Microglia-based Therapies for Brain Diseases

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ABSTRACT

Microglia are the resident immune cells of the Central Nervous System (CNS), and as such, they fulfill their role by constantly scanning the CNS environment and maintaining its homeostasis. For a long time, their chronic activation was considered detrimental in the pathology of neurodegenerative diseases and glioblastoma, as they were conceived as a driving factor of neuroinflammation. This perspective has nowadays changed as far as neurodegenerative diseases are concerned, and their more complex role as inflammatory mediators has been revealed, thanks to state-of-the-art techniques unveiling the existence of multiple subpopulations of microglia, some of them acting neuroprotectively. In this review, we highlight the multifaceted functions of microglia in the healthy CNS and in brain disorders, namely neurodegenerative diseases and glioblastoma. Based on the most recent findings, we propose microglia replacement as a novel treatment for neurodegenerative diseases, a method still in its developmental phase but showing promising potential for clinical applicability.

Plain Language Summary

The CNS has its own line of immune defense, and that is a resident type of immune cells called microglia. Microglia, besides acting as defenders against pathogens, are necessary for the maintenance of CNS homeostasis. Their functions also involve cleaning up debris and shaping neuronal circuits. In order to fulfill all these functions, microglia are highly plastic, and able to change their response based on environmental stimuli. They are quite numerous in the brain, accounting for approximately 10% of the brain cells. Not all of them exist in the same state, but they rather form a heterogeneous population, comprised of different subpopulations. Unfortunately, for many years researchers did not know about the different microglia subpopulations. Instead, it was thought that microglia are either in a resting state, during which they are inactive, or they exist in an activated state, during which they fight infections and cause inflammation. Moreover, in human brain disorders such as Alzheimer's and Parkinson's disease, or brain cancer, microglia were thought to stay for long periods of time in this activated state and worsen the progression of the disease. This view is now dismissed, and the current knowledge supports the concomitant existence of different subpopulations of microglia in the CNS. More precisely, the range of microglia subpopulations found in the brain increases in the case of neurological diseases. Some of these subpopulations are protective for the brain, acting against inflammation or supporting regeneration of the CNS tissue, while other subpopulations are harmful, releasing inflammatory agents and enhancing brain damage. Based on recent findings regarding the beneficial role of microglia in some brain disorders, scientists believe that they can help fight off these diseases by replacing the microglia in the brain with new, better equipped microglia. Different suggested methods to repopulate the CNS with microglia exist, the most popular one being microglia depletion in the CNS followed by repopulation. This involves the elimination of native microglia in the CNS, using pharmaceutical substances. Afterwards, new similar to microglia cells can be introduced into the body of the patient. The latter cells can either by extracted from the patient themselves, or they can be donated by healthy people. The replacement strategy is already being tested in animals suffering from neurological disorders, and the first results appear to be promising. However, future work needs to be done before the treatment reaches humans, as an in-depth examination of the therapy is necessary, to determine the safety, long-term efficacy, and relevance of microglia replacement for patients.

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Introduction

Although microglia were first identified by del Río-Hortega in the early 20th Century Pérez-Cerdá et al. (2015), their multifaceted and fascinating role in the CNS is still being explored. They are described as the resident immune cells of the CNS, and are a part of the primary innate immune system Nimmerjahn et al. (2005). These cells account for approximately 10% of the glial population, and their distribution in the brain and spinal cord differs depending on the region, ranging from 5% to 12% Lawson et al. (1990). A continued debate characterizes their origin, and many hypotheses have risen. The current consensus establishes that microglia are of myeloid nature, and their erythromyleoid precursor cells are found in the embryonic yolk sac Ginhoux et al. (2010); Kierdorf et al. (2013). As postnatal haematopoiesis does not provide the CNS with new precursors due to the existence of the Blood-Brain Barrier (BBB), during adulthood the population of microglia is sustained by the microglial progenitors that had previously migrated into the brain Ginhoux et al. (2010). Moreover, it has been proved that microglia have the ability of local self-renewal, as they can autonomously repopulate the adult brain when a small portion of them remains after ablation Ajami et al. (2007); Elmore et al. (2014).

Many key factors are necessary for microglial development and homeostasis, such as the interferon regulatory factor 8 and the transcription factor PU.1 Kettenmann et al. (2013). Colony stimulating factor 1 and its receptor, Colony Stimulating Factor 1 Receptor (CSF1R), are needed for the development of microglia Ginhoux et al. (2010). CSF1R is also crucial for the maintenance of microglia in the adult brain Elmore et al. (2014).

The diverse roles of microglia have not been fully elucidated yet, but important steps to perceive their full potential have been made. First of all, microglia exhibit more than just their immune functionality. They also have physiological roles: they are involved in the development of the CNS, in synaptic plasticity Schafer et al. (2012); Salter and Stevens (2017) and in synaptic pruning Paolicelli et al. (2011). Under homeostatic condition, microglia are always alert and surveilling the environment of the CNS. Although this is considered their resting state, it does not imply that they are inactive. Instead, they are characterized by their high motility and dynamic scanning of their microenvironment Nimmerjahn et al. (2005). Homeostatic microglia exhibit a ramified and highly branched morphology Kettenmann et al. (2011), and a unique gene expression including the purinergic receptor P2Y12 (*P2ry12*) and P2Y13 (*P2ry13*), the C-X3-C motif chemokine receptor 1 (CX3CR1) (*Cx3cr1*), the myeloid transmembrane receptor CD33, and the Transmembrane Protein 119 (TMEM119) (*Tmem119*) Butovsky et al. (2013).

The occurrence of any disturbance in brain homeostasis, such as an injury, infection, disease, or changes in neuronal activity, leads to what is called microglia activation or responsiveness. This response to pathological events is characterized in detail, and refers to morphological, phenotypical and functional alterations microglia undergo when they sense a danger in the CNS. From their ramified morphology they transform into an amoeboid form, enabling them to phagocytose debris, injured cells and pathogens. Their gene expression also changes Hanisch and Kettenmann (2007); Kettenmann et al. (2011). A subsequent difference in surface marker expression is observed, such as the upregulation of CD45 and Major Histocompatibility Complex (MHC) proteins Cartier et al. (2014).

In Neurodegenerative Diseases (NDs), microglia can act beneficially or detrimentally Hanisch and Kettenmann (2007), making many wonder if they are a friend or a foe in the CNS regarding human diseases Cartier et al. (2014). Microglia exhibit neuroprotective functions, but they are also able to induce serious damage to the CNS, when they remain reactive for prolonged periods of time and thus contribute to the vicious cycle of chronic inflammation. The involvement of microglia as a causative or ameliorating factor in NDs is not easily clarified. Moreover, recent findings highlighted the existence of different subpopulations of microglia in NDs, with each subpopulation exhibiting different markers and functionality Muzio et al. (2021).

This review aims to shed light on the complex role microglia play in Glioblastoma (GB) and some NDs, namely Alzheimer's Disease (AD), Parkinson's Disease (PD), Multiple Sclerosis (MS), Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Moreover, based on microglial involvement in these disorders, microglia-based cell therapy to mitigate them is discussed. Microglia replacement strategies are on the rise and could potentially be of great clinical use.

Microglial Functions in the Healthy Brain and in CNS Disorders

The roles of microglia in the developing and adult brain are many and diverse, as can be seen in figure 1. Their functions prove to be necessary for the survival and health of neurons Nayak et al. (2014). They are involved in synaptogenesis and the regulation of (adult) neurogenesis, they modulate synaptic plasticity Kettenmann et al. (2013),

they contribute to axon formation Ueno et al. (2013), while during development they participate in synaptic pruning and thus in the (re)modelling of synaptic circuits Schafer et al. (2012); Paolicelli et al. (2011). Besides being engaged in the survival of neurons, microglia are also implicated in controlled neuronal death by phagocytosis during development Marin-Teva et al. (2004). The important crosstalk between microglia and the rest of glial cells and neurons happens through the release of various factors. Some released neurotrophic factors, the Insulin-like Growth Factor-1 (IGF1) Ueno et al. (2013), the nerve growth factor, and the brain-derived neurotrophic factor, are crucial for the development of neurons during early life stages and neuronal maintenance through adulthood Nayak et al. (2014). Moreover, microglia exhibit physiological role in learning and memory formation Parkhurst et al. (2013).

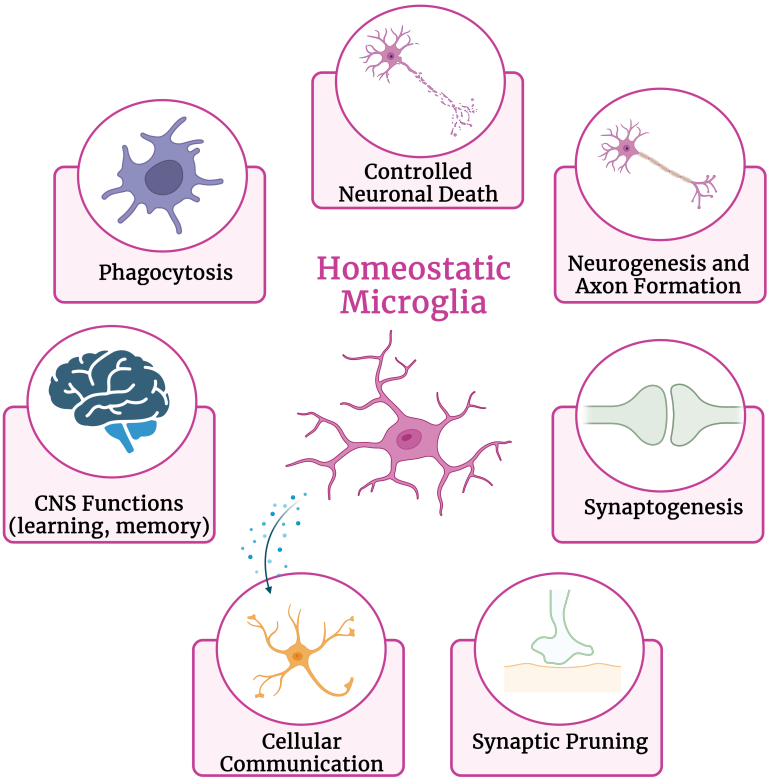

Figure 1: The main roles of microglia in maintaining CNS homeostasis. These include phagocytosis and controlled neuronal cell death, neurogenesis, axon formation, synaptogenesis, synaptic pruning, cellular communication with astrocytes through the secretion of factors, and supporting physiological functions such as memory formation and learning. Created with BioRender.com

Microglia exert their effects in the brain either through the secretion of pro-inflammatory, anti-inflammatory and neuroprotective mediators, or through removing toxic elements by phagocytosis Kettenmann et al. (2013). Microglia respond to any sign of threat to the CNS by changing form and becoming "activated". Microglial activation is more accurately described as a shift in activity and function rather than a transition from quiescence to activation, and the term reactive or responsive microglia is preferred over activated microglia. The initial microglial response concerns their conversion to an amoeboid form, a change in gene expression and the release of pro-inflammatory mediators. Microglia are able to phagocytose debris, damaged cells Hanisch and Kettenmann (2007); Kettenmann et al. (2011) and protein aggregates Zhang et al. (2023). The classic concept of microglia activation regards their activation towards different phenotypes, a phenomenon called microglia polarization, and these phenotypes are traditionally called M1 phenotype (pro-inflammatory) (M1) and M2 phenotype (anti-inflammatory) (M2). The M1 phenotype refers to classically activated microglia, which are pro-inflammatory and neurotoxic, secreting factors such as Tumour Necrosis Factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β). Phenotype M2 refers to alternatively activated microglia, which are anti-inflammatory and neurotrophic, and are releasing for example IGF1 David and Kroner (2011); Gupta et al. (2018). Nowadays, this old paradigm of M1 and M2 division is disregarded, as the diversity in microglial phenotypes is

much higher than that Muzio et al. (2021). It is generally accepted that one subtype of microglia can express both pro- and anti-inflammatory markers, secrete different kind of factors, and that microglia characterization should be based on their wider range of expressed markers. Microglia respond in CNS threats by acquiring a phenotype belonging in a range between the M1 and M2 phenotype Paolicelli et al. (2022), as can be seen in figure 2.

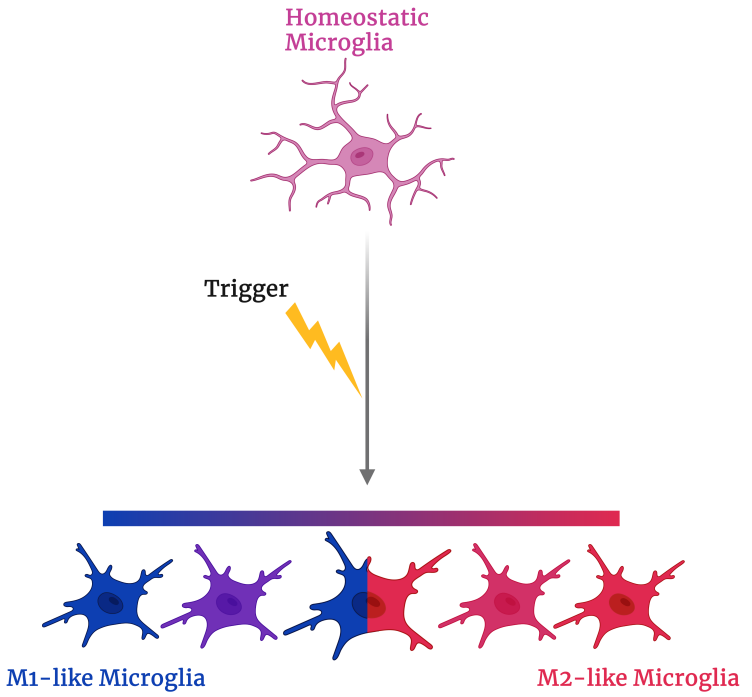

Figure 2: A clear division between the pro- and anti-inflammatory phenotype of the previously-called activated microglia is no longer advocated for, but rather a spectrum of inflammatory responses is used to describe more accurately the illicited response from microglia under any stimuli. M2-like and M1-like are terms used to correspond with the old classification of microglia polarization, but microglia do not adopt a specific marginal phenotype. Created with BioRender.com

In the state of acute inflammatory triggers, microglia respond to the trigger by releasing pro-inflammatory cytokines and proceeding with the neuronal repair process. In contrast, in chronic states of CNS unresolved inflammation their chronic responsiveness has deleterious effects to the CNS, leading to damage and neurodegeneration Graeber et al. (2011). This responsiveness is caused by the microenvironment in the CNS. The injured neurons and cells release Disease-Associated Molecular Patterns (DAMPs), which bind to various surface molecules in microglia, the most implicated in neurodegeneration being the Toll-Like Receptors (TLRs). DAMPs downstream effect is related to neuronal cell dysfunction and demise, while some commonly found DAMPs are Interleukin (IL) 33, IL-1α, and Adenosine Triphosphate (ATP). The overly reactive microglia and the constant release of neurotoxic factors, such as TNF-α, IL-1β, Reactive Oxygen Species (ROS), and glutamate Gupta et al. (2018) sustains the chronic inflammatory response and is implicated in many diseases, such as NDs Lull and Block (2010), stroke Olson and McKeon (2004), hypoxia Morioka et al. (1993) and neuropathic pain Tsuda et al. (2003). The vicious cycle of continuous CNS damage due to the microenvironment and microglial response to it is simplistically depicted in figure 3.

Other subpopulations of microglia are also being identified, as our knowledge for these cells expands. Recent single-cell RNA-sequencing experiments revealed a novel subset of microglia, namely the Disease-Associated Microglia (DAM) Keren-Shaul et al. (2017). Disease-Associated Microglial cells (DAMs) are characterized by the downregulation of the homeostatic genes Butovsky et al. (2013), and by the upregulation of genes involved in lysosomal, phagocytic, and lipid metabolism pathways, such as *Apolipoprotein E (ApoE)* and the *Triggering Receptor Expressed on Myeloid cells-2 (TREM2)*. These genes are also known to be risk factors in AD Lambert et al. (2013). Other noteworthy clusters of microglia are the injury-responsive microglia Hammond et al. (2019) and the aged microglia Safaiyan et al. (2016).

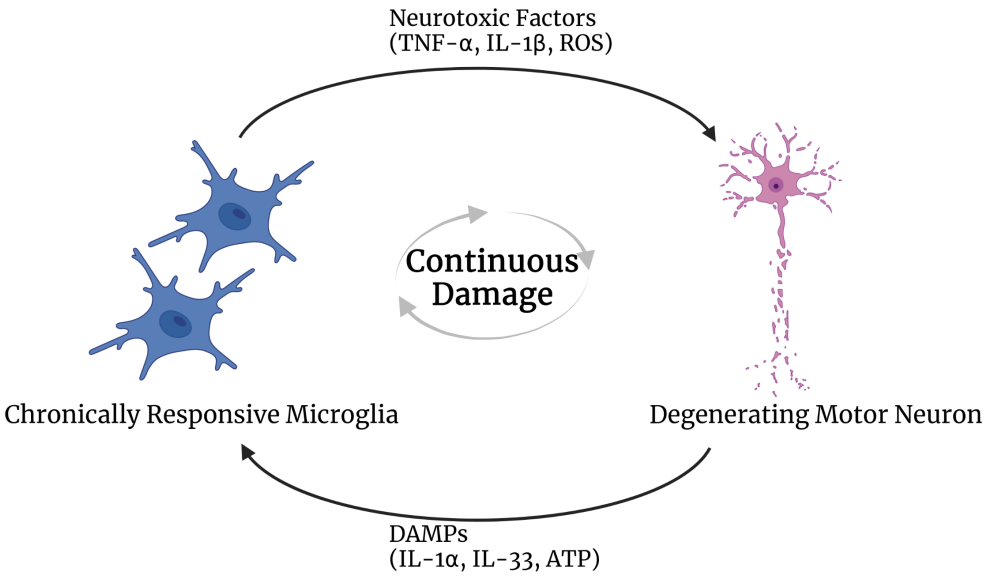

Figure 3: Part of the vicious cycle of continuous damage and inflammation observed in the CNS during neurodegenerative diseases: injured neurons secrete DAMPs, which lead to chronic responsiveness of microglia and the release of neurotoxic factors that further damage neurons. Created with BioRender.com

Microglia are susceptible to the aging process, which causes alterations in their function and state Muzio et al. (2021). Driving factors behind the senescence observed in microglia are the accumulated damage in the cell DNA, either nuclear or mitochondrial in the form of mutations, the elevated production of ROS and the consequent oxidative stress. These in turn lead to disturbed iron homeostasis and phagocytosis, and the accumulation of lipofuscin Kwon (2022). The question regarding the number of microglia in aged brains remains unanswered, as the conducted studies led to contradictory results. What is known about aged microglia is their distinguishable dystrophic state. Their morphology is altered: the soma is enlarged, while the branches are shorter and fragmented. It is probable that these alterations in morphology are depicting also a divergence in gene expression.

Immune-related pathways usually activated in pathological conditions can be upregulated in aging microglia, making the distinction between the different subpopulations even more difficult Muzio et al. (2021). In mice, the genes detected to be differentially expressed in aged microglia are concerning mitochondria-related and energy producing pathways, signalling pathways in lysosomes and phagosomes and antigen presentation. The markers TMEM119 and P2Y12 are also downregulated in aged mouse microglia. Moreover, the NLR family pyrin domain containing 3 (NLRP3) plays an important role in inducing an inflammatory response in aged microglia.

Aged human microglia are shown to downregulate the gene expression of proteins related with the actin and cytoskeleton system, cell surface receptors -generic or more microglia-specific such as P2Y12, the receptor of IL6, TLR10 Brawek et al. (2021), and CD36 Rawji et al. (2020)- and cell adhesion molecules. Genes of other molecules are upregulated, as for example the one of vascular endothelial growth factor A, of some integrin regulators, and of the CD22 and CD163 receptors. Of note, CD22 is a negative regulator of phagocytosis. As it is usually the case, the sets of differentially expressed genes in mouse and human aged microglia are not fully identical, but there is some overlap Brawek et al. (2021). But, in both organisms, half of the population of microglia in older brains accumulate lipid droplets Brawek et al. (2021), and these microglia are called Lipid-Droplet-Accumulating Microglial cells (LDAMs). These cells exhibit defects in phagocytosis and elevated production of ROS and pro-inflammatory cytokines. Their lipid processing and formation pathways are driven by a distinct gene expression, thus LDAMs are characterized by their unique transcriptional signature Marschallinger et al. (2020).

Regarding the epigenetic alterations imposed on aged microglia, they are mostly examined in animal models. The microglial deletion of a histone deacetylase involved in senescence, namely Sirtuin 1, leads to DNA hypomethylation and in turn to higher levels of IL-1β in animal models of aging. These animals exhibited cognitive decline. Another group also highlighted the importance of methylation in the promoter of the *IL-1β*. Other enzymes involved in

epigenetic modifications are also found to be implicated in the regulation of microglia, such as a histone demethylase called Jumonji domain-containing protein-3. Noncoding RNAs are also observed to be differentially expressed in microglia Wang et al. (2021).

Moreover, aged microglia may contribute to maintaining an aging-related inflammation in the brain, which is called "inflammaging" and refers to the constant existence of low-grade inflammation in the brain Muzio et al. (2021); Brawek et al. (2021). As NDs are present in older populations, aged microglia could also worsen the progression of the diseases by supporting the inflammaging Muzio et al. (2021), while rejuvenating or replacing these microglia may be beneficial Kwon (2022).

Microglia in Alzheimer's Disease

AD is a progressive Neurodegenerative Disease (ND), and the most common cause of dementia worldwide. The risk of suffering from AD increases with aging. The histopathological aspect of AD is described by extracellular amyloid plaques and intracellular neurofibrillary tangles, and their accumulation leads to neuronal loss and reactive gliosis. The plaques consist of Amyloid beta (aβ) peptide aggregates, while the tangles are composed of hyperphosphorylated tau protein aggregates Hansen et al. (2017).

In recent years, the implication of microglia in the pathogenesis of AD is being heavily investigated. Microglia-mediated exacerbation of inflammation and reactive gliosis play a key role in the disease. It has become evident that microglia can exhibit protective or harmful effects regarding the progression of AD Hansen et al. (2017); Leng and Edison (2020). However, elucidating the exact role and level of involvement of microglia in AD is not an easy task. Researchers have shed light on different aspects regarding AD microglia characterization. As an example, it has been observed that gene variants influencing the disease progression are also expressed in microglia, such as the E4 variant of *ApoE*, and the rare R74H variant of *TREM2* Hansen et al. (2017). Meanwhile, our knowledge about microglial functions in AD stems from animal mouse models and postmortem brain tissues of AD patients, and in those two discrepancies are detected Muzio et al. (2021). Murine microglia react differently than human microglia in the context of AD pathology. Fortunately, new chimeric models have been developed, in which human microglia progenitors are transplanted in the postnatal brain of immunodeficient AD mice. As mice age, the introduced microglia adapt to the brain environment while keeping the characteristics of human microglia responses, thus providing new models to study the interactions between human microglia and AD pathology *in vivo* Hasselmann et al. (2019)

Regarding the role of TREM2 in AD mice, DAMs with higher expression of TREM2 are localized near amyloid plaques Muzio et al. (2021). Diminishing its expression in 5XFAD mice leads to no detection of the DAM subpopulation Keren-Shaul et al. (2017). It should be noted that the 5XFAD mouse model is not an ideal representation of AD. In another animal model of AD, namely the APPPS1 mice, knocking out *TREM2* seemed to have protective effects against AD. More contrasting studies exist in literature. In mice expressing humanized TREM2, the administration of an antibody that acts as an TREM2 agonist led to increased phagocytosis of amyloid plaques from microglia, thus highlighting the protective role of reactive microglia and of stimulated TREM2. These animal models lack tau pathology, and thus the complete response of microglia in the AD environment Muzio et al. (2021). In mouse models of tau pathology, TREM2 is again exhibiting contradictory roles, with the absence of the receptor leading to either neuroprotective or neurotoxic effects dependent on the animal model. Although it is probable that microglia promote AD by exacerbating the damage caused in neurons by the neurofibrillary tangles, microglia may also be implicated in the delay of tauopathy progression Hansen et al. (2017).

Concerning the immunological aspect of microglia in AD mice, various observations have been made. It is known that the CD14 molecule expressed in microglia interacts with amyloid fibrils Muzio et al. (2021). Stimulation from aβ aggregates of the microglial upregulated surface markers, namely the CD14 molecule and TLRs, can result in increased clearance of aβ peptides Zhang et al. (2023). The stimulation can also result in the release of inflammatory cytokines, such as TNF-α and IL-1β. The latter reaction may be harmful and promote AD, as evidenced by a worsened tau and amyloid pathology in animal models Hansen et al. (2017). Blocking TNF-α activity using either genetic or pharmacological manipulation can ameliorate AD pathology. The same effect is detected when administrating antagonists for the IL-1 receptor Zhang et al. (2023). Furthermore, reactive microglia tend to accumulate around newly forming amyloid plaques, walling the aggregates off and constricting them in a denser form, possibly protecting the brain from additional damage and plaque production Hansen et al. (2017).

A spectrum of genetic variations and gene expression is observed in human microglia. As for the role of TREM2 in humans, analysis of postmortem brain tissues of AD patients revealed that loss-of-function *TREM2* variants advance the disease, possibly because they lead to reduced or less efficient uptake and degradation of the amyloid aggregates

Hansen et al. (2017). Moreover, human AD microglia express a variety of genes, some of which are also seen in mouse DAMs. Meanwhile, single cell sequencing and transcriptome analyses revealed the existence of various subsets of microglia in human brains, with some clusters expressing homeostatic genes while others expressing genes related to immune response. The gene expression characteristic of mouse DAM is not detected only in one cluster of human AD microglia but is distributed in different subpopulations, thus a direct correlation of mouse DAM with human AD microglia cannot be hypothesized Muzio et al. (2021). Regarding the shared downregulated markers in human AD microglia and DAM, they are the P2Y12 and CX3CR1, and the upregulated ones include ApoE, AXL, SPP1, and TREM2. Furthermore, tau-associated microglial subpopulations are detected in humans Paolicelli et al. (2022).

In conclusion, in early stages of AD microglia act beneficially, by phagocytosing aβ peptide aggregates, thus halting the formation of plaques. In contrast, it is possible that in later stages of the disease these cells act detrimentally by advancing the progression of AD due to their chronic activation and the consequent constant release of pro-inflammatory factors Gupta et al. (2018). Thus, the term of double-edged sword for microglia in AD progression is a well-deserved one Hansen et al. (2017); Zhang et al. (2023). Moreover, the different subpopulations of human microglia and their different role are still not illuminated fully.

Microglia in Parkinson's Disease

Following Alzheimer's disease, PD is the second most common neurodegenerative disorder. The movement disorder in PD is caused by a chronic and progressive loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) of patients, while the risk of suffering from the disease is related to environmental and genetic factors, and to aging. The histopathology of PD is characterized by the intracellular aggregation of α-synuclein, which forms protein inclusions named Lewy bodies and Lewy neurites, and by the demise of dopaminergic neurons. Microglial activation is also detected in PD patients Muzio et al. (2021); Badanjak et al. (2021).

It is still not elucidated if neuroinflammation[1] is a result or a fundamental contributor in the neurodegeneration characteristic of PD. It has been observed that microglia are in a responsive state and their numbers are elevated in the brain of PD patients Badanjak et al. (2021). In the first stages of the disease, microglia seem to adopt a more anti-inflammatatory phenotype, acting protectively against tissue damage. Unfortunately, the progression of the disease leads to over-activation of microglia, and consequently to the release of pro-inflammatory factors and finally to the vicious cycle of CNS damage seen in figure 3 Gupta et al. (2018).

Neurotoxins are most commonly used in animal models to induce parksinsonism. In a mouse model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD, reactive microglia levels are elevated. Blocking microglial responsiveness using minocycline in this animal model rescues dopaminergic neurons and reduces the amount of inflammatory factors, such as TNF-α and ROS Badanjak et al. (2021). Confirming the finding that inflammation is a leading cause behind neuronal death, another study found that MPTP-treated transgenic mice lacking TNF-α receptors exhibited decreased dopaminergic damage Muzio et al. (2021). To further prove these findings, in another animal model, in which rotenone is used as the neurotoxic agent, Interferon gamma (IFN-γ) acting synergistically with microglia induces neuronal cell death Badanjak et al. (2021).

Aggregates of α-synuclein are inflammatory triggers, and could be considered a Disease-Associated Molecular Pattern (DAMP) for PD. α-synuclein can trigger microglia responsiveness in animal models and cell cultures, and results in upregulation of specific genes and in increased microglial release of pro-inflammatory factors Badanjak et al. (2021). More specifically, α-synuclein fibrils can activate a number of receptors present in microglia, including the NLRP3. In consequent experiments on MPTP mice, admninistrating a BBB-penetrating NLRP3 inhibitor resulted in reduced dopaminergic neurotoxicity Muzio et al. (2021). In PD patients, amoeboid shaped microglia are related to misfolded protein pathology. But, as can also be seen in AD, the morphology and transcriptome heterogeneity of human PD microglia is higher than in animal models of PD Badanjak et al. (2021). In mice models, PD-DAMs are detected, and the upregulated markers in this subpopulation are included in figure 4.

In postmortem tissue studies of PD patients, MHC-II-positive microglia exist in higher numbers than in healthy controls, and especially the amoeboid type related to inflammation. In addition, some brain regions such as the SNpc, the putamen, and the temporal, cingulate and trans-entorhinal cortexes are more enriched in this specific population of microglia Badanjak et al. (2021). In the substantia nigra, higher numbers of reactive microglia, and higher levels of IFN-γ, TNF-α, and IL-1β are detected Gupta et al. (2018). These findigs indicate a region- and disease-specific accumulation of microglia Badanjak et al. (2021). Remarkably, elevated levels of inflammatory cytokines are also

[1] The term neuroinflammation is conventionally used throughout the text to signify the chronic inflammation of the CNS as a response of resident and circulating immune cells to local damage, observed in neurodegeneration.

detected in the serum of PD patients, and higher levels of TNF-α are associated with a more severe symptomatology of PD Badanjak et al. (2021). Moreover, Human Leukocyte Antigen – DR isotype, type of MHC-II receptor (HLA-DR) has emerged as a genetic risk factor for PD, as a single nucleotide polymorphism in one of its subunits is a causative factor in some cases of late-onset PD. HLA-DR-positive microglia can be detected in the substantia nigra of PD patients Zhang et al. (2023).

The already existing results from transcriptomic analyses of PD patients' tissues are not based on a vast variety of samples. Still, it is evident that PD microglia exhibit a different transcriptome when compared to healthy controls. Of note is the fact that in reactive PD microglia, various pathways are differentially activated, the most interesting ones being the inflammation-related aldosterone, the ROS metabolism and the synaptic transmission pathway Badanjak et al. (2021). Single nuclei RNA sequencing experiments revealed that reactive microglia in PD can follow different activation pathways and thus end up being divided in subpopulations. One such cluster expresses high levels of transmembrane glycoprotein NMB and another one expresses high levels of IL-1β . Moreover, possible genetic risk factors of PD linked with microglia have emerged, such as the variants of *LRRK2* Badanjak et al. (2021), and some variants of *TREM2*, for example the rs75932628 Gupta et al. (2018).

The adaptive immune system plays a key role in PD, as active peripheral T-cells can be found inside the brain tissues of patients. In line with these findings, in a rat model of PD, T-cells that have intruded the brain led to microglia activation and a pro-inflammatory response against α-synuclein Badanjak et al. (2021).

Overall, our current knowledge regarding microglia and Parkinson's disease is not as extended as in Alzheimer's disease. Microglia are upregulated in the brains of PD patients, and the consequent inflammation in the CNS is evidently deteriorating the damage impacted on dopaminergic neurons. Further research should shed light on the different subpopulations of microglia existing in PD, paving the way to manipulate microglia as a neuroprotective agent against this disorder.

Microglia In Multiple Sclerosis

MS is a chronic neurodegenerative inflammatory disease of the CNS, and is mainly affecting young adults. It is a complex disease, and the causes are still not known. Some genes are possibly implicated, and environmental factors are contributors. Pathophysiologically, the disorder is described by focal lesions in the brain and spinal cord, inflammation, demyelination of neurons in the white and grey matter, loss of oligodendrocytes and gradually escalating axonal loss. The activation of immune cells, namely T-cells, B-cells and microglia, is related to the lesions. Multiple sclerosis can be divided into different types. Most patients are suffering from the relapsing-remitting type of MS (Relapsing-Remitting Multiple Sclerosis (RRMS)), in which the relapses are inflammatory episodes. The rest of the patients experience primary progressive MS, in which no relapses or remissions are present. Finally, Secondary Progressive Multiple Sclerosis (SPMS) is a second progressive course of RRMS, characterized by less inflammation. In all types, neurodegeneration occurs since the beginning of the disorder Zia et al. (2020); Muzio et al. (2021).

The exact level of microglial implication regarding tissue destruction in MS is not fully clarified. In MS patients, reactive microglia are detected in the brain. These can be found at the rim of active demyelinating lesions, when the disease is in its progressive form (SPMS), and not at the rim of old inactive lesions. In postmortem brain tissues of SPMS patients, phagocytic microglia are found Muzio et al. (2021). In lesions of early stage MS, 40% of phagocytic cells are microglia, detected by the expression of TMEM119 Guerrero and Sicotte (2020). In RRMS, no correlation has been found between microglia activation and disability Muzio et al. (2021).

As is the case for most NDs, preclinical models for MS are not representative of the disease complexity. Experimental Autoimmune Encephalomyelitis (EAE) mice are most commonly used in preclinical studies of MS, an animal model that is characterized by neuroinflammation and demyelination caused by immunization. The severity of EAE is ameliorated when microglial activation is impaired using various methods, such as genetic manipulation, CSF1R inhibitors Zia et al. (2020); Muzio et al. (2021), or minocycline Guerrero and Sicotte (2020), highlighting the harmful aspect of microglia in MS pathogenesis. In another study, in which the EAE model used was indicative of SPMS, inhibition of CSF1R led to a worsened progression of the disease, thus emphasizing the protective role of microglia in MS Muzio et al. (2021). Ablation of all myeloid cells reduces EAE symptomatology, and various manipulations showed that the interplay between macrophages and microglia is a driving force behind MS progression Zia et al. (2020).

The explanation behind this diversity in the role of microglia lies in the existence of different subpopulations. It has been proved that microglial subpopulations that release pro-inflammatory factors are present in MS, while subpopulations also secreting anti-inflammatory factors and promoting remyelination exist. The microglial clusters

that are first detected near demyelinating lesions are damaging, characterized by their neurodegenerative phenotype. When those cells die, they are replaced by a neuroprotective, and remyelination-promoting population of microglia. This is indicative of the microglial ability to shift from a detrimental to a immunoregulatory type in MS Zia et al. (2020); Muzio et al. (2021). At least four different subpopulations of microglia in EAE models were found, and three different subsets in MS patients. The identity of microglia in human patients is possibly indicative of the lesion's evolution stage and microenvironment. In addition, in preclinical mouse models similarities between MS-microglia and AD-DAM gene expression profiles were found Zia et al. (2020). A specific to MS microglial subpopulation is identified in animal models, namely the Microglia Inflamed in MS (MIMS) Paolicelli et al. (2022). Its gene expression profile is indicated in figure 4. Moreover, even in normally appearing white and grey matter, microglia exhibit upregulation of genes involved in lipid and iron homeostasis, marking their more responsive state. Elevated expression of genes that are considered risk factors for MS in microglia are detected in MS patients Guerrero and Sicotte (2020).

Microglia can be detrimental or neuroprotective in MS progression, while research should be pointed towards illuminating the complex role of these cells in MS pathogenesis and progression. Some microglia populations in MS seem to limit the progression of the disease and help in remyelination, but others are exacerbating the damage caused in neurons.

Microglia in Amyotrophic Lateral Sclerosis

ALS is a fatal neurodegenerative disease, exhibiting a rapid progression after its onset. The neurodegeneration concerns the motor neurons in the motor cortex, spinal cord and brainstem. 90% of ALS cases are considered sporadic. Mutations in more than thirty genes, with *Superoxide Dismutase 1 (SOD1), TARDBP, FUS* and *C9orf72* being the most common, have been implicated in familial ALS, which accounts for the remaining 10% of patients. Motor neurons may be the affected cells, but non-neuronal cells are also involed in the progression of ALS, among them being microglia, astrocytes and peripheral immune cells. The demise of motor neurons and the following pathological hallmarks result in neuroinflammation, as evidenced from analysis of ALS patients' samples Muzio et al. (2021); Clarke and Patani (2020).

The general consensus regards that reactive microglia can be found in patients suffering from ALS. Discrepancies between studies exist. Some report that only a group of ALS patients has reactive microglia, while others argue that reactive microglia can be found not only in motor regions, but also in the spinal cord. The factors possibly affecting these findings include the limited number of samples analyzed, the different genotype of the patients, the fact that analysis of postmortem tissue means that only data regarding the end-stage of the disease can be collected while also the tissue could be decayed, technical variations between the studies and finally the age of each individual patient, as microglial gene expression changes with aging Clarke and Patani (2020); Muzio et al. (2021).

More information can be extracted from animal models of the disease, keeping in mind that microglial gene expression differs between humans and mice Clarke and Patani (2020). In the $SOD1^{G93A}$ mouse model, alterations in microglial phenotype are detected even before the disease onset when compared to the control group. During the disease, microglia can act detrimentally and beneficially against the disease at the same time. Single-cell RNA sequencing experiments using samples from $SOD1^{G93A}$ mice revealed a subpopulation of microglia having a gene expression profile similar with AD-DAM, and more precisely demonstrating higher levels of *TREM2* expression, and of genes related to phagocytosis and lipid metabolism Muzio et al. (2021). Although, the ALS-DAM subpopulation has a distinct gene expression signature compared with AD-DAM, as can be seen in figure 4.

Various manipulations in animal models to understand the complex role of microglia in ALS have already been done. Mutations in the *SOD1* gene can lead to protein misfolding, and mutant SOD1 tends to aggregate, the aggregates contributing to neurodegeneration. These aggregates can activate microglia towards secreting pro-inflammatory factors by binding with the CD14 molecule or TLRs, such as the Toll-Like Receptor 4 (TLR4). Inhibition of this protein-driven activation can be done either pharmacologically, or by genetical deprivation of the receptors. Correspondingly, TLR4 inhibition led only to a slighlty reduced degeneration of motor neurons, while TLR4 knock out extended mice survival by 2 weeks Muzio et al. (2021). Inhibition of CSF1R results in increased survival time of mice and prolongs the survival of motor neurons. Depleting half of proliferating microglia by other means does not attenuate the disease progression nor extend survival time, although this may implicate that the remaining proliferating microglia are adequate to induce damage. Using IL-4 gene therapy in $SOD1^{G93A}$ mice led to a shift in microglia phenotype, but resulted only in delayed onset of the disease and symptomatic improvement and had no effect in neurodegeneration and survival time. More similar studies have been conducted, manipulating IL-10 levels and its receptor, the granulocyte-colony stimulating

factor and some purinergic receptors, and their results are not the most promising, as none seemed to ameliorate all aspects of the disease.

In conclusion, neurodegeneration in ALS is not prevented by manipulating microglia. More research is needed to have concrete results and to understand the exact implication of microglia in the disease. It is highly probable that microglia are not a protagonist in ALS pathogenesis, but rather a secondary responder to neurodegeneration Clarke and Patani (2020); Muzio et al. (2021). Another factor which should be further investigated upon is the crosstalk between microglia and the rest of glial and peripheral immune cells, as this interaction determines the function of microglia Clarke and Patani (2020). Regardless, the contribution of microglia to the disease outcome and their potential to be exploited in a clinically relevant therapy should not be neglected, as they exhibit neuroprotective effects in ALS.

Microglia in Frontotemporal Dementia

FTD is a neurodegenerative disease, which shares similarities regarding genetic risk factors, clinical symptoms and pathology with ALS. It comes right after Alzheimer's disease as the second most prevalent cause of dementia, and it is lethal, without any cure in the market. The neurodegeneration is localized in the frontotemporal lobes. Genes expressed in microglia are implicated in the disease, and these genes are involved in phagocytosis, autophagy, and inflammation pathways, or their mutations lead to protein misfolding.

Some of these genes are *C9orf72*, *Progranulin (PGRN)*, and *TBK1* Lall and Baloh (2017); Haukedal and Freude (2019). PGRN regulates the inflammatory response and is involved in phagocytosis and lysosomal physiology. Lack of *PGRN* influences negatively the complement gene exression in microglia, and has a negative impact on synaptic pruning. In mice, loss of *PGRN* leads to aberrant complement secretion and microglia activation Lall and Baloh (2017); Sirkis et al. (2021). Haploinsufficiency in *PGRN* is a causative factor of FTD. Furthermore, microglia lacking *PGRN* form a distinct subpopulation, expressing higher levels of disease-associated genes and downregulating the expression of the homeostatic genes. Other proteins, whose function is related to PGRN, have also been implicated in the pathogenesis of FTD and are expressed in microglia, such as the TMEM106B and the SORT1 Sirkis et al. (2021).

Concerning the levels, responsiveness status and morphology of microglia in the brains of FTD patients, a small cohort study comparing patients with a control group revealed that microglia seem to increase in numbers and are more dystrophic in FTD patients. Regional variations are detected, dependent on the FTD subtype. Microglia can be found in the white matter, and those are phagocytic or antigen-presenting and dystrophic, while the microglia in grey matter are more reactive. The located in white matter microglia could possibly be senescent, as indicated by their dystrophic state, and thus dysfunctional, enhancing the susceptibility of this region to neurodegeneration. Another finding regards the higher levels of phagocytic microglia in patients, specifically CD68-positive microglia in frontotemporal regions. These results are far from conclusive, and further research is needed to understand the role of microglia in FTD, but these early findings indicate that a possible therapy for FTD would involve the anti-senescence Woollacott et al. (2020) or replacement of microglia in frontotemporal region, or the correction of the genes related to FTD and expressed in microglia.

Microglia in Glioblastoma

GB is the most frequent primary brain tumour and is characterized as the most aggresive one. Currently, no curative therapy exists. The therapeutic approach lies in a combination of surgery, radiotherapy, chemotherapy and tumour treating fields, while immunotherapy and targeted therapy methods are being investigated Geribaldi-Doldán et al. (2021); Khan et al. (2023). In the Tumour Microenvironment (TME) typical of GB, Tumour-associated Macrophages and Microglia (TAMs) are found in abundance, contributing to the immunosuppresive character of TME and tumour progression. TAMs exhibit high heterogeneity and plasticity. The identification of their different subpopulations is crucial for gaining insight into GB progression and treatment Khan et al. (2023).

TAMs seem to act detrimentally in GB. These cells are able to promote tumour progression, by inactivating T-cells through the CD274 pathway, by secreting cytokines and factors that promote GB angiogenesis and cancer cell proliferation, and by contributing to resistance to radiotherapy Andersen et al. (2021). Reactive microglia can be found spreaded throughout the tumour, and are the main type of TAMs in recently formed tumours. Macrophages are detected in vascular structures of GB tumours and are the majority of TAMs in recurrent tumours. The heterogeneity in TAMs subpopulations is wide Khan et al. (2023). Some studies have shown that the presence of M2-like microglia correlates with decreased survival time, as for example they promote angiogenesis favorable for the tumour, while others support that microglia act mainly pro-inflammatory and macrophages pro-tumourigenicly Andersen et al. (2021). A better description of the TAMs' phenotype is a continuous state of plasticity, and the co-existence in TME of both pro-

and anti-inflammatory types of cells has been proved. Interestingly, new microglial subpopulations found only in GB tumours of patients or in animal models have been identified, and such microglial subpopulations may express genes related to MHC. These microglia can be of the pro-inflammatory type, or can have immunosuppresive abilities by exhausting T-cells. Therapeutic strategies involving TAMs manipulation are being considered, such as blocking the recruitment of TAMs to the tumour, mediating their reprogramming or ability to phagocytose, and controlling newly identified subpopulations. Inhibition of CSF1R can either ablate TAMs or affect their phenotype, and is a promising strategy in preclinical trials, but only as part of a combination therapy Khan et al. (2023).

TAMs contribute to the poor prognosis in glioblastoma. These immune cells act detrimentally against the patient, as they support tumour progression, enhance the immunosuppresive TME and contribute to treatment resistance. Some subpopulations of TAMs seem to have a protective role against glioblastoma. Further research is needed in order to gain insight into the different subpopulations and their localization in the brains of GB patients, thus allowing us to manipulate them therapeutically against GB.

Therapeutic Approaches for Brain Diseases based on Microglia Replacement Strategies

The treatment of the previously mentioned brain diseases should be neuroprotective and neurorestorative, ideally not only inhibiting but also reversing the progression of the disease. As discussed, microglia are implicated in the pathogenesis of NDs due to common driving factors in these diseases, as can be seen in figure 4, and are also implicated in the progression of glioblastoma. They can possibly be used against NDs as a treatment themselves and against glioblastoma as a target. The method we suggest for microglia-based therapies for NDs would involve repopulating the brain with exogenous, but not necessarily allogeneic, microglia, as this has already been proved to ameliorate neuroinflammation Rice et al. (2017). Moreover, replacing the native microglia would be beneficial as the senescent subpopulation of microglia that may worsens neurodegeneration will be diminished.

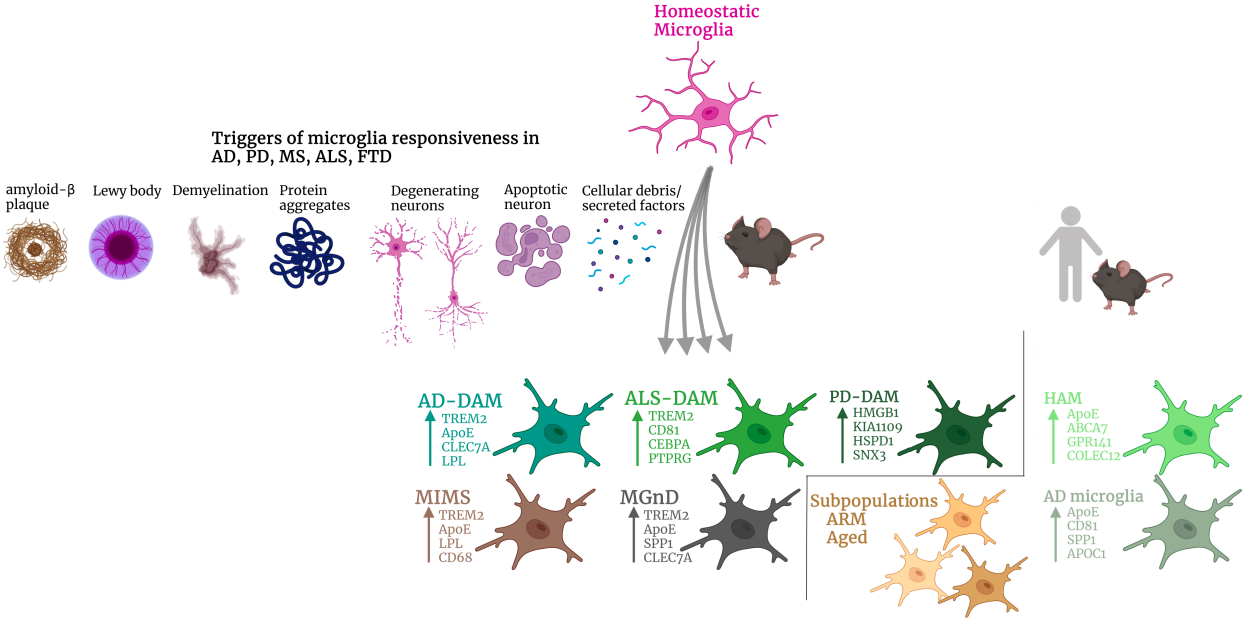

Figure 4: Driving factors in Alzheimer's and Parkinson's disease, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, and Frontotemporal Dementia that lead to microglia responsiveness, and depiction of the divergence of microglia into clusters in the environment of those diseases. The most distinct subpopulations that can be found in mouse brains are illustrated, namely DAM in AD, ALS and PD, MIMS in MS, and MGnD generally in neurodegeneration. HAM, ARM, AD microglia and aged microglia are commonly found in humans and mice. The most important upregulated markers in each phenotype except aged and ARM microglia are specified. Created with BioRender.com and partially adapted from Paolicelli et al. (2022).

Microglia Replacement and Rejuvenation Strategies

Various methods of microglial repopulation have been reported. The replacement of resident microglia can be achieved by endogenous microglia, or by (exogenous) Microglia-Like Cells (MLCs). In order for the new microglia or MLCs to repopulate the brain, the depletion of native microglia in order to create a microglia-free niche is required, so the new cells can occupy the available space Walsh and Lukens (2023).

The first general method of microglia replacement involves the depletion of endogenous microglia by short-term treatment with CSF1R inhibitors, usually administered for less than a month, which leads to 90% microglial depletion in the mouse brain. Afterwards, endogenous microglia can repopulate the created niche. These newly generated cells are reported to be healthy. This repopulation can have a positive impact on many diseases. It should be noted that the CSF1R inhibition may be the iniating factor leading to the observed reduced neuroinflammation, as CSF1R antagonists affect other immune cells. This method offers little control over the outcome of microglia replacement, and its ameliorating impact on diseases is possibly dependent on the stage of the disease and the functionality of microglia Kwon (2022).

Another method of microglia replacement refers to MLCs infiltration in the brain. These MLCs can be host-produced peripherally derived MLCs, occuring after microglial ablation using tamoxifen. These cells exhibit different marker signature compared to microglia, such as the lack of P2Y12. Moreover, these mononuclear cells can also be donor-derived, and, being influenced by the CNS and the microglia ablation, can resemble microglia Han et al. (2020). Hematopoietic Stem Cell Transplantation (HSCT), or Bone Marrow Transplantation (BMT), using healthy donor cells seems to be the preferred method of MLCs production and infiltration. Unfortunately, the efficiency of this method reaches only 5 to 20%, but it can be increased when microglia are depleted using a CSF1R inhibitor. These inhibitors are also proved to be relatively safe in humans, as evidenced by clinical trials Kwon (2022). Allogeneic HSCT could be beneficial for patients suffering from genetic disorders, such as CSF1R-related leukoencephalopathy, because the donor MLCs would carry the correct gene variant Han et al. (2020).

To battle the unfortunately low efficiency of BMT, Xu *et al.* introduced three different strategies of microglia replacement:

1. Microglia replacement by bone marrow transplantation (Mr BMT), which refers to transplantation of allogeneic Bone Marrow Cells (BMCs) able to differentiate into microglia-like cells (Bone Marrow-Derived Microglia-like (BMDML) cells) and engraft in the CNS. The replacement efficiency in the brain and the spinal cord is approximately 92.60% Xu et al. (2020, 2021a).
2. Microglia replacement by peripheral blood (Mr PB), which refers to the induction of peripheral blood cells (native or donor-derived) into MLCs able to engraft in the CNS. The efficiency reaches 80.74% in the brain. The main benefit of this method is the higher availability of donor cells compared to bone marrow Xu et al. (2020, 2021b).
3. Microglia replacement by Microglia Transplantation (MT) (Mr MT), which refers to direct microglial administration into the brain and offers control of the engraftment region instead of being CNS-wide. This method has only approximately 50% efficiency. The main benefit is that the introduced microglia resemble the most the endogenous microglia, and the preciseness regarding the engraftment region Xu et al. (2020, 2021c).

For a more detailed comparison between the three methods, readers are encouraged to refer to the work of Xu et al. (2020). Importantly, all three strategies offer the option of *in vivo* gene correction, either by introducing wild-type donor cells or by modifying *ex vivo* the autologous cells Xu et al. (2020).

Regarding the rejuvenation strategies of microglia, they either aim to ameliorate or reverse the aged phenotype. A variety of different methods are still being examined in order to achieve this. Roughly, there are four different methodologies:

1. Microglia replacement
2. Anti-inflammatory and anti-oxidant strategies
3. Prevention or reversion of physiological changes in aged microglia
4. Modulation of the senescent microglia milieu Wong (2013)

The second, third and fourth methodology are analyzed in depth in the review of Wong (2013).

The most straightforward strategy to rejuvenate microglia fully is to replace them, and the replacement by CSF1R inhibition and endogenous repopulation is shown to improve cognitive function in aged mice Elmore et al. (2018). Other methods include the administration of agents with anti-inflammatory activity, such as minocycline and antagonists for

the receptor of IL1, or anti-oxidating activity, such as flavonoids and other dietary supplements. Measures to correct the pathways which are impaired with aging are also reported. Examples include decreasing the accumulation of lipofuscin using fenretinide, enhancing autophagy using rapamycin and inhibiting the accumulation of mitochondrial DNA mutations by enhancing the activity or expression of mitochondrial transcription factor A Wong (2013).

Other methods involve restoring the expression of markers that are differentially expressed in aged microglia. For instance, CD36 is a molecule crucial for microglial phagocytosis and its downregulated in aged microglia. In the pathology of MS, recruitment of microglia in demyelinating axons is crucial, in order for microglia to phagocytose the myelin debris and secrete factors necessary for remyelination. This recruitment is regulated partly by CD36. Administration of niacin in mice restores the expression of CD36, deeming this rejuvenation strategy a possible strategy for the treatment of MS Rawji et al. (2020). In a similar concept, administration of a CD22 antagonist and the consequent improvement in phagocytosis aims to improve cognitive deficits and microglial aging Kwon (2022). Inhibition of NLRP3, for example with the molecule MCC950, could also be a strategy to alleviate the inflammatory response in aged microglia Dema et al. (2021).

Another study proved the importance of prostaglandin E2 and its receptor EP2 in aged microglia. The production of this prostaglandin is increased with aging, and results in reduced metabolic rate in microglia, while the EP2 receptor is the main responsible receptor for the effects of prostaglandin E2. Inhibition of the EP2 receptor led to better energy production in microglia, improved cognition and synaptic plasticity, and alleviated inflammaging, thus pinpointing antagonists of the EP2 receptor as a possible microglia rejuvenation strategy Minhas et al. (2021).

Application of Microglia Replacement in Preclinical Models

Many researchers have proved the effectiveness of microglia replacement in animal models of neurological diseases. The most promising results from preclinical trials in animal models of neurodegeneration are summarized in table 1. In a mouse model with extensive neuronal loss, microglia depletion using the CSF1R antagonist PLX5622 followed by endogenous microglia repopulation led to significant resolution of neuroinflammation and, more significantly, to improvement of the functional deficits Rice et al. (2017). Similar results were obtained from brains of aged mice, in which endogenous microglia replacement resulted in memory improvement, shifting of the microglial morphology, microglial characteristics and neuronal gene expression towards that of young mice, and general amelioration of aged-related impairment in the brain Elmore et al. (2018).

Microglia replacement strategies have already been tested in preclinical models of AD. BMDML cells injected into the hippocampi of AD mice accumulate around aβ plaques, and phagocytose aβ, minimizing the amount of plaques. An improvement in cognitive function is also observed Kawanishi et al. (2018). In another study, it has been observed that BMDML cells possibly condition endogenous microglia to phagocytose aβ by secreting Transforming Growth Factor-β1 (TGF-β1) Kuroda et al. (2020a). The injection of *ex vivo* produced peripheral blood-derived MLCs, stemming from differentation of Hematopoietic Stem Cells (HSCs), into the hippocampi of AD mice had similar outcomes with the previously mentioned study, namely the reduction of aβ burden and improvement in cognition. The latter study appears as a more clinically relevant method, as it uses autologous cells Kuroda et al. (2020b).

In familial ALS, microglia replacement is also beneficial. The first supporting evidence regarding this comes from a PU.1 knockout mouse model carrying the $SOD1^{G93A}$ mutation. BMT performed in this mouse model resulted in brain engraftment of wild-type microglia, which led to retardation of disease progression and extended survival time Beers et al. (2006). In the $SOD1^{G93A}$ mouse model, clodronate liposome was used as an agent to deplete microglia, and BMT was performed afterwards using cells expressing the wild-type *SOD1*. This experimental design resulted in replacement of endogenous microglia with BMDML cells expressing wild type SOD1, postponing of ALS progression, and extended survival time Lee et al. (2012). The Kobashi laboratory found bone marrow-derived mononuclear cells exhibiting high ability of proliferation and of differentiation into microglia, namely the BM-iMG cells. BM-iMG cells were transplanted into the spinal cord of $SOD1^{G93A}$ mice. The outcomes were increased survival time, amelioration of motor symptoms, reduced neuron demise and microgliosis, upregulated expression of beneficial genes and downregulated expression of pro-inflammatory genes Kobashi et al. (2022).

Furthermore, embryonic stem cell-derived microglia can serve as a delivery vehicle for neuroprotective therapeutics, when injected intravenously, as examined in a mouse model of EAE. The transplanted MLCs engrafted around the lesions. Even without the inclusion of a therapeutic agent in the transplanted cells, these MLCs acted benecifically against EAE when localized in the spinal cord, but the improvement was not significant Beutner et al. (2013).

A newly developed method managed to achieve microglia replacement in the brain nearing 90% efficiency, by treating mice with CSF1R inhibitors after BMT. This resulted in incorporation of peripheral blood-derived MLCs,

Table 1
The most imporant preclinical trials of microglia replacement strategies in neurological diseases, showing promising results. Glucosylceramidase beta (GBA), Knock-in (KI), Knock-out (KO), Prosaposin (Psap), Prosaposin Transgene Shibuya et al. (2022) (PS-NA)

Disease	Animal Model	Intervention	Outcome	Reference
Neuronal Loss	CaM/Tet-DTAxThy1-GFP-M mice	Endogenous Microglia Repopulation	Reduction of Inflammatory Response, Functional Recovery	Rice et al. (2017)
Aging	Aged mice	Endogenous Microglia Repopulation	Reversal of age-related deficits	Elmore et al. (2018)
Progressive neurodegeneration	$GBA1^{D409H}$xPsap KOxPS-NA mice	BMT	Amelioration of pathology, slowed progression, extended survival time	Shibuya et al. (2022)
GBA-associated PD	GBA^{D409V} KI mice	BMT	Successful Gene Delivery by MLCs	Plasschaert et al. (2022)
PGRN-associated FTD	Grn^{R493X} mice	BMT	Successful Gene Delivery by MLCs	Plasschaert et al. (2022)
AD	APP/PS1 mice	BMT	Decreased aβ deposition	Li et al. (2011)
AD	APdE9 mice	MT using BMDML cells	Increased aβ clearance, improved cognitive function	Kawanishi et al. (2018)
AD	APdE9 mice	MT using BMDML cells	TGF-β1 secretion, increased aβ clearance	Kuroda et al. (2020a)
AD	APdE9 mice	MT using peripheral blood-derived MLCs	Increased aβ clearance, improved cognitive function	Kuroda et al. (2020b)
MS	EAE mice (myelin oligodendrocyte glycoprotein, incomplete Freunds adjuvant, Pertussis toxin)	Engineered MLCs, intravenous administration	Successful Delivery Vehicle	Beutner et al. (2013)
ALS	$PU.1^{-/-}$x$SOD1^{G93A}$ mice	BMT	Extended survival, slowed disease progression	Beers et al. (2006)
ALS	$SOD1^{G93A}$ mice	BMT	Extended survival, slowed disease progression	Lee et al. (2012)
ALS	$SOD1^{G93A}$ mice	MT using BM-iMG cells	Extended survival, amelioration of symptoms	Kobashi et al. (2022)

called by the authors Circulation-Derived Myeloid Cells (CDMCs), in the brain. Applying this methodology in a mouse model characteristic of neurodegenerative conditions, namely prosaposin-mutant mice, when the symptoms of the disease were already evident, resulted in amelioration of CNS pathology, halting of neurodegeneration and extended survival time of the mice. Noteworthy, CDMCs are distinct from microglia. They exhibit different gene expression, morphology and functions, while they still have similarities to microglia, such as the expression of TMEM119, a similarly branched morphology, and the ability to phagocytose Shibuya et al. (2022).

Clinical Translation of Microglia Replacement Therapy

While the benefit of microglia replacement therapy in NDs is made clear by preclinical trials, in order for microglia-based therapies to reach humans several key limitations have first to be examined. To start, MLCs are similar but not identical with native microglia, their differences laying in their origin and characteristics. BMDML cells exhibit high similarity regarding gene expression with microglia, of approximately 90%. Other shared traits are the observed self-renewal of MLCs, and their response to alterations in CNS environment, as they are able to change their phenotype. Remarkably, their full functionality is not established, and possible deviations from microglia functions may exist, especially regarding the effects on neurons or their ability to scan the CNS environment Zhang et al. (2023).

Besides the need to determine if MLCs have unexpected effects and interactions in the CNS, several other considerations need to be taken into account. In order for microglia replacement therapies to be utilized, we need to determine the ideal timing for the depletion and repopulation of microglia regarding the stage of each disease. It is probable that if the disease has already progressed too far, the replacement will not be beneficial. This can be investigated in preclinical models, examining the efficacy and safety of the therapy in different timepoints, preferably when symptoms are already present. Moreover, long-term effects and long-term efficacy of the therapy should be studied.

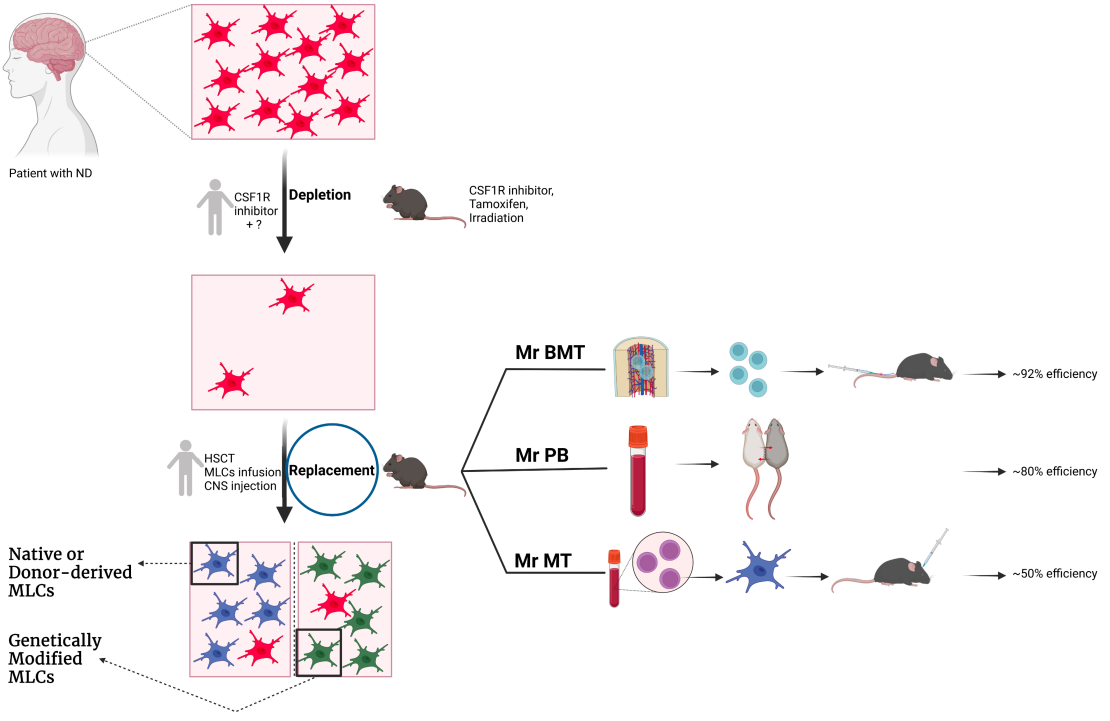

Figure 5: The most up-to-date microglia replacement strategies in mice, and a simplification of the envisioned future strategies applicable in humans. The native microglia are depicted with red color, while the blue colored are transplanted MLCs and the green colored are *ex vivo* genetically modified and transplanted MLCs. Both types of MLCs can be donor or patient-derived, either differentiated in the patient's body after BMT or *ex vivo* differentiated and then blood infused or injected in the CNS, the later administrative method being relevant to MT. In mice, CSF1R inhibitor and 9 Gy head irradiation, or tamoxifen, are usually administrated to produce a migrolial niche Xu et al. (2020). Afterwards, the replacement can occur throught BMT (Mr BMT), parabiosis and peripheral blood transfusion (Mr PB), or direct CNS injection (Mr MT), with the methods exhibiting different efficiencies in microglia replacement. Created with BioRender.com

The existing strategies for microglia replacement refer only to animal models, and their translation to humans remains difficult, partially because of the need to use harsh methods (irradiation, chemotherapeutics) to create the necessary microglial niche. These methods have serious adverse events such as immunosuppression or higher risk of cancer. Moreover, the mainly relevant method for humans up to now is HSCT, which also has drawbacks, such as

the need to harvest cells from donors, the necessary immunosuppression of the patient, the high mortality and low efficacy of the treatment, the unknown long-term effects, and the distinction between native microglia and the MLCs that inhabit the brain Walsh and Lukens (2023). In addition, the BMT outcome is characterized by high variability, because the whole bone marrow is too miscellaneous and ill-defined. The severity of BMT renders it an option only for the treatment of end-stage diseases. For these reasons, primary HSCs can be utilized instead of BMT, a method more clinically revelant Shibuya et al. (2022). The transplantation of genetically engineered MLCs appears as the most promising strategy. It can either introduce cells with the correct genetic variant, such as the wild-type *SOD1* in the case of ALS, or it can enhance the neuroprotective effect of MLCs, for example by introducing in them a beneficial mutation in *TREM2* in the case of AD, to increase the aβ clearance. In advance, these cells can be autologous and thus the infusion would be safer for the patient due to lack of immune response. If coupled with human-applicable Mr MT instead of blood infusion, which would lead to a regional specific integration of exogenous microglia, these settings seem to conclude in the most controllable outcome. Unfortunately, the invasive administration of MT cannot be overlooked. A depiction of the probable human and current mouse microglia replacement methodology is depicted in figure 5, with the questions about human depletion and replacement strategies being unanswered.

In support of using genetically modified MLCs for microglia replacement, a recent finding paves the way for their clinical application. This is based on the work of Chadarevian et al. (2022), who engineered a CSF1R variant altered only in one amino acid position but rendered resistant to inhibitors. Introducing this mutant form of CSF1R into MLCs enables them to be unaffected by the common depletion strategy that uses CSF1R inhibitors. Thus, the depletion of native microglia by administration of a CSF1R inhibitor can occur concomitantly with the replacement by the engineered MLCs. Inducing this mutation in human-induced pluripotent stem cell–derived microglia and transplanting these cells into mouse brains after CSF1R inhibitor treatment resulted in the exogenous microglia being engrafted for more than a month after ceasement of the CSF1R inhibitor administration. Some brain regions were characterized by lower engraftment efficiency. Furthermore, the transplanted microglia resembled native microglia regarding gene expression and immune response. This methodolody foreshadows future clinical applications of engineered MLCs without the use of harsh microglia depletion strategies. Further proof-of-concept studies, as also complete characterization of the effects of the mutated CSF1R in cell function, especially *in vivo*, are some of the next necessary steps to validate this strategy.

Noteworthy, various groups have been developing more representing for human diseases preclinical models, by transplanting human microglia into the brain of mice. These microglia keep their human "signature" while adapting to the mouse brain environment, allowing us to examine *in vivo* the response of microglia more realistically. These models can be further used to study the effects of the transplantation of genetically modified microglia, or even patient-derived MLCs Hasselmann et al. (2019); Fattorelli et al. (2021).

Other Possible Microglial Therapeutics

The aim of this review is to highlight the complex pathological role of microglia in some NDs, namely AD, PD, MS, ALS and FTD, and also in GB. Based on the detection of some beneficial against those diseases subpopulations of microglia, on the need to rejuvenate the senescent microglia in neurodegeneration, and also based on the promising results of preclinical trials, we propose microglia replacement strategy as a therapeutic option against NDs. As for glioblastoma treatment, microglia can either be used as a target to be eliminated, or the microglia replacement strategy could be utilized in combination with a method that favors the induction of only beneficial subpopulations, if possible. While further research is needed to determine the plausibility of microglia replacement, encouraging steps have already been made towards that direction.

Including microglia replacement and rejuvenation strategies for the treatment of NDs, microglia-based therapeutic options are being considered in the scientific community. The first generation regarding these therapies is the anti-inflammatory strategy, in which it was hypothesized that anti-inflammatory agents would battle the neuroinflammation observed in NDs Kwon (2022), and immunotherapy against TAMs would alleviate glioblastoma progression Rivera et al. (2021). The results from clinical trials from this first generation therapeutics were not positive. The second generation involves shifting the balance of microglia phenotype towards the oversimplified M2 phenotype, either by direct administration of agents favoring this shifting, or by transplantation of stem cells that indirectly support the shifting. This second-generation therapeutic option is exhibiting better results. Following is the third generation of microglia-based therapies, which refers to influencing the phagocytosis pathway, in a correcting or enhancing manner, but does not have any results from clinical trials. Lastly, the fourth generation regards rejuvenation of aged microglia

and the microglia replacement strategies Kwon (2022). Regardless, advanced therapeutic strategies could involve the combination of different microglia-based and conventional treatments, a method highly relevant for all NDs and glioblastoma, but applicable when additional research has revealed the unknown aspects of microglia replacement strategies.

Abbreviations

aβ Amyloid beta. 6, 7, 13, 14, 16

AD Alzheimer's Disease. 2, 4, 6, 7, 9, 11, 13, 14, 16, 17

ALS Amyotrophic Lateral Sclerosis. 2, 9–11, 13, 14, 16

ApoE Apolipoprotein E. 4, 6, 7

ARM Activated Response Microglia. 11

ATP Adenosine Triphosphate. 4

BBB Blood-Brain Barrier. 2, 7

BM-iMG Bone Marrow-derived inducible Microglia-like cells. 13, 14

BMCs Bone Marrow Cells. 12

BMDML Bone Marrow-Derived Microglia-like. 12–15

BMT Bone Marrow Transplantation. 12–16

CDMCs Circulation-Derived Myeloid Cells. 14

CNS Central Nervous System. 1–5, 7, 8, 12, 14, 15

CSF1R Colony Stimulating Factor 1 Receptor. 2, 8, 9, 11–13, 15, 16

CX3CR1 C-X3-C motif chemokine receptor 1. 2, 7

DAM Disease-Associated Microglia. 4, 6, 7, 9, 11

DAMP Disease-Associated Molecular Pattern. 7

DAMPs Disease-Associated Molecular Patterns. 4, 5

DAMs Disease-Associated Microglial cells. 4, 6, 7

EAE Experimental Autoimmune Encephalomyelitis. 8, 9, 13, 14

FTD Frontotemporal Dementia. 2, 10, 14, 16

GB Glioblastoma. 2, 10, 11, 16

GBA Glucosylceramidase beta. 14

HAM Human AD Microglia. 11

HLA-DR Human Leukocyte Antigen – DR isotype, type of MHC-II receptor. 8

HSCs Hematopoietic Stem Cells. 13, 16

HSCT Hematopoietic Stem Cell Transplantation. 12, 15

IFN-γ Interferon gamma. 7

IGF1 Insulin-like Growth Factor-1. 3

IL Interleukin. 4–6, 9, 13

IL-1β Interleukin-1 beta. 3–8

KI Knock-in. 14

KO Knock-out. 14

LDAMs Lipid-Droplet-Accumulating Microglial cells. 5

M1 M1 phenotype (pro-inflammatory). 3, 4

M2 M2 phenotype (anti-inflammatory). 3, 4, 10, 16

MGnD Microglial Neurodegenerative Phenotype. 11

MHC Major Histocompatibility Complex. 2, 7, 11

MIMS Microglia Inflamed in MS. 9, 11

MLCs Microglia-Like Cells. 12–16

MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. 7

MS Multiple Sclerosis. 2, 8, 9, 11, 13, 14, 16, 18

MT Microglia Transplantation. 12, 14–16

ND Neurodegenerative Disease. 6

NDs Neurodegenerative Diseases. 2, 4, 6, 8, 11, 15–17

NLRP3 NLR family pyrin domain containing 3. 5, 7, 13

PD Parkinson's Disease. 2, 7, 8, 11, 14, 16

PGRN Progranulin. 10, 14

PS-NA Prosaposin Transgene Shibuya et al. (2022). 14

Psap Prosaposin. 14

ROS Reactive Oxygen Species. 4, 5, 7, 8

RRMS Relapsing-Remitting Multiple Sclerosis. 8

SNpc Substantia Nigra pars compacta. 7

SOD1 Superoxide Dismutase 1. 9, 13, 14, 16

SPMS Secondary Progressive Multiple Sclerosis. 8

TAMs Tumour-associated Macrophages and Microglia. 10, 11, 16

TGF-β1 Transforming Growth Factor-β1. 13, 14

TLR4 Toll-Like Receptor 4. 9

TLRs Toll-Like Receptors. 4, 6, 9

TME Tumour Microenvironment. 10, 11

TMEM119 Transmembrane Protein 119. 2, 5, 8, 14

TNF-α Tumour Necrosis Factor-alpha. 3, 4, 6–8

TREM2 Triggering Receptor Expressed on Myeloid cells-2. 4, 6–9, 16

References

F. Pérez-Cerdá, M. V. Sánchez-Gómez, C. Matute, Pío del río hortega and the discovery of the oligodendrocytes, Frontiers in Neuroanatomy 9 (2015).

A. Nimmerjahn, F. Kirchhoff, F. Helmchen, Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo, Science 308 (2005) 1314–1318.

L. Lawson, V. Perry, P. Dri, S. Gordon, Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain, Neuroscience 39 (1990) 151–170.

F. Ginhoux, M. Greter, M. Leboeuf, S. Nandi, P. See, S. Gokhan, M. F. Mehler, S. J. Conway, L. G. Ng, E. R. Stanley, I. M. Samokhvalov, M. Merad, Fate mapping analysis reveals that adult microglia derive from primitive macrophages, Science 330 (2010) 841–845.

K. Kierdorf, D. Erny, T. Goldmann, V. Sander, C. Schulz, E. G. Perdiguero, P. Wieghofer, A. Heinrich, P. Riemke, C. Hölscher, D. N. Müller, B. Luckow, T. Brocker, K. Debowski, G. Fritz, G. Opdenakker, A. Diefenbach, K. Biber, M. Heikenwalder, F. Geissmann, F. Rosenbauer, M. Prinz, Microglia emerge from erythromyeloid precursors via pu.1- and irf8-dependent pathways, Nature Neuroscience 16 (2013) 273–280.

B. Ajami, J. L. Bennett, C. Krieger, W. Tetzlaff, F. M. V. Rossi, Local self-renewal can sustain CNS microglia maintenance and function throughout adult life, Nature Neuroscience 10 (2007) 1538–1543.

M. R. Elmore, A. R. Najafi, M. A. Koike, N. N. Dagher, E. E. Spangenberg, R. A. Rice, M. Kitazawa, B. Matusow, H. Nguyen, B. L. West, K. N. Green, Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain, Neuron 82 (2014) 380–397.

H. Kettenmann, F. Kirchhoff, A. Verkhratsky, Microglia: New roles for the synaptic stripper, Neuron 77 (2013) 10–18.

D. P. Schafer, E. K. Lehrman, A. G. Kautzman, R. Koyama, A. R. Mardinly, R. Yamasaki, R. M. Ransohoff, M. E. Greenberg, B. A. Barres, B. Stevens, Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner, Neuron 74 (2012) 691–705.

M. W. Salter, B. Stevens, Microglia emerge as central players in brain disease, Nature Medicine 23 (2017) 1018–1027.

R. C. Paolicelli, G. Bolasco, F. Pagani, L. Maggi, M. Scianni, P. Panzanelli, M. Giustetto, T. A. Ferreira, E. Guiducci, L. Dumas, D. Ragozzino, C. T. Gross, Synaptic pruning by microglia is necessary for normal brain development, Science 333 (2011) 1456–1458.

H. Kettenmann, U.-K. Hanisch, M. Noda, A. Verkhratsky, Physiology of microglia, Physiological Reviews 91 (2011) 461–553.

O. Butovsky, M. P. Jedrychowski, C. S. Moore, R. Cialic, A. J. Lanser, G. Gabriely, T. Koeglsperger, B. Dake, P. M. Wu, C. E. Doykan, Z. Fanek, L. Liu, Z. Chen, J. D. Rothstein, R. M. Ransohoff, S. P. Gygi, J. P. Antel, H. L. Weiner, Identification of a unique TGF-$TEST$–dependent molecular and functional signature in microglia, Nature Neuroscience 17 (2013) 131–143.

U.-K. Hanisch, H. Kettenmann, Microglia: active sensor and versatile effector cells in the normal and pathologic brain, Nature Neuroscience 10 (2007) 1387–1394.

N. Cartier, C.-A. Lewis, R. Zhang, F. M. V. Rossi, The role of microglia in human disease: therapeutic tool or target?, Acta Neuropathologica 128 (2014) 363–380.

L. Muzio, A. Viotti, G. Martino, Microglia in neuroinflammation and neurodegeneration: From understanding to therapy, Frontiers in Neuroscience 15 (2021).

D. Nayak, T. L. Roth, D. B. McGavern, Microglia development and function, Annual Review of Immunology 32 (2014) 367–402.

M. Ueno, Y. Fujita, T. Tanaka, Y. Nakamura, J. Kikuta, M. Ishii, T. Yamashita, Layer v cortical neurons require microglial support for survival during postnatal development, Nature Neuroscience 16 (2013) 543–551.

J. L. Marin-Teva, I. Dusart, C. Colin, A. Gervais, N. van Rooijen, M. Mallat, Microglia promote the death of developing purkinje cells, Neuron 41 (2004) 535–547.

C. N. Parkhurst, G. Yang, I. Ninan, J. N. Savas, J. R. Yates, J. J. Lafaille, B. L. Hempstead, D. R. Littman, W.-B. Gan, Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor, Cell 155 (2013) 1596–1609.

Y.-D. Zhang, T. Jiang, S.-Y. Wang, X.-X. Fu, R. Duan, B. Wei, H.-M. Cao, E. Yan, S.-Y. Chen, The alzheimer's disease-associated gene TREML2 modulates inflammation by regulating microglia polarization and NLRP3 inflammasome activation, Neural Regeneration Research 18 (2023) 434.

S. David, A. Kroner, Repertoire of microglial and macrophage responses after spinal cord injury, Nature Reviews Neuroscience 12 (2011) 388–399.

N. Gupta, S. Shyamasundar, R. Patnala, A. Karthikeyan, T. V. Arumugam, E.-A. Ling, S. T. Dheen, Recent progress in therapeutic strategies for microglia-mediated neuroinflammation in neuropathologies, Expert Opinion on Therapeutic Targets 22 (2018) 765–781.

R. C. Paolicelli, A. Sierra, B. Stevens, M.-E. Tremblay, A. Aguzzi, B. Ajami, I. Amit, E. Audinat, I. Bechmann, M. Bennett, F. Bennett, A. Bessis, K. Biber, S. Bilbo, M. Blurton-Jones, E. Boddeke, D. Brites, B. Brône, G. C. Brown, O. Butovsky, M. J. Carson, B. Castellano, M. Colonna, S. A. Cowley, C. Cunningham, D. Davalos, P. L. D. Jager, B. de Strooper, A. Denes, B. J. Eggen, U. Eyo, E. Galea, S. Garel, F. Ginhoux, C. K. Glass, O. Gokce, D. Gomez-Nicola, B. González, S. Gordon, M. B. Graeber, A. D. Greenhalgh, P. Gressens, M. Greter, D. H. Gutmann, C. Haass, M. T. Heneka, F. L. Heppner, S. Hong, D. A. Hume, S. Jung, H. Kettenmann, J. Kipnis, R. Koyama, G. Lemke, M. Lynch, A. Majewska, M. Malcangio, T. Malm, R. Mancuso, T. Masuda, M. Matteoli, B. W. McColl, V. E. Miron, A. V. Molofsky, M. Monje, E. Mracsko, A. Nadjar, J. J. Neher, U. Neniskyte, H. Neumann, M. Noda, B. Peng, F. Peri, V. H. Perry, P. G. Popovich, C. Pridans, J. Priller, M. Prinz, D. Ragozzino, R. M. Ransohoff, M. W. Salter, A. Schaefer, D. P. Schafer, M. Schwartz, M. Simons, C. J. Smith, W. J. Streit, T. L. Tay, L.-H. Tsai, A. Verkhratsky, R. von Bernhardi, H. Wake, V. Wittamer, S. A. Wolf, L.-J. Wu, T. Wyss-Coray, Microglia states and nomenclature: A field at its crossroads, Neuron 110 (2022) 3458–3483.

M. B. Graeber, W. Li, M. L. Rodriguez, Role of microglia in CNS inflammation, FEBS Letters 585 (2011) 3798–3805.

M. E. Lull, M. L. Block, Microglial activation and chronic neurodegeneration, Neurotherapeutics 7 (2010) 354–365.

E. Olson, R. McKeon, Characterization of cellular and neurological damage following unilateral hypoxia/ischemia, Journal of the Neurological Sciences 227 (2004) 7–19.

T. Morioka, A. N. Kalehua, W. J. Streit, Characterization of microglial reaction after middle cerebral artery occlusion in rat brain, The Journal of Comparative Neurology 327 (1993) 123–132.

M. Tsuda, A. Mizokoshi, Y. Shigemoto-Mogami, S. Koizumi, K. Inoue, Activation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury, Glia 45 (2003) 89–95.

H. Keren-Shaul, A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T. K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz, I. Amit, A unique microglia type associated with restricting development of alzheimer's disease, Cell 169 (2017) 1276–1290.e17.

J.-C. Lambert, , C. A. Ibrahim-Verbaas, D. Harold, A. C. Naj, R. Sims, C. Bellenguez, G. Jun, A. L. DeStefano, J. C. Bis, G. W. Beecham, B. Grenier-Boley, G. Russo, T. A. Thornton-Wells, N. Jones, A. V. Smith, V. Chouraki, C. Thomas, M. A. Ikram, D. Zelenika, B. N. Vardarajan, Y. Kamatani, C.-F. Lin, A. Gerrish, H. Schmidt, B. Kunkle, M. L. Dunstan, A. Ruiz, M.-T. Bihoreau, S.-H. Choi, C. Reitz, F. Pasquier, P. Hollingworth, A. Ramirez, O. Hanon, A. L. Fitzpatrick, J. D. Buxbaum, D. Campion, P. K. Crane, C. Baldwin, T. Becker, V. Gudnason, C. Cruchaga, D. Craig, N. Amin, C. Berr, O. L. Lopez, P. L. D. Jager, V. Deramecourt, J. A. Johnston, D. Evans, S. Lovestone, L. Letenneur, F. J. Morón, D. C. Rubinsztein, G. Eiriksdottir, K. Sleegers, A. M. Goate, N. Fiévet, M. J. Huentelman, M. Gill, K. Brown, M. I. Kamboh, L. Keller, P. Barberger-Gateau, B. McGuinness, E. B. Larson, R. Green, A. J. Myers, C. Dufouil, S. Todd, D. Wallon, S. Love, E. Rogaeva, J. Gallacher, P. S. George-Hyslop, J. Clarimon, A. Lleo, A. Bayer, D. W. Tsuang, L. Yu, M. Tsolaki, P. Bossù, G. Spalletta, P. Proitsi, J. Collinge, S. Sorbi, F. Sanchez-Garcia, N. C. Fox, J. Hardy, M. C. D. Naranjo, P. Bosco, R. Clarke, C. Brayne, D. Galimberti, M. Mancuso, F. Matthews, S. Moebus, P. Mecocci, M. D. Zompo, W. Maier, H. Hampel, A. Pilotto, M. Bullido, F. Panza, P. Caffarra, B. Nacmias, J. R. Gilbert, M. Mayhaus, L. Lannfelt, H. Hakonarson, S. Pichler, M. M. Carrasquillo, M. Ingelsson, D. Beekly, V. Alvarez, F. Zou, O. Valladares, S. G. Younkin, E. Coto, K. L. Hamilton-Nelson, W. Gu, C. Razquin, P. Pastor, I. Mateo, M. J. Owen, K. M. Faber, P. V. Jonsson, O. Combarros, M. C. O'Donovan, L. B. Cantwell, H. Soininen, D. Blacker, S. Mead, T. H. Mosley, D. A. Bennett, T. B. Harris, L. Fratiglioni, C. Holmes, R. F. A. G. de Bruijn, P. Passmore, T. J. Montine, K. Bettens, J. I. Rotter, A. Brice, K. Morgan, T. M. Foroud, W. A. Kukull, D. Hannequin, J. F. Powell, M. A. Nalls, K. Ritchie, K. L. Lunetta, J. S. K. Kauwe, E. Boerwinkle, M. Riemenschneider, M. Boada, M. Hiltunen, E. R. Martin, R. Schmidt, D. Rujescu, L.-S. Wang, J.-F. Dartigues, R. Mayeux, C. Tzourio, A. Hofman, M. M. Nöthen, C. Graff, B. M. Psaty, L. Jones, J. L. Haines, P. A. Holmans, M. Lathrop, M. A. Pericak-Vance, L. J. Launer, L. A. Farrer, C. M. van Duijn, C. V. Broeckhoven, V. Moskvina, S. Seshadri, J. Williams, G. D. Schellenberg, P. Amouyel, , and, Meta-analysis of 74, 046 individuals identifies 11 new susceptibility loci for alzheimer's disease, Nature Genetics 45 (2013) 1452–1458.

T. R. Hammond, C. Dufort, L. Dissing-Olesen, S. Giera, A. Young, A. Wysoker, A. J. Walker, F. Gergits, M. Segel, J. Nemesh, S. E. Marsh, A. Saunders, E. Macosko, F. Ginhoux, J. Chen, R. J. Franklin, X. Piao, S. A. McCarroll, B. Stevens, Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes, Immunity 50 (2019) 253–271.e6.

S. Safaiyan, N. Kannaiyan, N. Snaidero, S. Brioschi, K. Biber, S. Yona, A. L. Edinger, S. Jung, M. J. Rossner, M. Simons, Age-related myelin degradation burdens the clearance function of microglia during aging, Nature Neuroscience 19 (2016) 995–998.

M.-S. Kwon, Advanced therapeutic strategies targeting microglia: beyond neuroinflammation, Archives of Pharmacal Research 45 (2022) 618–630.

B. Brawek, M. Skok, O. Garaschuk, Changing functional signatures of microglia along the axis of brain aging, International Journal of Molecular Sciences 22 (2021) 1091.

K. S. Rawji, A. M. Young, T. Ghosh, N. J. Michaels, R. Mirzaei, J. Kappen, K. L. Kolehmainen, N. Alaeiilkhchi, B. Lozinski, M. K. Mishra, A. Pu, W. Tang, S. Zein, D. K. Kaushik, M. B. Keough, J. R. Plemel, F. Calvert, A. J. Knights, D. J. Gaffney, W. Tetzlaff, R. J. M. Franklin, V. W. Yong, Niacin-mediated rejuvenation of macrophage/microglia enhances remyelination of the aging central nervous system, Acta Neuropathologica 139 (2020) 893–909.

J. Marschallinger, T. Iram, M. Zardeneta, S. E. Lee, B. Lehallier, M. S. Haney, J. V. Pluvinage, V. Mathur, O. Hahn, D. W. Morgens, J. Kim, J. Tevini, T. K. Felder, H. Wolinski, C. R. Bertozzi, M. C. Bassik, L. Aigner, T. Wyss-Coray, Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain, Nature Neuroscience 23 (2020) 194–208.

L. Wang, C.-C. Yu, X.-Y. Liu, X.-N. Deng, Q. Tian, Y.-J. Du, Epigenetic modulation of microglia function and phenotypes in neurodegenerative diseases, Neural Plasticity 2021 (2021) 1–13.

D. V. Hansen, J. E. Hanson, M. Sheng, Microglia in alzheimer's disease, Journal of Cell Biology 217 (2017) 459–472.

F. Leng, P. Edison, Neuroinflammation and microglial activation in alzheimer disease: where do we go from here?, Nature Reviews Neurology 17 (2020) 157–172.

J. Hasselmann, M. A. Coburn, W. England, D. X. F. Velez, S. K. Shabestari, C. H. Tu, A. McQuade, M. Kolahdouzan, K. Echeverria, C. Claes, T. Nakayama, R. Azevedo, N. G. Coufal, C. Z. Han, B. J. Cummings, H. Davtyan, C. K. Glass, L. M. Healy, S. P. Gandhi, R. C. Spitale, M. Blurton-Jones, Development of a chimeric model to study and manipulate human microglia in vivo, Neuron 103 (2019) 1016–1033.e10.

K. Badanjak, S. Fixemer, S. Smajić, A. Skupin, A. Grünewald, The contribution of microglia to neuroinflammation in parkinson's disease, Int. J. Mol. Sci. 22 (2021).
S. Zia, K. S. Rawji, N. J. Michaels, M. Burr, B. J. Kerr, L. M. Healy, J. R. Plemel, Microglia diversity in health and multiple sclerosis, Frontiers in Immunology 11 (2020).
B. L. Guerrero, N. L. Sicotte, Microglia in multiple sclerosis: Friend or foe?, Frontiers in Immunology 11 (2020).
B. E. Clarke, R. Patani, The microglial component of amyotrophic lateral sclerosis, Brain 143 (2020) 3526–3539.
D. Lall, R. H. Baloh, Microglia and c9orf72 in neuroinflammation and ALS and frontotemporal dementia, Journal of Clinical Investigation 127 (2017) 3250–3258.
H. Haukedal, K. Freude, Implications of microglia in amyotrophic lateral sclerosis and frontotemporal dementia, Journal of Molecular Biology 431 (2019) 1818–1829.
D. W. Sirkis, L. W. Bonham, J. S. Yokoyama, The role of microglia in inherited white-matter disorders and connections to frontotemporal dementia, The Application of Clinical Genetics Volume 14 (2021) 195–207.
I. O. C. Woollacott, C. E. Toomey, C. Strand, R. Courtney, B. C. Benson, J. D. Rohrer, T. Lashley, Microglial burden, activation and dystrophy patterns in frontotemporal lobar degeneration, Journal of Neuroinflammation 17 (2020).
N. Geribaldi-Doldán, C. Fernández-Ponce, R. N. Quiroz, I. Sánchez-Gomar, L. G. Escorcia, E. P. Velásquez, E. N. Quiroz, The role of microglia in glioblastoma, Frontiers in Oncology 10 (2021).
F. Khan, L. Pang, M. Dunterman, M. S. Lesniak, A. B. Heimberger, P. Chen, Macrophages and microglia in glioblastoma: heterogeneity, plasticity, and therapy, Journal of Clinical Investigation 133 (2023).
R. S. Andersen, A. Anand, D. S. L. Harwood, B. W. Kristensen, Tumor-associated microglia and macrophages in the glioblastoma microenvironment and their implications for therapy, Cancers 13 (2021) 4255.
R. A. Rice, J. Pham, R. J. Lee, A. R. Najafi, B. L. West, K. N. Green, Microglial repopulation resolves inflammation and promotes brain recovery after injury, Glia 65 (2017) 931–944.
A. E. Walsh, J. R. Lukens, Engineering mighty microglia, Journal of Experimental Medicine 220 (2023).
J. Han, H. Sarlus, Z. K. Wszolek, V. D. Karrenbauer, R. A. Harris, Microglial replacement therapy: a potential therapeutic strategy for incurable CSF1r-related leukoencephalopathy, Acta Neuropathologica Communications 8 (2020).
Z. Xu, Y. Rao, Y. Huang, T. Zhou, R. Feng, S. Xiong, T.-F. Yuan, S. Qin, Y. Lu, X. Zhou, X. Li, B. Qin, Y. Mao, B. Peng, Efficient strategies for microglia replacement in the central nervous system, Cell Reports 32 (2020) 108041.
Z. Xu, X. Zhou, B. Peng, Y. Rao, Microglia replacement by bone marrow transplantation (mr BMT) in the central nervous system of adult mice, STAR Protocols 2 (2021a) 100666.
Z. Xu, Y. Rao, B. Peng, Protocol for microglia replacement by peripheral blood (mr PB), STAR Protocols 2 (2021b) 100613.
Z. Xu, B. Peng, Y. Rao, Microglia replacement by microglia transplantation (mr MT) in the adult mouse brain, STAR Protocols 2 (2021c) 100665.
W. T. Wong, Microglial aging in the healthy CNS: phenotypes, drivers, and rejuvenation, Frontiers in Cellular Neuroscience 7 (2013).
M. R. P. Elmore, L. A. Hohsfield, E. A. Kramár, L. Soreq, R. J. Lee, S. T. Pham, A. R. Najafi, E. E. Spangenberg, M. A. Wood, B. L. West, K. N. Green, Replacement of microglia in the aged brain reverses cognitive, synaptic, and neuronal deficits in mice, Aging Cell 17 (2018) e12832.
M. Dema, H. Eixarch, L. M. Villar, X. Montalban, C. Espejo, Immunosenescence in multiple sclerosis: the identification of new therapeutic targets, Autoimmunity Reviews 20 (2021) 102893.
P. S. Minhas, A. Latif-Hernandez, M. R. McReynolds, A. S. Durairaj, Q. Wang, A. Rubin, A. U. Joshi, J. Q. He, E. Gauba, L. Liu, C. Wang, M. Linde, Y. Sugiura, P. K. Moon, R. Majeti, M. Suematsu, D. Mochly-Rosen, I. L. Weissman, F. M. Longo, J. D. Rabinowitz, K. I. Andreasson, Restoring metabolism of myeloid cells reverses cognitive decline in ageing, Nature 590 (2021) 122–128.
S. Kawanishi, K. Takata, S. Itezono, H. Nagayama, S. Konoya, Y. Chisaki, Y. Toda, S. Nakata, Y. Yano, Y. Kitamura, E. Ashihara, Bone-marrow-derived microglia-like cells ameliorate brain amyloid pathology and cognitive impairment in a mouse model of alzheimer's disease, Journal of Alzheimer's Disease 64 (2018) 563–585.
E. Kuroda, K. Nishimura, S. Kawanishi, M. Sueyoshi, F. Ueno, Y. Toji, N. Abo, T. Konishi, K. Harada, S. Satake, C. Shima, Y. Toda, Y. Kitamura, S. Shimohama, E. Ashihara, K. Takata, Mouse bone marrow-derived microglia-like cells secrete transforming growth factor-$TEST$1 and promote microglial a$TEST$ phagocytosis and reduction of brain a$TEST$, Neuroscience 438 (2020a) 217–228.
E. Kuroda, K. Takata, K. Nishimura, H. Oka, M. Sueyoshi, M. Aitani, A. Kouda, S. Satake, C. Shima, Y. Toda, S. Nakata, Y. Kitamura, E. Ashihara, Peripheral blood-derived microglia-like cells decrease amyloid-$TEST$ burden and ameliorate cognitive impairment in a mouse model of alzheimer's disease, Journal of Alzheimer's Disease 73 (2020b) 413–429.
Y. Shibuya, K. K. Kumar, M. M.-D. Mader, Y. Yoo, L. A. Ayala, M. Zhou, M. A. Mohr, G. Neumayer, I. Kumar, R. Yamamoto, P. Marcoux, B. Liou, F. C. Bennett, H. Nakauchi, Y. Sun, X. Chen, F. L. Heppner, T. Wyss-Coray, T. C. Südhof, M. Wernig, Treatment of a genetic brain disease by CNS-wide microglia replacement, Science Translational Medicine 14 (2022).
R. N. Plasschaert, M. P. DeAndrade, F. Hull, Q. Nguyen, T. Peterson, A. Yan, M. Loperfido, C. Baricordi, L. Barbarossa, J. K. Yoon, Y. Dogan, Z. Unnisa, J. W. Schindler, N. P. van Til, L. Biasco, C. Mason, High-throughput analysis of hematopoietic stem cell engraftment after intravenous and intracerebroventricular dosing, Molecular Therapy 30 (2022) 3209–3225.
B. Li, M. E. Gonzalez-Toledo, C.-S. Piao, A. Gu, R. E. Kelley, L.-R. Zhao, Stem cell factor and granulocyte colony-stimulating factor reduce $TEST$-amyloid deposits in the brains of APP/PS1 transgenic mice, Alzheimer's Research & Therapy 3 (2011) 8.
C. Beutner, V. Lepperhof, A. Dann, B. Linnartz-Gerlach, S. Litwak, I. Napoli, M. Prinz, H. Neumann, Engineered stem cell-derived microglia as therapeutic vehicle for experimental autoimmune encephalomyelitis, Gene Therapy 20 (2013) 797–806.
D. R. Beers, J. S. Henkel, Q. Xiao, W. Zhao, J. Wang, A. A. Yen, L. Siklos, S. R. McKercher, S. H. Appel, Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis, Proceedings of the National Academy of Sciences 103 (2006) 16021–16026.
J. C. Lee, J. Seong, S. H. Kim, S. J. Lee, Y. J. Cho, J. An, D.-H. Nam, K. M. Joo, C. I. Cha, Replacement of microglial cells using clodronate liposome and bone marrow transplantation in the central nervous system of SOD1g93a transgenic mice as an in vivo model of amyotrophic lateral sclerosis, Biochemical and Biophysical Research Communications 418 (2012) 359–365.

S. Kobashi, T. Terashima, M. Katagi, M. Urushitani, H. Kojima, Bone marrow-derived inducible microglia-like cells ameliorate motor function and survival in a mouse model of amyotrophic lateral sclerosis, Cytotherapy 24 (2022) 789–801.

J. P. Chadarevian, S. I. Lombroso, G. C. Peet, J. Hasselmann, C. Tu, D. E. Marzan, J. Capocchi, F. S. Purnell, K. M. Nemec, A. Lahian, A. Escobar, W. England, S. Chaluvadi, C. A. O'Brien, F. Yaqoob, W. H. Aisenberg, M. Porras-Paniagua, M. L. Bennett, H. Davtyan, R. C. Spitale, M. Blurton-Jones, F. C. Bennett, Engineering an inhibitor-resistant human CSF1r variant for microglia replacement, Journal of Experimental Medicine 220 (2022).

N. Fattorelli, A. Martinez-Muriana, L. Wolfs, I. Geric, B. D. Strooper, R. Mancuso, Stem-cell-derived human microglia transplanted into mouse brain to study human disease, Nature Protocols 16 (2021) 1013–1033.

M. Rivera, E. D. Bander, B. Cisse, Perspectives on microglia-based immune therapies against glioblastoma, World Neurosurgery 154 (2021) 228–231.