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# Microglial Depletion, a New Tool in Neuroinflammatory Disorders: Comparison of Pharmacological Inhibitors of the CSF-1R

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## ABSTRACT

A growing body of evidence highlights the importance of microglia, the resident immune cells of the CNS, and their pro-inflammatory activation in the onset of many neurological diseases. Microglial proliferation, differentiation, and survival are highly dependent on the CSF-1 signaling pathway, which can be pharmacologically modulated by inhibiting its receptor, CSF-1R. Pharmacological inhibition of CSF-1R leads to an almost complete microglial depletion whereas treatment arrest allows for subsequent repopulation. Microglial depletion has shown promising results in many animal models of neurodegenerative diseases (Alzheimer's disease (AD), Parkinson's disease, or multiple sclerosis) where transitory microglial depletion reduced neuroinflammation and improved behavioral test results. In this review, we will focus on the comparison of three different pharmacological CSF-1R inhibitors (PLX3397, PLX5622, and GW2580) regarding microglial depletion. We will also highlight the promising results obtained by microglial depletion strategies in adult models of neurological disorders and argue they could also prove promising in neurodevelopmental diseases associated with microglial activation and neuroinflammation. Finally, we will discuss the lack of knowledge about the effects of these strategies on neurons, astrocytes, and oligodendrocytes in adults and during neurodevelopment.

## 1 | Introduction

Microglia are the resident immune cells of the central nervous system (CNS). They derive from erythromyeloid precursors in the yolk sac that migrate towards the neuroepithelium during the embryo's development, where they play important developmental and homeostatic roles (Kierdorf et al. 2013; Ginhoux et al. 2010, 2013; Tay et al. 2017). Colony stimulating factor 1 (CSF-1) is the primary growth factor in the erythromyeloid lineage and therefore has a crucial responsibility in microglial proliferation, differentiation, and survival.

Microglial activation participates in the onset of neuroinflammation. These cells express a wide variety of receptors (e.g., receptors to cytokines/chemokines, to so called damage associated molecular patterns (DAMPs) to pathogen-associated molecular patterns (PAMPs), or Toll-like receptors (TLR)) enabling them to perform a broad analysis of their environment. However, the

roles of microglia are diverse, and their description cannot be limited to an inflammatory perspective. Microglia, indeed, are also involved in crucial neurodevelopmental milestones and homeostatic processes. In the white matter, microglia promote fasciculation and myelination (Wlodarczyk et al. 2017) at developmental stages before allowing for the maintenance of oligodendrocyte (OL) progenitors in adults (Hagemeyer et al. 2017). Finally, microglia are involved in neurogenesis. They regulate the number of neuronal progenitor cells (NPCs) by removing excess and apoptotic NPCs (Prinz et al. 2021) and participate in synaptic pruning through their phagocytic capabilities (Sierra et al. 2010).

Unfortunately, the pro-inflammatory activation of microglia keeps them further away from these roles (Krishnan et al. 2017) and leads to the secretion of molecules that can prove deleterious to their environment. More and more data from the literature tend to confirm the implication of activated microglia in numerous neurological diseases such as Alzheimer's (Wang et al. 2015; Martin et al. 2017), Parkinson's (Tang and Le 2016; Liu et al. 2022; Smajić et al. 2022), Huntington's (Palpagama et al. 2019), or multiple sclerosis (Yong 2022; Nissen et al. 2018; Absinta et al. 2021).

Targeting this activated pro-inflammatory microglial population could be a promising therapeutic strategy in these pathologies. The blocking of the CSF-1 signaling pathway through the pharmacological inhibition of the CSF-1 receptor (CSF-1R) allows for the negative modulation of the population of microglia up to 99% depletion. The amplitude and duration of this depletion can be modulated depending on dose and time of drug administration. Adding such molecules to the therapeutic arsenal of juvenile and adult neurological diseases could be a significant step forward.

In this review, we will describe three main pharmacological inhibitors of CSF-1R (PLX3397, PLX5622, and GW2580) and the results obtained when they were used for microglial depletion. We will further highlight the promising neuroprotective results obtained by pharmacological microglial depletion in adults, but also discuss the limits of our current knowledge regarding the effects of these strategies on surrounding cells. Understanding these potential repercussions is crucial to the extension of microglial depletion strategies to neurodevelopmental diseases associated with microglial activation and neuroinflammation.

## 2 | The CSF-1 Pathway

### 2.1 | CSF-1R Ligands

The growth factor CSF-1 (also known as Macrophage Colony Stimulating Factor or M-CSF) is the main effector of homeostasis in the erythromyeloid lineage. Its associated pathway is therefore responsible for the proliferation, differentiation, and survival, of the cells of the mononuclear system (monocytes, dendritic cells, macrophages, microglia, osteoclast) and their medullary progenitors (Stanley et al. 1976). The ability of CSF-1 to stimulate the proliferation of these cells after administration has been demonstrated in vivo in mice (Hume et al. 1988), rats (Ulich et al. 1990), primates (Munn, Garnick, and Cheung 1990) and humans (VandePol and Garnick 1991).

Because of proteolysis and alternative splicing, the CSF-1 protein has three homodimeric active isoforms: a glycoprotein located in the surface membrane (csCSF-1) and two secreted isoforms: a proteoglycan (spCSF-1) and a glycoprotein (sgCSF-1) (Pixley and Stanley 2004). They all share a N-terminal region, including the active area of 149 amino acids structured in 4 alpha helices. Blood circulating concentrations of spCSF-1 and sgCSF-1 are similar, and they elevate endoneurial macrophages to a similar degree (Groh et al. 2016). Studies on genetically engineered isoform specific mice showed that these isoforms had different, although overlapping, roles regarding development and macrophage proliferation (Ryan et al. 2001; Dai et al. 2004). A study in a mouse model of Charcot–Marie-Tooth, highlighted the opposite roles of CSF-1 isoforms regarding macrophage activation with spCSF-1 mediating macrophage activation and csCSF-1 inhibiting it (Groh et al. 2016). Using transgenic mice expressing spCSF-1, csCSF-1 or all isoforms, they indeed showed that, while having similar influences on macrophage overall population, spCSF-1 expression led to an increase in the expression of the activation markers CD206 and CD86 while mice expressing csCSF-1 demonstrated the opposite effect (Groh et al. 2016). In this sense, csCSF-1, being located at the cell membrane, shows a local regulatory role opposed to the secretory isoforms spCSF-1 and sgCSF-1 that act on distant cells.

Many cells produce CSF-1, such as fibroblasts, endothelial cells, monocytes, macrophages, lymphocytes, microglia, osteoblasts, neurons, or astrocytes (Chitu and Stanley 2006; Pollard 2009). In normal conditions, this production is balanced by the endocytosis of CSF-1R upon ligation and the following degradation of CSF-1 (Bartocci et al. 1987; Hamilton 1997).

Nevertheless, its production, as well as circulating and tissue concentrations, increase as part of many biological or pathological events. A transitory increase is found in mice during pregnancy and placental formation (Pollard et al. 1987) but has also been described in inflammatory diseases, cancers, or auto-immune diseases (Chitu and Stanley 2006; Sweet and Hume 2003; Hamilton 2008). In these pathological conditions, pharmacological modulation of the CSF-1/CSF-1R axis could be a promising path to explore.

A second ligand of CSF-1R, interleukin (IL)-34, has recently been identified. Even though they both act similarly upon binding to CSF-1R; CSF-1 and IL-34 do not have any sequence homology with each other, even at their active site (Chihara et al. 2010). Nakamichi et al. and Freuchet et al. demonstrated that the binding of IL-34 to CSF-1R has, although close, different biological effects than CSF-1 (Nakamichi, Udagawa, and Takahashi 2013; Freuchet et al. 2021). A growing body of evidence supports that they bind to different subunits within CSF-1R (Wei et al. 2010). This coexistence of two different ligands with different properties led to the development of specific antibodies for the bonding of each ligand (Chihara et al. 2010). It could furthermore explain the differences between knock-out CSF-1R ($Csfr^{-/-}$) mice and CSF-1 deficient mice ($Csf1^{op/op}$), where $Csfr^{-/-}$ exhibited a more severe macrophage depletion than $Csf1^{op/op}$, suggesting the action of an alternative ligand compensating for the

absence of CSF-1, therefore IL-34 (Nakamichi, Udagawa, and Takahashi 2013).

## 2.2 | CSF-1R

The action of CSF-1 against targeted cells is done by linking to its receptor: CSF-1R, a type III kinase protein receptor located at the extracellular membrane (Stanley and Chitu 2014; Chen et al. 2008). CSF-1 links to the extracellular domain of CSF-1R to induce its homodimerization and the autophosphorylation of tyrosine residues (Figure 1A) (Stanley and Chitu 2014; Guo and Ikegawa 2021; Mun, Park, and Park-Min 2020; Hu et al. 2021), leading to a complex cascade of intracellular signals (Figure 1) well described by Hu et al. (2021).

The structure of CSF-1R is highly conserved between mice and humans. The extracellular domain of CSF-1R contains immunoglobulin (Ig)-like domains to which ligands bind. Three N-terminal Ig domains (D1–D3) contribute to ligand recognition, while the next two Ig domains (D4–D5) are involved in stabilizing the ligand-receptor complex. The cytoplasmic domain consists of two kinase domains, a kinase insert, a juxtamembrane domain, and a carboxy-terminal tail. CSF-1R also undergoes

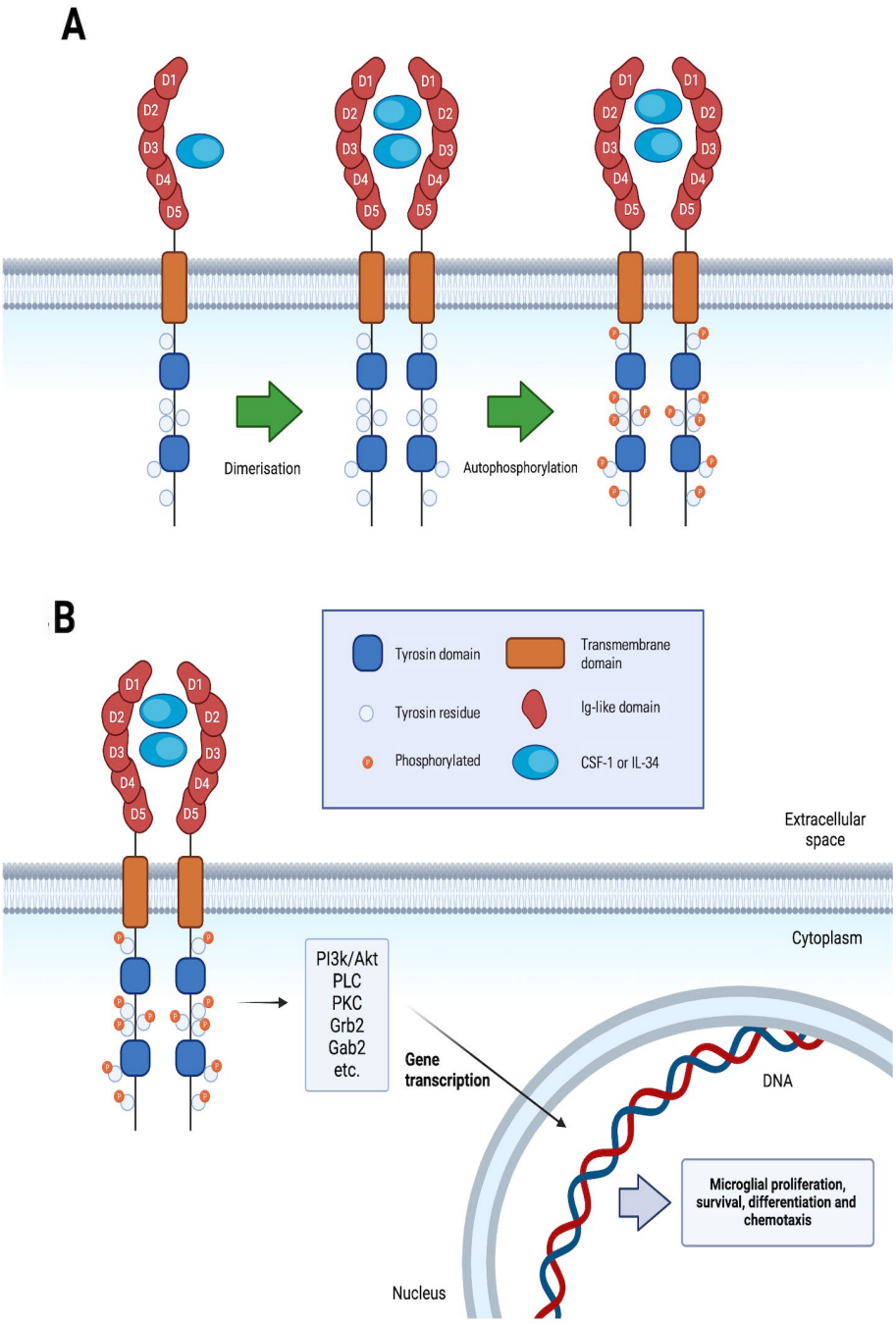


**FIGURE 1** | Activation of CSF1-R upon binding of CSF1, or IL-4 (A) and its signaling cascade leading to microglia proliferation, survival, differentiation and chemotaxis (B).

post-translational modifications such as phosphorylation and glycosylation. In the absence of ligand, CSF-1R is in an inactive autoinhibitory state. Upon ligand binding, the juxtamembrane domain moves from an autoinhibitory position to an activated conformation.

### 2.3 | Roles of the CSF-1 Pathway During Neurodevelopment

The CSF-1 pathway ends up in the production of factors promoting proliferation, differentiation, chemotaxis, and survival of cells of the erythromyeloid lineage (Figure 1B) (Stanley and Chitu 2014). CSF1-R is expressed in a wide variety of cells such as paneth cells (PC) (Huynh et al. 2009), epithelial intestinal cells of the colon (Huynh et al. 2013), renal proximal tubule epithelial cells (Menke et al. 2009), neural progenitor cells (NPCs) (Nandi et al. 2012), and several subpopulations of neurons (Nandi et al. 2012; Luo et al. 2013; Wang, Berezovska, and Fedoroff 1999). Therefore, the relevance of the CSF-1 pathway is not limited to these cells, especially during embryonic and early brain development. This is particularly apparent in studies using $Csf1^{op/op}$ (Kubota et al. 2009) and $Csf1r^{-/-}$ (Gordon et al. 2010) mice where the lack of CSF1 expression leads to impaired angiogenesis and lymphangiogenesis.

During neurodevelopment, the CSF-1/CSF-1R axis is primordial to the differentiation and proliferation of microglia. CSF-1R expression first occurs in erythromyeloid precursor cells (EMPs) at embryonic day (E)8 (Gomez Perdiguero et al. 2015) and in A2 progenitors at E9 (Kierdorf et al. 2013). These progenitors are involved in the second wave (in regard to a first wave of maternally derived macrophages between E7.5–8) of macrophage generation starting from the yolk sac and colonize different tissues. EMPs colonize the fetal liver giving rise to erythrocytes, granulocytes, and monocytes (Gomez Perdiguero et al. 2015) while A2 progenitors colonize the brain and are responsible for the generation of microglia and brain associated macrophages (Kierdorf et al. 2013; Hagemeyer et al. 2017). The IL-34/CSF-1R pathway plays a crucial role in the migration of microglial progenitors towards the CNS, as described in zebrafishes (Wu et al. 2018), and CSF-1R (Erblich et al. 2011) as well as IL-34 (Wang et al. 2012) deficient mice display a drastic reduction in microglial numbers.

In addition to its key roles in microglial development, CSF-1 signaling is also involved in neurogenesis. In vitro experiments, CSF-1 and IL-34 have been shown to regulate NPC self-renewal, differentiation, and survival (Nandi et al. 2012). These properties, conjointly with the ones of microglia in regard to neurogenesis, synaptic pruning, and oligodendrogenesis further put forward the roles of CSF-1 signaling in neurodevelopment. In humans, inactivating mutations of *Csf1r* lead to the onset of progressive dementia in adults (Nicholson et al. 2013; Konno et al. 2014).

## 3 | Pharmacological Modulation of the CSF-1/CSF-1R Axis

Pharmacological modulation of the CSF-1/CSF-1R axis can serve two important scientific and therapeutic purposes. Firstly, it enables the study of the CSF-1 pathway and its targeted cells. Secondly, it offers a potential treatment option for certain cancer types.

### 3.1 | CSF-1R Inhibition in Glioblastoma

The role of the immune system in oncogenesis is ambivalent. While at first targeting cancerous cells, it can later encourage cancer progression by favorizing the infiltration of tumor-associated macrophages (TAMs) (Pan et al. 2020). TAMs play a crucial role in the tumoral microenvironment, therefore promoting tumoral growth and angiogenesis through the secretion of growth factors active on endothelial cells (Lamagna, Aurrand-Lions, and Imhof 2006). CSF-1 is overexpressed in many solid tumors, facilitating the differentiation of monocytes into TAMs and their survival in the tumoral microenvironment (Stafford et al. 2016; Wesolowski et al. 2019; Shi, Yang, et al. 2019). The presence of such infiltrates is associated with adverse prognosis in most cancers (Pedersen et al. 2014) making TAMs perfect therapeutic targets in oncology. Two main pharmacological tools have been developed to act on the CSF-1/CSF-1R axis in TAMs: antibodies targeting either CSF-1R or one of its ligands (CSF-1 or IL-34) and CSF-1R specific tyrosine-kinase inhibitors (Lin 2021; Vaynrub et al. 2022).

These constatations also apply to glioblastoma (GBM), the most aggressive (14.6 months median survival (Dubrow and Darefsky 2011)) and common (15.6% of all primary brain tumors, 54% of primary malignant brain tumors (Ostrom et al. 2013)) type of cancer in the CNS. Similarly to other types of cancers, the progression of GBM is importantly affected by the tumor microenvironment and specifically by the action of resident microglia/macrophages through the production of growth factors (e.g., STI1, (Carvalho da Fonseca et al. 2014), EGF (Coniglio et al. 2012), TGF-β (Wesolowska et al. 2008)) (Watters, Schartner, and Badie 2005). It is that glioma associated microglia/macrophages (GAMs) can represent 30%–50% of the tumor mass (Gutmann et al. 2013). In GBM, several studies have reported the overexpression of CSF-1 (but not of IL-34) and CSF-1R (Bender et al. 2010; Komohara, Jinushi, and Takeya 2014; De et al. 2016; Sun et al. 2019) and their correlation with tumor progression (Komohara et al. 2008). De et al.'s study on a genetically engineered mouse model (FAP-V12Ha-ras-IRESLacZ (Ras*)) designed to modulate CSF-1 expression levels further corroborated the role of CSF-1 in gliomagenesis. Their results showed that CSF-1 deficiency diminished glioma formation while its overexpression accelerated the formation of high-grade gliomas. This accelerated formation was associated with an increased density of glioma associated microglia/macrophages (GAM) and decreased survival rates (De et al. 2016).

The CSF-1/CSF-1R axis plays both a role in the recruitment of GAMs as well as in their acquisition of specific phenotypes. In the tumor microenvironment, CSF-1, overexpressed by glioma cells, acts as a chemoattractant (Coniglio et al. 2012) toward GAMs where CSF-1R-mediated signaling is associated with the acquisition of a pro-tumorigenic, anti-inflammatory M2-like phenotype (Komohara et al. 2008; Pyonteck et al. 2013). More precise description of GAM phenotypes, going beyond the simplistic M1/M2 dichotomy, has been made possible by modern

omic approaches. In this sense, a single-cell RNA sequencing study by Ochocka et al. (Ochocka et al. 2021) highlighted the differential repartition of microglia and macrophages within the tumor mass with microglia being more peripheral to the tumor than infiltration monocytes/macrophages, usually situated at the core of the tumor. However, they demonstrated that these cells adopt a similar phenotype, showing a phenotypic continuum making their precise identification furthermore difficult. This continuum of phenotypes seemed to be partially driven by a differential expression of pro-inflammatory markers and specially of genes encoding components of the MHCII protein (*H2Aa*, *H2-Ab1*, and *H2-Eb1*). This increase was, indeed, more important in microglia, and so at the periphery of the tumor, than in macrophages. Finally, in adequation with the description of M2 microglia and macrophages, *Lgals3* expression was also augmented (Ochocka et al. 2021).

In view of the ineffectiveness of current therapeutic strategies, targeting the microenvironment of gliomas, and especially GAMs, through the inhibition of CSF-1 signaling appears, as in other solid tumors, as a promising therapeutic approach. Pyonteck et al. explored this path using the CSF-1R inhibitor BLZ945 (sotuletinib) to target GAMs in a mouse model of proneural glioblastoma multiforme and showed a significant regression of established tumors and an increase in survival. CSF-1R inhibition led to increased apoptosis of tumorous cells as well as an arrest of their proliferation but, surprisingly, did not cause GAM depletion. This preservation of GAMs was linked to the secretion of factors such as granulocyte-macrophage colony stimulating factor (GM-CSF) and interferon gamma (INFγ) in the tumor microenvironment that promoted their survival. Finally, the beneficial effects of CSF-1 inhibition regarding tumoral growth were correlated with a reduction in the expression of pro-inflammatory markers in GAMs (Pyonteck et al. 2013). In humans, however, the oral administration of PLX3397 showed no efficacy during a phase II trial in patients suffering from recurrent glioblastoma with a 6-month progression-free survival (PFS6) of only 8.6% (Butowski et al. 2016).

These therapeutic strategies targeting the CSF-1/CSF-1R axis have been transposed to specifically target microglia using anti-CSF-1/CSF-1R antibodies or inhibitors of the tyrosine-kinase activity of CSF-1R. The next focus of our review will be on this second approach, particularly on three CSF-1R inhibitors: PLX3397 (pexidartinib), PLX5622, and GW2580. We chose pexidartinib (PLX3397) because of its well-known safety profile as it was approved by the Food and Drug Administration (FDA) in August 2019 for the treatment of tenosynovial tumors (Lamb 2019; U.S. Food and Drug Administration 2019). PLX5622 and GW2580 were selected because of their growing importance in microglia-focused research. Other microglial depletion strategies such as monoclonal antibodies, viral vectors, or clodronate, were not included as they were judged less adaptable to the treatment of neurodegenerative and neurodevelopmental diseases impacted by microglial activation such as Alzheimer's, Parkinson's, multiple sclerosis, or encephalopathy of prematurity. Finally, we will limit the extent of this review to microglial depletion strategies implying direct administration of the molecule to the subject animal and not to gestating mothers, as it has been done in some neurodevelopmental studies (Niiyama, Fujimoto, and Imai 2023; Rosin, Vora, and Kurrasch 2018). These studies highlight the important roles of microglia and macrophages during development as well as the adverse effects of CSF-1R inhibitors on osteoclasts (Rosin, Vora, and Kurrasch 2018). In a study conducted by Rosin et al., treatment using PLX5622 in dams starting at E3.5 led to a 99% microglial depletion in pups by E15.5. These pups later displayed weight loss, craniofacial deformity, difficulty to open their eyes, and a poorer global health status up until P28 (Rosin, Vora, and Kurrasch 2018). Finally, these strategies furthermore imply concomitant microglial depletion, as well as possible adverse effects, in dams, which could hinder their translation to clinical settings.

### 3.2 | Pharmacological Properties of PLX3397, PLX5622, and GW2580

PLX3397, PLX5622, and GW2580 possess facilitating pharmacological characteristics when considering their further investigation as potential treatments. First, these molecules cross the blood–brain barrier (BBB) and are bioavailable orally, making their oral administration possible (Elmore et al. 2014; Valdearcos et al. 2014; Conway et al. 2005). Their pharmacokinetic and pharmacodynamic properties are detailed in Table 1. Second, they all display a strong and selective inhibition of the tyrosine kinase-dependent activation of CSF-1R with no major effect on other tyrosine kinases in the same range of concentrations (with the exception of the dual inhibitor PLX3397 as discussed below) (Conway et al. 2005; Spangenberg et al. 2019; Benner et al. 2020).

PLX3397 binds to the juxtamembrane region of CSF-1R and selectively and powerfully inhibits the receptor. As most tyrosine kinase inhibitors, PLX3397 possesses inhibitory proprieties on other tyrosine kinase receptors, and especially on c-kit, a receptor for the growth factor Stem Cell Factor expressed by hematopoietic stem cells. PLX3397 highly binds to plasma proteins (> 99%) (Lamb 2019) and is metabolized in the liver by the cytochrome P450 3A4 (CYP3A4) and the UDP-glucuronosyltransferase 1–4 (UGT1A4) (Zahir et al. 2023, 2022). Pharmacokinetic studies using radiolabeled PLX3397 showed it was eliminated at 65% in feces (44% of which was unmetabolized) and 27% in urine. The half-life of the molecule was determined at 26.6 h (Zahir et al. 2023).

PLX3397 has been FDA approved in 2019 (U.S. Food and Drug Administration 2019) under the trade name Turalio for the treatment of tenosynovial giant cell tumors. This rare form of cancer, though mostly benign, is associated with highly incapacitating aggressive tumor growth correlated with the infiltration of TAMs overexpressing CSF-1. The antiproliferative action of PLX3397 on TAMs allows for the diminution of the size of the tumors in patients and constitutes the main alternative to surgery in this disease (Lamb 2019; Cassier et al. 2015). In 2016, the phase 3 ENLIVEN study established that the treatment response rate (evaluated with the Response Evaluation Criteria in Solid Tumors or RECIST method (Eisenhauer et al. 2009)) was 39% (and up to 53% when treatment was prolonged) against 0% in the placebo group (Tap et al. 2019). Beyond this first successful study, the molecule is currently being tested in many other types of cancer such as melanoma, leukemia, lymphoma,

**TABLE 1** | Key pharmacokinetic and pharmacodynamic properties of PLX3397, PLX5622, and GW2580.

| Properties (IV) | PLX3397 | PLX5622 | GW2580 |
|---|---|---|---|
| Human approval | FDA (U.S. Food and Drug Administration 2019; FDA and "Prescribing Information—TURALIO." 2024) | No | No |
| Half-life (h) | Human: 26.6 (FDA and "Prescribing Information—TURALIO." 2024) | Mouse: 2.6 (Spangenberg et al. 2019) | NA |
| Clearance | 5.1 L/h (FDA and "Prescribing Information—TURALIO." 2024) | Mouse: 2.1 mL/min/kg (Spangenberg et al. 2019) | NA |
| Distribution volume | 187 L (FDA and "Prescribing Information—TURALIO." 2024) | NA | NA |
| Elimination | 65% fecal 27% renal (FDA and "Prescribing Information—TURALIO." 2024) | NA | NA |
| Plasma protein binding | Human: 99% (FDA and "Prescribing Information—TURALIO." 2024) | NA | Human: 98% (Conway et al. 2005) |
| $IC_{50}$ CSF-1R (μM) | 0.017 (Benner et al. 2020) | 0.016 (Spangenberg et al. 2019) | 0.03 (Conway et al. 2005) |
| $IC_{50}$ c-kit (μM) | 0.012 (Benner et al. 2020) | 0.86 (Spangenberg et al. 2019) | > 13 (Conway et al. 2005) |

Abbreviations: CSF-1R, colony stimulating factor 1 receptor; $IC_{50}$, half maximal inhibitory concentration; IV, intravenous; NA: non-available.

glioblastoma, and many forms of solid tumors (Cannarile et al. 2017).

It is worth noticing that the peripheral effects of PLX3397 on monocytes and macrophages are already visible at doses used to deplete microglia (Lei et al. 2020). This forecasted setback to its utilization in neurological diseases remains to be evaluated (Han et al. 2020). Safety-wise, it has also been reported that the molecule could present a risk of liver toxicity (Tap et al. 2019). and patients' liver function surveillance has been imposed by the FDA throughout the treatment (U.S. Food and Drug Administration 2019). Furthermore, as PLX3397 is primarily eliminated in urine, dosage should be adapted to the patient's kidney function (FDA and "Prescribing Information—TURALIO." 2024). The pharmacodynamics of PLX3397 is not impacted by age, sex, race, or liver insufficiency.

Chemically close to PLX3397, PLX5622 displays similar characteristics and allows for broad microglial depletion. However, PLX5622 has a reduced inhibiting potential on c-kit compared to PLX3397 with a calculated $IC_{50}$ more than 50 times superior for this receptor (Spangenberg et al. 2019). IPLX5622, furthermore, displays a greater passage of the BBB than PLX3397 (Spangenberg et al. 2019). At doses used for microglial depletion, Lei et al. measured a strong and long-lasting effect of PLX5622 on peripheral and circulating macrophages and especially a suppression of C-C chemokine receptor type 2 ($CCR2^+$) monocyte progenitors and C-X3-C motif chemokine receptor 1 ($CX3CR1^+$) macrophages derived from the bone marrow (Lei et al. 2020). CX3CR1 expression in macrophages has been identified as a crucial regulator of their function at sites of inflammation and mediates skin wound healing (Burgess et al. 2019; Ishida, Gao, and Murphy 2008). Furthermore, Lei et al. noted these depletions were long lasting and not always recovered by treatment arrest. This was notably observed in the spleen, where $CX3CR1^+$ cells were still depleted 3 weeks after the end of the treatment. Overall, after treatment by PLX5622, bone marrow-derived macrophages displayed diminished phagocytosis abilities and IL-1β expression.

GW2580 acts as a competitive inhibitor towards the fixation of adenosine triphosphate (ATP) to the CSF-1R, therefore inhibiting the tyrosine kinase activity of the receptor. GW2580 does not affect mouse lymphoid cells, human fibroblasts, endothelial cells, or five different human tumor lineages (Dev et al. 2004) while inhibiting the development of myeloid cells and monocytes. In mice, the use of GW2580 reduces the CSF-1 mediated production of Tumor Necrosis Factor (TNF) and IL-6 induced by the injection of lipopolysaccharide (LPS). Similarly, GW2580 inhibits the growth of CSF-1-dependent tumorous cells in the peritoneal cavity. However, some properties have not been confirmed in vitro: effects on TNF, IL-6, and prostaglandin (PG) E2 following LPS in human and mouse monocytes and macrophages (Wadsworth et al. 1999; Gaul et al. 2003). Finally, in vitro, GW2580 inhibited bone degradation in cultures of human and rat osteoclasts.

## 4 | Microglial Depletion

In neurobiology, the main goal of using CSF-1R inhibitors is microglial depletion. This was first described in 2014 by Elmore et al. who achieved a 99% microglial depletion using PLX3397 (Elmore et al. 2014). In the literature, this new tool has been of great interest into deciphering the different roles of microglia during normal development and in the adult brain (Hagemeyer et al. 2017).

It is worth noting that microglia depletion strategies targeting the CSF-1R are not exclusive to rodents and have been used

successfully in other models. For example, in a study from 2012 (Huang et al. 2012), Huang et al. illustrated in zebrafish that the movement of macrophages into the CNS hinges on the signaling facilitated by CSF-1R. Their findings revealed that the targeted suppression of CSF-1R using morpholino oligonucleotides slows down the migration of macrophages from the yolk sac to the retina. The consequent late macrophage migration leads to microphthalmia, a delay in the withdrawal of retinal progenitor cells from the cell cycle, and a lack of neuronal differentiation. Subsequent observation reveals that when embryos are allowed to survive beyond the period when morpholino-induced translation inhibition diminishes, microglia repopulate the retina leading to a partial recovery of neuronal differentiation. These results underscore the indispensable role of microglia in facilitating normal retinal growth and neurogenesis.

In rodents, multiple microglia depletion strategies have been used. Table 2 describes these different strategies displaying the molecules used, their dose, time, and means of administration. CSF-1R inhibitor-based strategies most commonly revolve on the administration of the drug directly in the chow, although gavage and even intraperitoneal (i.p.) injections have been used. The choice of different means of administration mainly differs on ease of use rather than on efficacy (Table 2) and is most importantly driven by the imperative of each animal model, and notably by the age of animals. While directly feeding animals with chow containing the molecule of interest is easier, i.p. injections become relevant when studying microglial depletion before weaning, allowing for direct administration to the pups. Moreover, i.p. and gavage allow for the precise dosage of drugs that can prove precious when complete microglial depletion is not targeted. In most studies, such almost complete microglial depletion could be achieved after 1–2 weeks of treatment at different ages using PLX3397 or PLX5622 (Liu et al. 2019; Ma et al. 2020; Feng et al. 2017; Riquier and Sollars 2022; Soto-Diaz et al. 2021; Neal et al. 2020), the latter being most effective. Unlike PLX3397 and PLX5622, GW2580 did not induce microglial depletion. However, in a study by Neal et al., GW2580, while not reducing cell counts of Iba1+ (Ionized calcium-binding adapter molecule 1) stained microglia, had a noticeable mitigating effect on neuroinflammation by causing a decrease in microglial mRNA expression of pro-inflammatory factors such as *Nos2*, *Il-1b* or *Il-6*, without affecting mRNA levels of anti-inflammatory mediators (Neal et al. 2020). These data further confirm the direct role of CSF1 signaling in microglial activation beyond sole microglial depletion. Moreover, the inability of GW2580 to cause significative microglial depletion could be explained by its selectivity on CSF-1R and its very low inhibitory capacity on c-kit (Conway et al. 2005), a receptor also involved in the survival of microglia (Zhang and Fedoroff 1998).

### 4.1 | Effects of Microglial Depletion in Neonates and Adults

Microglial depletion can be achieved in a variety of contexts, at different ages, in both physiological situations and pathological models. Approaching the consequences of these depletory strategies outside of any pathological situation has been used to better understand the roles of microglia and is important to understand the ramifications of microglial depletion. Because of the roles held by microglia in neurodevelopment, their depletion at early stages, and especially in the neonatal period, are of the upmost importance when studying the feasibility of microglial depletion strategies in neurodevelopmental disorders.

**TABLE 2** | Microglial depletion strategies using PLX3397, PLX5622, or GW2580 and their results.

| Molecule | Administration | Animal | Dose and duration | Age of animal | Depletion |
|---|---|---|---|---|---|
| PLX3397 | Chow (Liu et al. 2019) | Mice | 275 mg/kg for 7 days and 21 days | 2–3 months | 50%–70% to D7<br>90% to D21 |
| | Gavage (Ma et al. 2020) | Mice | 50 mg/kg for 14 days, once a day | P14 | 55% to D7 (P21)<br>78% to D14 (P28) |
| | Gavage (Zhang et al. 2021) | Mice | 25 mg/kg for 7 days, twice daily | P4 | 90% to D5 |
| | Intraperitoneal (Kuse et al. 2018) | Mice | 0.25 and 1 mg/kg, twice daily | P0 | 33% to D7 |
| PLX5622 | Chow (Liu et al. 2019) | Mice | 275 mg/kg for 7 days | 2–3 months | > 95% to D3 |
| | Chow (Feng et al. 2017) | Mice | 1200 mg/kg for 7 days | 5 weeks | 90% to D7<br>95% to D21 |
| | Intraperitoneal (Riquier and Sollars 2022) | Rat | 50 mg/kg, once daily (P1–P11), then 50 mg/kg, twice daily until P40 | P1 | > 97% to P3, ≈ 99% to P14, remain stable through P21 and P40 |
| GW2580 | Gavage (Soto-Diaz et al. 2021) | Mice | 50 mg/kg for 8 days | 8–12 weeks | No significant effect |
| | Intraperitoneal (Neal et al. 2020) | Mice | 80 mg/kg for 6 days | P7 | No significant effect |

Abbreviations: D, day of treatment; P, postnatal day.

The consequences of microglial depletion during the neonatal period were underlined in several studies that highlighted the developmental roles of microglia. In a 2017 study focused on the behavioral consequences of microglial depletion, Nelson et al., showed an increase in anxiety-like behavior and in motor activity in P30 and P80 rats following intracerebroventricular injections of liposomal clodronate between P1 and P4 (Nelson and Lenz 2017). These results highlight the long-term consequences of microglial depletion (70% depletion at P6 in this case), most probably through the perturbation of neurodevelopmental processes. However, only few studies studied some of the mechanisms behind these behavioral outcomes using microglial depletion strategies based on CSF-1R tyrosine kinase inhibitors in neonate pups. Among this narrow sample of the literature, Kuse and colleagues highlighted the regulatory effects of microglia on the proliferation of mice retinal precursor cells (Kuse et al. 2018). Their study notably shows the decrease of the proliferative capacity of these cells when microglia is depleted using PLX3397, forecasting potential impairment of the retinal layer in these animals. In another example, Riquier et al., demonstrated the important role of microglia during development in the pruning of the glossopharyngeal nerve. PLX5622-induced microglial depletion indeed impeded synaptic pruning at the terminal field of the glossopharyngeal nerve and cause an expansion of this region (Riquier and Sollars 2022). It can therefore be expected that this effect on synaptic pruning impacts all the CNS and therefore that perinatal microglial depletion strategies could be responsible for abnormal establishment of neural circuits.

In adult mice, the perspective of microglial depletion strategies using CSF-1R inhibitors, however, appears to face not as many hurdles. Feng et al. notably reported no adverse effects of a 98% microglial depletion, 5 weeks after its induction using PLX5622. Their study, focused on the hippocampus, did not report any consequences in tasks regarding learning or memory (Feng et al. 2017). On the contrary, microglia depleted mice had better results than controls when assessing their susceptibility to develop postoperative cognitive decline (POCD) in a Morris water maze test. These improvements were linked to an important reduction of surgery associated neuroinflammation and, especially showed a decrease in the secretion of pro-inflammatory mediators such as IL-1β, IL-6, TNFα, and chemokine ligand 2 (CCL2), as well as an arrest in the recruitment of CCR2+ leukocytes. Moreover, in adult mouse models of cerebral ischemic strokes, the use of CSF-1R inhibitors highlighted the protective roles of microglia. In such models, microglial depletion led to an exacerbation of stroke severity through the pro-inflammatory activation of astrocytes, the dysregulation of neuronal calcium responses, and increased neuronal cell death (Szalay et al. 2016; Jin et al. 2017).

### 4.2 | Microglial Depletion, a Promising Therapeutic Strategy in Neurodegenerative Diseases?

The interest for the therapeutic potential of CSF-1R inhibitors in neurological diseases linked to microglial activation is quite recent. However, the number of these publications constantly increased over the last decade as the central role of microglial activation in many neurological diseases was progressively highlighted (Paolicelli et al. 2022). Indeed, through a wide variety of receptors (e.g., TLRs, receptors to cytokines/chemokines, to so called DAMPs and PAMPs) microglia scan their environment for any homeostatic change. In the event of an activation signal, microglia can adopt a range of activation phenotypes, implying morphological modifications (Paolicelli et al. 2022). Pro-inflammatory microglial activation and its associated neuroinflammation are more and more found to be common denominators in neurodegenerative diseases (86) such as multiple sclerosis (Perry, Nicoll, and Holmes 2010), Alzheimer's disease (AD) (Baik et al. 2019; Olah et al. 2020), Parkinson's disease (Su et al. 2008; Kim et al. 2013; Croisier et al. 2005) or Huntington's disease (Tai et al. 2007; Sapp et al. 2001; Pavese et al. 2006). In these pathologies, microglial depletion strategies promise reduced neuroinflammation and showed positive effects on its behavioral consequences (Dwyer et al. 2020; Spiteri et al. 2022).

Among neurodegenerative diseases, the effects of CSF-1R tyrosine kinase inhibitors have most importantly been studied in rodent models of AD. In AD, expression levels of CSF-1 and CSF-1R are increased (Walker, Tang, and Lue 2017; Olmos-Alonso et al. 2016) and further justify the use of these strategies. Moreover, microglia have been shown to be implicated in the processes leading to the aggregation and abnormal phosphorylation of the Tau protein (pTau) as well as in the accumulation of amyloid-β (Aβ) plaques, two hallmarks of the disease (Serrano-Pozo et al. 2011). Asai and colleagues explored the involvement of microglia in pTau propagation using PLX5622-induced microglial depletion in a mouse model of tauopathy and showed that microglial depletion was responsible for a reduction of 86% in AT8+ pTau in the granular layer (Asai et al. 2015). Furthermore, some authors, such as Shi et al. implied that microglial activation could directly mediate neurodegeneration in AD. Their research focused on a transgenic mouse model of tauopathy demonstrated that PLX3397 administered through chow to 6-month-old mice, a critical timeframe regarding neurodegeneration in the model, drastically reduced neurodegeneration and AT8+ pTau accumulation (Shi, Manis, et al. 2019). In this study, brain atrophy was highly correlated with CD68 expression, highlighting the role of activated microglia. The positive effects of microglial depletion on Aβ aggregation where studied by Spangenberg and colleagues using PLX5622 or PLX3397 in a mouse model (5xFAD) of AD (Spangenberg et al. 2019). Their research, concluded in a several positive outcomes regarding Aβ plaques formation. Microglial depletion led to a 33% reduction in plaque formation and prevented the downregulation of synaptic genes such as *Dlk2*, *Dync1l1*, *Gls*, *Kcnq3*, *Nrg3*, and *Scn1b* in the hippocampus.

Similarly to what is observed in AD, CSF-1R expression is 15% higher in Parkinson patients than in controls (Walker, Tang, and Lue 2017) and positive outcomes of treatments using CSF-1R inhibitors where found. In a 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP) induced model of Parkinson's, GW2580 treatment showed a decrease in the expression of genes associated with microglial activation, such as *IL-1β*, *Nos2*, *Gp91*, or *Il-6* (Neal et al. 2020). However, no positive regulatory effect was shown regarding genes associated with anti-inflammatory phenotypes of microglia. These signs of a decrease in the pro-inflammatory activation of microglia were linked to better behavioral test

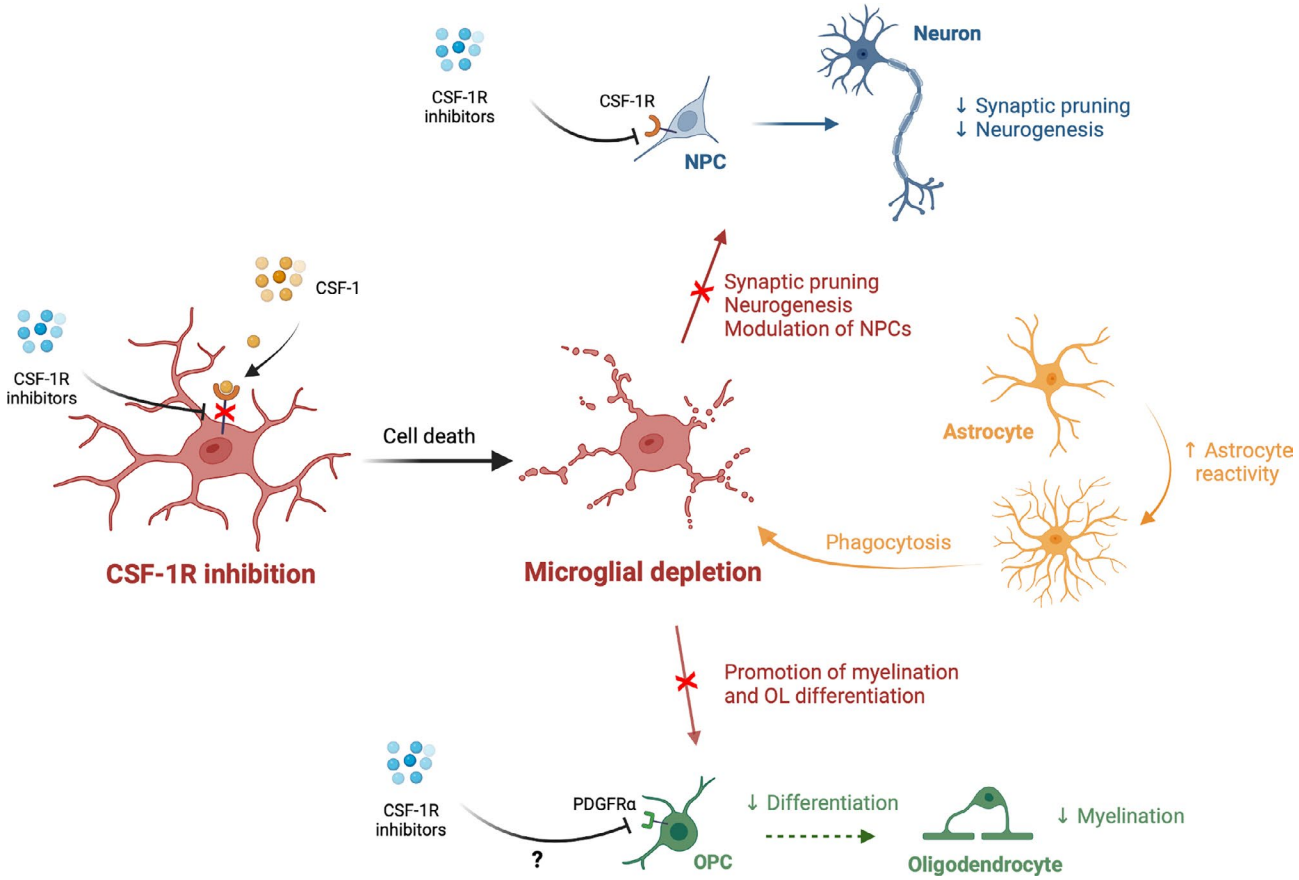


**FIGURE 2** | The possible impacts of microglial depletion using CSF-1R inhibitors on neurons, astrocytes and oligodendrocytes. The inhibition of the CSF1 signaling pathway by CSF-1R tyrosine kinase inhibitors leads to the depletion of up to 99% of microglia. The depletion of these cells could prove to be deleterious on other cell types in the CNS through the arrest of key homeostatic and neurodevelopmental functions of these cells or through the rupture of homeostasis the accumulation of microglia debris could cause. Moreover, CSF-1R is expressed by NPCs, where its signaling is important to their differentiation and proliferation. Finally, like other tyrosine kinase inhibitors, the specificity of PLX3397, PL5622, and GW2580 is not directed towards the active site of the CSF-1R but rather towards the intracellular tyrosine kinase domains. These molecules could therefore show inhibitory capabilities on other tyrosine kinase receptors such as PDGFRα, a receptor involved in the differentiation of OPCs. Abbreviations: NPC, neural progenitor cell; OPC, oligodendrocyte progenitor cell.

results and reduced neurodegeneration of dopaminergic neurons. GW2580 treatment was responsible for a diminution of neuronal loss from 60% to 17% and a significant amelioration of the use of the animal hind legs in behavioral tests.

In multiple sclerosis, Marzan et al., explored the possibilities offered by microglial depletion using PLX3397 in a cuprizone model, known to induce white matter lesions (Marzan et al. 2021). Using fate-mapping strategies, they first identified microglia to be present at the site of cuprizone-induced lesions and demonstrated that microglial activation induced by CSF-1 injection, was sufficient to cause demyelinating injuries. These results directly make microglia the effector of these demyelinating lesions. Accordingly, microglial depletion delayed cuprizone-induced demyelination as well as astrogliosis.

Benefits were also observed in a mouse model of Huntington's disease, where microglial depletion by PLX3397 led to better results in object recognition trials and had a positive effect on the reduction of the striatum volume that is characteristic of the disease (Crapser et al. 2020). In addition, positive behavioral outcomes were observed in models of traumatic brain injury (TBI), where neuroinflammatory processes mediate long-term neurodegenerative deleterious effects (Henry et al. 2020).

The link between microglial activation and neuronal loss moreover paves the way for microglial repopulating strategies such as the one explored by Chadarevian et al. (2023). The strategy they propose is based upon the selective depletion of native microglia to allow for the repopulation of the CNS by CSF-1R inhibitors-resistant microglia originating from hematopoietic stem cell grafts. It could indeed ensure a direct reduction in the activation phenotype of microglia while allowing for the maintenance of their homeostatic functions.

## 5 | Impact of CSF-1R Inhibitors on Other Brain Cell Types

Before considering microglial depletion as a sustainable therapeutic option, it must show a positive benefits/risks balance and the absence of deleterious effects. This question applies especially to the cells surrounding microglia: neurons and other glial cells such as astrocytes, and oligodendrocytes (Figure 2).

### 5.1 | Neurons

The impact of microglial depletion on neurons is hard to determine, and its evaluation outside of pathological models has yet to be done, especially during neurodevelopment. Neurons indeed express the CSF-1R (Wang, Berezovska, and Fedoroff 1999) and the CSF-1 pathway has been linked to neurodevelopmental processes such as neural progenitors' differentiation and axons' capacity to cross the corpus callosum midline in CSF-1R$^{ko}$ mice embryos (Nandi et al. 2012). CSF-1R inhibition during CNS development could therefore prove to be deleterious, especially when performed at young age and regarding synapse development. The existence of synapse development impairments following early microglial depletion was assessed by several authors at different times and regions such as the cortex (Ma et al. 2020), the auditory nervous system (Chokr et al. 2022), or the glossopharyngeal nerve (Riquier and Sollars 2022). On the other hand, in several adult models of disease where neuroinflammation is at the heart of neuronal loss, such as AD, amyotrophic lateral sclerosis or TBI, CSF-1R inhibition-mediated microglial depletion showed positive results regarding neurodegeneration (Han et al. 2019). This was notably observed in an adult model of TBI where a diminution in the size of observed lesions, a reduction in neuronal loss, as well as improvements in the recovery of sensorimotor and cognitive function were observed 3 months after TBI (Henry et al. 2020). However, the effects of CSF-1R inhibition on neurons remain to be studied in healthy conditions.

### 5.2 | Astrocytes

Astrocytes are the most represented cells among glia. They have multiple essential functions, from trophic and supportive roles to neurons to BBB integrity and even play a role at the synapse where they actively participate in the modulation of neurotransmission (Halassa, Fellin, and Haydon 2007). In the healthy brain, astrocytes produce immunomodulatory factors such as transforming growth factor beta (TGF-β) and favor the persistence of an anti-inflammatory environment (Norden et al. 2014). However, following CNS insult, astrocytes show their own reactivity and participate alongside microglia to the neuroinflammatory response notably through the production of numerous pro-inflammatory cytokines (Escartin et al. 2021) such as IL-6 and CCL2 (Linnerbauer, Wheeler, and Quintana 2020).

As for neurons, only a few studies focused on the effects of CSF-1R inhibitors on astrocytes. A recent study by Yang et al. demonstrated that the ablation of microglia using PLX3397 led to increased astrocyte reactivity and inflammatory response but did not increase the total number of astrocytes in a mouse model of Parkinson's disease (Yang et al. 2018). This increased astrocyte reactivity could be the consequence of the homeostatic rupture caused by the death of microglia and the accumulation of their debris. A 2022 study indeed showed that microglial debris are mostly phagocyted by astrocytes through C4b opsonization although microglia showed in vitro capabilities to phagocyte microglia debris as well (Zhou et al. 2022). On the other hand, Riquier and Sollars (2020) while also noticing constant total numbers of astrocytes after microglial depletion, showed a reduced astrocyte response to brain injury after microglial depletion. Again, the effects of these molecules on astrocytes in the healthy brain remain to be investigated.

### 5.3 | Oligodendrocytes

OLs are the myelinating cells of the CNS. They differentiate from oligodendrocyte progenitor cells (OPC) deriving from neural stem cells. In adults, the presence of a pool of OPCs ensures the renewal and replacement of dead OLs. The pre-oligodendrocyte (pre-OL) stage is intermediate to OPCs and mature OLs and is characterized by the engagement of the pre-OL towards an axon. Only mature OLs have myelinating capabilities. In addition to their main myelinating function, OLs play an important metabolic function and support axonal integrity (as deeply discussed in a review by Nave, Asadollahi, and Sasmita (2023)).

In many models of white matter lesions, OLs are the first cells to suffer from microglial activation and would therefore be the first beneficiaries of microglial depletion during neuroinflammation. This is particularly true in neurodevelopmental diseases linked to neuroinflammation where OL maturation arrest leads to white matter lesions such as encephalopathy of prematurity (Volpe et al. 2011; Verney et al. 2012; Favrais et al. 2011; Shiow et al. 2017). Bocazzi et al. highlighted the interplay between OLs and microglia in the context of perinatal inflammation. In their study, immature OLs displayed an upregulation of several inflammatory genes such as *Il1b, Ifnb1, Ccl2 or Cxcl10*. Moreover, they showed in vitro the capacity of OLs to modulate microglial activation and concluded that the response of oligodendrocytes can play an autonomous role in blocking their own differentiation (Boccazzi et al. 2021).

However, as for the other cells in the CNS, the amount of data we have on the effect of CSF-1R inhibitors on oligodendrocytes is not sufficient. Contradictory elements tend to show that the impact on these cells would depend on the molecule rather than on microglial depletion itself. In a study from 2019, PLX5622 did not significantly affect OPCs at low doses while PLX3397 caused a significant OPC loss in the brain of adult mice (Liu et al. 2019). These results may also highlight the different unwanted cross-inhibitory capacities of CSF-1R tyrosine kinase inhibitors on PDGFRα (Platelet-derived growth factor receptor A), another typer 3 kinase protein receptor, involved in the regulation OPC migration and proliferation (Zhu et al. 2014; Woodruff et al. 2004).

## 6 | Conclusion

CSF-1R tyrosine kinase inhibitors allow for the depletion of microglial populations by up to 99%. Compared with other molecules used in depletory strategies, their capacity to cross the BBB and their oral bioavailability make them promising therapeutic candidates in pathologies where microglial activation is at fault. This is notably the case in many neurodegenerative and neurodevelopmental disorders. In several models of neurological diseases such as AD or Parkinson's disease, both characterized by the pro-inflammatory activation of microglia, the application of these depletory strategies led to a reduction of neuroinflammation and neurodegeneration.

However, there is an important lack of knowledge regarding the effects of such molecules and strategies on neurons, astrocytes and oligodendrocytes, the three other major cell types in the CNS. These effects need to be evaluated to ensure the absence of deleterious effects of microglial depletion, especially at neurodevelopmental stages. Indeed, the importance of microglial activation in neurological disorders is not limited to neurodegenerative diseases but is also of particular importance in neurodevelopmental disorders, such as encephalopathy of prematurity and hypoxic–ischemic injury. As microglia displays important neurodevelopmental roles in the arrangement of the neural circuitry, in oligodendrocyte differentiation and myelination, the importance of such research appears pivotal to the implementation of microglial depletion strategies in the therapeutic arsenal of these diseases.

### Author Contributions

**David Guenoun, Nathan Blaise** and **Alexandre Sellam:** conceptualization, writing – original draft, writing – review and editing. **Julie Roupret-Serzec** and **Alice Jacquens:** writing – original draft, writing – review and editing. **Juliette Van Steenwinckel**, **Pierre Gressens** and **Cindy Bokobza:** conceptualization, project administration, supervision, validation, visualization, writing – original draft, writing – review and editing.


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This article does not involve human or animal subjects; therefore, an ethics approval statement and patient consent statement are not applicable.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The authors have nothing to report.

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