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Small molecule therapeutics for neuroinflammation-mediated neurodegenerative disorders

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Chronically activated microglia and the resulting cascade of neuroinflammatory mechanisms have been postulated to play a critical role in neurodegenerative disorders. Microglia are the main component of the brain's innate immune system and become activated by infection, injury, misfolded proteins or a multitude of other stimuli. Activated microglia release pro-inflammatory and cytotoxic factors that can damage neurons and transform astrocytes to become toxic to neurons as well. Therapeutic approaches aiming to modulate microglia activation may be beneficial to mitigate the progression of inflammatory-mediated neurodegenerative diseases. In this literature review, we provide an overview of recent progress on key microglia targets and discovery of small molecule compounds advancing in clinical trials to minimize neuroinflammation.

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Dr. Silke Miller obtained her MSc. in Biology from the Leibnitz University of Hannover, Germany, and her PhD. in Neuroscience from the ETH in Zurich. After being awarded a postdoctoral fellowship in neuropharmacology at UC Irvine by the Swiss and German Research Foundations, and a short tenure as assistant professor at the University of Erlangen, she began her industry career in 2001 by joining Silence Therapeutics (formerly Atugen) in Berlin as research scientist. In the following years, she held lab head positions in Neuroscience Drug Discovery at Lundbeck and Amgen and produced key data supporting the successful transition of several programs into clinical testing, two of which culminated in approved drugs for depression and migraine. Currently, she is the head of the biochemistry and molecular biology group at Sage Therapeutics. Her research passion is to find novel drug targets and for psychiatric and neurodegenerative diseases.

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Maria-Jesus Blanco is a highly experienced and enthusiastic medicinal chemist focused on bringing meaningful medicines to patients. She is currently Head of Chemistry and Senior Director at Sage Therapeutics in Cambridge, MA. Previously, she was Director of Discovery Chemistry Research & Technologies at Eli Lilly. She has been working in drug discovery and development for >21 years, playing leadership and strategic roles in Neuroscience as well as emerging fields of drug discovery as PET ligands, chemical biology, protein–protein interactions and translational research. Maria was part of the team that discovered Reyvow (approved by the FDA in 2019 for the acute treatment of migraine). She has actively contributed to the delivery of 14 compounds to clinical studies. Maria obtained her Bachelor and Ph.D. degree in Synthetic Organic Chemistry from the University of Santiago de Compostela, Spain. She held postdoctoral positions at Massachusetts Institute of Technology (MIT) with Prof. Masamune and University of Strasbourg, France with Nobel Laureate Prof. Sauvage before joining Lilly headquarters in Indianapolis. Maria has >100 scientific publications, presentations, patent and patent applications. Maria is an involved science advocate, mentor and member of the executive committee of ACS National Division of Medicinal Chemistry.

1. Introduction – the role of microglia in neuroinflammation

Neuroinflammation has been considered as contributing factor to the onset and progression of neurodegenerative diseases attracting much interest from the scientific community conscious of the aging population. In the past two decades, we have seen an exponential increase in neuroinflammation research and potential therapeutic treatments, reflected in a rapid increase of number of publications (Fig. 1).

Inflammatory processes underpin many diseases as diverse as autoimmune diseases, cancer, cardiovascular diseases and neurodegenerative disorders. Steroids and non-steroidal anti-inflammatory drugs (NSAIDS) are effective in treating symptoms and pain associated with inflammation, but have no impact on the disease itself and are limited by side-effects. The discovery of biological drugs shifted the way inflammatory diseases are treated, for example with monoclonal antibodies that inhibit tumor necrosis factor (TNF) receptors, not only symptoms can be ameliorated, but the course of rheumatoid arthritis can be halted.[1] While biological drugs against cytokines had a great impact on improving therapy of many of these diseases, the treatment of inflammatory mechanisms in neurodegenerative disorders presents additional challenges due to compartmentalization of the central nervous system (CNS) by the blood–brain barrier. In this review, we focus on recent small molecule approaches that modulate microglia receptors or downstream signaling cascades involved in neuroinflammation associated with neurodegenerative disorders.

Neuroinflammatory processes in the CNS are mediated by glial cells – astrocytes, oligodendrocyte precursor cells, oligodendrocytes, and microglia – which are present throughout the CNS in high numbers. Microglia are innate immune cells and directly respond to endogenous stimuli, injury and pathogens.[2] In the normal healthy brain, microglia play a role in synapse elimination,[3] axon guidance and clearance of apoptotic cells in neurogenic zones.[4,5] Cytokines released by microglia under baseline conditions may contribute to plasticity such as synaptic scaling.[6] These homeostatic functions of microglia are considered essential for cognition and plasticity.[7] When encountering injury or pathogens, microglia become activated and release an array of mediators including pro- and anti-inflammatory cytokines, neurotoxic proteins, chemokines and neurotrophic factors[8–10] that in turn activate astrocytes and oligodendrocytes.[11–13] Microglia activity is restrained by inhibitory pathways that suppress unwanted inflammatory responses and tissue destruction associated with immune activation.[14] An imbalance of pro- and anti-inflammatory mechanisms is proposed to underlie the chronic, unresolved low-grade inflammation that promotes disease progression in neurodegenerative diseases.[15–17]

The presence of glial cells associated with neuritic plaques, a hallmark of Alzheimer's disease pathology, was described by Alois Alzheimer himself[18–20] and activated microglia specifically has been described in brains from patients with Parkinson's disease or Alzheimer's dementia more than thirty years ago.[21,22] However, microglia became an intense focus of research in neurodegeneration only in the past decade.[23] Neuroinflammation was initially dismissed as being too non-specific to provide an effective target for treating neurodegenerative disorders, supported by clinical findings on lack of evidence of efficacy of NSAIDS,

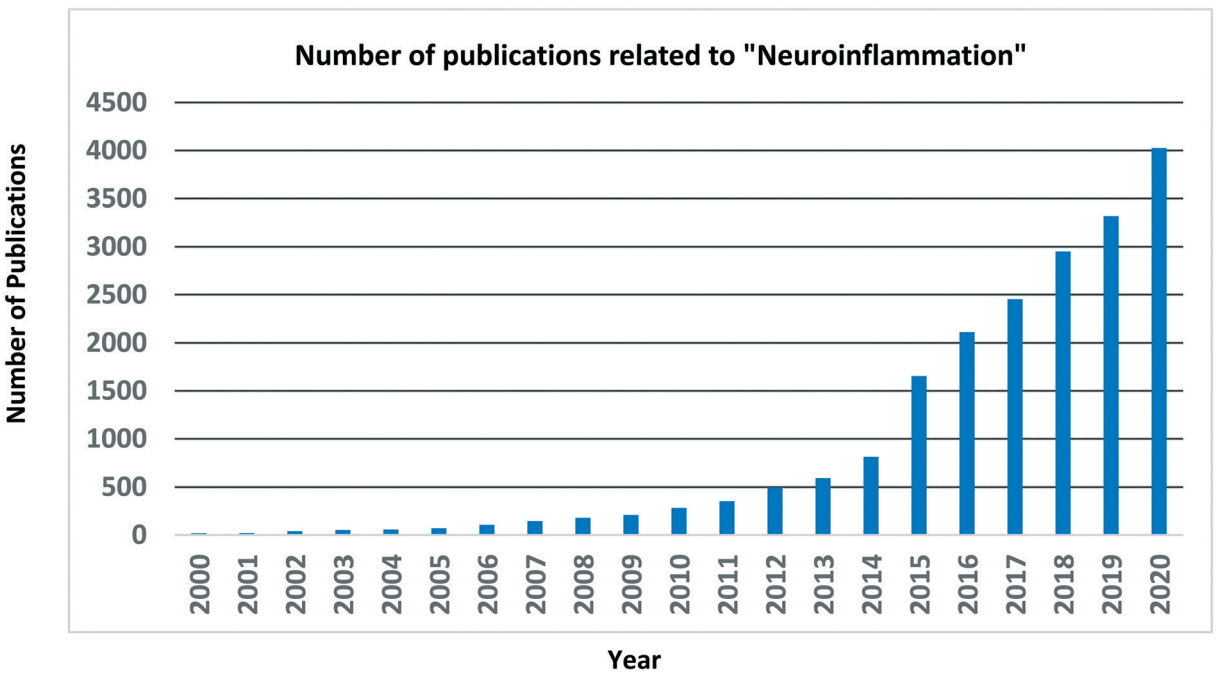


Fig. 1 Increasing number of publications from 2000–2020 obtained from a search of the term "neuroinflammation" in SciFinder®.

glucocorticoids or selective cyclooxygenase-2 inhibitors in Alzheimer's disease.[24,25] Large scale genome-wide association studies challenged this view by strongly implicating genes related to the innate immune response in Alzheimer's disease,[26–29] many of which were expressed in or even specific for human microglia.[30] In fact, the largest risk factor for Alzheimer's disease, apolipoprotein E (APOE), is mainly expressed by astroglia and activated microglia.[31] Since these initial publications, numerous genetic studies in humans corroborated by findings from animal models have highlighted a connection between immunity and neurodegeneration in other diseases including Parkinson's disease[32,33] and amyotrophic lateral sclerosis.[34]

A blunt approach to ameliorate neuroinflammation is to deplete microglia by reducing proliferation *via* inhibition of colony stimulating factor 1 receptor (CSFR1).[35] Masitinib, a dual CSF1R and tyrosine-protein kinase c-kit inhibitor met the primary endpoint in a pilot phase 2A study in progressive multiple sclerosis,[36] as add-on to riluzole in a phase 2/3 clinical trial for amyotrophic lateral sclerosis,[37] and in combination with acetylcholine esterase inhibitors in a phase 2/3 trial in Alzheimer's disease.[38] However, in 2018 the European Medicine Agency's Committee for Medicinal Products for Human Use did not recommend marketing authorization for amyotrophic lateral sclerosis due to concerns about safety and the reliability of the efficacy data[39] and additional studies are required for approval. CSFR1 inhibition also has shown to exert long-lasting, possibly detrimental effects on peripheral macrophages.[40]

More recent approaches to target microglia involve analysis of microglia sub-types to find effective targets for modulating neuroinflammation: comprehensive single-cell sequencing and proteomics techniques enabled a breakthrough in understanding the complexity of microglia phenotypes associated with onset and disease progression in neurodegeneration.[41,42] These studies defined a gene expression signature characterizing a subset of disease-associated microglia (DAM) in animal models of Alzheimer's disease, ALS, and aging[43,44] and markers of the DAM signature were also detected in human postmortem Alzheimer's brains.[45,46] The DAM gene expression signature is characterized by upregulation of genes involved in lysosomal, phagocytic, and lipid metabolism pathways, including several known Alzheimer's disease risk factors, such as APOE and triggering receptor expressed on myeloid cells 2 (TREM2).[47] However, it is not clear whether the DAM gene expression signature is protective or deleterious.[48,49] Other microglia gene expression modules, such as the interferon-related and classical inflammatory gene sets, are also induced in bulk cortex tissue from Alzheimer's.[46] These additional gene sets are not evident in mouse models of Alzheimer's disease, but can be robustly induced after lipopolysaccharide (LPS) treatment or virus infection and also in a mouse model of severe neurodegeneration.[50]

Mechanisms that modulate these gene signatures and restore an appropriate balance of inflammatory and immune suppressive signaling in microglia may offer new targets for drug discovery in neurodegeneration. Potential targets are receptors and signaling cascades that are associated with inflammatory mechanisms. Microglia express various immunoreceptors including toll-like receptors (TLRs), TREM2, chemokine receptors, TNF receptors, Fc receptors, complement receptors, scavenger receptors, nucleotide binding oligomerization domains (NODs) and NOD-like receptors, and inflammasomes.[17] These receptors enable microglia to recognize and respond to both acute and chronic CNS injury and infections by converging on downstream signaling cascades that culminate in changing gene expression patterns impacting proliferation, migration and the production of pro- or anti-inflammatory factors. TLR and TNF signaling culminating in stimulation of nuclear factor (NF)-κB transcription has been shown to be upregulated across human neurodegenerative diseases.[51]

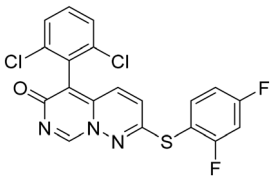
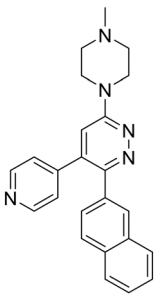
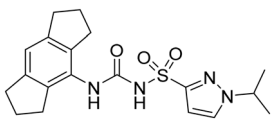
Despite the escalating number of publications about neuroinflammation (Fig. 1), no new small molecules have been approved for neuroinflammation associated with neurodegeneration. Known barriers for drug discovery are species differences in immune functions and the resulting poor translatability of animal models.[52] The development of cell reprogramming methods offers the possibility to generate human pluripotent cell lines, which have recently been successfully differentiated into microglia.[53] While overcoming the problems arising from species differences, this *in vitro* approach harbors other pitfalls, such as the need to generate isogenic cell lines to eliminate confounding factors due to differences in genetic background between patient and healthy donor cells, and artifacts arising from the *in vitro* culture conditions.[54]

In the following paragraphs we discuss recent developments in the discovery of small molecules modulating key microglia receptors and kinases that are advancing to or in early stages of clinical testing for neurodegenerative disorders (Table 1). We present a concise overview of representative chemical scaffolds that have influenced the field and the most recent preclinical compounds published. In each of the figures, we have included a few calculated physicochemical properties[55] (molecular weight, MW; calculated partition coefficient log *P*, clog *P*; total polar surface area, tPSA) to illustrate the chemical diversity across targets, chemotypes and for relevant descriptors for CNS compounds.[56] In addition to the targets and small molecule modulators discussed below, other mechanisms are being tested in clinical trials for Alzheimer's or Parkinson's disease or amyotrophic lateral sclerosis, that may also impact microglia, but are beyond the scope of the current review.[57–60]

2. Microglia membrane receptors – TLRs

TLRs have a high affinity to bind to foreign microbial pathogens, which are hypothesized to contribute to neurodegenerative diseases.[73] TLRs can also be activated by brain-endogenous proteins associated with Alzheimer's or Parkinson's disease

Table 1 Novel small molecule drug candidates targeting specific inflammatory mechanisms in/near clinical development for neurodegenerative diseases

Target	Drug candidate name	Structure	Company	Stage/clinical trial	Ref.
TLR-2 inhibitor	NPT1220-312	Undisclosed	Neuropore	Advancing to phase 1	61, 62
P38 MAPK inhibitor	Neflamapimod (VX-745)		EIP Pharma	Phase 2 Alzheimer's disease (missed primary endpoint) Phase 2 Lewy body dementia (met primary endpoint) Phase 2 in Huntington's disease Imaging study with [^{18}F]-DPA714 in Alzheimer's patients	63 64 65 66
	MW150 (MW01-18-150SRM)		NeuroKine Therapeutics	Advancing to phase 1	67
RIPK1 inhibitor	DNL747	Undisclosed	Sanofi/Denali	Phase 1 in Alzheimer's disease completed Phase 1 in amyotrophic lateral sclerosis paused	68 69
	DNL788 (back up)	Undisclosed	Sanofi/Denali	Phase 1a planned for early 2021	70
Inflammasome inhibitor	Inzomelid (MCC-7840)		Roche/Inflazome	Phase 2 Parkinson's disease	71, 72

pathology: TLR4s have been shown to mediate binding and internalization of the main species of amyloid peptide found in Alzheimer's disease brain (Aβ1–42) *in vitro*[74] and TLR2 has been shown to bind human synuclein and activate mouse microglia *in vitro* and *in vivo*.[75] Stimulation of TLRs activates microglia towards an inflammatory state characterized by a round shape and release of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, interferon (IFN)-γ, TNF-α, and various chemokines.[76] These pro-inflammatory cytokines can damage neurons either directly or indirectly by activating astrocytes and stimulating release of neurotoxic factors.[11] Knockout of genes encoding downstream inflammatory cytokines such as TNF-α, IL-6, and IL-1β has been generally reported to have beneficial effects on neurons.[77] However, inhibition of this pathway through knockout of TLR2, or through heterozygous knockout of the signaling adapter protein Myd88 has been shown to ameliorate Alzheimer's or Parkinson's disease pathology and amyloid plaque formation in animal models, but accelerate behavioral deficits.[78–80] Several small molecule TLR agonists and antagonists have been described.[81–83] Neuropore Therapeutics has reported the advancement of TLR2 modulators towards clinical testing, the structure of the clinical candidate has not yet been disclosed.[61,62]

From the medicinal chemistry perspective, there have been multiple efforts to identify potent TLR2 inhibitors for different diseases, in particular related to inflammation.[84] The structural knowledge from the X-ray crystal structure of the mouse and human TLR2 heterodimers has contributed to the identification of selective ligands. TLR2 forms a highly flexible horse-shoe-like ectodomain that comprises the ligand binding domain, characterized by a hydrophobic pocket where lipophilic ligands bind. Once ligands are bound, TLR2 is in the activated state, generating M-shaped hetero- or homodimers with TLR1 or TLR6 connecting to the extracellular C-terminal domain. Using computer-aided drug design to screen a large library of FDA-approved compounds, Mistry *et al.*[84] discovered a TLR2 inhibitor C29 (**1**, Fig. 2) that inhibited TLR2/1 and TLR2/6 signaling induced by synthetic and bacterial TLR2 agonists. One of the first competitive inhibitors discovered was CU-CPT22 (ref. 85) (**2**, Fig. 2). The compound exhibited modest micromolar activity (IC_{50} = 3.13 μM displacing the synthetic triacylated lipoprotein, Pam3CSK4 binding to TLR2/1 dimer) with high selectivity towards TLR2/1 heterodimers in mice, but no selectivity towards human TLR2 heterodimers. Compound MMG-11 (**3**) is a pyrogallol derivative TLR2 inhibitor identified through structure-based virtual screening.[86] MMG-11

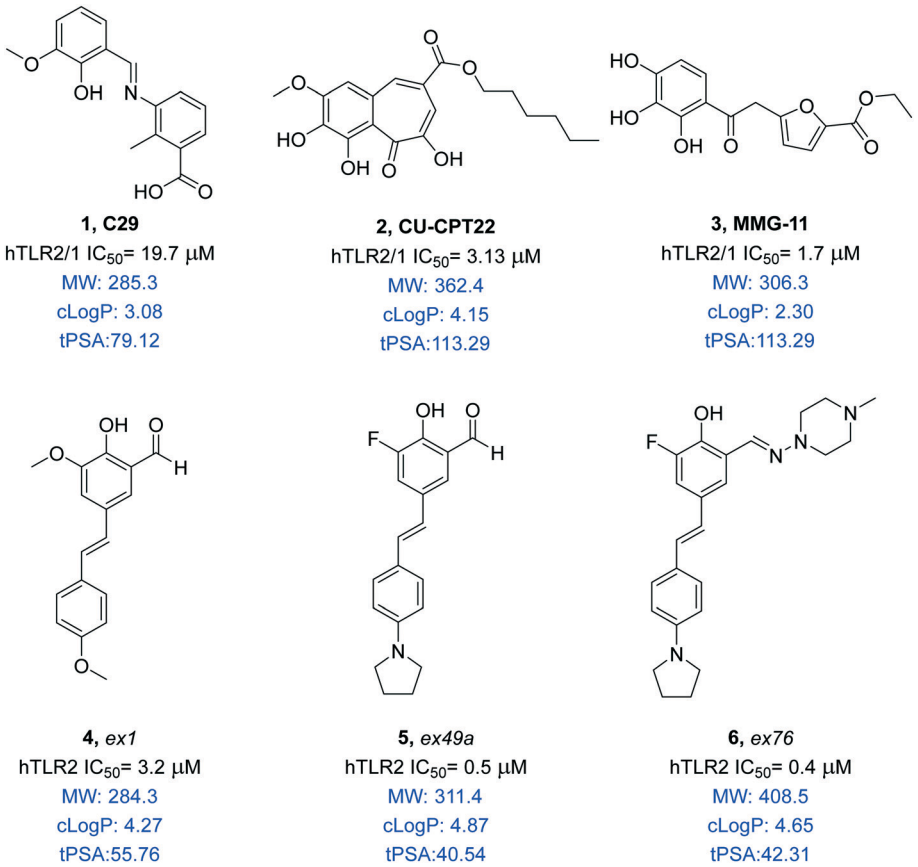


Fig. 2 Small molecule TLR2 inhibitors.

demonstrated the competitive inhibition of both Pam3CSK4-induced TLR2/1 and Pam2CSK4-induced TLR2/6 signaling with IC_{50} of 1.7 μM and 5.7 μM, respectively. Although CU-CPT22 (**2**) and MMG-11 (**3**, Fig. 2) are interesting tool compounds to interrogate the pharmacology of TLR2 inhibition, there are opportunities to further improve potency as well as druggability. The presence of an ester moiety in both analogs raise questions regarding their metabolic stability.

Although the molecular structure of Neuropore's development candidate has not been disclosed, a recent patent highlights a new scaffold for TLR2 inhibitors.[87] Substituted benzaldehydes like compounds **4**–**6** (Fig. 2) demonstrated inhibition of TLR2 against Pam2CSK4 (TLR2/6 agonist) and Pam3CSK4 (TLR2/1 agonist). Compounds **5** and **6** were the most potent *in vitro* with IC_{50} around 0.5 μM for both assays.

Cyclohexene derivative TAK-242 (also known as resatorvid, **7**, Fig. 3) is a selective inhibitor of TLR4.[88] It blocked the production of TNF-α and IL-6 cytokines in LPS-stimulated human peripheral blood mononuclear cells (PBMCs) with IC_{50} values ranging 11–33 nM. Further studies indicated that the mechanism of action of TAK-242 might be through a disruption of protein–protein interactions of TLR4 with other adaptor molecules after the initial binding to TLR4.[89] This destabilization of the protein–protein interaction inhibits the TLR4 signal transduction and its corresponding downstream effects. In a recent publication, treatment with TAK-242 demonstrated a

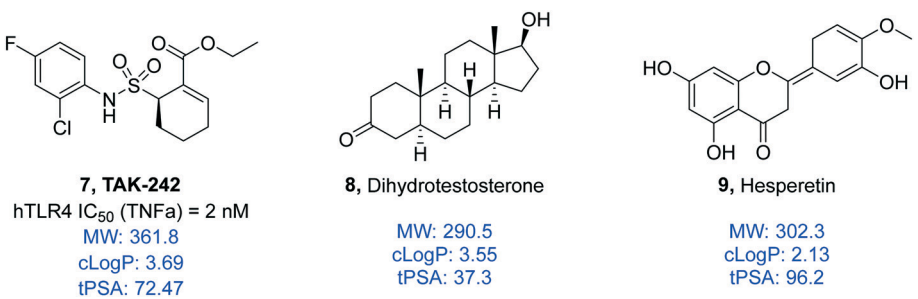


Fig. 3 TLR4 inhibitors.

decrease in $A\beta_{25-35}$ mediated upregulation of inflammatory cytokine production, as well as stimulating a shift from an inflammatory to a protective microglia phenotype in mouse models of Alzheimer's disease.[90] In a randomized placebo-controlled and double-blind phase 2 trial for sepsis, TAK-242 failed to significantly decrease cytokine levels.[91] Prodrug[92] and deuteration[93] approaches have been pursued to improve the pharmacokinetic and distribution profile of TAK-242.

Dihydrotestosterone (**8**, Fig. 3) has been reported as a TLR4 antagonist that ameliorated neuroinflammation and exhibited neuroprotective properties. In LPS-induced primary microglia and the BV-2 mouse microglial cell line, dihydrotestosterone inhibited the secretion of pro-inflammatory factors as TNF-α, IL-1β, IL-6, inducible nitic oxide (NO) synthase, NO, COX-2 and prostaglandin E2.[94]

Another known TLR4 inhibitor is hesperetin (**9**, Fig. 3), a natural flavanone and the aglycone of hesperidin which is produced by citrus fruits. In LPS-stimulated BV-2 microglial cells, hesperetin strongly inhibited the release of NO, IL-6 and IL-1β cytokines.[95] Additionally, hesperetin blocked astrocyte and microglia activation in LPS-challenged mouse brain, suggesting the inhibition of microglia-mediated neuroinflammation.

3. Kinases – p38 MAPK and RIPK1

TLR and other microglia receptor signaling pathways converge on P38 mitogen-associated protein kinase (MAPK) and phosphoinositide 3 kinase pathways leading to the activation of nuclear factor (NF)-κB transcription and production of cytokines including IL-1β, IFN-γ and TNF-α.[96] Cytokines bind to their respective receptors on the same microglia and neighboring cells and amplify inflammatory signaling pathways in an auto-and paracrine fashion. Signaling from multiple cytokine receptors converges on a few kinases which has made those potential targets to treat undesired inflammatory processes.[97] However, the complexity of each signaling pathway in different cell types including microglia is not well understood in humans. In addition, signal transduction for kinases can be mediated by non-catalytic functions, such as structural scaffolding.[98,99] The success rate for the generation of selective small – molecule kinase inhibitors is low, because most kinase inhibitors target the adenosine-triphosphate (ATP)-binding site, which is highly conserved across the kinome.

MAPKs represent key signaling nodes expressed by many different cell types throughout the body and found in all major cell types in the brain.[100] Microglial p38 MAPKs regulate microglial activation upon exposure to proinflammatory cytokines and amyloid-β and have been implied in mediating neuroinflammation in multiple contexts and models.[101] Numerous p38 MAPK small molecule inhibitors not optimized for CNS disorders have failed in CNS-related clinical trials mainly due to poor brain penetration or low kinase selectivity leading to some dose-limiting toxicities.[102] The only p38 MAPK inhibitor currently in clinical trials for neuroinflammation

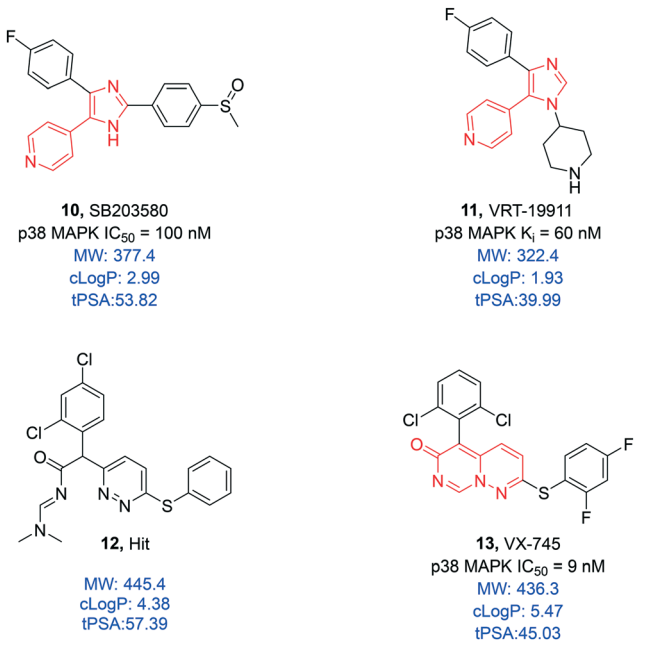
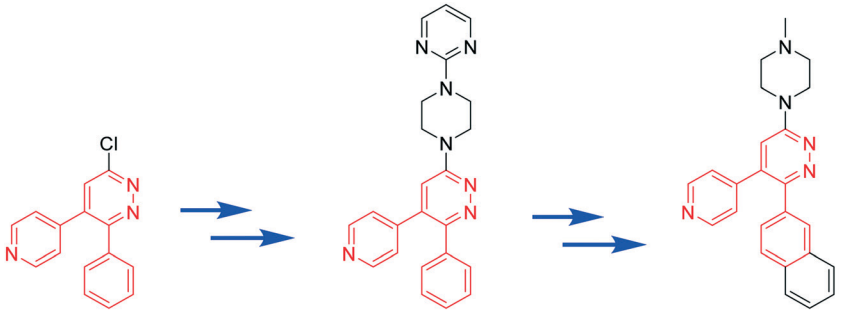


Fig. 4 p38/MAPK inhibitors.

associated with neurodegenerative disorders is Neflamapimod (VX-745)[103] (Table 1).

The discovery of VX-745 (**13**, Fig. 4) has been reported back in 2011 by Duffy and colleagues to treat inflammation.[104] The initial scientific rationale was to treat rheumatoid arthritis based on the success of TNF-α mediated approaches. Initial pyridinylimidazole leads SB203580 (**10**) and VRT-19911 (**11**, Fig. 4) demonstrated p38 MAPK activity by halting the release of TNF-α and IL-1β in an LPS induced assay. Compound **10** is an inhibitor of p38α (IC_{50} = 100 nM), with <5-fold selectivity over p38β. Compound **11** is a potent and selective p38α inhibitor with a K_i = 60 nM. A common issue for both compounds was the presence of the pyridyl moiety believed to be the culprit for significant hepatic cytochrome P450 (CYP) inhibition and preventing further development for chronic diseases. Applying a virtual screening to identify novel p38 inhibitors starting points, compound **12** was recognized as a screening hit. During the resynthesis and hit validation process, it was discovered that the actual structure included a core bicyclic 6*H*-pyrimido[1,6-*b*]pyridazin-6-one and not **12**. Further optimization onto the aromatic domains of the core led to the discovery of VX-745 (**13**), as a potent p38α inhibitor (IC_{50} = 9 nM), demonstrating PBMC IL-1β activity (IC_{50} = 45 nM) and PBMC TNF-α (IC_{50} = 51 nM). In addition, VX-745 showed approx. 20-fold selectivity over p38β and no significant inhibition of other MAP kinases and an improved CYP profile in comparison to previous leads (CYPs 3A4, 2D6, 2C19, 2C9, and 1A2 were IC_{50} > 40 μM). The originally planned clinical studies for rheumatoid arthritis were discontinued and the finding of higher drug concentrations in the CNS (in comparison to the periphery) led to a repurposing approach. In animal models, VX-745 has been reported to change the microglial activation



14
(*Fragment*)
MW: 267.7
cLogP: 2.26
tPSA:37.08

15
(*Hit*)
p38α IC_{50} = 0.60 μM
MW: 395.5
cLogP: 2.08
tPSA:68.28

16, MW150
(*Development Candidate*)
p38α IC_{50} = 0.23 μM
MW: 381.5
cLogP: 3.53
tPSA:43.56

Fig. 5 Evolution from initial fragment to development candidate for p38α/MAPK MW150.

state from pro-inflammatory to phagocytic, resulting in improved mitochondrial function, synaptic transmission and memory.[105]

In 2019, EIP Pharma began additional phase 2 trials in patients with Lewy body dementia and early-stage Huntington's disease. In November of the same year, the FDA granted a fast-track designation for VX-745 for dementia with Lewy bodies. In October 2020, it was announced that VX-745 (**13**) achieved its primary endpoint improving the cognitive composite score compared to placebo.[106] Another phase 2 clinical study is ongoing to assess memory impairment in patients with prodromal Alzheimer's disease (completion expected in January 2021).[66]

Another p38α MAPK drug candidate recently disclosed is MW01-18-150SRM (or MW150, Table 1) which is advancing to phase 1 human studies. In a recent publication,[102] Watterson and colleagues described the discovery of MW150 (**16**, Fig. 5) using a structure-based approach starting from an initial fragment (**14**). Through a methodical medicinal chemistry campaign using secondary pharmacology profiling of the initial scaffolds, the researchers de-risked potential dose-limiting toxicology preclinical liabilities. A simplified northern domain version of the initial hit (**15**, Fig. 5) mitigated the narrow therapeutic index observed with this compound while maintaining desired activity and selectivity over p38βMAPK. Further optimization guided by crystallography in the southern domain of the molecule to improve human liver microsomes led to the selection of compound **16** (MW-150). This rigorous approach led to an enhanced kinome-wide selectivity of the pyridazine series and enabled a suitable CNS pharmacokinetic profile for the candidate MW-150. The candidate was profiled in several pharmacodynamic models, and in particular, in the LPS treated BV2 microglia cell line, it demonstrated endogenous kinase inhibition as measured by phosphorylated substrate pMK2 with an IC_{50} of 332 nM and inhibition of IL-1β overproduction with an IC_{50} of 936 nM. Interestingly, screening for CYP inhibition (CYPs 1A2, 2B6, 2D6, 2C8, 2C9, 2C19, and 3A4) provided negative results.

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a key mediator of cell death and inflammation. RIPK1 is an exception among the kinase targets due to its unique allosteric pocket that led to the discovery of RIPK1 – specific kinase inhibitors (necrostatins, GNE684 and other classes, Fig. 6).[107] RIPK1 is highly expressed in microglia in mouse and human brain samples.[108] The levels of RIPK1, at both the mRNA and protein levels, in postmortem samples from individuals with late-onset Alzheimer's disease are increased compared with controls and positively correlate with the reduction in brain weights and pathological stages.[109] RIPK1 mediates transcription of neuroinflammatory genes in microglia[110] and inhibition of RIPK1 kinase activity has been shown to promote the ability of microglia to degrade Aβ and reduce the levels of amyloid plaques in APP/PS1 mice.[108] The lack of detrimental phenotypes observed in RIP1 kinase-dead mice have suggested that RIPK1 is a promising clinical target.[111] Three molecules, R552,[112] GSK2982772 (ref. 113) and DNL747 successfully completed phase I trials, the latter is intended to be superseded by DNL788 and developed for amyotrophic lateral sclerosis.[70]

Necrostatins, and in particular Nec-1 (**17**, Fig. 6) were first characterized as inhibitors of necroptosis, blocking TNF-α-induced necrotic cell death.[107] Additional studies led to the identification of RIPK1 as key target modulating TNF signaling and Nec-1 as a RIPK1 inhibitor. An X-ray co-crystal structure of one of the necrostatins enabled the discovery of an allosteric binding mode without the usual hinge-binding domain found for most kinase inhibitors and facilitating maximum kinome selectivity for RIPK1. These features attracted a significant enthusiasm to develop highly selective small-molecule inhibitors of RIPK1, which have demonstrated safety in preclinical models and clinical trials.[114]

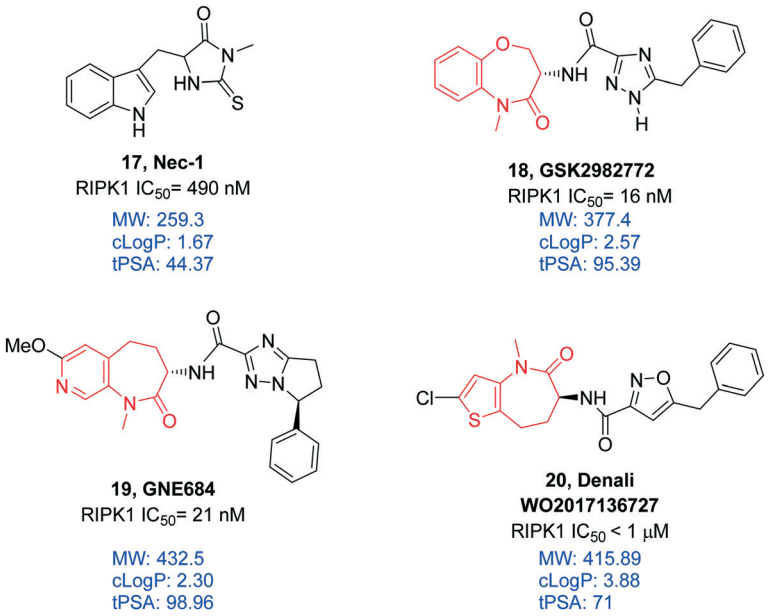


Fig. 6 RIPK1 inhibitors.

The discovery of clinical candidate GSK2982772 (ref. 115) (**18**, Fig. 6) has been recently reported. An initial benzoxazepinone hit identified using a DNA-encoded library approach, was further optimized to provide GSK2982772 (**18**) as a development candidate. Compound **18** has advanced to phase 2a for the treatment of peripheral inflammatory diseases, such as psoriasis, rheumatoid arthritis and ulcerative colitis. Genentech has been another key player in this area, exploring benzoxazepinone derivatives and eventually disclosing evaluation of the inhibition of RIPK1 in inflammatory preclinical models using GNE684 (**19**, Fig. 6) as a tool compound.[111,116]

Denali Therapeutics was the first company to focus on the development of brain penetrant RIPK1 inhibitors. Fig. 7 shows some examples (**21**–**24**) from patent literature published in 2017–2018.[117–120] Based on the structures, it

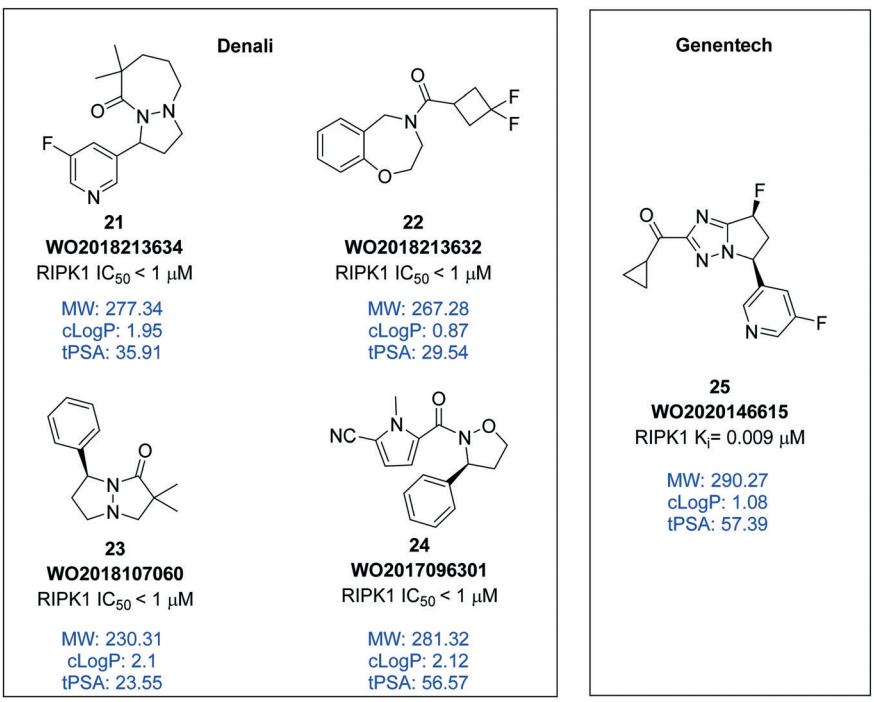


Fig. 7 Examples of RIPK1 inhibitors from Denali[119–122] and Genentech.[123]

seems that there was an effort designing compounds with low MW and tPSA to allow for significant brain penetration. An initial development candidate DNL104 (structure not reported) was advanced to clinical studies.[118] DNL104 was characterized as brain penetrant and selective for RIPK1 demonstrating activity against inflammation and cell death in multiple animal models. Assessment of safety and tolerability was conducted in phase 1 single-ascending dose (SAD) and multiple-ascending dose (MAD). While DNL104 was well tolerated in the SAD group, post-dose liver toxicity was observed in >30% of the MAD group.[118] The finding was determined to be drug-related. Additional discovery efforts led to DNL747, another brain penetrant inhibitor whose structure or preclinical studies have not been disclosed. After phase 1 studies, it was announced that DNL747 was well-tolerated and target engagement was achieved using a blood-based biomarker of RIPK1. However, in June 2020, adverse results in monkey toxicity studies halted further development of DNL747 in favor of DNL788, which might have a superior preclinical therapeutic window.[117]

A 2020 patent publication from Genentech[123] has also reported small molecules as RIPK1 inhibitors (Fig. 7, compound **25**) that might display brain penetration.

4. Inflammasome

The microglia inflammatory signaling pathways may culminate in the activation of the NOD-, leucine-rich repeat- and pyrin domain-containing protein 3 (NLRP3) inflammasome.[124] An inflammasome is defined as a multiprotein receptor complex generated by the innate immune system to eventually activate caspases. The NLRP3 inflammasome recognizes a wide range of stimuli ranging from bacteria and viruses to damage-associated endogenous factors including extracellular adenosine triphosphate (ATP), and β-amyloid plaques.[125,126] Activation of the NLRP3 inflammasome involves two steps, priming and activation.[127] In the priming step, TLRs and/or cytokine receptors recognize pathogen- or danger-associated molecular patterns and activate the gene expression of NLRP3 and inflammatory cytokine precursors such as pro-IL-1β and pro-IL-18 through the ATP pathway.[127] The activation step occurs as the primed cell recognizes another stimulus resulting in caspase-1 activation following by processing and secretion of IL-1β and IL-18.[128] In animal models of Alzheimer's disease, NLRP3 inflammasome activation contributes to memory impairment and amyloid pathology.[129,130] Currently, there are no approved drugs targeting NLRP3 selectively, although the initial publication of MCC950 (or CP-456773, Fig. 8) as a NLRP3 selective inflammasome inhibitor stirred an increasing number of drug discovery campaigns.[131] The most advanced inhibitor of the NLRP3 inflammasome (Inzomelid, or MCC7840) is now in phase 2 clinical testing for Parkinson's disease (Table 1). Inflammasome-associated caspases also directly cleave a 53 kDa protein substrate called gasdermin D that induces pyroptosis of the cell.[132] IL-1β and inflammasomes released from the dead cell can be engulfed by neighboring microglia and trigger further pyroptosis. Targeting gasdermin D may be a novel approach for inhibiting downstream consequences of the NLRP3 inflammasome activation.[133] Modulating purinergic P2X7 receptors upstream of NLRP3 activation is yet another therapeutic approach to restrain inflammasome activation (reviewed elsewhere[134]) and currently tested in clinical trials for depression with positive inflammatory biomarkers.[135]

An initial observation that the antidiabetic glyburide (**26**, Fig. 8) inhibited IL-1β (cell IC_{50} = 12 μM in human monocytes stimulated by LPS/ATP) by a post-translational process led to a broader medicinal chemistry effort around sulfonylureas (Fig. 8). The approach culminated in the discovery of potent MCC950 (**27**, cell IL-1β IC_{50} = 8 nM in human monocyte-derived macrophage stimulated by LPS/ATP).[125]

Additional SAR studies led to the identification of MCC7840 (**28**, Fig. 8), another potent derivative (cell IL-1β IC_{50} = 13 nM in human monocyte-derived macrophages stimulated by LPS/ATP) with a PK profile perhaps more suitable for development, with reduced clearance (0.62 mL/min/kg) in comparison with MCC950 (1.9 mL/min/kg) in mice.[136] As stated above, clinical compound MCC7840 (**28**) is currently in phase 2 studies for Parkinson's disease.

Lately, multiple pre-clinical sulfonyl-urea derivatives have been reported as NLRP3 inhibitors.[137] In particular, a series of *N*-cyano sulfoximine compounds (**29–31**, Fig. 9) exhibiting potency and selectivity towards NLRP3 inhibition, with suitable pharmacokinetic profiles for *in vivo* testing and potential further development.[138] Compounds **29–31** displayed IC_{50} values of 7, 5 and 12 nM, respectively, in a mouse IL-1β inhibition assay.

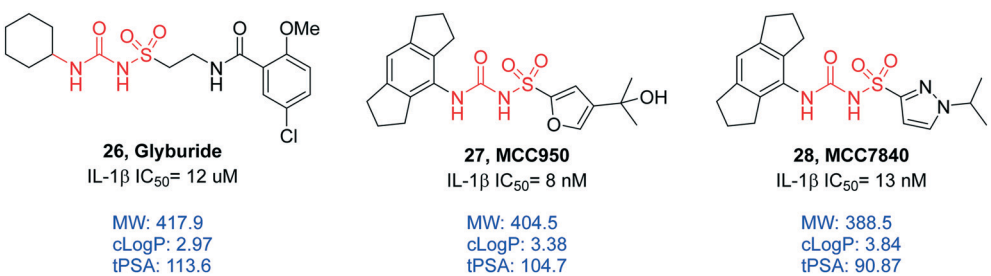


Fig. 8 Sulfonyl-urea NLRP3 inhibitors.

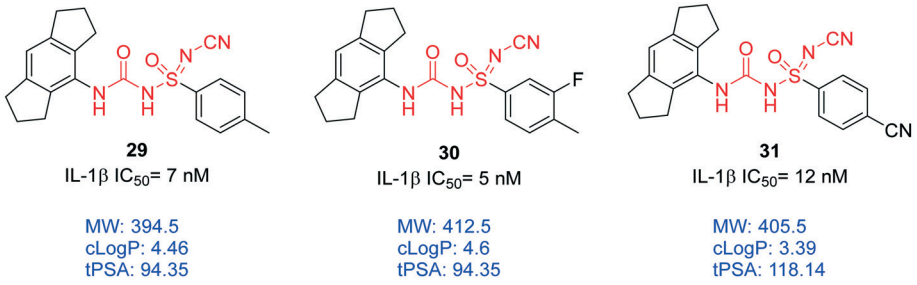


Fig. 9 *N*-Cyano sulfoximine NLRP3 inhibitors.

5. New targets for modulating microglia phenotypes

Human microglia express the components of the recently discovered cGAS–STING pathway, *i.e.* cyclic GMP–AMP synthase (cGAS) and its adapter stimulator of interferon genes (STING).[139] The STING-mediated type I interferon (IFN-I) response was shown to significantly alter microglial phenotype and promote disease progression in murine prion disease, a model for neurodegenerative disorders.[140] Double-stranded DNA released from mitochondria has been proposed to upregulate and activate this pathway in neurodegeneration and knock-out of STING rescued motor defects and neurodegeneration in a mouse model of Parkinson's disease.[141] However, while c-GAS–STING pathway inhibition and the resulting IFN-I blockade may be beneficial in neurodegenerative disorders, activation of this pathway by ganciclovir was shown to be beneficial in experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis.[142] These contradictory findings may reflect differences in the IFN-I/IFN-II balance in Parkinson's or Alzheimer's disease as compared to multiple sclerosis.[143] Future studies will be required to fully understand the potential of cGAS–STING pathway inhibition or activation in neuroinflammation associated with neurodegenerative disorders. Although a few molecules have been identified as modulators of the cGAS–STING pathway,[144] there are no advanced compounds in clinical development for neurodegenerative diseases.

In addition to therapies aimed at decreasing the inflammatory response, the emerging biology of microglia is fueling discovery of novel targets for neurodegeneration including modulation of lipid processing, lysosomal function and TREM2-mediated homeostatic mechanisms.[145] In the brain, TREM2 is exclusively expressed by microglia and recognizes lipids present in apoptotic cellular substances. Its activation promotes a microglia state characterized by proliferation, ramification and phagocytosis that is also necessary for expression of the DAM gene module.[146] Rare loss-of-function mutations in TREM2 are associated with an Alzheimer's disease risk similar to that of a single allele of the E4 isoform of APOE.[147,148] While the genetic findings suggest that boosting TREM2 function is beneficial in Alzheimer's disease, evidence from animal models is controversial and both, beneficial and deleterious effects of TREM2 have been reported.[48,149–151] Small molecule modulators for TREM2 are in drug discovery stages[152,153] and large molecule drugs are already in development[153–155] to assess the potential of TREM2 for ameliorating neurodegeneration.

6. Future directions

While the novel anti-neuroinflammatory small molecules described above provide hope that there are new treatment approaches on the horizon to ameliorate neurodegenerative diseases, there have been a number of failure clinical trials in this field already.[57,59,60] Therefore, in addition to dissecting microglia phenotypes and understanding their complex biology, the development of diagnostic and therapeutic biomarkers for neuroinflammation is key to support future drug development. Biomarkers of neuroinflammation, combined with disease-specific diagnostic biomarkers, will enable the molecular profiling of patients who could most benefit from treatments targeting neuroinflammatory mechanisms.[25] Structural and nuclear brain imaging are routinely used for diagnosis and/or outcome measures in neurodegenerative diseases and can provide information about the regional progression of neuroinflammation. The most widely used target for clinical positron emission tomography (PET) is the translocator protein 18 kDa (TSPO). Several PET tracers for TSPO have been developed, but their application is limited by lack of cellular specificity and inability to discriminate between beneficial and toxic microglia responses.[156] To overcome the limitations of TSPO PET, other imaging targets are being developed including ligands for purinergic receptors and colony-stimulating factor 1 receptor that are mainly expressed by microglia.[157,158]

Compared with non-invasive imaging techniques, collection of cerebrospinal fluid (CSF) or blood enables measurement of several biomarkers simultaneously. Several candidate fluid biomarkers of neuroinflammation are under validation:[159] clinical studies revealed that the level of soluble (s) TREM2 is elevated in the cerebrospinal fluid (CSF) of Alzheimer's disease patients and positively correlated with the levels of known CSF biomarkers of Alzheimer's pathology, *i.e.* total tau and phosphorylated tau. Therefore, sTREM2 is

linked to neuronal injury and may offer complementary information relevant for diagnostic purposes and novel treatment approaches targeting microglia in Alzheimer's disease.[160–163] Other fluid biomarkers under investigation are YKL-40 (chitinase-3-like protein 1), which is expressed by microglia and astrocytes in response to cytokines, and the cytokines themselves, such as monocyte chemoattractant protein-1 (MCP-1), IL-6 and 1β and TNF-α.[25]

Fluid biomarkers tracking different molecular pathways together with neuroimaging techniques capturing the regional evolution of neuroinflammation will be invaluable tools for future clinical trials targeting microglia in neurodegeneration.

From the chemistry perspective, we have shown a summary of the most recent medicinal chemistry approaches towards clinical candidates. Only few compounds were developed specifically for CNS indications and the clinical results will provide further insights to the scientific community. The high interest in this area will ensure a plethora of new scaffolds and even perhaps, new chemical modalities to address the unmet medical need on neurodegenerative disorders.[164]

Conclusion

The past years have seen an unprecedented increase in research aimed at phenotyping and targeting microglia to treat neurodegenerative diseases. Several small molecules modulating microglia receptors or kinase signaling pathways are currently entering or in early clinical trials. At the same time, biomarkers for neuroinflammatory mechanisms are being developed to improve identification of patients that may benefit from anti-inflammatory treatments and to measure treatment responses. Continued phenotyping of disease-associated microglia in animal models and advances in producing human microglia models from inducible pluripotent stem cells are supporting a future next wave of drugs targeting microglia in neurodegenerative diseases.

Conflicts of interest

There is no conflict of interest to declare.

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