeding, which produce diffuse lesions. In the lysolecithin model, Para.09, used at the same dose as in the AD models, drastically reduced microglia numbers, myelin debris clearance, oligodendrocyte precursor cell recruitment, and astrocyte activation, nearly completely halting remyelination (Etxeberria et al., Fig 14). This was confirmed using mAb5 in wild-type mice (Etxeberria et al., Fig 15). In the cuprizone model, Para.09 had no impact on microglial response, myelin debris clearance, or oligodendrocyte precursor cell recruitment and was associated with impaired remyelination (Etxeberria et al., Fig 16).

The conflicting findings reported by Etxeberria *et al.* indicate that TREM2 agonist antibody treatment is unable to significantly limit disease progression in AD models and unexpectedly produces detrimental effects in demyelination models. These findings align with an emerging view that targeting TREM2 with agonist antibodies to counteract AD and MS pathology produces mixed outcomes, often failing to produce the results expected from *Trem2* knockout models. While some inconsistencies can be attributed to technical variations between studies, such as differences in antibody design, the

more fundamental reason for this variability may be the phenotypic diversity of microglia and the complexity of their activation.

Key factors affecting antibody performance include binding affinity, off-target effects, and blood-brain barrier (BBB) permeation. Binding affinity, determined by valency (number of binding sites) and avidity (binding strength), controls how effectively an antibody activates its target (Damelang et al., 2024). Earlier TREM2 studies used bivalent antibodies, but Zhao et al. found a tetravalent antibody boosted activation 100-fold (Zhao et al., 2022). Etxeberria et al. tried increasing Para.09's valency through hexamerization, however this did not change the *in vitro* outcomes. The authors suggested that Para.09 may be incompatible with higher-valency complexes due to the proximity of its binding site to the cell membrane, limiting its activation compared to other studies (Etxeberria et al., 2024).

While all TREM2 agonist antibodies bind to TREM2, their off-target effects are largely determined by their backbones (Damelang et al., 2024). Early studies used IgG backbones (Etxeberria et al., 2024), which can activate effector cells via FcγR, leading to cytotoxic effects and interactions with other innate immune processes (Damelang et al., 2024). Etxeberria et al. found TREM2 and FcγR synergy *in vitro* and addressed this by using engineered effector-silenced IgG backbones. Regarding blood-brain barrier (BBB) penetration, Para.09 led to higher sTREM2 levels in the brain than in plasma, indicating limited targeted engagement within the brain (Etxeberria et al., 2024), likely due to restricted movement across the BBB. Other studies engineered antibodies for better

BBB crossing via transcytosis, introducing yet another potential source of variation (Etxeberria et al., 2024).

Notwithstanding these technical variations, microglia are strongly influenced by their environment, leading to diverse context-dependent phenotypes. While TREM2 is necessary for microglial activation, it alone cannot induce the DAM phenotype; additional signalling mechanisms are necessary (Ellwanger et al., 2021). This interplay may explain why TREM2 activation can result in other phenotypes beyond DAM, such as the senescent phenotype recently described in AD mouse models (Rachmian et al., 2024).

Etxeberria et al. confirmed that Para.09 effectively engages TREM2, as evidenced by downstream signalling in cell culture and reduced sTREM2 levels in mice. However, it remains uncertain if this engagement promotes the neuroprotective DAM phenotype in AD and MS (Zhang et al., 2023). Previous studies showed that some agonist antibodies stimulated microglial proliferation without inducing the DAM phenotype (Etxeberria et al., 2024). While the authors use transcriptomics to confirm upregulation of proliferation markers and downregulation of neurodegeneration markers in microglia responding to Para.09 (Etxeberria et al., Fig.5A), they did not characterise the specific microglial phenotypes induced. Given that microglial phenotypes vary with proximity to pathological features such as amyloid-β plaques (Ulland and Colonna, 2018), future studies could use spatially resolved and single-cell transcriptomics to give valuable insights into the determinants of activated microglial phenotypes and behaviour.

TREM2 agonism remains a promising target for future pharmacotherapies against AD and MS. To realise this potential, further research is needed to optimise antibody engagement with TREM2 and deepen our understanding of microglial phenotypes and behaviours following TREM2 agonism.

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