HHS Public Access

Author manuscript

Neurosci Biobehav Rev. Author manuscript; available in PMC 2022 August 01.

Published in final edited form as:

Neurosci Biobehav Rev. 2021 August ; 127: 958–978. doi:10.1016/j.neubiorev.2021.06.027.

INTERPLAY BETWEEN IMMUNITY AND AMYOTROPHIC LATERAL SCLEROSIS: CLINICAL IMPACT

Fabiola De Marchi[1], **Ivana Munitic**[2], **Amedeo Amedei**[3], **James D. Berry**[4], **Eva L. Feldman**[5], **Eleonora Aronica**[6], **Giovanni Nardo**[7], **Donatienne Van Weehaeghe**[8], **Elena Niccolai**[3], **Nikolina Prtenjaca**[2], **Stacey A. Sakowski**[5], **Caterina Bendotti**[7], **Letizia Mazzini**[1]

[1]Department of Neurology and ALS Centre, University of Piemonte Orientale, Maggiore della Carità Hospital, Corso Mazzini 18, Novara 28100, Italy

[2]Laboratory for Molecular Immunology, Department of Biotechnology, University of Rijeka, R. Matejcic 2, 51000 Rijeka, Croatia

[3]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy

[4]Sean M. Healey & AMG Center for ALS, Department of Neurology, Massachusetts General Hospital, 165 Cambridge Street, Suite 600, Boston, MA 02114, USA

[5]Department of Neurology, University of Michigan, Ann Arbor, MI 48109, USA

[6]Amsterdam UMC, University of Amsterdam, Department of (Neuro) Pathology, Amsterdam Neuroscience, Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

[7]Laboratory of Molecular Neurobiology, Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Via Mario Negri 2, Milan 20156, Italy

[8]Division of Nuclear Medicine, Department of Imaging and Pathology, University Hospitals Leuven, KU Leuven, Leuven, Belgium

Abstract

Amyotrophic lateral sclerosis (ALS) is a debilitating and rapidly fatal neurodegenerative disease. Despite decades of research and many new insights into disease biology over the 150 years since the disease was first described, causative pathogenic mechanisms in ALS remain poorly understood, especially in sporadic cases. Our understanding of the role of the immune system in ALS pathophysiology, however, is rapidly expanding. The aim of this manuscript is to summarize the recent advances regarding the immune system involvement in ALS, with particular attention to clinical translation. We focus on the potential pathophysiologic mechanism of the immune system in ALS, discussing local and systemic factors (blood, cerebrospinal fluid, and microbiota) that influence ALS onset and progression in animal models and people. We also explore the potential of Positron Emission Tomography to detect neuroinflammation *in vivo,* and discuss ongoing clinical trials of therapies targeting the immune system. With validation in human patients, new

Corresponding Author: Letizia Mazzini, MD, Department of Neurology and ALS Centre, University of Piemonte Orientale, Maggiore della Carità Hospital, Corso Mazzini 18, 28100 Novara, Tel +39-0321-3733834; Fax +39-0321-3733298, letizia.mazzini@uniupo.it.

DECLARATIONS

Conflicts of interest: FDM, IM, AA, JDB, ELF, EA, GN, DVW, EN, NP, SAS, CB and LM declare no disclosures.

evidence in this emerging field will serve to identify novel therapeutic targets and provide realistic hope for personalized treatment strategies.

Keywords

immunity; inflammation; amyotrophic lateral sclerosis; biomarkers; targeted therapies; target

Introduction

Amyotrophic lateral sclerosis (ALS), the most common form of motor neuron disease (MND), is a debilitating and rapidly fatal neurodegenerative disease. It is characterized by progressive degeneration of upper and lower motor neurons with severe functional impairment (Hardiman et al., 2017). While marked phenotypic variability exists, ALS commonly begins insidiously with focal weakness and spreads to involve most skeletal muscles, including the diaphragm (Goutman, 2017). The incidence of ALS is between 0.6 and 3.8 per 100,000 person-years, and the prevalence is between 4.1 and 8.4 per 100,000 persons (Chiò et al., 2013; Longinetti and Fang, 2019; Nelson et al., 2018). Incidence increases with age. Most cases of ALS are adult-onset (median age between 51 and 66 years) and sporadic, with proven evidence of familial disease in less than 20% of cases (Hardiman et al., 2017). Effective treatments remain elusive, and there is no available treatment capable of stopping the neurodegeneration or clinical progression of ALS. In the European Union (EU), only riluzole is currently approved for ALS disease modification, and in the United States of America (USA), only riluzole and edaravone are approved. Other candidates have failed to show efficacy in prior trials, though a number of candidate drugs are currently in clinical trials (Chiò et al., 2020).

Jean-Martin Charcot first described ALS in 1869, but exploration of the pathogenesis of ALS made a leap forward in 1993 with the discovery of ALS-causative mutations in the copper/zinc superoxide dismutase (SOD1) gene and the generation of reliable transgenic animal models (Gurney et al., 1994; Rosen et al., 1993). Nowadays, it is well established that some pathogenic mechanisms, such as protein misfolding, oxidative stress, impaired axonal transport, alterations in RNA metabolism and protein homeostasis, mitochondrial and cytoskeletal dysfunction, and defects in nucleocytoplasmic transport are central mechanisms underlying ALS. As with other neurodegenerative diseases, ALS pathogenesis is at least partially non-cell-autonomous, involving astrocytes, oligodendrocytes, microglia, and various peripheral immune cells (Mejzini et al., 2019; Taylor et al., 2016).

In recent years, considerable evidence has begun to support the hypothesis of an important role of neuroinflammation in ALS pathophysiology. Overall, the data from human studies suggest that we can detect imbalances in components of the immune system that allow: 1) differentiation of people with ALS from controls, 2) identification of subsets of patients with specific genotype/phenotype features, 3) improved prognostication, and 4) monitoring of disease progression (Beers and Appel, 2019). The role of the immune response in ALS has been demonstrated on several levels, from *in vivo* imaging studies in mouse ALS models to *ex vivo* analyses that revealed changes in activation of astrocytes and microglia already

in presymptomatic phases, as well as to recent discoveries showing involvement of several genes directly linked to the immune response (Béland et al., 2020; Cirulli et al., 2015; Freischmidt et al., 2015; Lai and Ichida, 2019; Maruyama et al., 2010). Early evidence of neuroinflammatory responses in human ALS brain has also been reported in autopsy studies for both sporadic and chromosome 9 open reading frame 72 gene (C9orf72) mutated patients (Brettschneider et al., 2013; Kawamata et al., 1992; Sasaki, 2011). Likewise, neuroimaging studies utilizing Positron Emission Tomography (PET) with 18 kDa translocator protein (TSPO) tracers have allowed the identification of *in vivo* neuroinflammatory responses and microglial activation (Turner et al., 2004; Zürcher et al., 2015).

A better understanding of the role of immunity and inflammation in ALS pathogenesis can have several positive repercussions. Not only can it support the identification of new biomarkers for diagnosis and for the precise tracking of immune responses from preclinical to clinical disease stages, but it can also offer an innovative avenue to identify therapeutic drugs and precision medicine opportunities. This paper is based on an extensive review of the literature and our multidisciplinary knowledge on this topic. Herein, we summarize the molecular mechanisms of immune dysregulation in ALS, the role of the major immune cell players, and the insights from *in vivo* modulators of inflammation and *in vivo* biomarkers of immune activation. We finally present an overview of emerging immune-targeted emerging drugs and cellular therapies for ALS.

Molecular mechanisms of immune dysregulation in ALS

The biological mechanisms underpinning immune dysregulation in ALS are still being elucidated. Of particular interest is the unresolved chicken-egg dilemma of the link between neurodegeneration and neuroinflammation. The predominant understanding is that neuroinflammation is secondary to neurodegeneration. Neuroinflammation then in turn serves to amplify damage, particularly at advanced disease stages when proinflammatory factors prevail (Chiot et al., 2019). Indeed, the evidence from >25 ALS-linked genes supports the idea that the aforementioned pathogenic mechanisms, such as dysfunction in proteostasis, axonal transport, RNA metabolism, mitochondria, the cytoskeleton, and nucleocytoplasmic transport, originate in neurons (Brown and Al-Chalabi, 2017; Hardiman et al., 2017). Subsequently, neuronal pathology, even at the very early stages of the disease, serves to activate the immune system. For example, damaged neurons or their products such as aggregated TAR DNA-binding protein 43 (TDP-43), a pathological hallmark in >95% of ALS cases irrespective of genetic background, and aggregated SOD1, found in ALS patients carrying mutated SOD1, induce microglial activation, which can become neurotoxic by itself (Boillée et al., 2006; Swarup et al., 2011; Roberts et al. 2013).

The second hypothesis that dysregulated immune and/or inflammatory responses could in some cases be an initial trigger and precede neurodegeneration has only recently begun to be explored in greater detail. This is particularly due to the fact that several recently researched ALS-linked genes have been shown to be direct immunomodulators. These include genes that encode for *C9orf72*, cylindromatosis protein (*CYLD*), TANK-binding kinase 1 (*TBK-1*), and optineurin (*OPTN*) (Cirulli et al., 2015; Freischmidt et al., 2015; Maruyama et al., 2010; Renton et al., 2011).

Similarly, an interleukin (IL)-6 receptor (IL-6R) polymorphism (Asp358Ala), has been linked with higher IL-6 and IL-6R levels, potentially acting as a disease modifier (Wosiski-Kuhn et al., 2019). It is notable that both excessive and ineffective immune responses have been linked with these genes, and that most of them also participate in some other cellular pathways such as autophagy, so it is still difficult to discern which of their functions is most important for ALS pathogenesis. A more detailed discussion of the dilemma on primary or secondary role of immune system dysfunction in ALS is out of the scope of this review, but can be found in our previous article (Béland et al., 2020). Here it is important to emphatize that the evidence on the involvement of the immune system in ALS exists at multiple levels: 1) protein aggregates associated with ALS can directly activate innate immune damage sensors, 2) most ALS-linked genes encode for multifunctional proteins, and some of their functions affect immune pathways, 3) there is a strong positive feedback between various ALS-linked pathogenic processes and inflammation, including mitochondrial damage, ER stress, and lack of proteasomal and/or autophagic degradation, 4) inflammaging, one of the most important ALS risk factors linked to advanced age, contributes to proinflammatory skewing, 5) gut microbiota influence immunity and subsequently the neurodegenerative process, and finally, 6) some of the newly discovered ALS-linked genes are direct modulators of innate immune responses and are expressed at the highest levels in myeloid cells (Beers and Appel, 2019; Béland et al., 2020; Franceschi et al., 2018; Lyon et al., 2019; McCauley and Baloh, 2019). Therefore, although it is sometimes difficult to unequivocally pinpoint immune dysfunction as primary, secondary or concomitant to neuronal damage, it is evident that it occurs at multiple levels and early in the pathogenic process, thus opening potentially important therapeutic avenues.

Key immune cell players

A large body of evidence in ALS patients and animal models demonstrates a prominent role of innate and adaptive immunity in modulating the course of the disease (Beers and Appel, 2019; Béland et al., 2020; Lyon et al., 2019; McCauley and Baloh, 2019). The principal immune cells in the central nervous system (CNS) are microglia - the only resident parenchymal immune cells (Kettenmann et al., 2011). Microglia arise from yolk-sack hematopoietic progenitors, rely on signaling through colony stimulating factor 1 receptor (CSFR1) for their development, maintenance, and renewal, and are not exchanged by blood-derived monocytes in healthy CNS (Askew et al., 2017; Elmore et al., 2014; Ginhoux et al., 2010; Weimer et al., 2019). Microglia are usually described in two states – resting and activated. The resting microglia, contrarily to their name, are highly active in screening the microenvironment and participating in synaptic pruning, neurogenesis, and modulation of neuronal networks, which do not induce inflammatory responses (Nimmerjahn et al., 2005). Microglial activation and subsequent inflammatory responses are triggered in response to CNS damage (neuronal debris, extracellular ATP, and/or aggregated proteins) detected through numerous damage sensors, such as Toll-like receptors (TLRs), cytosolic DNA/RNA sensors, triggering receptor expressed on myeloid cells 2 (TREM2), receptor for advanced glycation endproducts (RAGE), P2 purinergic receptors, or complement and scavenger receptors. Activation includes shortening of protrusions, an increase in cell body size, proliferation, and higher phagocytic ability, a state collectively known as microgliosis. Microgliosis is mediated through nuclear factor κB (NF-κB), interferon regulatory factor

(IRF3), mammalian target of rapamycin (mTOR), and other signaling pathways. Depending on the extent and duration of damage, microglia get polarized to predominantly a pro-inflammatory or anti-inflammatory phenotype. Pro-inflammatory microglia, traditionally known as M1, secrete cytokines, such as tumor necrosis factor (TNF), IL-1β and IL-6, interferon-β (IFN-β), and chemokines CCL2 and IL-8 (chemoattractants for monocytes and neutrophils), upregulate damage sensors, and produce reactive oxygen species (ROS) and nitric oxide (NO) (Beers and Appel, 2019; Béland et al., 2020). In contrast, anti-inflammatory microglia, also known as M2, suppress inflammation, downregulate some damage sensors, promote tissue regeneration via cytokines IL-4, IL-13, and IL-10, and phagocytose cell debris and aggregated proteins, in part due to upregulation of scavenger receptors YM1 and CD206 (Gravel et al., 2016). Apart from microglia, several other macrophage populations populate meninges, perivascular spaces, and the choroid plexus (Goldmann et al., 2016). Meninges and abluminal perivascular spaces also harbor mast cells, whose number increases in ALS (Graves et al., 2004). Due to their granules prepacked with histamine, serotonin, and other mediators, and their ability to rapidly secrete cytokines, mast cells represent the early sentinel cells in the CNS that crosstalk with microglia to orchestrate an inflammatory response (Jones et al., 2019). While the fine role that would distinguish macrophages and mast cells from microglia in CNS tissue during ALS is still unclear, macrophages and mast cells clearly exert more prominent roles in peripheral nerves, as they are located in close proximity to axons and nerve terminals (Gupta and Harvima, 2018).

Although not immune cells *per se,* astrocytes and blood brain barrier (BBB) endothelial cells also represent an important source of immunologically relevant factors. Microglia have been shown to influence the functions of reactive astrocytes, driving astrocyte phenotype from neuroprotective to neurotoxic (Ferraiuolo et al., 2011; Filipi et al., 2020; Johann, 2017; Liddelow and Barres, 2017; Pehar et al., 2017; Sofroniew, 2015). Conversely, astrocytes release molecules (including TGF-β) that regulate microglial functions. Specifically, in response to activated microglia, astrocytes downregulate neuroprotective growth factor production, decrease glutamate excitatory amino acid transporter 2 (EAAT2), decrease lactate supply, and secrete proinflammatory cytokines, chemokines, and components of the complement pathway, thus amplifying pathological inflammatory signalling. This leads to astrogliosis and triggers excitotoxicity and disease progression. As homeostatic cells in the CNS, astrocytes are also involved in the modulation of oxidative stress, which is pivotal for inducing or perpetuating astrocyte-mediated inflammation (Filipi et al., 2020; Johann, 2017; Lee et al., 2016). Therefore, the bidirectional crosstalk between microglia and astrocytes has been shown to be crucial for the maintenance of a pro-inflammatory environment under pathological conditions (Burda and Sofroniew, 2017; Jha et al., 2019; Liddelow and Barres, 2017; Vainchtein and Molofsky, 2020). However, despite the attention given to the pro-inflammatory roles for astrocytes, there is mounting evidence supporting their ability to mediate a wide range of compensatory (protective) responses, including those aimed at mitigating inflammation (anti-inflammatory response), protecting against redox stress (antioxidant response), increasing small heat shock protein expression, and promoting protective and repair processes (Apolloni et al., 2017; Brambilla et al., 2016; Filipi et al., 2020; Gorter et al., 2019; Johann, 2017). Astrocytes form the BBB together with CNS endothelial cells, and astrocyte-derived growth factors, cytokines, and extracellular vesicles

directly influence endothelial cells. Endothelial cells are the source of various angiogenic factors, adhesion molecules, and chemokines important for regulating CNS oxygen and nutrient delivery, as well as selective entrance of blood-derived cells. In response to proinflammatory cytokines derived from astrocytes and microglia during ALS progression, endothelial cells upregulate adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule (VCAM), and E-selectin, and expose chemokines at their surface, thus facilitating peripheral cell infiltration (Alexianu et al., 2001; Evans et al., 2013). ALS is also marked by damage to the BBB and/or brain spinal cord barrier (BSCB), as evidenced by endothelial cell degeneration, loss of tight junction proteins, astrocyte end-feet swelling, microhemorrhages, and increased permeability, with some reports suggesting that this precedes motor neuron damage (Garbuzova-Davis et al., 2019, 2007; Zhong et al., 2008).

The damage encountered in chronic neurodegenerative disorders exceeds the capacity to be repaired by the resident CNS cells. Therefore, during the neurodegenerative process, microglia and astrocytes orchestrate a network of peripheral immune cells (Beers and Appel, 2019; Béland et al., 2020). Some of these cells then infiltrate the CNS, such as T cells and natural killer (NK) cells, although the infiltration in ALS is limited in comparison to autoimmune CNS diseases, such as multiple sclerosis, and predominantly occurs late in the disease course. NK cells eliminate stressed cells via cytotoxic granules or Fas ligand (Orr and Lanier, 2010). Furthermore, by producing IFN-γ, they can influence microglia and macrophage polarization toward a proinflammatory phenotype, and suppress regulatory T cells (Tregs), thus influencing both innate and adaptive immunity. NK cells, which are absent from healthy CNS, in ALS accumulate in motor cortex, spinal cord, and muscles, contributing to disease progression (Garofalo et al., 2020). Other notable immune subsets that take part in ALS pathogenesis, such as monocytes, neutrophils, and dendritic cells (DCs), have no access to the CNS in health and have limited access during ALS. In contrast, they exert important roles in peripheral nerves and meninges. The role of DCs in ALS is perhaps less clear due to difficulties in telling them apart from other myeloid lineage cells, especially macrophages, given their shared origin from blood monocytes. DCs are also absent from healthy CNS parenchyma, but can be found in meninges. Some reports suggested that DC numbers decrease in peripheral blood and increase in ALS lesions (Henkel et al., 2004; Rusconi et al., 2017; Sta et al., 2011), but this needs to be confirmed by fate-mapping studies.

In the adaptive immune system, specific roles in ALS pathogenesis have been ascribed to several T cell subsets. Usually, T cells have limited access to healthy CNS, so it was initially presumed that they are invariably noxious in ALS. However, when mouse ALS models were rendered T cell-deficient, this led to accelerated disease, demonstrating their overall protective role (Beers et al., 2008a; Chiu et al., 2008). It is notable though that individual T cell subsets have distinct functions. Neuroprotective functions have been ascribed to $CD4^+CD25^+$ Tregs and $CD4^+$ Th2 subsets, whereas $CD4^+$ Th1, $CD4^+$ Th17, and $CD8^+$ cytotoxic T cell subsets are linked to an accelerated disease course (Russo and McGavern, 2016; Schwartz and Shechter, 2010). It is clear now that, in contrast to microglia that get activated in the earliest stages of ALS, infiltrating T cells contribute to later stages, with $CD8^+$ T cells being linked exclusively to end stage disease. It is still uncertain if T cells

in ALS can exhibit some of their effects without crossing the BBB, as was previously described for protective effects of $CD4^+$ T cells in cognitive performance (Kipnis et al., 2012). Protective effects of Tregs and Th2 T cells are at least in part due to promoting the differentiation of anti-inflammatory microglia through IL-4 and IL-10 (Appel et al., 2010). In contrast, a direct neurotoxic role of $CD8^+$ T cells, executed by classical cytotoxic mechanisms via granzymes, Fas ligand, and IFN-γ, has been recently demonstrated in co-cultures of motor neurons with $CD8^+$ T cells taken from a mouse ALS model at a late disease stage (Coque et al., 2019). However, in the face of a toxic effect of $CD8^+$ T cells on motor neurons at late stage, these cells as well as macrophages may exert protective effect in the peripheral nerves at the early stage of the disease (Chiu et al., 2009; Nardo et al., 2018, 2016) as discussed in next section. B cells do not appear to contribute to CNS homeostasis, and their role in ALS is still puzzling. Lack of B cells does not affect progression in mouse ALS models (Naor et al., 2009). Intriguingly, immunoglobulin G (IgG) has been reported to accumulate in and/or around motor neurons (Donnenfeld et al., 1984; Engelhardt and Appel, 1990). Moreover, administration of IgG from ALS patients can be taken up and/or stimulate motor neurons, astrocytes, and microglia, increase serum and spinal cord cytokine levels, and elicit motor neuron degeneration (Appel et al., 1991; Bataveljic et al., 2014; Milošević et al., 2017; Obál et al., 2016; Pagani et al., 2011; Pullen et al., 2004). Some of the antibodies recognize aggregated proteins and neurofilaments, but their exact pathogenic mechanism is unclear (Malaspina et al., 2015; May et al., 2014).

Effector functions of antibodies are commonly linked to activation of complement, and depositions of activated complement components are present together with antibodies in the motor cortex of ALS patients (Donnenfeld et al., 1984; Sta et al., 2011). In healthy CNS, complement proteins are tightly linked to CNS homeostasis by participating in microglia-mediated synapse pruning and other remodeling, which represent non-immune functions of the complement system (Schafer et al., 2012; Stevens et al., 2007). Whereas peripheral complement system proteins are primarily produced by liver, in the CNS neurons, reactive microglia, and astrocytes are the main source of various complement proteins in the CNS (Lee et al., 2013; Luchena et al., 2018). Complement activation, antibody-dependent or independent, amplifies microglial activation, and contributes to a broad scope of neurodegenerative diseases, including ALS (Dalakas et al., 2020). Many of the immune cells and factors listed here will be further discussed in the upcoming sections as potential therapeutic targets and diagnostic biomarkers.

Immunity insights from animal models

Due to the inaccessibility of the CNS in patients, animal ALS models continue to be crucial for understanding the real timing of immune cell activation, its causal relation to neuropathology, and potential for therapeutic targeting. Among available animal ALS models (De Giorgio et al., 2019; Picher-Martel et al., 2016), those that best recapitulate most of the features of human pathology are the transgenic rodents overexpressing human SOD1 mutations (mSOD1), most commonly $SOD1^{G93A}$. The multiple facets of immune cell activation, from the presymptomatic to end stage of ALS, in mSOD1 mouse models at three key locations – CNS, peripheral nerves and muscle – are summarized in Figure 1.

In brief, the first immune hallmark in the spinal cord of mSOD1 mice, which precedes symptom appearance, is microglial activation, occurring in concomitance with the upregulation of chemokines (Nardo et al.2013) and release of danger stimuli (misfolded mSOD1, HMGB1 and ATP) from damaged neurons (Lo Coco et al., 2007; Urushitani et al., 2006; Volonté et al., 2003); this is followed shortly thereafter by astroglial activation (Gandelman et al., 2010). The initial predominantly anti-inflammatory microglial phenotype advances into a proinflammatory phenotype as the disease progresses (Beers et al., 2011), leading to a self-propagating hyperinflammatory and neurotoxic environment that accelerates motor neuron loss and precipitates death (Appel et al., 2010). Notably, during the disease course there is a coexistence of a continuum of the aforementioned opposite microglial fingerprints – M1 and M2 – and their prevalence depends on the surrounding cytokine milieu, which is modulated by astrocytes (Endo et al., 2015), and/or the interaction with infiltrating T cells, NK cells, and eventually monocytes and DCs (Appel et al., 2010; Chiot et al., 2020; Garofalo et al., 2020; Henkel et al., 2004).

The resulting neuroprotective or neurotoxic immune functions can be manipulated in animal models. For example, CNS delivery of IL-4 or IL-10 via viral vectors in mSOD1 mice at the presymptomatic stage enhanced the M2 phenotype in the spinal cord and delayed disease onset (Rossi et al. 2018; Beland et al. 2016). On the other hand, the deletion of TLR4, known to promote the M1 phenotype and induce motor neuron death (De Paola et al., 2016), extended survival in $SOD1^{G93A}$ mice (Lee et al., 2015). Transplantation of astroglial precursor cells into the spinal cord of mSOD1 mice attenuated motor neuron loss, improved motor function, and extended survival of these mice (Lepore et al., 2008). The real role of monocytes within the CNS is still unclear due to difficulties in distinguishing them from microglia and other myeloid cells. However, a progressive monocyte infiltration and their differentiation to macrophages has been consistently demonstrated within peripheral motor axons and at the neuromuscular junctions of mSOD1 mice from an early disease stage, due to the release of chemoattractants (CCL2/monocyte chemoattractant protein-1 (MCP-1)), complement (C3)-mediated opsonization of motor axons, and immunoglobulins (IgM) deposition (Chiu et al. 2009; Kano et al. 2012; Nardo et al. 2013; 2016; Wang et al. 2017). This correlated with a significant delay of symptom onset and slower disease progression in $SOD1^{G93A}$ mice (Nardo et al., 2018, 2016), and is consistent with the association between peripheral nervous system (PNS) inflammation and longer disease duration in ALS patients carrying SOD1 mutations (Schreiber et al., 2019). According to the hypothesis that muscle denervation precedes motor neuron death and disease symptoms (Fischer et al., 2004), it is believed that the initial axonal-muscle immune infiltration facilitates the removal of the degenerating axonal debris to allow a compensatory reinnervation of the muscles (Chiu et al., 2009; Nardo et al., 2018, 2016). In addition to denervation, skeletal muscle might also play an active role in NMJ dismantling and the ensuing inflammation in ALS in line with the dying back hypothesis. For example, the complement activation products C3/C3b and C1q may precedes end-plate denervation in human ALS and mSOD1 mice (Bahia El Idrissi et al., 2016; Heurich et al., 2011). On the other hand, an early immune infiltration and a macrophage skewing from a proinflammatory (M1) to anti-inflammatory (M2) in the denervated/damaged muscle might trigger an initial regenerative stage through the activation, expansion and differentiation of satellite cells (Forcina et al., 2020).

If inflammatory mSOD1 macrophages are replaced with those overexpressing wild type SOD1 at symptom onset, disease progression is further slowed down, leading to a prolonged mouse survival (Chiot et al., 2020). Similarly, deletion or pharmacological inhibition of C5a complement receptor C5aR1 after disease onset attenuated overall inflammation in the CNS, blood, and muscle of $SOD1^{G93A}$ mice, and extended their lifespan (Lee et al., 2017; Wang et al., 2017). Mast cells and neutrophils have also been shown to progressively infiltrate and exacerbate inflammation around degenerating motor nerve endings and in skeletal muscle of ALS patients and the $SOD1^{G93A}$ rat model after the onset of paralysis (Trias et al., 2018). Interestingly, the inhibition of mast cell degranulation induced by the pluripotent tyrosine kinase inhibitor masitinib (Trias et al., 2018, 2017), or by the mast cell stabilizer cromolyn (Granucci et al., 2019), ameliorated the clinical phenotype and CNS pathology and reduced the immune cell-mediated inflammatory response within axons and skeletal muscles of mSOD1 rodent models. Therefore, while an efficient immune response in the periphery is crucial to maintain axonal integrity and maximize connectivity with the muscle during early disease, at the later disease stages this response needs to be suppressed.

T cells have gained particular attention in ALS, as they comprise a significant proportion of the immune cells infiltrating the CNS and PNS of ALS patients and animal models (Beers et al., 2008b; Chiu et al., 2008; Coque et al., 2019; Henkel et al., 2013; Nardo et al., 2018). Tregs proved to be the most attractive T cell subset for therapeutic manipulation since their declining effector functions over the disease course affect both adaptive and innate immune responses, hampering immune tolerance and stimulating a proinflammatory phenotype that, in a vicious circle, induces further Treg dysfunction and rapid disease progression (Beers et al., 2018). Lower Treg numbers are in fact a recognized immunological fingerprint in rapidly progressing ALS patients (Beers et al., 2017; Henkel et al., 2013). Interestingly, adoptive transfer of Tregs in $SOD1^{G93A}$ mice (Banerjee et al., 2008) or their expansion following treatment with IL-2/anti-IL-2 monoclonal antibody complexes (IL-2c) with rapamycin, induces M2 microglia in the spinal cord and prolongs mouse survival (Sheean et al., 2018). Presymptomatic depletion of NK cells using an antibody against NK1.1 delayed the onset of paralysis and increased survival of mSOD1 mice through the activation of Treg cells (Garofalo et al., 2020). However, the same procedure applied at symptom onset was ineffective, suggesting a narrow temporal window of intervention in targeting these cells. Cytotoxic $CD8^+$ T cell infiltrations have been reported in both ALS patients (Holmøy, 2008; Sta et al., 2011) and mSOD1 mice (Coque et al., 2019; Nardo et al., 2018), although the systemic alterations in circulating $CD8^+$ T cells were inconsistent in ALS patients, indicating either a decrease (Mantovani et al., 2009), increase (Gustafson et al., 2017; Jin et al., 2020), or no changes (Zhang et al. 2005). Notably, the depletion of $CD8^+$ T cells and diminished major histocompatibility complex class I expression in ALS mice reduced inflammation and motor neuron loss in the CNS (Coque et al., 2019; Nardo et al., 2018), but in the periphery exacerbated the denervation-mediated atrophy, accelerating symptom onset. These data strengthen the hypothesis that activating the immune response in the PNS in the early phase is crucial for maintaining muscle innervation and delaying disease progression (Chiu et al., 2009; Nardo et al., 2018, 2016).

The immune system has been studied in a limited number of other animal ALS models as well; however, a major caveat is the paucity of neurologic symptoms in many models.

Particularly, among the mouse models carrying ALS mutant genes associated with the immune response, including *TARDBP* (encoding for TDP-43), *C9orf72*, *TBK1*, and *OPTN,* only the TDP-43 or C9orf72 transgenic models exhibit some neurologic symptoms, and often to a variable degree (De Giorgio et al., 2019; Hayes and Rothstein, 2016). Notable similarities to $SOD1^{G93A}$ mice were reported in transgenic mice carrying the patient $TDP\text{-}43^{Q331K}$ mutation under a mouse prion protein promoter (Lee et al. 2018). These mice show microgliosis, astrocytosis, and functional and morphological neuromuscular junction abnormalities well before the loss of spinal motor neurons (Chand et al., 2018; Lee et al., 2018). Importantly, like $SOD1^{G93A}$ mice, they also exhibit an upregulation of complement C5a-C5aR1 in the spinal cord and tibialis muscle during disease progression, validating the C5aR1 as a potential therapeutic target for ALS. Interestingly, in a reversible neuron-specific model carrying a TDP-43 deletion in the nuclear localization signal, the neuroprotective role of microglia was unleashed only upon suppression of transgene expression, whereas prior to that, neuronal TDP-43 expression inhibited microglial functions and proliferation (Spiller et al., 2018). In accordance with the newly discovered role of STING in ALS, genetic deletion or pharmacological inhibition of the STING pathway in transgenic $TDP43^{A315T}$ mice led to a marked reduction of proinflammatory cytokines in the spinal cord and cortex, ameliorated symptoms, and increased survival (Yu et al., 2020).

An immunologic phenotype is practically absent in unmanipulated *TBK1* ($Tbk1^{+/-}$) and *OPTN* models (knockouts and loss-of-function mutants) (Brenner et al., 2019; Dermentzaki et al., 2019; Gerbino et al., 2020; Markovinovic et al., 2018; Munitic et al., 2013; Slowicka et al., 2016), whereas complete loss of *C9orf72* leads to an autoimmune disease marked by splenomegaly, lymphadenopathy, and widespread peripheral myeloid and lymphoid immune cell activation in the absence of neurodegeneration (O'Rourke et al. 2016; Atanasio et al. 2016; Burberry et al. 2016). Instead, mice expressing 500 G4C2 repeats in bacterial artificial chromosomes, which more accurately mimic potential gain-of-function patient *C9orf72* mutations (BAC-C9orf72), were initially reported to exhibit motor abnormalities and decreased survival associated with motor neuron loss, without reactive gliosis, in about 30% of females (Liu et al., 2016). However, this ALS phenotype was not confirmed by other groups (Mordes et al., 2020) and was not observed in other BAC-C9orf72 mice (Jiang et al., 2016; O'Rourke et al., 2015; Peters et al., 2015). An elevated type I interferon response mediated by the STING pathway has also been found in myeloid cells from *C9orf72* knockout mice and blood macrophages and brain tissue from ALS/frontotemporal dementia patients carrying *C9orf72* G4C2 hexanucleotide repeats (McCauley et al., 2020). Mice haploinsufficient for TBK1 do not develop clinical or histological signs of motor neuron degeneration up to 2 years of age (Bruno et al., 2020; Gerbino et al., 2020). However, TBK1 haploinsufficiency in $SOD1^{G93A}$ mice led to an acceleration of symptom onset and muscle denervation, but surprisingly in later disease stages decelerated disease progression and increased life span (Brenner et al., 2019; Gerbino et al., 2020). Overall, these data further support the hypothesis that altered function of immune cells, while not directly starting neurodegenerative processes, may substantially contribute to disease pathogenesis and progression, and/or act in synergy with other ALS risk factors.

In conclusion, studies from animal models have identified key aspects of immune system involvement in the progressive damage of the neuromuscular system in ALS. Although these

models do not fully recapitulate human disease, they have advanced our understanding of the temporal and regional impact of both local and systemic immune changes in modulating neuroimmune interactions in the CNS and PNS. Such investigations hold promise in characterizing the immune response at different disease stages, not only for the development of the right therapy at the right moment, but also to identify diagnostic and prognostic biomarkers to improve clinical trials.

Immunity insight from neuropathology in ALS

Most of the earliest observations regarding inflammatory changes in ALS are based on studies of patient autopsy material. Several studies in the early 1990s immunohistochemically analyzed the cellular composition of inflammatory infiltrate in cases of sporadic ALS, showing scattered T cell infiltration (mainly represented by a cytotoxic $CD8^+$ T cell subset) associated with an abundant presence of reactive microglia/macrophages, as well as reactive astrocytes throughout the degenerating areas (Troost, Van den Oord, and Jong 1990; Smitt et al. 1992; Engelhardt, Tajti, and Appel 1993; Schiffer et al. 1996; Kawamata et al. 1992). Besides T lymphocytes, DCs and NK cells involved in the crosstalk between innate and adaptive immunity have been identified in human ALS tissues (Garofalo et al., 2020; Sta et al., 2011). Additional studies confirmed the persistent and prominent activation of both innate and adaptive immunity in human ALS spinal cord and motor cortex samples, documenting the activation of the complement system, TLR signaling, and TNF-α related pathways (Brambilla et al., 2016; Casula et al., 2011; Gorter et al., 2019; Henkel et al., 2004; Sta et al., 2011; Tortarolo et al., 2017). These and other studies have also highlighted the role of astrocytes as emerging key regulators of the inflammatory responses in several human CNS diseases, including ALS (Johann, 2017; Pehar et al., 2017; Sofroniew, 2015).

Further supporting the relationship between inflammation and ALS, several transcriptomic studies in human ALS samples have been performed to study the molecular changes associated with motor neuron degeneration in ALS spinal cord and motor cortex (Aronica et al., 2015; Dols-Icardo et al., 2020; Krokidis and Vlamos, 2018; Morello et al., 2017). A recent study using machine learning algorithms stratified the transcriptomes of a large cohort of ALS post-mortem cortex samples into three distinct molecular subgroups, including one with a predominant signature of glial activation (Tam et al., 2019). The use of recently developed spatial transcriptomic techniques further allows the study of single-cell gene expression *in situ* (Gregory et al., 2020; Maniatis et al., 2019). Moreover, single nucleus RNA sequencing (snRNASeq) using droplet technology can be applied to frozen brain tissue to elucidate cellular heterogeneity in human tissue (Habib et al., 2017), offering the possibility to achieve a more detailed understanding of the heterogeneous and dynamic glial phenotypes and other cellular components contributing to the inflammatory/immune response in ALS. Finally, given the rapid advances in genetic technology and knowledge in ALS, recent studies describing the glial pathology associated with specific genetic mutations emphasize aberrant interglial communication as a contributor to immune activation and related clinical phenotype (McCauley and Baloh, 2019; Rojas et al., 2014; Thangavelu et al., 2011; Velebit et al., 2020; Wallis et al., 2018).

Ex vivo analysis of immune profiles as marker of disease course variability

Clinical heterogeneity in ALS is exemplified by the significant difference in survival from symptoms onset, with a percentage of ALS individuals reaching end stage disease in less than a year and others progressing over more than a decade (Ravits and La Spada, 2009). The complexity of clinical heterogeneity is also seen in the way different outcomes can be linked to the disease phenotype at onset. For example, bulbar onset ALS is more common in women with cognitive impairment and in elderly patients and is associated with a rapidly progressive disease course (Watanabe et al., 2015), while longer survival is seen in limb onset patients (Benjaminsen et al., 2018; Longinetti and Fang, 2019; Watanabe et al., 2015). The rate of progression from disease onset is possibly the feature of ALS that has more bearing on the inflammatory response observed systemically and at a tissue level in this condition. In fact, it has been shown that the immune response has a significant influence in governing the severity in the disease progression (Beers et al., 2018; Choi et al., 2020; Murdock et al., 2016). As described above, a key point of neuroinflammation is glial activation and associations with increased levels of inflammatory mediators. Several ILs have been found elevated in CSF and/or blood of ALS patients compared to controls and/or patients with other non-inflammatory neurological disorders.

- Systemic biomarkers—Blood is an excellent biofluid for the discovery and validation of disease biomarkers. First, blood biomarkers are easily accessible, and blood analyses are repeatable over time, in contrast with CSF and imaging biomarkers due to invasiveness and high costs. In addition, the ethical implications of collecting blood samples are minor relative to CSF collection. These features further allow consideration of blood biomarkers as an excellent tool for monitoring disease-related changes. Several immunological pathways can be investigated at the serum level, and differences in the immunological asset between ALS patients and healthy controls and among ALS patients in different disease stages have been reported. However, the agreement between blood and CSF biomarkers is still debated. Overall, some studies showed a significant correlation between blood and CSF for some biomarkers, such as for growth factors, IFN-γ (Guo et al., 2017), and neurofilaments (Gagliardi et al., 2019; Wilke et al., 2019), while other works failed to detect blood biomarker changes corresponding to those in CSF (Steinacker et al., 2008). Furthermore, some blood biomarkers are generated only peripherally, and thus are not detectable in CSF.

TGF-β1 is a pleiotropic cytokine able to regulate proliferation, survival, and differentiation of many types of cells. TGF-β1 and related pathways are one of the most studied inflammatory mechanism in ALS, but the role of this cytokine during the progressive degeneration of motor neurons, and in glutamate-mediated excitotoxicity, is debated (Katsuno et al., 2011). It seems that a chronically upregulated TGF-β1 system may promote disease progression by inducing an imbalance between neurogenesis and neurodegeneration (Peters et al. 2017). Independent studies showed higher plasma levels of TGF- β1 in ALS compared to controls, and a positive correlation between TGF-β1 levels and disease duration (Houi et al. 2002; Peters et al. 2017). Coherently, the group of De Carvalho (Duque et al., 2020) observed that TGF-β1 and TGF-β3 levels had a significant negative correlation with ALS Functional Rating Scale-Revised (ALSFRS-R) scores, a marker of disease severity. Likewise, an early and progressive increase of TGF-β1 and TGF-β3 mRNA was found in the

skeletal muscle of ALS patients and mSOD mice in correlation with disease severity (Si et al., 2015). However, other studies using proteomics simultaneously in brain and blood failed to detect changes in blood TGF-β1 levels between fast and slow progressing ALS patients (Zubiri et al., 2018).

In ALS, the dysregulation of plasma inflammatory biomarkers may involve several ILs, with both pro- and anti-inflammatory functions. Ehrhart and colleagues showed significant downregulation of IL-5 and upregulation of IL-6 in blood samples of ALS patients compared to controls when collected at the first evaluation, while at the follow-up evaluation, a normalization of IL-5 and IL-6 levels associated with a decrement in IL-2 and increase in IL-8 levels (Ehrhart et al., 2015). This finding showing correlation with disease duration suggests an evolving system where specific humoral factors represent specific inflammatory and oxidative stress responses during disease progression.

Another interesting study showed significantly increased IL-6 levels, along with other ILs such as TNF-α, IL-4, IL-13, in ALS patients when compared with controls at baseline (Lu et al., 2016). In the longitudinal analysis, IL-6 increased in some subgroups of patients, including the slow progressors (showing less functional impairment), males, patients with spinal onset, and patients treated with riluzole (Lu et al., 2016).

On the contrary, another report showed that serum IL6 levels, in most patients carried the IL6R358Ala variant, negatively correlate both with the patient's functional (ALSFRS-R and subscores), and with respiratory function as measured by the percent predicted forced vital capacity (FVC) (Wosiski-Kuhn et al., 2021).

The role of TNF-α can be more controversial. Indeed, other studies showed normal or lower levels in ALS patients when compared with controls (Andrés-Benito et al., 2017; Martinez-Merino et al., 2018). These inconsistent results may be explained by the pleiotropic role of TNF-α, which can act in both pro- and anti-inflammatory responses. Contrasting results have been reported also regarding IFN-γ, which can participate in both innate and adaptive immunity. Lu and colleagues documented that IFN-γ was significantly decreased in plasma of ALS patients compared with controls. On the contrary, other studies highlighted elevated IFN-γ in ALS blood compared to controls, showing association also with faster progression and shorter survival (Guo et al., 2017; Liu et al., 2015; Saresella et al., 2013). Finally, IL-13, a profibrotic cytokine responsible for Th2 responses in humans, has been related to worse functional performances in ALS patients, showing a negative correlation with functional abilities measured by ALRFSR-R and a significant positive correlation with the disease progression rate (Shi et al., 2007).

To date, despite the large number of cytokines that participate in the interplay of neuroinflammation in ALS pathogenesis, there is no definitive biomarker that can be singularly used in clinical evaluation. However, blood-based biomarkers have the potential to improve diagnosis and monitoring of disease progression, by increasing convenience, offering ease of testing, and reducing costs.

As previously reported in ALS animal models, modulation of specific leukocyte or myeloid populations can be involved in disease progression, making blood leukocytes attractive as

biomarkers and targets for ALS drug development. Several alterations in T lymphocyte populations have been described in ALS patients as compared with controls. While differences on the percentage of different lymphocyte populations between patients and controls are discrepant among studies, changes in Tregs are more consistent and their levels inversely correlate with the rate of disease progression. Specifically, patients with minor disease severity have higher levels of Tregs, supporting a model where Tregs actively contribute to neuroprotection through their interactions with microglia (Beers et al., 2017, 2011; Henkel et al., 2017; Thonhoff et al., 2018b). Thus, the percentage of $CD4^+$ T lymphocytes and Tregs in the blood of patients with ALS was proposed as a biomarker for differentiating slowly progressing from rapidly progressing patients (Beers et al., 2011; Henkel et al., 2013). However, a reduction of Tregs and $CD14^+$ monocytes levels in ALS patients with a less severe disease has been also reported, suggesting a possible early recruitment of these cells in the damaged regions (Mantovani et al., 2009).

The complexity of peripheral immune responses may be supported by the shift from an anti-inflammatory phenotype (Th2 and Treg) to a pro-inflammatory profile (Th1 and Th17), as revealed by several studies investigating T cell changes in ALS patients (Beers et al., 2011; Henkel et al., 2013; Saresella et al., 2013). Jin and colleagues recently confirmed this pro-inflammatory shift, showing negative correlations between Th1 and Th17 with the ALSFRS-R score and FVC (Jin et al., 2020). Murdock and colleagues analyzed peripheral blood leukocytes from ALS patients, observing a significant increase in the percentage of neutrophils and a significant decrease in the percentage of $CD4^+$ T cells and $CD16^-$ monocytes in the blood of patients compared to controls. However, only $CD16^-$ monocyte levels positively correlated with the disease progression (Murdock et al., 2016). The same group showed increased mean counts of total leukocytes per millimeter of blood, with a prevalence of neutrophils, $CD16^+$ and $CD16^-$ monocytes, and NK cells, and further reported that early changes in immune cell numbers (neutrophils and $CD4^+$ T cells) had a significant direct correlation with disease progression measured by the change in ALSFRS-R score (Murdock et al., 2017). A recent study on the quantification of myeloid cell populations showed that patients with greater disease severity had a reduction in non-classical monocytes, and patients with greater bulbar involvement had a reduction in the proportions of classical, intermediate, and non-classical monocyte populations (McGill et al., 2020). Finally, the Choi group (Choi et al., 2020), hypothesizing that the neutrophil-to-lymphocyte ratio can reflect the degree of neuroinflammation in patients with ALS, confirmed a shorter survival duration in subjects with higher baseline neutrophil-to-lymphocyte ratio.

- CSF markers—CSF is considered the most dependable fluid for biological ALS markers due to its anatomical contiguity with the CNS. Several CSF biomarkers have been proposed for ALS diagnosis and monitoring disease progression, but currently, it is not entirely clear the exact added diagnostic and/or prognostic value of these markers.

In CSF, as was already shown in blood, the overall trend is an imbalance between pro- and anti- inflammatory cytokines, chemokines, and other mediators of the inflammatory response. Among them, the most investigated immunological biomarkers are IFN-γ, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), MCP-1, and IL-8 showing an increased level, and IL-10, angiogenin, and follistatin showing a decreased level (Guo

et al., 2017; Kuhle et al., 2009; Lind et al., 2016; Mitchell et al., 2009). In support of this, Mishra et al. measured various inflammatory markers in rat astroglial cultures exposed to ALS-CSF and compared with the diseased or normal controls. The ALS-CSF enhanced the production and release of inflammatory cytokines, such as IL-6, TNF-α, COX-2, and PGE-2, and induced a down-regulation of anti-inflammatory cytokines, such as IL-10, vascular endothelial growth factor (VEGF), and glial cell-derived neurotrophic factor (GDNF) (Mishra et al., 2016). Other interesting factors are IL-17, basic fibroblast growth factor (bFGF), macrophage inflammatory proteins (MIP)-1β and −1α, MCP-1β (Guo et al., 2017), IL-1α, follistatin, and kallikrein-5 (Lind et al., 2016).

Only a few studies have investigated a possible prognostic value for these biomarkers. Higher levels of IL-4, IL-10, bFGF, VEGF, and MIP-1α have been reported to associate with slower disease progression (Furukawa et al., 2015; Guo et al., 2017), and IL-4 and IL-10 were also higher in patients with prevalent lower motor neuron phenotype (Furukawa et al., 2015). Alternatively, both CSF and serum IFN-γ levels were reported to be increased in ALS patients relative to controls (Guo et al., 2017; Tateishi et al., 2010) and to directly correlate with disease duration (> 12 months) (Liu et al., 2015), with higher IFN-γ levels directly correlating with the interval from the disease onset to diagnosis, disease progression rate (Liu et al., 2015), and shorter survival (Guo et al., 2017). Likewise, higher MCP-1 levels were associated with worse disease severity and faster progression (Zhang et al., 2017). A recent study by Yang et al. described the role of the milk fat globule-EGF factor 8 (MFG-E8), an inflammatory modulator, in ALS, reporting a relatively higher MFG-E8 level in ALS patients compared to healthy controls and a negative correlation between MFG-E8 and the ALSFRS-R score (Yang et al., 2020). The group of Tateishi (Tateishi et al., 2010) discovered, among cytokines/chemokines elevated in ALS, that CCL2 and CXCL8 levels were also negatively correlated with the ALSFRS-R score, while CCL4 and CXCL10 showed a positive correlation. Additionally, CCL4 and CXCL10 showed negative correlations with disease progression rate. The concentration of TGF-β1 in CSF, however, is still debated. One previous report indicated that the TGF-β1 concentration was significantly higher in the CSF of patients with a long disease duration compared to patients with a short duration of ALS, and that there was a significant positive correlation between CSF TGF-β1 and ALS duration (Iłzecka et al., 2002). Conversely, Masuda et al. found that TGF-β1 levels in the CSF were not significantly different between ALS patients and normal controls (Masuda et al., 2017) and did not correlate with the disease course or stages. Finally, elevated IL-6 levels in CSF and serum seemingly correlate with respiratory clinical severity and hypoxia, suggesting that the cytokine levels are dependent on oxygenation and highlighting a link between hypoxia and inflammation in ALS (Moreau et al., 2005).

Recently, several researchers have focused on chitinase levels in patients with ALS. Indeed, the level of macrophage-derived chitinases, as a signal of microglia/macrophage activation, seems to be clearly increased in ALS patients, gaining interest as potential disease biomarker (Swash, 2020). In 2013, Varghese et al. first reported chitinases in the context of ALS, showing that CSF levels of chitotriosidase-1 (CHIT1), chitinase-3-like protein 1 (CHI3L1/YKL40), and chitinase-3-like protein 2 (CHI3L2) were significantly elevated in ALS patients relative to healthy controls (Varghese et al., 2013). Increased levels of YKL40 in CSF, expressed in inflammatory astrocytes, apart from a significant increase in the

CSF of patients compared to healthy controls, correlated with disease progression (Andrés-Benito et al., 2017; Gille et al., 2019) and degree of cognitive dysfunction (Thompson et al., 2019). Similarly, the levels of CHIT1 and CHIT3L2 were elevated in patients compared to controls and to ALS-mimics, and correlated with disease progression (Gille et al., 2019; Thompson et al., 2019). However, Gille et al. reported that elevated CSF chitinases were only weakly specific to ALS compared to a control group of patients with other neurological diseases (Gille et al., 2019). CHIT1 and YKL40 are also correlated with phosphorylated neurofilament heavy chain, considered a strong marker of axonal neurodegeneration, confirming the link between neuroinflammation and neurodegeneration. Importantly, all chitinase levels are longitudinally stable and do not change over time (Thompson et al., 2019). However, a flaw of these biomarkers is the absence of correlation with blood markers, due to limited elevation in blood.

Although these studies, even with a different list of inflammatory markers tested, have individually demonstrated the potential role of a combination of fluid biomarkers for diagnosis and progression of ALS, to date we cannot conclude which represents the best one, nor we can hypothesize that a unique CSF biomarker can capture all the pathogenic processes involved in the disease. However, we contend that the idea of hypothesizing a panel of immune markers should be considered for use in both clinical practice and as a common outcome in clinical trials.

- Imaging inflammation—PET allows for imaging of neuroinflammation *in vivo* by administration of radioligands targeting activated glial cells. The majority of these radioligands designed to visualize neuroinflammation target TSPO (Narayanaswami et al., 2018), a protein located in the outer mitochondrial membrane that is upregulated on activated glial cells. In ALS, a temporal pattern of TSPO expression on glial cells has been suggested, with TSPO expression mainly located on microglia during early disease stages and shifting to astrocytes in a later phase (Guilarte, 2019). A multitude of TSPO radioligands have been developed and will be briefly explained in the next paragraphs.

[^{11}C]PK11195 is the first generation TSPO radioligand and has been adopted in sporadic and familial ALS patients (Turner et al. 2004; Tondo et al. 2020). In sporadic ALS patients, increased [^{11}C]PK11195 binding was apparent in the motor cortex, pons, frontal cortex, and thalamus (Turner et al., 2004). Similarly, in symptomatic and asymptomatic patients with SOD1 mutations, higher [^{11}C]PK11195 binding was observed in the motor cortex, supplementary motor area, thalamus, medulla oblongata, and the occipital and temporal lobes (Tondo et al. 2020). The latter demonstrates that neuroinflammation can be discerned during the presymptomatic stage, important for future therapeutic studies. Despite these significant findings, [^{11}C]PK11195 is hampered by a low signal-to-noise ratio and low brain extraction (Best et al., 2019). These limitations led to the development of second-generation TSPO radioligands; however, while the second generation radioligands have a higher signal-to-noise ratio, they are hindered by their sensitivity to a polymorphism (rs 6971, Alanine/Threonine) (Best et al., 2019). This polymorphism divides the human population into high (approx. 50%), medium (approx. 40%) and low (approx. 10%) binding affinity groups. Recently, it has also been shown that the first-generation TSPO radioligand [^{11}C]PK1195 is influenced by this polymorphism, although in a more limited manner (Fujita et al., 2017).

[^{11}C]PBR28 and [^{18}F]DPA714, two second-generation TSPO radioligands, have been successfully applied in ALS studies to detect glial activation in the primary motor cortex, supplementary motor area, and frontotemporal lobes (Albrecht et al. 2018; Alshikho et al. 2018; Ratai et al. 2018; Corcia et al. 2012; Van Weehaeghe et al. 2020; Van Weehaeghe et al. 2020; Zürcher et al. 2015). Glial activation in the primary motor cortices correlated with clinical parameters of functional decline, such as ALSFRS-R, and structural and metabolic measures of neurodegeneration and axon loss, such as motor cortical thickness, fractional anisotropy, and spectroscopy changes (myoinositol/creatinine and N-acetyl-aspartate/creatinine ratios) (Alshikho et al., 2018; Ratai et al., 2018). Longitudinal evaluation of glial activation by [^{11}C]PBR28 uptake in ten ALS patients over a 6-month time window revealed a stable glial activation (Alshikho et al., 2018). However, the authors acknowledged that this longitudinal evaluation was performed in a biased selection of slow progressors (decline of 0.5 points on the ALSFRS-R scale/month), which might obscure a significant difference in this limited timeframe (Alshikho et al., 2018). Future studies including fast progressors and over longer time periods are needed to answer this clinically relevant question. Importantly, the feasibility of pooling multicenter multitracer and multiscanner TSPO scans has been demonstrated for two second generation radioligands, [^{11}C]PBR28 and [^{18}F]DPA714 (Van Weehaeghe et al., 2020a). Pooling data is crucial to facilitate the multicenter therapeutic studies needed to boost sample size in future therapeutic neuroinflammation studies.

Ideally, a TSPO radioligand without the need for genotyping is required. Therefore, two recent third generation TSPO radioligands, [^{18}F]GE180 and [^{11}C]ER176, have been developed (Best et al., 2019). A head-to-head comparison study of [^{11}C]PBR28 and [^{18}F]GE180 disclosed low brain penetration of [^{18}F]GE180 in 4 healthy volunteers and one ALS patient (Zanotti-Fregonara et al., 2018). Therefore, [^{11}C]PBR28 is preferred to [^{18}F]GE180 for TSPO imaging in the brain. In contrast, a head-to-head comparison study of [^{11}C]PBR28 and [^{11}C]ER176 in seven healthy volunteers demonstrated that [^{11}C]ER176 had a smaller intersubject variability compared to [^{11}C]PBR28, and that specific binding of [^{11}C]ER176 in low affinity binders exceeds the specific binding of [^{11}C]PBR28 in high affinity binders (Zanotti-Fregonara et al., 2019). Accordingly, [^{11}C]ER176 studies would need fewer subjects to obtain a similar statistical power and obviate the need for genotyping prior to scanning, as all subjects can be included. Currently, no [^{11}C]ER176 studies have been performed in ALS patients.

Aside from the two previously described drawbacks of TSPO radioligands, they also have measurable endothelial binding which complicates quantification (Best et al., 2019). These downsides encouraged researchers to explore other promising neuroinflammation targets. At present, to our knowledge, two other neuroinflammatory markers have been examined in ALS: monoamine oxidase B (MAOB) and the P2X7 receptor (P2X7R). MAOB is an enzyme located on the outer mitochondrial membrane and is mainly located in astrocytes. Therefore, in contrast to TSPO radioligands that visualize both activated microglia and astrocytes, MAOB radioligands detect activated astrocytes (Narayanaswami et al., 2018). L-[^{11}C]deprenyl, an irreversible MAOB inhibitor, was increased in the pons, white matter, spinal cord, and motor cortex of ALS patients (Johansson et al., 2007).

The P2X7R is an ATP-activated ion-channel, and is predominantly expressed on the cell membrane of activated microglia (Narayanaswami et al., 2018). Nonetheless, neurons, astrocytes, and oligodendrocytes also express P2X7R, as the P2X7R mRNA signal dropped by only 83% in hippocampal slice cultures depleted of microglia (Masuch et al., 2016). A head-to-head comparison between [^{11}C]JNJ717, a P2X7R radioligand, and TSPO ligand [^{18}F]DPA714 *in vitro* and *in vivo* in symptomatic ALS patients demonstrated increased [^{18}F]DPA714 binding in the motor cortex on an individual level, whereas no increased [^{11}C]JNJ717 binding was discernible (Van Weehaeghe et al. 2020). Moreover, this study suggested that frontotemporal dysfunction in ALS patients is associated with increased glial activation in the frontal cortex (Van Weehaeghe et al., 2020c). Notably, the lack of increased P2X7R expression in this head-to-head study could be attributed to limited or even lack of P2X7R upregulation in symptomatic stages, as the P2X7R initiates microglial activation (Monif et al., 2010). It has also been hypothesized that the P2X7R is important around the switch from M2 neuroprotective neuroinflammation to M1 neurotoxic neuroinflammation (Cieślak et al., 2019). Future studies in presymptomatic ALS patients are required to clarify whether P2X7R radioligands may have value as a biomarker in ALS.

To this point, no studies have investigated *in vivo* neuroinflammation in the spinal cord; however, hypermetabolism suggested to be caused by neuroinflammation has been observed (Marini et al. 2016; 2018; Van Weehaeghe et al. 2020). Spinal cord imaging has been hindered by the small structure, although a recent study in chronic radicular pain patients demonstrated the feasibility of TSPO quantification in the spinal cord.

Altogether, these studies demonstrate the biomarker potential of neuroinflammation PET scans. First, they may allow patient stratification based on the amount and pattern of glial activation (Albrecht et al. 2018; Alshikho et al. 2018; Ratai et al. 2018; Zürcher et al. 2015; Corcia et al. 2012; Van Weehaeghe et al. 2020; Van Weehaeghe et al. 2020). Second, they may provide prognostic information, as glial activation in the frontotemporal regions is associated with frontotemporal dementia (Zhang 2015; Van Weehaeghe et al. 2020). Finally, they can enable direct therapeutic response monitoring (Dupont et al., 2018; Werry et al., 2019).

- Microbiota-immunity axis—An important modulator of the immune responses is the gut microbiota. Increasing data document that, in addition to circulating neuropeptides and other immune cells playing in concert through the gut-brain axis, the gut microbiota work by forming immune tolerance and controlling Treg number and suppressive activity. Therefore, the gut and brain are connected and mutually regulated through microbiota-immune system crosstalk, and the alteration of the normal gut microbial composition (dysbiosis) may contribute to the onset and exacerbation of various neurodegenerative disorders. Previous mouse models and recent human data have shown an altered enteric flora in early-stage ALS, pointing to a possible gut microbiota role in ALS pathogenesis. First, the Sun group examined the gut of SOD1^{G93A} transgenic mice, a common ALS model, proposing a link between gut microbiota and ALS onset; the transgenic mice showed a leaky gut, increased number of intestinal Paneth cells, and a dysbiotic flora before ALS onset, suggesting the gut microbiota had a role in ALS pathogenesis (Wu et al., 2015). Interestingly, the administration of butyrate in these mice restored the

intestinal microbial homeostasis, reduced Paneth cell number, improved gut integrity, and prolonged mouse life span compared to control mice (Zhang et al. 2017). In addition, Blacher et al. observed a distinct microbiota profile in pre-symptomatic $SOD1^{G93A}$ mice, observing a vivarium-dependent gut microbiota composition, suggesting that a combination of genetic susceptibility and environmental factors driving early pre-clinical dysbiosis potentially contribute to ALS modulation (Blacher et al., 2019). Moreover, antibiotic treatment exacerbates the disease course in mice, and distinct commensal bacteria have been correlated with ALS severity. In particular, Akkermansia muciniphila ameliorates ALS symptoms (possibly due to nicotinamide production), whereas Ruminococcus torques and Parabacteroides distasonis exacerbate ALS symptoms. The authors identified distinct microbiome and metabolite configurations also in a cohort of 37 ALS patients compared with household controls. Additionally, Figueroa-Romero et al. confirmed the alterations in the gut microbiota composition early in the life of $SOD1^{G93A}$ mice, looking also at immune response changes and at the temporal evolution relative to symptom onset (Figueroa-Romero et al., 2020). Specifically, immune cell expansion and activation, particularly in the spinal cord, and global brain cytosine hydroxymethylation were noted in transgenic mice at disease end stage compared to control mice. The correlation analysis confirmed the microbiome-immune system interplay, suggesting that gut flora could influence the epigenome by secreting metabolites and modulating the immune system.

Very recently, Burberry et al. tried to enhance current knowledge on the microbiota contribution to motor neuron degeneration, studying a mouse model of ALS carrying a mutant version of the *C9orf72* gene (Burberry et al., 2020). In particular, the authors analyzed two vivariums of *C9orf72* mice, housed in different facilities, which display discrepancy in ALS progression and severity. They demonstrated that the animals in the two facilities showed distinct microbial profiles, and that the alterations in the gut microbiota modulated how ALS-related symptoms manifested. More interestingly, they found that inflammation and autoimmune responses were attenuated by reducing the microbial burden in mutant mice with broad-spectrum antibiotic treatment, or by transplanting gut microflora from a healthy environment, even after their onset, suggesting that microbial modulation of inflammation outside the brain regulates the disease course.

Regarding human ALS research, few studies have compared the fecal microbiota of ALS patients to healthy subjects and have led to different and controversial conclusions, probably due to study limitations, such as the number of patients and other confounding factors (diet, secondary disease effects, dysphagia, etc.). Different groups comparing the fecal microbiota of a few ALS patients to controls have observed differences of some bacteria genera abundances (in particular Ruminococcus) and documented a lower Firmicutes/Bacteroidetes (F/B) ratio (a dysbiotic indicator) in ALS patients (Fang et al., 2016; Rowin et al., 2017; Zhai et al., 2019). Brenner and colleagues, comparing the fecal microbiota of 25 ALS patients with 32 healthy subjects, did not find substantial taxonomic or metagenomics differences, apart from different proportions of Ruminococcaceae (Brenner et al., 2018). Likewise, Ngo et al. have more recently confirmed, enrolling a wider number of patients, that the fecal microbiota of ALS patients does not differ significantly from healthy subjects. The authors did however find increased risk of earlier death in ALS patients with higher microbiome richness and diversity, and in those with a greater F/B ratio. Given the well-

known heterogeneity of ALS disease, they suggest that larger studies are needed to explore the correlation between microbiota and ALS progression/prognosis. Nevertheless, there are manifold recent evidence of intestinal dysbiosis in ALS patients (Di Gioia et al., 2020; Nicholson et al., 2020; Zeng et al., 2020). Zeng and colleagues have shown a marked change in microbial structure in 20 ALS patients, where the Bacteroidetes phylum and several bacterial genera were up-regulated and the Firmicutes and Megamonas genus were down-regulated compared to healthy controls. Overall, ALS patients seem to be characterized by the reduction of butyrate-producing bacteria (Di Gioia et al., 2020; Nicholson et al., 2020), which are important for gut integrity and regulation of inflammation. In particular, since butyrate favors the expression of Foxp3 gene and regulates Treg/Th17 balances (Zhang et al., 2016), this observation provides additional proof of the microbiota and immune dysfunction link in ALS.

As such, the ascertained gut microbiota role in ALS pathogenesis could open innovative therapeutic opportunities to shape the microbiota composition to favorably modulate its functionality. Of relevance to this concept is the prospective longitudinal study addressing the impact of 6 months of probiotic supplementation on the gut microbiota on ALS progression. Fifty ALS patients and 50 matched controls were enrolled, with the former randomized to receive probiotic treatment or placebo. The results demonstrated that the gut microbiota composition of patients differed compared to controls, and that the gut microbiota changes during disease progression (Di Gioia et al., 2020). Additionally, while the probiotic treatment influenced the gut microbiota composition, it did not bring the biodiversity of intestinal microbiota of ALS patients closer to that of control subjects, nor did it affect the progression of the disease as measured by ALSFRS-R.

Since fecal microbial transplantation (FMT) seems to be the most efficacious therapeutic intervention to restore entire microbial communities and their metabolic products to modulate systemic immunity, a multicenter randomized double-blind clinical trial employing FMT as a therapeutic intervention for ALS is underway (ClinicalTrials.gov identifier: NCT03766321). The study will evaluate the effect of FMT on immunological features, in particular Treg numbers and the Th17/Treg ratio, and on the clinical response, in terms of safety and efficacy. Ultimately, detailed study of immune cell populations, levels of cytokines, and microbiota has potential to shed light on early processes that may lead to degenerative ALS (Mandrioli et al. 2019).

Finally, there are various theoretical reasons supporting the role of the microbiota-immunity axis in ALS pathogenesis that are supported by evidence in animal and human studies. In some cases, human studies have limitations due to the small number of enrolled patients or to the large genetic and phenotypical heterogeneity displayed in ALS. Moreover, it must be clarified whether the gut microbiota alterations observed in ALS patients precedes the disease or is a consequence of dietary changes due to dysphagia and anorexia that typically occur in during ALS progression. Larger, well-conducted clinical trials will be required to provide important information on the pathogenic mechanisms underlying ALS, and to define the effectiveness of therapies targeting the microbiota-immunity axis.

Clinical trials and therapeutic options

The recent pathogenic insights presented in this review are providing several therapeutic opportunities. Briefly, immune cells in both the CNS and the periphery have an inflammatory phenotype in ALS (Beers et al., 2017; Murdock et al., 2015; Zhao et al., 2017, 2013). Immune cell populations such as microglia are both more activated and cytotoxic during ALS (Zhao et al., 2010, 2004), and as the disease progresses, cytokine production in the CNS becomes increasingly pro-inflammatory (Beers et al., 2011; Henkel et al., 2006). In ALS mouse models, however, targeting populations of immune cells has induced both positive and negative effects on disease progression (Beers et al. 2006; Butovsky et al. 2012; Finkelstein et al. 2011; Beers et al. 2008), supporting the contention that the immune response in ALS is not purely destructive to CNS motor neurons. This likely explains why clinical trials utilizing global immune suppression have failed or accelerated disease (Cudkowicz et al. 2006; Gordon et al. 2007; V Meininger et al. 2006; Meininger et al. 2009): beneficial cells are being suppressed along with detrimental cells. These realizations have prompted development and translation of both pharmacologic and cell-based strategies that target specific immune populations and pro-inflammatory immune polarization in disease progression.

- Emerging drug therapies—Numerous drugs that modulate the immune system have been developed. Yet, several therapies targeting immune cell types, pathways, and regulatory factors that have shown promise in preclinical ALS studies ultimately had limited success in human studies (Crisafulli et al., 2018; Filipi et al., 2020; Khalid et al., 2017). While this lack of clinical efficacy may be explained by advanced disease progression in enrolled patients beyond a point where targeting neuroinflammation or immune activation could be effective, recently completed and ongoing studies, as well as novel drug repurposing strategies, are continuing to provide insight regarding the utility of immunomodulatory therapeutic strategies.

In one recently completed clinical trial, the NIPALS2013 study (NCT01884571; (Fournier et al., 2018)), 31 ALS subjects received a 6-month combination immunosuppression regimen consisting of basiliximab, tacrolimus, mycophenylate, and prednisone. This regimen mirrored that administered in a previous first-in-human trial of a stem cell transplantation therapy in ALS subjects where unanticipated improvement was seen in one subject (Feldman et al., 2014; Glass et al., 2016; Goutman et al., 2018). No subjects receiving the immunosuppression regimen in the NIPALS2013 trial, however, met the predefined criteria of a 6-point ALSFRS-R increase over 6 months. These findings are not surprising given the previously mentioned fact that global immune suppression studies have failed or accelerated disease (Cudkowicz et al. 2006; Gordon et al. 2007; V Meininger et al. 2006; Meininger et al. 2009), but additional studies are required to tease out how related immunomodulatory paradigms may affect ALS progression.

Another recently completed study, the TCZALS-001 study (NCT0246896), assessed the safety and tolerability of tocilizumab in 22 ALS subjects across 5 Northeast ALS Consortium (NEALS) study sites in the USA. The intravenously-administered drug prevents immune cell stimulation by blocking IL-6 (Sebba, 2008). The primary study outcomes were

safety and tolerability of administration every 4 weeks for 8 weeks, and secondary endpoints included assessment of proinflammatory genes in peripheral blood mononuclear cells and CSF, evaluation of cytokines in CSF, and MRI-PET in a subset of subjects to examine immunomodulatory efficacy and CNS penetration. Results were posted to ClinicalTrials.gov in late 2019, but published reports are not yet available.

The Modifying Immune Response and OutComes in ALS (MIROCALS) study (NCT03039673), a Phase II, placebo-controlled, double-blind, parallel group study based in the United Kingdom and France, assessed the efficacy and safety of Treg enhancement using low-dose (ld)-IL-2 (Tang et al., 2008; Zorn et al., 2006) in 304 subjects treated for 18 months. Secondary objectives included validation of a new Phase II study design, with the addition of exploratory assessments of immune and inflammatory phenotype, brain biomarkers, and genomics and transcriptomics. Recruiting has completed but results are not yet reported; however, this trial is a follow-on study of the earlier Immuno-modulation in ALS (IMODALS) trial where the safety, dosing, and activity of ld-IL-2 was assessed in 36 subjects randomized to placebo or one of two treatment doses (NCT02059759). Results demonstrated that the treatment was well-tolerated and revealed a dose-dependent increase in Tregs (Camu et al., 2020), thus informing and supporting the ongoing MIROCALS trial.

Rapamycin is being evaluated in Italy in the Phase II RAP-ALS trial, where 63 ALS patients randomized to placebo or one of two rapamycin doses were treated for 18 months and followed for an additional 36 months (NCT03359538; (Mandrioli et al. 2018)). The orally-administered drug inhibits mTOR signaling, which enhances protein degradation and exerts immunomodulatory effects, thereby having multiple potential benefits in ALS (Barmada et al., 2014; Caccamo et al., 2009; Cheng et al., 2015). mTOR acts on the homeostasis of the naive CD4+ T cells, which can develop into Th1, Th2, or Th17 effectors using promoted by mTOR pathways. Conversely, mTOR inhibits the induction of Tregs. Rapamycin inhibits mTORC1, which targets regulatory proteins in cell signaling and regulates autophagy by inhibiting the unc-51-like kinase 1 complex. Inhibition of mTORC1 by the tested drug stimulates autophagy through the formation of autophagosome from the phagophore. Inhibition of mTORC1 by Rapamycin expands Tregs and, in a mouse model, increased Tregs and induction of M2 microglia, associated with a stable phase of the disease (Mandrioli et al., 2018). The primary outcome of the RAPALS study is enhanced Treg numbers, with secondary outcomes including safety, CNS penetrance, mTOR pathway inhibition, immune phenotyping, and other functional and survival assessments. Final follow-up is in progress, and results are anticipated in early 2021.

Another immunomodulatory treatment that is advancing through clinical trial stages is RNS60, a novel agent that upregulates Tregs and activates protective cells (Vallarola et al., 2018). Drug administration involves weekly infusions along with daily nebulization on remaining weekdays. A pilot study (NCT02525471) in 24 ALS patients showed that a 23-week regimen was safe, tolerable, and capable of slowing disease progression (Paganoni et al., 2019), thus supporting a larger multicenter, randomized, double-blind, placebo-controlled, parallel group, add-on Phase II study designed to assess the effect of RNS60. ALS biomarkers, including Tregs and other pharmacodynamics markers, were the primary outcome, and ALSFRS-R scores, FVC, safety, quality of life, and survival were the

secondary outcomes. A total of 142 subjects received drug for 24 weeks, and an additional 24-week follow-up is in progress; anticipated study completion is targeted for mid-2021.

Immune-targeted therapies are also included in the recently launched Healey ALS Platform Trial. This innovative trial paradigm, the first of its kind in ALS, involves recruitment of subjects into a Master study protocol (NCT04297683) that randomizes subjects into one of several treatment arms evaluating potential therapeutic compounds or into one placebo group which is shared across arms, thus increasing the percentage of subjects receiving a study drug while still maintaining power. At present, there are 3 drug regimens included in the platform trial, with the prospect for addition of new drugs as approved. Two of the three current drugs and one planned drug modulate immune mechanisms. Zilucoplan is a small-molecule inhibitor of complement protein C5 that has anti-inflammatory and cell protection properties through a mechanism that involves inhibition of membrane attack complex (MAC) assembly. The drug is administered by daily subcutaneous self-injection and had a favorable safety profile in trials for myasthenia gravis (Beecher et al., 2019; Howard et al., 2020). Verdiperstat, alternatively, is an orally-administered drug, but it also has a favorable safety profile and is well tolerated. Its antioxidative properties, potential to reduce microglial activation, and effects on mitigating brain inflammation are driven by inhibition of myeloperoxidase, a pro-oxidant enzyme present in activated microglia and other immune cells (Beecher et al., 2019; Howard et al., 2020; Jucaite et al., 2015). Finally, one of the planned therapies for this trial is IC14 immunotherapy. Initially screened in a Phase I pilot study (NCT03487263), this treatment targets CD14, a master regulator of the immune response, particularly microglia, and is proposed as a strategy to “rebalance” the immune response in ALS. Recruiting for this platform trial commenced in late 2020, and results are highly anticipated.

In a similar mechanism to one of the platform trial drugs, the Phase III CHAMPION-ALS trial evaluating the safety and efficacy of ravulizumab (Lee et al. 2019), a complement C5 inhibitor, has recently commenced (NCT04248465). This multi-site study involving 49 worldwide sites is designed to include 354 subjects who will receive a single intravenous loading dose followed by regular maintenance dosing of ravulizumab or placebo for 50 weeks, with a planned open-label extension period for all subjects. ALSFRS-R is the primary outcome measure, and survival, change in FVC, muscle strength, and serum neurofilament, as well as safety are secondary outcomes. Likewise, an open-label, adaptive design Phase II study of BLZ945 (NCT04066244), a compound that impacts microglia (Wies Mancini et al., 2019), also began within the past year in the USA, Finland, and Sweden to evaluate safety, repeated dosing, and microglial response, as measured by PET imaging for TSPO binding, to inform future study planning. The study will include 20 subjects and completion is targeted for 2021.

A Phase III trial is also in the planning phases in Germany to evaluate two ascending dose titrations of masitinib, an oral tyrosine kinase inhibitor that exerts effects via CNS and PNS microglia, macrophages, and mast cells (Harrison and Rafuse, 2020; Trias et al., 2018), as an add-on therapy to riluzole (NCT03127267). The estimated enrollment for the study is 495 participants, with ALSFRS-R over the 48-week evaluation period as the primary outcome and muscle strength, FVC, quality of life, and progression-free survival

as secondary outcomes. This trial is based on promising results following a previous double-blind, randomized study that demonstrated safety and beneficial effects of masitinib versus placebo in 394 ALS subjects (Mora et al., 2020). Interestingly, palmitoylethanolamide, an endocannabinoid and potent inhibitor of mast cell activation (Skaper et al., 2013), was also reported to clinically improve pulmonary function in ALS patients (Palma et al., 2016).

Finally, there are several emerging immune-based strategies in development, including evaluation of compounds that modulate glial cells and repurposing drugs utilized in other disorders with immune components (Filipi et al., 2020; Khalid et al., 2017). One example includes recently developed inhibitors of the JAK/STAT pathway, a signaling pathway crucial to initiating inflammatory responses. These drugs inhibit Janus kinase (JAK) and thus alter immune polarization, preventing pro-inflammatory Th1 responses while preserving regulatory function (Kubo et al., 2014; Maeshima et al., 2012; Sonomoto et al., 2014). A Phase I study in renal transplant patients demonstrated that pharmacologically targeting JAK/STAT signaling can reduce peripheral NK cell levels in a dose-dependent manner (Van Gurp et al., 2008), and we have unpublished *in vitro* data showing that inhibiting JAK/STAT signaling can also reduce NK cell activity and cytotoxicity. Importantly, JAK/STAT inhibitors that have passed Phase III clinical trials to treat multiple diseases (Azevedo and Torres, 2018; Gladman et al., 2017; Sandborn et al., 2017) are commercially available, and others are already under development (He et al., 2017; Thorarensen et al., 2017). Repurposing such inhibitors therefore represents a novel approach to reduce peripheral NK cell levels and NK cell activity in ALS patients that is amenable to rapid clinical translation.

- Emerging cell-based therapies—The maturation of cell-based therapeutic approaches provides a new opportunity to reduce abnormal inflammation with cell-based therapies. Across medicine, cell therapy applications are expanding rapidly, with more and more cell types being employed for their therapeutic potential. Research in ALS has focused primarily, if not exclusively, on four types of cells: 1) neural stem cells (NSCs) and glial progenitor cells (GPCs), 2) induced-pluripotent stem cells (iPSCs), 3) mesenchymal stromal cells (MSCs), and 4) Tregs. The first three are progenitor cells whose actions are predicted to be broad, including neurotrophic factor elaboration, preservation of neuromuscular function, and (importantly) reduction in neuroinflammation. The last are immune cells, aimed at reducing both systemic inflammation and neuroinflammation.

Neural stem cells (NSCs) and glial progenitor cells (GPCs): NSCs have been investigated not for their potential to replace motor neurons, but rather for their ability to exert their influence to protect existing motor neurons. Their benefit in a variety of animal models of neurologic disease, from spinal cord injury (Teng et al., 2002) to models of Parkinson's and Huntington's disease (Cho et al., 2007; Redmond et al., 2007), have acted as the basis for critical early work examining their effect in ALS. In addition, non-neuronal cells in chimeric mice have long been demonstrated to have a beneficial impact on mSOD1 ALS neurons (Clement et al., 2003). A multi-center consortium of investigators examining the effect of NSCs on $SOD1^{G93A}$ mice demonstrated engraftment of transplanted cells, reduced astrogliosis and neuroinflammation, and even improved survival and function (Teng et al.,

2012). The experiments suggested that broader spread of the cells met with better outcomes, as did a higher number of non-neuronal NSC-derived cells engrafted in close proximity to motor neurons. There was, however, variability in the functional and survival benefit of the cells. Other studies recapitulated the beneficial effect of GPCs in SOD1^{G93A} ALS rats (Lepore et al., 2008). However, again, not all research in GPCs demonstrated the same benefit in murine models. One study in SOD1^{G93A} mice failed to demonstrate a functional or survival benefit with transplanted human GPCs (Lepore et al., 2011). These results could be providing important insight into limitations of GPCs as a therapy, or experimental design issues, such as problems with the immune suppression regimens used to dosage or spread of cells within the CNS, may have hampered the results.

The first FDA-approved clinical trial to treat ALS with allogenic human spinal cord-derived neural progenitor cells used a purpose-built catheter system for delivery into the ventral horn of the spinal cord (Glass et al., 2016). The trial demonstrated safety, albeit with a few notable adverse events of sensory disturbance and pain attributed to the surgical procedure. Fluorescence in-situ hybridization identified cells surviving up to 2.5 years later, with some differentiating into neurons and others remaining stem cells (Tadesse et al., 2014). Post-hoc long-term analysis of this small open-label cohort relative to historical controls suggested a potential slowing of functional decline, but no effect on survival (Goutman et al., 2018). Further trials are not yet underway. A trial of human GPCs is indexed on ClinicalTrials.gov, but has not begun startup activities or enrollment (NCT02478450).

Mesenchymal Stromal Cells (MSCs): *In vitro* work using MSCs suggests an anti-inflammatory effect in primary motor neuron cultures (Sun et al., 2013), providing evidence that therapy with MSCs may have an anti-inflammatory, and thus beneficial, effect on motor neurons. In ALS murine models, treatments with human MSCs intramuscularly or intrathecally have been safe and paved the way for clinical trials (Gothelf et al., 2014; Kim et al., 2010). Mesenchymal stromal cells have a robust ability to secrete neurotrophic factors and reduce the inflammatory milieu in the CNS, as measured by cytokine levels in the CSF (Kwon et al., 2014). Although MSC survival in animal models has been shown to be limited to weeks, their effect has frequently outlived the cells themselves (Sadan et al., 2009). Furthermore, human MSCs appeared to induce Tregs and anti-inflammatory cytokines in the blood of treated SOD1^{G93A} mice (Kwon et al., 2014), suggesting that the effect they exert is both anti-inflammatory and systemic. Subsequently, numerous trials have explored the effect of MSC in people with ALS.

An open label trial of autologous intrathecal transplantation of bone marrow-derived MSCs in ten participants in India demonstrated safety, but failed to show any statistical or clinically meaningful reduction in post-transplant rate of decline compared to baseline decline on the ALSFRS-R (Prabhakar et al., 2012). Additionally, a Phase I trial of bone-marrow derived MSCs (Neuronata-R) transplanted intrathecally demonstrated safety with longitudinal injections (Oh et al. 2015). Preliminary responder analyses have suggested that people whose MSCs secreted higher levels of neurotrophic factors and were more robustly anti-inflammatory had better clinical responses in one of these early trials (Kim et al. 2014). The Phase II trial demonstrated a slowing of decline of the ALSFRS-R in the treated group at four (2.98 points) and six months (3.38 points) post-treatment, without a difference in

long-term survival. TGF-β1 was increased in those reaching a threshold for benefit (Oh et al. 2018).

Phase I and IIa trials of MSC-NTF (NurOwn) in humans delivered intramuscularly and/or intrathecally demonstrated safety and suggested hints of possible efficacy (Berry et al., 2019; Petrou et al., 2016). In the subsequent Phase IIa trial, only a pre-specified subgroup analysis excluding slow progressors demonstrated a significant slowing of ALSFRS-R decline at four- and eight-weeks following treatment with MSC-NTF. Importantly, the pro-inflammatory biomarker MCP-1 was significantly reduced in CSF in the MSC-NTF group. The top-line Phase III trial (NCT03280056) results were recently reported in a press-release (https://ir.brainstorm-cell.com/2020-11-17-BrainStorm-Announces-Topline-Results-from-NurOwn-R-Phase-3-ALS-Study). According to the release, the trial demonstrated no statistically significant effect in the overall trial population, though reportedly showed statistically significant increases in neurotrophic factors and decreases in inflammatory markers in the CSF of those treated with MSC-NTF. Future analysis will likely focus on determining any correlations of the biomarker response with clinical response.

Additional ongoing trials may shed further light on the ability of MSCs to reduce neuroinflammation and improve clinical outcomes for people with ALS. A Phase I trial of adipose-derived MSCs expanded *ex vivo* and delivered intrathecally demonstrated safety (Staff et al., 2016), and a Phase II trial is currently underway (NCT03268603). A multi-center Phase I/II trial is also underway in Spain (NCT02290886). More recently, a case-control study in 67 patients that received three intrathecal injections of human umbilical MSCs (hUC-MSCs) every two months at a dose of 30 $\times 10^6$ cells showed reduction in the rate of disease progression and a two-fold extension of survival without adverse reactions, compared with 67 paired matched reference patients from the PRO-ACT database (Barczewska et al., 2020). Interestingly, the same cells injected into the cerebral ventricles of $SOD1^{G93A}$ mice protected lumbar motor neurons and upregulated anti-inflammatory and neurotrophic factors in the spinal cord, although this response was insufficient to prevent muscle denervation and prolong survival (Sironi et al., 2017). Even beyond the well-recognized glial activation, broad immune dysregulation and motor neuron loss, stem cell therapies have been shown to have beneficial effects on lesser-studied potential disease pathologies, such as endothelial cell damage (Garbuzova-Davis et al., 2021; Garbuzova-Davis and Borlongan, 2021) and microvasculature damage to lung tissue seen in SOD1 mouse models, both of which were shown to improve with intravenous delivery of human bone marrow-derived stem cells (Garbuzova-Davis et al., 2020a). Even cell-free extra-cellular vesicles derived from bone marrow endothelial progenitor cells have been shown to exert a beneficial effect on the microvasculature in ALS and could be shown to be of therapeutic value in the future (Garbuzova-Davis et al., 2020b).

Induced Pluripotent Stem Cells (iPSCs): In the $SOD1^{G93A}$ rat model of ALS, neural progenitor cells transfected with lentivirus to secrete high levels of human GDNF were used to explore glial replacement in the spinal cord (Klein et al., 2005). While the primary focus of these experiments was on delivery of neurotrophic factors, the successes in cell survival and integration, and neurotrophic factor secretion, were a step forward for the field and have

led both directly and indirectly to more cell-based therapies in ALS. The Phase I human trial is now complete (NCT02943850), though results have not been published yet.

Regulatory T Cells (Tregs): Under normal circumstances, Tregs exhibit an anti-inflammatory effect when the immune system is activated, leading to the eventual diminution of the inflammatory response. In ALS, a reduction in Treg suppressive function correlates with more rapid disease progression (Henkel et al., 2013). Furthermore, monocytes are more pro-inflammatory in people with ALS than controls (Butovsky et al., 2012; Zhao et al., 2017), providing more rationale for seeking therapeutic strategies, like boosting Treg function, that can reduce the inflammatory state in people with ALS to slow disease progression. In an open-label Phase I trial, three people with ALS were treated with autologous Tregs harvested by leukapheresis, expanded *ex vivo*, and given as weekly intravenous infusions alongside concomitant ld-IL-2 therapy aimed at maintaining Treg phenotype (Thonhoff et al., 2018a). The Treg infusions appeared to slow decline of the Appel ALS scale, and Treg suppressive function increased in parallel. A Phase IIa trial is presently underway to further characterize these clinical and biomarker effects in the setting of a small randomized controlled trial (NCT04055623). In addition, a Phase I trial of autologous Tregs with slightly different derivation is set to begin enrollment soon (NCT04220190) and will extend the evidence from the first trials of autologous Tregs in people with ALS. And, while not a trial of cell therapy, per se, the MICROALS multicenter trial of ld-IL-2 mentioned above (NCT03039673) is likewise aimed at bolstering the number and function of circulating Tregs in ALS patients and will provide additional insight.

Certainly, one of the benefits of cell therapies is that they can target multiple dysregulated pathways at the same time. Yet, these four categories of cell therapies are just beginning to generate data that will drive a rich understanding of novel ways to reduce neuroinflammation and protect motor neurons in people with ALS.

Future prospective and conclusions

The recent developments in understanding ALS pathophysiology have focused on the role of the immune system, and this field of research is rapidly expanding. This review summarizes many of the recent advances regarding immune involvement in ALS, with particular attention to clinical translation. With validation in human patients, the knowledge from this expanding field will serve to identify novel therapeutic targets.

Importantly, there is clear evidence of an extensive overlap in immune system activation in ALS patients and animal models of disease (Figure 2), providing important molecular insight into potential therapeutic targets and diagnostic biomarkers. Recent results have pinpointed T cells and NK cells, as well as other peripheral cells, such as monocytes, in disease pathology. Moreover, several ALS risk genes have been identified that directly modulate the immune system, and the immune system itself is also profoundly affected by gut microbiota, providing a broad array of potential therapeutic targets.

Given that the immune system has both harmful and beneficial effects, there is a need to focus research efforts on enhancing the beneficial effects of protective immunity. It also remains to be clarified if inflammation is of similar importance in all forms of ALS.

Taking into account the heterogeneity of the disease, not necessarily all individuals with ALS exhibit the same degree of neuroinflammation, or at all time points in the course of the disease. Hence, patients need to be stratified into subgroups based on the importance of inflammatory pathways to pathogenesis. PET might aid in patient stratification, as it provides *in vivo* information about neuroinflammation. This will facilitate identifying patients who may benefit most from anti-inflammatory and/or other immunomodulatory interventions. Improving our knowledge on the immune and inflammatory biomarkers, including but not limited to soluble factors, peripheral monocytes/macrophages, Tregs, and other T cells, and the composition of microbiota in relation with ALS phenotypes and progression may improve diagnosis and speed the development of immune-modulating treatments. An exciting novel and realistic route for the diagnosis and monitoring of the progression of neuroinflammation in ALS is represented by advanced neuroimaging techniques, and particularly PET, of the brain and spinal cord. However, some technological boundaries of imaging, particularly for the spinal cord, and the lack of specific ligands that can differentiate anti-inflammatory from proinflammatory microglia still limit the use of these methodologies in clinical practice.

The developments in understanding the role of immunity in the pathophysiology of ALS, however, have already encouraged new treatment approaches. Clinical trials using both pharmacologic and cell-based strategies that target specific immune cell subsets, pro-inflammatory mediators, and polarization in disease progression have been concluded and others are still ongoing. Although the initial broad-scope anti-inflammatory therapies were generally disappointing due to the incomplete understanding of the complexity of the immune response in ALS, the ongoing and upcoming selective and/or timely therapies provide a realistic hope for prospective, more specific personalized treatment strategies. To this end, both basic and clinical researchers must focus on understanding the interplay between innate and adaptive immunity that leads to immune disbalance and/or hyperinflammation. This is relevant at all stages of ALS progression in the different clinical phenotypes and genotypes for designing the best treatment trials.

Acknowledgments:

EA is supported by Stichting ALS Nederland.

Funding:

The paper was realized with the support of the AGING Project for Department of Excellence at the Department of Translational Medicine (DIMET), Università del Piemonte Orientale, Novara, Italy; with the contribution of Regione Lombardia, Italy, “POR FESR 2014-2020 resources Call HUB Ricerca Innovazione. IM is supported by the Croatian Science Foundation (IP-2018-01-8563) and University of Rijeka grants (18-211-1369). ELF is supported by the National Institutes of Health (R01ES030049), the Centers for Disease Control and Prevention/ Agency for Toxic Substances and Disease Registry (R01TS000289), and the University of Michigan NeuroNetwork for Emerging Therapies.

REFERENCES

Albrecht DS, Normandin MD, Shcherbinin S, Wooten DW, Schwarz AJ, Zürcher NR, Barth VN, Guehl NJ, Akeju O, Atassi N, 2018. Pseudoreference regions for glial imaging with 11C-PBR28: investigation in 2 clinical cohorts. J. Nucl. Med. 59, 107–114. [PubMed: 28818984]

Alexianu ME, Kozovska M, Appel SH, 2001. Immune reactivity in a mouse model of familial ALS correlates with disease progression. Neurology57, 1282–1289. [PubMed: 11591849]

Alshikho MJ, Zürcher NR, Loggia ML, Cernasov P, Reynolds B, Pijanowski O, Chonde DB, Izquierdo Garcia D, Mainero C, Catana C, 2018. Integrated magnetic resonance imaging and [11C]-PBR28 positron emission tomographic imaging in amyotrophic lateral sclerosis. Ann. Neurol. 83, 1186–1197. [PubMed: 29740862]

Andrés-Benito P, Moreno J, Domínguez R, Aso E, Povedano M, Ferrer I, 2017. Inflammatory Gene Expression in Whole Peripheral Blood at Early Stages of Sporadic Amyotrophic Lateral Sclerosis. Front. Neurol. 8, 546. 10.3389/fneur.2017.00546 [PubMed: 29081763]

Apolloni S, Fabbrizio P, Amadio S, Napoli G, Verdile V, Morello G, Iemmolo R, Aronica E, Cavallaro S, Volonté C, 2017. Histamine regulates the inflammatory profile of SOD1-G93A microglia and the histaminergic system is dysregulated in amyotrophic lateral sclerosis. Front. Immunol. 8, 1689. [PubMed: 29250069]

Appel SH, Beers DR, Henkel JS, 2010. T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening?Trends Immunol. 31, 7–17. [PubMed: 19879804]

Appel SH, Engelhardt JI, Garcia J, Stefani E, 1991. Immunoglobulins from animal models of motor neuron disease and from human amyotrophic lateral sclerosis patients passively transfer physiological abnormalities to the neuromuscular junction. Proc. Natl. Acad. Sci. 88, 647–651. [PubMed: 1988960]

Aronica E, Baas F, Iyer A, ten Asbroek ALMA, Morello G, Cavallaro S, 2015. Molecular classification of amyotrophic lateral sclerosis by unsupervised clustering of gene expression in motor cortex. Neurobiol. Dis. 74, 359–376. [PubMed: 25500340]

Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S, 2017. Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Rep. 18, 391–405. [PubMed: 28076784]

Atanasio A, Decman V, White D, Ramos M, Ikiz B, Lee H-C, Siao C-J, Brydges S, LaRosa E, Bai Y, Fury W, Burfeind P, Zamfirova R, Warshaw G, Orengo J, Oyejide A, Fralish M, Auerbach W, Poueymirou W, Freudenberg J, Gong G, Zambrowicz B, Valenzuela D, Yancopoulos G, Murphy A, Thurston G, Lai K-MV, 2016. C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production, and glomerulonephropathy in mice. Sci. Rep. 6, 23204. 10.1038/srep23204 [PubMed: 26979938]

Azevedo A, Torres T, 2018. Tofacitinib: a new oral therapy for psoriasis. Clin. Drug Investig. 38, 101–112.

Bahia El Idrissi N, Bosch S, Ramaglia V, Aronica E, Baas F, Troost D, 2016. Complement activation at the motor end-plates in amyotrophic lateral sclerosis. J. Neuroinflammation13, 72. 10.1186/s12974-016-0538-2 [PubMed: 27056040]

Banerjee R, Mosley RL, Reynolds AD, Dhar A, Jackson-Lewis V, Gordon PH, Przedborski S, Gendelman HE, 2008. Adaptive immune neuroprotection in G93A-SOD1 amyotrophic lateral sclerosis mice. PLoS One3, e2740. 10.1371/journal.pone.0002740 [PubMed: 18648532]

Barczewska M, Maksymowicz S, Zdolińska-Malinowska I, Siwek T, Grudniak M, 2020. Umbilical Cord Mesenchymal Stem Cells in Amyotrophic Lateral Sclerosis: an Original Study. Stem cell Rev. reports16, 922–932. 10.1007/s12015-020-10016-7

Barmada SJ, Serio A, Arjun A, Bilican B, Daub A, Ando DM, Tsvetkov A, Pleiss M, Li X, Peisach D, 2014. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. Nat. Chem. Biol. 10, 677–685. [PubMed: 24974230]

Bataveljic D, Milosevic M, Radenovic L, Andjus P, 2014. Novel molecular biomarkers at the blood-brain barrier in ALS. Biomed Res. Int. 2014.

Beecher G, Putko BN, Wagner AN, Siddiqi ZA, 2019. Therapies directed against B-cells and downstream effectors in generalized autoimmune myasthenia gravis: current status. Drugs79, 353–364. [PubMed: 30762205]

Beers DR, Appel SH, 2019. Immune dysregulation in amyotrophic lateral sclerosis: mechanisms and emerging therapies. Lancet Neurol. 18, 211–220. [PubMed: 30663610]

Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH, 2006. Wild-type microglia extend survival in PU. 1 knockout mice with familial amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. 103, 16021–16026. [PubMed: 17043238]

Beers DR, Henkel JS, Zhao W, Wang J, Appel SH, 2008a. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc. Natl. Acad. Sci. 105, 15558–15563. [PubMed: 18809917]

Beers DR, Henkel JS, Zhao W, Wang J, Appel SH, Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, Brown RH, Carroll MC, Henkel JS, Beers DR, Wen S, Rivera AL, Toennis KM, Appel JE, Zhao W, Moore DH, Powell SZ, Appel SH, 2008b. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. Proc. Natl. Acad. Sci. 105, 64–79. [PubMed: 18165312]

Beers DR, Zhao W, Appel SH, 2018. The Role of Regulatory T Lymphocytes in Amyotrophic Lateral Sclerosis. JAMA Neurol. 10.1001/jamaneurol.2018.0043

Beers DR, Zhao W, Liao B, Kano O, Wang J, Huang A, Appel SH, Henkel JS, 2011. Neuroinflammation modulates distinct regional and temporal clinical responses in ALS mice. Brain. Behav. Immun. 25, 1025–1035. [PubMed: 21176785]

Beers DR, Zhao W, Wang J, Zhang X, Wen S, Neal D, Thonhoff JR, Alsuliman AS, Shpall EJ, Rezvani K, 2017. ALS patients' regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity. JCI insight2.

Béland L-C, Markovinovic A, Jakovac H, de Marchi F, Bilic E, Mazzini L, Kriz J, Munitic I, 2020. Immunity in amyotrophic lateral sclerosis: blurred lines between excessive inflammation and inefficient immune responses. Brain Commun.

Benjaminsen E, Alstadhaug KB, Gulsvik M, Baloch FK, Odeh F, 2018. Amyotrophic lateral sclerosis in Nordland county, Norway, 2000–2015: prevalence, incidence, and clinical features. Amyotroph. Lateral Scler. Frontotemporal Degener. 19, 522–527. 10.1080/21678421.2018.1513534 [PubMed: 30265157]

Berry JD, Cudkowicz ME, Windebank AJ, Staff NP, Owegi M, Nicholson K, McKenna-Yasek D, Levy YS, Abramov N, Kaspi H, 2019. NurOwn, phase 2, randomized, clinical trial in patients with ALS: Safety, clinical, and biomarker results. Neurology.

Best L, Ghadery C, Pavese N, Tai YF, Strafella AP, 2019. New and old TSPO PET radioligands for imaging brain microglial activation in neurodegenerative disease. Curr. Neurol. Neurosci. Rep. 19, 24. [PubMed: 30941587]

Blacher E, Bashiardes S, Shapiro H, Rothschild D, Mor U, Dori-Bachash M, Kleimeyer C, Moresi C, Harnik Y, Zur M, 2019. Potential roles of gut microbiome and metabolites in modulating ALS in mice. Nature572, 474–480. [PubMed: 31330533]

Boillée S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW, 2006. Onset and progression in inherited ALS determined by motor neurons and microglia. Science (80-.). 312, 1389–1392.

Brambilla L, Guidotti G, Martorana F, Iyer AM, Aronica E, Valori CF, Rossi D, 2016. Disruption of the astrocytic TNFR1-GDNF axis accelerates motor neuron degeneration and disease progression in amyotrophic lateral sclerosis. Hum. Mol. Genet. 25, 3080–3095. [PubMed: 27288458]

Brenner D, Hiergeist A, Adis C, Mayer B, Gessner A, Ludolph AC, Weishaupt JH, 2018. The fecal microbiome of ALS patients. Neurobiol. Aging61, 132–137. [PubMed: 29065369]

Brenner D, Sieverding K, Bruno C, Lüningschrör P, Buck E, Mungwa S, Fischer L, Brockmann SJ, Ulmer J, Bliederhäuser C, Philibert CE, Satoh T, Akira S, Boillée S, Mayer B, Sendtner M, Ludolph AC, Danzer KM, Lobsiger CS, Freischmidt A, Weishaupt JH, 2019. Heterozygous Tbk1 loss has opposing effects in early and late stages of ALS in mice. J. Exp. Med. 216, 267–278. 10.1084/jem.20180729 [PubMed: 30635357]

Brettschneider J, Del Tredici K, Toledo JB, Robinson JL, Irwin DJ, Grossman M, Suh E, Van Deerlin VM, Wood EM, Baek Y, 2013. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann. Neurol. 74, 20–38. [PubMed: 23686809]

Brown RH, Al-Chalabi A, 2017. Amyotrophic lateral sclerosis. N. Engl. J. Med. 377, 162–172. [PubMed: 28700839]

Bruno C, Sieverding K, Freischmidt A, Satoh T, Walther P, Mayer B, Ludolph AC, Akira S, Yilmazer-Hanke D, Danzer KM, Lobsiger CS, Brenner D, Weishaupt JH, 2020. Haploinsufficiency of TANK-binding kinase 1 prepones age-associated neuroinflammatory changes without causing motor neuron degeneration in aged mice. Brain Commun. 2, fcaa133. 10.1093/braincomms/fcaa133 [PubMed: 33005894]

Burberry A, Suzuki N, Wang J-Y, Moccia R, Mordes DA, Stewart MH, Suzuki-Uematsu S, Ghosh S, Singh A, Merkle FT, Koszka K, Li Q-Z, Zon L, Rossi DJ, Trowbridge JJ, Notarangelo LD, Eggan K, 2016. Loss-of-function mutations in the C9ORF72 mouse ortholog cause fatal autoimmune disease. Sci. Transl. Med. 8, 347ra93. 10.1126/scitranslmed.aaf6038

Burberry A, Wells MF, Limone F, Couto A, Smith KS, Keaney J, Gillet G, van Gastel N, Wang J-Y, Pietilainen O, 2020. C9orf72 suppresses systemic and neural inflammation induced by gut bacteria. Nature1–6.

Burda JE, Sofroniew MV, 2017. Seducing astrocytes to the dark side. Cell Res. 27, 726–727. [PubMed: 28303889]

Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, Doykan CE, Wu PM, Gali RR, Iyer LK, 2012. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J. Clin. Invest. 122, 3063–3087. [PubMed: 22863620]

Caccamo A, Majumder S, Deng JJ, Bai Y, Thornton FB, Oddo S, 2009. Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. J. Biol. Chem. 284, 27416–27424. [PubMed: 19651785]

Camu W, Mickunas M, Veyrune J-L, Payan C, Garlanda C, Locati M, Juntas-Morales R, Pageot N, Malaspina A, Andreasson U, 2020. Repeated 5-day cycles of low dose aldesleukin in amyotrophic lateral sclerosis (IMODALS): A phase 2a randomised, double-blind, placebo-controlled trial. EBioMedicine59, 102844. [PubMed: 32651161]

Casula M, Iyer AM, Spliet WGM, Anink JJ, Steentjes K, Sta M, Troost D, Aronica E, 2011. Toll-like receptor signaling in amyotrophic lateral sclerosis spinal cord tissue. Neuroscience179, 233–243. [PubMed: 21303685]

Chand KK, Lee KM, Lee JD, Qiu H, Willis EF, Lavidis NA, Hilliard MA, Noakes PG, 2018. Defects in synaptic transmission at the neuromuscular junction precede motor deficits in a TDP-43(Q331K) transgenic mouse model of amyotrophic lateral sclerosis. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 32, 2676–2689. 10.1096/fj.201700835R

Cheng C-W, Lin M-J, Shen C-KJ, 2015. Rapamycin alleviates pathogenesis of a new Drosophila model of ALS-TDP. J. Neurogenet. 29, 59–68. [PubMed: 26219309]

Chiò A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, White LA, 2013. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology41, 118–130. [PubMed: 23860588]

Chiò A, Mazzini L, Mora G, 2020. Disease-modifying therapies in amyotrophic lateral sclerosis. Neuropharmacology167, 107986. [PubMed: 32062193]

Chiot A, Lobsiger CS, Boillée S, 2019. New insights on the disease contribution of neuroinflammation in amyotrophic lateral sclerosis. Curr. Opin. Neurol. 32, 764–770. [PubMed: 31306211]

Chiot A, Zaïdi S, Iltis C, Ribon M, Berriat F, Schiaffino L, Jolly A, de la Grange P, Mallat M, Bohl D, Millecamps S, Seilhean D, Lobsiger CS, Boillée S, 2020. Modifying macrophages at the periphery has the capacity to change microglial reactivity and to extend ALS survival. Nat. Neurosci. 23, 1339–1351. 10.1038/s41593-020-00718-z [PubMed: 33077946]

Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, Brown RH, Carroll MC, 2008. T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. Proc. Natl. Acad. Sci. 105, 17913–17918. [PubMed: 18997009]

Chiu IM, Phatnani H, Kuligowski M, Tapia JC, Carrasco MA, Zhang M, Maniatis T, Carroll MC, 2009. Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. Proc. Natl. Acad. Sci. U. S. A. 106, 20960–20965. 10.1073/pnas.0911405106 [PubMed: 19933335]

Cho S-R, Benraiss A, Chmielnicki E, Samdani A, Economides A, Goldman SA, 2007. Induction of neostriatal neurogenesis slows disease progression in a transgenic murine model of Huntington disease. J. Clin. Invest. 117, 2889–2902. [PubMed: 17885687]

Choi S-J, Hong Y-H, Kim S-M, Shin J-Y, Suh YJ, Sung J-J, 2020. High neutrophil-to-lymphocyte ratio predicts short survival duration in amyotrophic lateral sclerosis. Sci. Rep. 10, 428. 10.1038/s41598-019-57366-y [PubMed: 31949271]

Cieślak M, Roszek K, Wujak M, 2019. Purinergic implication in amyotrophic lateral sclerosis—from pathological mechanisms to therapeutic perspectives. Purinergic Signal. 15, 1–15. [PubMed: 30430356]

Cirulli ET, Lasseigne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, Couthouis J, Lu Y-F, Wang Q, Krueger BJ, 2015. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science (80-.). 347, 1436–1441.

Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, 2003. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science (80-.). 302, 113–117.

Coque E, Salsac C, Espinosa-Carrasco G, Varga B, Degauque N, Cadoux M, Crabé R, Virenque A, Soulard C, Fierle JK, 2019. Cytotoxic CD8+ T lymphocytes expressing ALS-causing SOD1 mutant selectively trigger death of spinal motoneurons. Proc. Natl. Acad. Sci. 116, 2312–2317. [PubMed: 30674678]

Corcia P, Tauber C, Vercoullie J, Arlicot N, Prunier C, Praline J, Nicolas G, Venel Y, Hommet C, Baulieu J-L, 2012. Molecular imaging of microglial activation in amyotrophic lateral sclerosis. PLoS One7, e52941. [PubMed: 23300829]

Crisafulli SG, Brajkovic S, Mis MSC, Parente V, Corti S, 2018. Therapeutic strategies under development targeting inflammatory mechanisms in amyotrophic lateral sclerosis. Mol. Neurobiol. 55, 2789–2813. [PubMed: 28455693]

Cudkowicz ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD, Drachman DB, Consortium NALS, 2006. Trial of celecoxib in amyotrophic lateral sclerosis. Ann. Neurol. 60, 22–31. [PubMed: 16802291]

Dalakas MC, Alexopoulos H, Spaeth PJ, 2020. Complement in neurological disorders and emerging complement-targeted therapeutics. Nat. Rev. Neurol. 16, 601–617. 10.1038/s41582-020-0400-0 [PubMed: 33005040]

De Giorgio F, Maduro C, Fisher EMC, Acevedo-Arozena A, 2019. Transgenic and physiological mouse models give insights into different aspects of amyotrophic lateral sclerosis. Dis. Model. Mech. 12.

De Paola M, Sestito SE, Mariani A, Memo C, Fanelli R, Freschi M, Bendotti C, Calabrese V, Peri F, 2016. Synthetic and natural small molecule TLR4 antagonists inhibit motoneuron death in cultures from ALS mouse model. Pharmacol. Res. 103, 180–187. 10.1016/j.phrs.2015.11.020 [PubMed: 26640075]

Dermentzaki G, Politi KA, Lu L, Mishra V, Pérez-Torres EJ, Sosunov AA, McKhann GM 2nd, Lotti F, Shneider NA, Przedborski S, 2019. Deletion of Ripk3 Prevents Motor Neuron Death In Vitro but not In Vivo. eNeuro6. 10.1523/ENEURO.0308-18.2018

Di Gioia D, Cionci NB, Baffoni L, Amoruso A, Pane M, Mogna L, Gaggìa F, Lucenti MA, Bersano E, Cantello R, 2020. A prospective longitudinal study on the microbiota composition in amyotrophic lateral sclerosis. BMC Med. 18, 1–19. [PubMed: 31898501]

Dols-Icardo O, Montal V, Sirisi S, López-Pernas G, Cervera-Carles L, Querol-Vilaseca M, Muñoz L, Belbin O, Alcolea D, Molina-Porcel L, 2020. Motor cortex transcriptome reveals microglial key events in amyotrophic lateral sclerosis. Neurol. Neuroinflammation7.

Donnenfeld H, Kascsak RJ, Bartfeld H, 1984. Deposits of IgG and C3 in the spinal cord and motor cortex of ALS patients. J. Neuroimmunol. 6, 51–57. [PubMed: 6368581]

Dupont A-C, Largeau B, Guilloteau D, Santiago Ribeiro MJ, Arlicot N, 2018. The place of PET to assess new therapeutic effectiveness in neurodegenerative diseases. Contrast Media Mol. Imaging2018.

Duque T, Gromicho M, Pronto-Laborinho AC, de Carvalho M, 2020. Transforming growth factor-β plasma levels and its role in amyotrophic lateral sclerosis. Med. Hypotheses139, 109632. 10.1016/j.mehy.2020.109632 [PubMed: 32085981]

Ehrhart J, Smith AJ, Kuzmin-Nichols N, Zesiewicz TA, Jahan I, Shytle RD, Kim S-H, Sanberg CD, Vu TH, Gooch CL, Sanberg PR, Garbuzova-Davis S, 2015. Humoral factors in ALS patients

during disease progression. J. Neuroinflammation12, 127. 10.1186/s12974-015-0350-4 [PubMed: 26126965]

Elmore MRP, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, Kitazawa M, Matusow B, Nguyen H, West BL, 2014. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron82, 380–397. [PubMed: 24742461]

Endo F, Komine O, Fujimori-Tonou N, Katsuno M, Jin S, Watanabe S, Sobue G, Dezawa M, Wyss-Coray T, Yamanaka K, 2015. Astrocyte-derived TGF-β1 accelerates disease progression in ALS mice by interfering with the neuroprotective functions of microglia and T cells. Cell Rep. 11, 592–604. 10.1016/j.celrep.2015.03.053 [PubMed: 25892237]

Engelhardt JI, Appel SH, 1990. IgG reactivity in the spinal cord and motor cortex in amyotrophic lateral sclerosis. Arch. Neurol. 47, 1210–1216. [PubMed: 2122877]

Engelhardt JI, Tajti J, Appel SH, 1993. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. Arch. Neurol. 50, 30–36. [PubMed: 8093428]

Evans MC, Couch Y, Sibson N, Turner MR, 2013. Inflammation and neurovascular changes in amyotrophic lateral sclerosis. Mol. Cell. Neurosci. 53, 34–41. [PubMed: 23110760]

Fang X, Wang Xin, Yang S, Meng F, Wang Xiaolei, Wei H, Chen T, 2016. Evaluation of the microbial diversity in amyotrophic lateral sclerosis using high-throughput sequencing. Front. Microbiol. 7, 1479. [PubMed: 27703453]

Feldman EL, Boulis NM, Hur J, Johe K, Rutkove SB, Federici T, Polak M, Bordeau J, Sakowski SA, Glass JD, 2014. Intraspinal neural stem cell transplantation in amyotrophic lateral sclerosis: phase 1 trial outcomes. Ann. Neurol. 75, 363–373. [PubMed: 24510776]

Ferraiuolo L, Higginbottom A, Heath PR, Barber S, Greenald D, Kirby J, Shaw PJ, 2011. Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. Brain134, 2627–2641. 10.1093/brain/awr193 [PubMed: 21908873]

Figueroa-Romero C, Guo K, Murdock BJ, Paez-Colasante X, Bassis CM, Mikhail KA, Raue KD, Evans MC, Taubman GF, McDermott AJ, 2020. Temporal evolution of the microbiome, immune system and epigenome with disease progression in ALS mice. Dis. Model. Mech. 13.

Filipi T, Hermanova Z, Tureckova J, Vanatko O, 2020. Glial Cells—The Strategic Targets in Amyotrophic Lateral Sclerosis Treatment. J. Clin. Med. 9, 261.

Finkelstein A, Kunis G, Seksenyan A, Ronen A, Berkutzki T, Azoulay D, Koronyo-Hamaoui M, Schwartz M, 2011. Abnormal changes in NKT cells, the IGF-1 axis, and liver pathology in an animal model of ALS. PLoS One6, e22374. [PubMed: 21829620]

Fischer LR, Culver DG, Tennant P, Davis AA, Wang M, Castellano-Sanchez A, Khan J, Polak MA, Glass JD, 2004. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp. Neurol. 185, 232–240. 10.1016/j.expneurol.2003.10.004 [PubMed: 14736504]

Forcina L, Cosentino M, Musarò A, 2020. Mechanisms Regulating Muscle Regeneration: Insights into the Interrelated and Time-Dependent Phases of Tissue Healing. Cells9. 10.3390/cells9051297

Fournier CN, Schoenfeld D, Berry JD, Cudkowicz ME, Chan J, Quinn C, Brown RH, Salameh JS, Tansey MG, Beers DR, 2018. An open label study of a novel immunosuppression intervention for the treatment of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Front. Degener. 19, 242–249.

Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A, 2018. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat. Rev. Endocrinol. 14, 576–590. [PubMed: 30046148]

Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Müller K, Marroquin N, Nordin F, Hübers A, Weydt P, 2015. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Nat. Neurosci. 18, 631–636. [PubMed: 25803835]

Fujita M, Kobayashi M, Ikawa M, Gunn RN, Rabiner EA, Owen DR, Zoghbi SS, Haskali MB, Telu S, Pike VW, 2017. Comparison of four 11 C-labeled PET ligands to quantify translocator protein 18 kDa (TSPO) in human brain:(R)-PK11195, PBR28, DPA-713, and ER176—Based on recent publications that measured specific-to-non-displaceable ratios. EJNMMI Res. 7, 1–5. [PubMed: 28058659]

Furukawa T, Matsui N, Fujita K, Nodera H, Shimizu F, Miyamoto K, Takahashi Y, Kanda T, Kusunoki S, Izumi Y, Kaji R, 2015. CSF cytokine profile distinguishes multifocal motor neuropathy from progressive muscular atrophy. Neurol. Neuroimmunol. neuroinflammation2, e138. 10.1212/NXI.0000000000000138

Gagliardi D, Meneri M, Saccomanno D, Bresolin N, Comi G. Pietro, Corti S, 2019. Diagnostic and Prognostic Role of Blood and Cerebrospinal Fluid and Blood Neurofilaments in Amyotrophic Lateral Sclerosis: A Review of the Literature. Int. J. Mol. Sci. 20. 10.3390/ijms20174152

Gandelman M, Peluffo H, Beckman JS, Cassina P, Barbeito L, 2010. Extracellular ATP and the P2X7 receptor in astrocyte-mediated motor neuron death: implications for amyotrophic lateral sclerosis. J. Neuroinflammation7, 33. 10.1186/1742-2094-7-33 [PubMed: 20534165]

Garbuzova-Davis S, Boccio KJ, Ehrhart J, Sanberg PR, Appel SH, Borlongan CV, 2021. Detection of endothelial cell-associated human DNA reveals transplanted human bone marrow stem cell engraftment into CNS capillaries of ALS mice. Brain Res. Bull. 170, 22–28. 10.1016/j.brainresbull.2021.01.020 [PubMed: 33545308]

Garbuzova-Davis S, Borlongan CV, 2021. Stem cell-derived extracellular vesicles as potential mechanism for repair of microvascular damage within and outside of the central nervous system in amyotrophic lateral sclerosis: perspective schema. Neural Regen. Res. 16, 680–681. 10.4103/1673-5374.294337 [PubMed: 33063723]

Garbuzova-Davis S, Haller E, Saporta S, Kolomey I, Nicosia SV, Sanberg PR, 2007. Ultrastructure of blood–brain barrier and blood–spinal cord barrier in SOD1 mice modeling ALS. Brain Res. 1157, 126–137. [PubMed: 17512910]

Garbuzova-Davis S, Kurien C, Haller E, Eve DJ, Navarro S, Steiner G, Mahendrasah A, Hailu S, Khatib M, Boccio KJ, 2019. Human bone marrow endothelial progenitor cell transplantation into symptomatic ALS mice delays disease progression and increases motor neuron survival by repairing blood-spinal cord barrier. Sci. Rep. 9, 1–20. [PubMed: 30626917]

Garbuzova-Davis S, Shell R, Mustafa H, Hailu S, Willing AE, Sanberg PR, Borlongan CV, 2020a. Advancing Stem Cell Therapy for Repair of Damaged Lung Microvasculature in Amyotrophic Lateral Sclerosis. Cell Transplant. 29, 963689720913494. 10.1177/0963689720913494 [PubMed: 32207340]

Garbuzova-Davis S, Willing AE, Ehrhart J, Wang L, Sanberg PR, Borlongan CV, 2020b. Cell-Free Extracellular Vesicles Derived from Human Bone Marrow Endothelial Progenitor Cells as Potential Therapeutics for Microvascular Endothelium Restoration in ALS. Neuromolecular Med. 22, 503–516. 10.1007/s12017-020-08607-1 [PubMed: 32820422]

Garofalo S, Cocozza G, Porzia A, Inghilleri M, Raspa M, Scavizzi F, Aronica E, Bernardini G, Peng L, Ransohoff RM, 2020. Natural killer cells modulate motor neuron-immune cell cross talk in models of Amyotrophic Lateral Sclerosis. Nat. Commun. 11, 1–16. [PubMed: 31911652]

Gerbino V, Kaunga E, Ye J, Canzio D, O'Keeffe S, Rudnick ND, Guarnieri P, Lutz CM, Maniatis T, 2020. The Loss of TBK1 Kinase Activity in Motor Neurons or in All Cell Types Differentially Impacts ALS Disease Progression in SOD1 Mice. Neuron106, 789–805.e5. 10.1016/j.neuron.2020.03.005 [PubMed: 32220666]

Gille B, De Schaepdryver M, Dedeene L, Goossens J, Claeys KG, Van Den Bosch L, Tournoy J, Van Damme P, Poesen K, 2019. Inflammatory markers in cerebrospinal fluid: independent prognostic biomarkers in amyotrophic lateral sclerosis?J. Neurol. Neurosurg. Psychiatry90, 1338–1346. 10.1136/jnnp-2018-319586 [PubMed: 31175169]

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science (80-.). 330, 841–845.

Gladman D, Rigby W, Azevedo VF, Behrens F, Blanco R, Kaszuba A, Kudlacz E, Wang C, Menon S, Hendrikx T, 2017. Tofacitinib for psoriatic arthritis in patients with an inadequate response to TNF inhibitors. N. Engl. J. Med. 377, 1525–1536. [PubMed: 29045207]

Glass JD, Hertzberg VS, Boulis NM, Riley J, Federici T, Polak M, Bordeau J, Fournier C, Johe K, Hazel T, 2016. Transplantation of spinal cord–derived neural stem cells for ALS: analysis of phase 1 and 2 trials. Neurology87, 392–400. [PubMed: 27358335]

Goldmann T, Wieghofer P, Jordão MJC, Prutek F, Hagemeyer N, Frenzel K, Amann L, Staszewski O, Kierdorf K, Krueger M, 2016. Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat. Immunol. 17, 797. [PubMed: 27135602]

Gordon PH, Moore DH, Miller RG, Florence JM, Verheijde JL, Doorish C, Hilton JF, Spitalny GM, MacArthur RB, Mitsumoto H, 2007. Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Lancet Neurol. 6, 1045–1053. [PubMed: 17980667]

Gorter RP, Stephenson J, Nutma E, Anink J, de Jonge JC, Baron W, Jahreiβ M, Belien JAM, van Noort JM, Mijnsbergen C, 2019. Rapidly progressive amyotrophic lateral sclerosis is associated with microglial reactivity and small heat shock protein expression in reactive astrocytes. Neuropathol. Appl. Neurobiol. 45, 459–475. [PubMed: 30346063]

Gothelf Y, Abramov N, Harel A, Offen D, 2014. Safety of repeated transplantations of neurotrophic factors-secreting human mesenchymal stromal stem cells. Clin. Transl. Med. 3, 21. [PubMed: 25097724]

Goutman SA, 2017. Diagnosis and clinical management of amyotrophic lateral sclerosis and other motor neuron disorders. Contin. Lifelong Learn. Neurol. 23, 1332–1359.

Goutman SA, Brown MB, Glass JD, Boulis NM, Johe K, Hazel T, Cudkowicz M, Atassi N, Borges L, Patil PG, 2018. Long-term Phase 1/2 intraspinal stem cell transplantation outcomes in ALS. Ann. Clin. Transl. Neurol. 5, 730–740. [PubMed: 29928656]

Granucci EJ, Griciuc A, Mueller KA, Mills AN, Le H, Dios AM, McGinty D, Pereira J, Elmaleh D, Berry JD, Paganoni S, Cudkowicz ME, Tanzi RE, Sadri-Vakili G, 2019. Cromolyn sodium delays disease onset and is neuroprotective in the SOD1(G93A) Mouse Model of amyotrophic lateral sclerosis. Sci. Rep. 9, 17728. 10.1038/s41598-019-53982-w [PubMed: 31776380]

Gravel M, Béland L-C, Soucy G, Abdelhamid E, Rahimian R, Gravel C, Kriz J, 2016. IL-10 controls early microglial phenotypes and disease onset in ALS caused by misfolded superoxide dismutase 1. J. Neurosci. 36, 1031–1048. [PubMed: 26791230]

Graves M, Fiala M, Dinglasan LA, Liu N, Sayre J, Chiappelli F, van Kooten C, Vinters H, 2004. Inflammation in amyotrophic lateral sclerosis spinal cord and brain is mediated by activated macrophages, mast cells and T cells. Amyotroph. Lateral Scler. Other Mot. Neuron Disord. 5, 213–219.

Gregory JM, McDade K, Livesey MR, Croy I, Marion de Proce S, Aitman T, Chandran S, Smith C, 2020. Spatial transcriptomics identifies spatially dysregulated expression of GRM3 and USP47 in amyotrophic lateral sclerosis. Neuropathol. Appl. Neurobiol.

Guilarte TR, 2019. TSPO in diverse CNS pathologies and psychiatric disease: A critical review and a way forward. Pharmacol. Ther. 194, 44–58. [PubMed: 30189290]

Guo J, Yang X, Gao L, Zang D, 2017. Evaluating the levels of CSF and serum factors in ALS. Brain Behav. 7, e00637. 10.1002/brb3.637 [PubMed: 28293476]

Gupta K, Harvima IT, 2018. Mast cell-neural interactions contribute to pain and itch. Immunol. Rev. 282, 168–187. [PubMed: 29431216]

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX, 1994. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science264, 1772–1775. 10.1126/science.8209258 [PubMed: 8209258]

Gustafson MP, Staff NP, Bornschlegl S, Butler GW, Maas ML, Kazamel M, Zubair A, Gastineau DA, Windebank AJ, Dietz AB, 2017. Comprehensive immune profiling reveals substantial immune system alterations in a subset of patients with amyotrophic lateral sclerosis. PLoS One12, e0182002. 10.1371/journal.pone.0182002 [PubMed: 28742871]

Habib N, Avraham-Davidi I, Basu A, Burks T, Shekhar K, Hofree M, Choudhury SR, Aguet F, Gelfand E, Ardlie K, 2017. Massively parallel single-nucleus RNA-seq with DroNc-seq. Nat. Methods14, 955–958. [PubMed: 28846088]

Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, Shaw PJ, Simmons Z, Van Den Berg LH, 2017. Amyotrophic lateral sclerosis. Nat. Rev. Dis. Prim. 3, 17071. [PubMed: 28980624]

Harrison JM, Rafuse VF, 2020. Muscle fiber-type specific terminal Schwann cell pathology leads to sprouting deficits following partial denervation in SOD1G93A mice. Neurobiol. Dis. 145, 105052. [PubMed: 32827689]

Hayes LR, Rothstein JD, 2016. C9ORF72-ALS/FTD: Transgenic Mice Make a Come-BAC. Neuron90, 427–431. 10.1016/j.neuron.2016.04.026 [PubMed: 27151634]

He L, Pei H, Lan T, Tang M, Zhang C, Chen L, 2017. Design and synthesis of a highly selective JAK3 inhibitor for the treatment of rheumatoid arthritis. Arch. Pharm. (Weinheim). 350, 1700194.

Henkel JS, Beers DR, Siklós L, Appel SH, 2006. The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. Mol. Cell. Neurosci. 31, 427–437. [PubMed: 16337133]

Henkel JS, Beers DR, Wen S, Rivera AL, Toennis KM, Appel JE, Zhao W, Moore DH, Powell SZ, Appel SH, 2013. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. EMBO Mol. Med. 5, 64–79. [PubMed: 23143995]

Henkel JS, Engelhardt JI, Siklós L, Simpson EP, Kim SH, Pan T, Goodman JC, Siddique T, Beers DR, Appel SH, 2004. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. Ann. Neurol. Off. J. Am. Neurol. Assoc. Child Neurol. Soc. 55, 221–235.

Henkel JS, Engelhardt JIJI, Siklós L, Simpson EP, Kim SH, Pan T, Goodman JC, Siddique T, Beers DR, Appel SH, Casula M, Iyer AM, Spliet WGM, Anink JJ, Steentjes K, Sta M, Troost D, Aronica E, Brambilla L, Guidotti G, Martorana F, Iyer AM, Aronica E, Valori CF, Rossi D, Tortarolo M, Lo Coco D, Veglianese P, Vallarola A, Giordana MT, Marcon G, Beghi E, Poloni M, Strong MJ, Iyer AM, Gorter RP, Stephenson J, Nutma E, Anink JJ, de Jonge JC, Baron W, Jahreiβ M, Belien JAM, van Noort JM, Mijnsbergen C, Sofroniew MV, Pehar M, Harlan BA, Killoy KM, Vargas MR, Johann S, Filipi T, Hermanova Z, Tureckova J, Vanatko O, Lee J, Hyeon SJ, Im H, Ryu HH, Kim Y, Ryu HH, Sta M, Sylva-Steenland RMR, Casula M, de Jong JMBV, Troost D, Aronica E, Baas F, Garofalo S, Cocozza G, Porzia A, Inghilleri M, Raspa M, Scavizzi F, Aronica E, Bernardini G, Peng L, Ransohoff RM, Smitt PAES, Blaauwgeers HGT, Troost D, de Jong JMBV, Lyon MS, Wosiski-Kuhn M, Gillespie R, Caress J, Milligan C, Engelhardt JIJI, Tajti J, Appel SH, McGeer PL, McGeer EG, Kawamata T, Akiyama H, Yamada T, McGeer PL, Troost D, Van den Oord JJ, JONG JMBVDE, Schiffer D, Cordera S, Cavalla P, Migheli A, 2017. Inflammation, Immunity, and amyotrophic lateral sclerosis: I. Etiology and pathology. Neuropathol. Appl. Neurobiol. 23, 401–410.

Heurich B, El Idrissi NB, Donev RM, Petri S, Claus P, Neal J, Morgan BP, Ramaglia V, 2011. Complement upregulation and activation on motor neurons and neuromuscular junction in the SOD1 G93A mouse model of familial amyotrophic lateral sclerosis. J. Neuroimmunol. 235, 104–109. 10.1016/j.jneuroim.2011.03.011 [PubMed: 21501881]

Holmøy T, 2008. T cells in amyotrophic lateral sclerosis. Eur. J. Neurol. 15, 360–366. 10.1111/j.1468-1331.2008.02065.x [PubMed: 18266871]

Houi K, Kobayashi T, Kato S, Mochio S, Inoue K, 2002. Increased plasma TGF-beta1 in patients with amyotrophic lateral sclerosis. Acta Neurol. Scand. 106, 299–301. 10.1034/j.1600-0404.2002.01301.x [PubMed: 12371924]

Howard JF, Nowak RJ, Wolfe GI, Freimer ML, Vu TH, Hinton JL, Benatar M, Duda PW, MacDougall JE, Farzaneh-Far R, 2020. Clinical Effects of the Self-administered Subcutaneous Complement Inhibitor Zilucoplan in Patients With Moderate to Severe Generalized Myasthenia Gravis: Results of a Phase 2 Randomized, Double-Blind, Placebo-Controlled, Multicenter Clinical Trial. JAMA Neurol. 77, 582–592. [PubMed: 32065623]

Iłzecka J, Stelmasiak Z, Dobosz B, 2002. Transforming growth factor-Beta 1 (tgf-Beta 1) in patients with amyotrophic lateral sclerosis. Cytokine20, 239–243. 10.1006/cyto.2002.2005 [PubMed: 12550109]

Jha MK, Jo M, Kim J-H, Suk K, 2019. Microglia-astrocyte crosstalk: an intimate molecular conversation. Neurosci. 25, 227–240.

Jiang J, Zhu Q, Gendron TF, Saberi S, McAlonis-Downes M, Seelman A, Stauffer JE, Jafar-Nejad P, Drenner K, Schulte D, Chun S, Sun S, Ling S-C, Myers B, Engelhardt J, Katz M, Baughn M, Platoshyn O, Marsala M, Watt A, Heyser CJ, Ard MC, De Muynck L, Daughrity LM, Swing DA, Tessarollo L, Jung CJ, Delpoux A, Utzschneider DT, Hedrick SM, de Jong PJ, Edbauer

D, Van Damme P, Petrucelli L, Shaw CE, Bennett CF, Da Cruz S, Ravits J, Rigo F, Cleveland DW, Lagier-Tourenne C, 2016. Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. Neuron90, 535–550. 10.1016/j.neuron.2016.04.006 [PubMed: 27112497]

Jin M, Günther R, Akgün K, Hermann A, Ziemssen T, 2020. Peripheral proinflammatory Th1/Th17 immune cell shift is linked to disease severity in amyotrophic lateral sclerosis. Sci. Rep. 10, 5941. 10.1038/s41598-020-62756-8 [PubMed: 32246039]

Johann S, 2017. Astrocytes pathology in ALS: A potential therapeutic target?Curr. Pharm. Des. 23, 5022–5036. [PubMed: 28619000]

Johansson A, Engler H, Blomquist G, Scott B, Wall A, Aquilonius S-M, Långström B, Askmark H, 2007. Evidence for astrocytosis in ALS demonstrated by [11C](L)-deprenyl-D2 PET. J. Neurol. Sci. 255, 17–22. [PubMed: 17346749]

Jones MK, Nair A, Gupta M, 2019. Mast Cells in Neurodegenerative Disease. Front. Cell. Neurosci. 13, 171. 10.3389/fncel.2019.00171 [PubMed: 31133804]

Jucaite A, Svenningsson P, Rinne JO, Cselenyi Z, Varnäs K, Johnström P, Amini N, Kirjavainen A, Helin S, Minkwitz M, 2015. Effect of the myeloperoxidase inhibitor AZD3241 on microglia: a PET study in Parkinson's disease. Brain138, 2687–2700. [PubMed: 26137956]

Kano O, Beers DR, Henkel JS, Appel SH, 2012. Peripheral nerve inflammation in ALS mice: cause or consequence. Neurology78, 833–835. 10.1212/WNL.0b013e318249f776 [PubMed: 22377817]

Katsuno M, Adachi H, Banno H, Suzuki K, Tanaka F, Sobue G, 2011. Transforming growth factor-β signaling in motor neuron diseases. Curr. Mol. Med. 11, 48–56. 10.2174/156652411794474356 [PubMed: 21189118]

Kawamata T, Akiyama H, Yamada T, McGeer PL, 1992. Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. Am. J. Pathol. 140, 691. [PubMed: 1347673]

Kettenmann H, Hanisch U-K, Noda M, Verkhratsky A, 2011. Physiology of microglia. Physiol. Rev. 91, 461–553. [PubMed: 21527731]

Khalid SI, Ampie L, Kelly R, Ladha SS, Dardis C, 2017. Immune modulation in the treatment of amyotrophic lateral sclerosis: a review of clinical trials. Front. Neurol. 8, 486. [PubMed: 28993751]

Kim H, Kim HY, Choi MR, Hwang S, Nam K-H, Kim H-C, Han JS, Kim KS, Yoon HS, Kim SH, 2010. Dose-dependent efficacy of ALS-human mesenchymal stem cells transplantation into cisterna magna in SOD1-G93A ALS mice. Neurosci. Lett. 468, 190–194. [PubMed: 19879334]

Kim HY, Kim H, Oh K, Oh S, Koh S, Baik W, Noh MY, Kim KS, Kim SH, 2014. Biological markers of mesenchymal stromal cells as predictors of response to autologous stem cell transplantation in patients with amyotrophic lateral sclerosis: an investigator-initiated trial and in vivo study. Stem Cells32, 2724–2731. [PubMed: 24966156]

Kipnis J, Gadani S, Derecki NC, 2012. Pro-cognitive properties of T cells. Nat. Rev. Immunol. 12, 663–669. [PubMed: 22903149]

Klein SM, Behrstock S, McHugh J, Hoffmann K, Wallace K, Suzuki M, Aebischer P, Svendsen CN, 2005. GDNF delivery using human neural progenitor cells in a rat model of ALS. Hum. Gene Ther. 16, 509–521. [PubMed: 15871682]

Krokidis MG, Vlamos P, 2018. Transcriptomics in amyotrophic lateral sclerosis. Front Biosci (Elite Ed)10, 103–121. [PubMed: 28930607]

Kubo S, Yamaoka K, Kondo M, Yamagata K, Zhao J, Iwata S, Tanaka Y, 2014. The JAK inhibitor, tofacitinib, reduces the T cell stimulatory capacity of human monocyte-derived dendritic cells. Ann. Rheum. Dis. 73, 2192–2198. [PubMed: 24013646]

Kuhle J, Lindberg RLP, Regeniter A, Mehling M, Steck AJ, Kappos L, Czaplinski A, 2009. Increased levels of inflammatory chemokines in amyotrophic lateral sclerosis. Eur. J. Neurol. 16, 771–774. 10.1111/j.1468-1331.2009.02560.x [PubMed: 19236470]

Kwon M, Noh M, Oh K, Cho K, Kang B, Kim K, Kim Y, Kim SH, 2014. The immunomodulatory effects of human mesenchymal stem cells on peripheral blood mononuclear cells in ALS patients. J. Neurochem. 131, 206–218. [PubMed: 24995608]

Lai JD, Ichida JK, 2019. C9ORF72 protein function and immune dysregulation in amyotrophic lateral sclerosis. Neurosci. Lett. 713, 134523. [PubMed: 31568865]

Lee J, Hyeon SJ, Im H, Ryu Hyun, Kim Y, Ryu Hoon, 2016. Astrocytes and microglia as non-cell autonomous players in the pathogenesis of ALS. Exp. Neurobiol. 25, 233–240. [PubMed: 27790057]

Lee JD, Kamaruzaman NA, Fung JNT, Taylor SM, Turner BJ, Atkin JD, Woodruff TM, Noakes PG, 2013. Dysregulation of the complement cascade in the hSOD1G93A transgenic mouse model of amyotrophic lateral sclerosis. J. Neuroinflammation10, 119. 10.1186/1742-2094-10-119 [PubMed: 24067070]

Lee JD, Kumar V, Fung JNT, Ruitenberg MJ, Noakes PG, Woodruff TM, 2017. Pharmacological inhibition of complement C5a-C5a(1) receptor signalling ameliorates disease pathology in the hSOD1(G93A) mouse model of amyotrophic lateral sclerosis. Br. J. Pharmacol. 174, 689–699. 10.1111/bph.13730 [PubMed: 28128456]

Lee JD, Levin SC, Willis EF, Li R, Woodruff TM, Noakes PG, 2018. Complement components are upregulated and correlate with disease progression in the TDP-43(Q331K) mouse model of amyotrophic lateral sclerosis. J. Neuroinflammation15, 171. 10.1186/s12974-018-1217-2 [PubMed: 29859100]

Lee JW, Sicre de Fontbrune F, Wong Lee Lee L, Pessoa V, Gualandro S, Füreder W, Ptushkin V, Rottinghaus ST, Volles L, Shafner L, 2019. Ravulizumab (ALXN1210) vs eculizumab in adult patients with PNH naive to complement inhibitors: the 301 study. Blood133, 530–539. [PubMed: 30510080]

Lee JY, Lee JD, Phipps S, Noakes PG, Woodruff TM, 2015. Absence of toll-like receptor 4 (TLR4) extends survival in the hSOD1 G93A mouse model of amyotrophic lateral sclerosis. J. Neuroinflammation12, 90. 10.1186/s12974-015-0310-z [PubMed: 25962427]

Lepore AC, O'Donnell J, Kim AS, Williams T, Tuteja A, Rao MS, Kelley LL, Campanelli JT, Maragakis NJ, 2011. Human glial-restricted progenitor transplantation into cervical spinal cord of the SOD1G93A mouse model of ALS. PLoS One6, e25968. [PubMed: 21998733]

Lepore AC, Rauck B, Dejea C, Pardo AC, Rao MS, Rothstein JD, Maragakis NJ, 2008. Focal transplantation–based astrocyte replacement is neuroprotective in a model of motor neuron disease. Nat. Neurosci. 11, 1294–1301. [PubMed: 18931666]

Liddelow SA, Barres BA, 2017. Reactive astrocytes: production, function, and therapeutic potential. Immunity46, 957–967. [PubMed: 28636962]

Lind A-L, Wu D, Freyhult E, Bodolea C, Ekegren T, Larsson A, Gustafsson MG, Katila L, Bergquist J, Gordh T, Landegren U, Kamali-Moghaddam M, 2016. A Multiplex Protein Panel Applied to Cerebrospinal Fluid Reveals Three New Biomarker Candidates in ALS but None in Neuropathic Pain Patients. PLoS One11, e0149821. 10.1371/journal.pone.0149821 [PubMed: 26914813]

Liu J, Gao L, Zang D, 2015. Elevated Levels of IFN-γ in CSF and Serum of Patients with Amyotrophic Lateral Sclerosis. PLoS One10, e0136937. 10.1371/journal.pone.0136937 [PubMed: 26332465]

Liu Y, Pattamatta A, Zu T, Reid T, Bardhi O, Borchelt DR, Yachnis AT, Ranum LPW, 2016. C9orf72 BAC Mouse Model with Motor Deficits and Neurodegenerative Features of ALS/FTD. Neuron90, 521–534. 10.1016/j.neuron.2016.04.005 [PubMed: 27112499]

Lo Coco D, Veglianese P, Allievi E, Bendotti C, 2007. Distribution and cellular localization of high mobility group box protein 1 (HMGB1) in the spinal cord of a transgenic mouse model of ALS. Neurosci. Lett. 412, 73–77. 10.1016/j.neulet.2006.10.063 [PubMed: 17196331]

Longinetti E, Fang F, 2019. Epidemiology of amyotrophic lateral sclerosis: an update of recent literature. Curr. Opin. Neurol. 32, 771. [PubMed: 31361627]

Lu C-H, Allen K, Oei F, Leoni E, Kuhle J, Tree T, Fratta P, Sharma N, Sidle K, Howard R, Orrell R, Fish M, Greensmith L, Pearce N, Gallo V, Malaspina A, 2016. Systemic inflammatory response and neuromuscular involvement in amyotrophic lateral sclerosis. Neurol. Neuroimmunol. neuroinflammation3, e244. 10.1212/NXI.0000000000000244

Luchena C, Zuazo-Ibarra J, Alberdi E, Matute C, Capetillo-Zarate E, 2018. Contribution of Neurons and Glial Cells to Complement-Mediated Synapse Removal during Development, Aging and in Alzheimer's Disease. Mediators Inflamm. 2018, 2530414. 10.1155/2018/2530414 [PubMed: 30533998]

Lyon MS, Wosiski-Kuhn M, Gillespie R, Caress J, Milligan C, 2019. Inflammation, Immunity, and amyotrophic lateral sclerosis: I. Etiology and pathology. Muscle Nerve59, 10–22. [PubMed: 29979464]

Maeshima K, Yamaoka K, Kubo S, Nakano K, Iwata S, Saito K, Ohishi M, Miyahara H, Tanaka S, Ishii K, 2012. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon-γ and interleukin-17 production by human CD4+ T cells. Arthritis Rheum. 64, 1790–1798. [PubMed: 22147632]

Malaspina A, Puentes F, Amor S, 2015. Disease origin and progression in amyotrophic lateral sclerosis: an immunology perspective. Int. Immunol. 27, 117–129. [PubMed: 25344935]

Mandrioli J, Amedei A, Cammarota G, Niccolai E, Zucchi E, Ricci F, Quaranta G, Spanu T, Masucci L, 2019. FETR-ALS study protocol: a randomized clinical trial of fecal microbiota transplantation in amyotrophic lateral sclerosis. Front. Neurol. 10, 1021. [PubMed: 31620079]

Mandrioli J, D'Amico R, Zucchi E, Gessani A, Fini N, Fasano A, Caponnetto C, Chiò A, Bella ED, Lunetta C, Mazzini L, Marinou K, Sorarù G, De Biasi S, Lo Tartaro D, Pinti M, Nichelli P, Vicini R, Cabona C, Calvo A, Moglia C, Manera U, Fuda G, Canosa A, Ilardi A, Lauria G, Dalla Bella E, Gerardi F, Scognamiglio A, De Marchi F, Mora G, Gizzi M, Cossarizza A, 2018. Rapamycin treatment for amyotrophic lateral sclerosis protocol for a phase II randomized, double-blind, placebo-controlled, multicenter, clinical trial (RAP-ALS trial). Med. (United States)97. 10.1097/MD.0000000000011119

Maniatis S, Äijö T, Vickovic S, Braine C, Kang K, Mollbrink A, Fagegaltier D, Andrusivová Ž, Saarenpää S, Saiz-Castro G, 2019. Spatiotemporal dynamics of molecular pathology in amyotrophic lateral sclerosis. Science (80-.). 364, 89–93.

Mantovani S, Garbelli S, Pasini A, Alimonti D, Perotti C, Melazzini M, Bendotti C, Mora G, 2009. Immune system alterations in sporadic amyotrophic lateral sclerosis patients suggest an ongoing neuroinflammatory process. J. Neuroimmunol. 210, 73–79. [PubMed: 19307024]

Marini C, Cistaro A, Campi C, Calvo A, Caponnetto C, Nobili FM, Fania P, Beltrametti MC, Moglia C, Novi G, 2016. A PET/CT approach to spinal cord metabolism in amyotrophic lateral sclerosis. Eur. J. Nucl. Med. Mol. Imaging43, 2061–2071. [PubMed: 27421971]

Marini C, Morbelli S, Cistaro A, Campi C, Caponnetto C, Bauckneht M, Bellini A, Buschiazzo A, Calamia I, Beltrametti MC, 2018. Interplay between spinal cord and cerebral cortex metabolism in amyotrophic lateral sclerosis. Brain141, 2272–2279. [PubMed: 30730551]

Markovinovic A, Ljutic T, Béland L-C, Munitic I, 2018. Optineurin Insufficiency Disbalances Proinflammatory and Anti-inflammatory Factors by Reducing Microglial IFN-β Responses. Neuroscience388, 139–151. 10.1016/j.neuroscience.2018.07.007 [PubMed: 30017954]

Martinez-Merino L, Iridoy M, Galbete A, Roldán M, Rivero A, Acha B, Irún P, Canosa C, Pocoví M, Mendioroz M, Jericó I, 2018. Evaluation of Chitotriosidase and CC-Chemokine Ligand 18 as Biomarkers of Microglia Activation in Amyotrophic Lateral Sclerosis. Neurodegener. Dis. 18, 208–215. 10.1159/000490920 [PubMed: 30134252]

Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, Kinoshita Y, Kamada M, Nodera H, Suzuki H, 2010. Mutations of optineurin in amyotrophic lateral sclerosis. Nature465, 223–226. [PubMed: 20428114]

Masuch A, Shieh C, van Rooijen N, van Calker D, Biber K, 2016. Mechanism of microglia neuroprotection: involvement of P2X 7, TNF α, and valproic acid. Glia64, 76–89. [PubMed: 26295445]

Masuda T, Itoh J, Koide T, Tomidokoro Y, Takei Y, Ishii K, Tamaoka A, 2017. Transforming growth factor-β1 in the cerebrospinal fluid of patients with distinct neurodegenerative diseases. J. Clin. Neurosci. Off. J. Neurosurg. Soc. Australas. 35, 47–49. 10.1016/j.jocn.2016.09.018

May C, Nordhoff E, Casjens S, Turewicz M, Eisenacher M, Gold R, Brüning T, Pesch B, Stephan C, Woitalla D, 2014. Highly immunoreactive IgG antibodies directed against a set of twenty human proteins in the sera of patients with amyotrophic lateral sclerosis identified by protein array. PLoS One9, e89596. [PubMed: 24586901]

McCauley ME, Baloh RH, 2019. Inflammation in ALS/FTD pathogenesis. Acta Neuropathol. 137, 715–730. [PubMed: 30465257]

McCauley ME, O'Rourke JG, Yáñez A, Markman JL, Ho R, Wang X, Chen S, Lall D, Jin M, Muhammad AKMG, Bell S, Landeros J, Valencia V, Harms M, Arditi M, Jefferies C, Baloh RH, 2020. C9orf72 in myeloid cells suppresses STING-induced inflammation. Nature585, 96–101. 10.1038/s41586-020-2625-x [PubMed: 32814898]

McGill RB, Steyn FJ, Ngo ST, Thorpe KA, Heggie S, Ruitenberg MJ, Henderson RD, McCombe PA, Woodruff TM, 2020. Monocytes and neutrophils are associated with clinical features in amyotrophic lateral sclerosis. Brain Commun. 2, fcaa013. 10.1093/braincomms/fcaa013 [PubMed: 33033799]

Meininger V, Asselain B, Guillet P, Leigh PN, Ludolph A, Lacomblez L, Robberecht W, 2006. Pentoxifylline in ALS: a double-blind, randomized, multicenter, placebo-controlled trial. Neurology66, 88–92. [PubMed: 16401852]

Meininger V, Drory VE, Leigh PN, Ludolph A, Robberecht W, Silani V, 2009. Glatiramer acetate has no impact on disease progression in ALS at 40 mg/day: a double-blind, randomized, multicentre, placebo-controlled trial. Amyotroph. Lateral Scler. 10, 378–383. [PubMed: 19922128]

Mejzini R, Flynn LL, Pitout IL, Fletcher S, Wilton SD, Akkari PA, 2019. ALS genetics, mechanisms, and therapeutics: Where are we now?Front. Neurosci. 13. [PubMed: 30760975]

Milošević M, Milićević K, Božić I, Lavrnja I, Stevanović I, Bijelić D, Dubaić M, Živković I, Stević Z, Giniatullin R, 2017. Immunoglobulins G from sera of amyotrophic lateral sclerosis patients induce oxidative stress and upregulation of antioxidative system in BV-2 microglial cell line. Front. Immunol. 8, 1619. [PubMed: 29218049]

Mishra P-S, Dhull DK, Nalini A, Vijayalakshmi K, Sathyaprabha TN, Alladi PA, Raju TR, 2016. Astroglia acquires a toxic neuroinflammatory role in response to the cerebrospinal fluid from amyotrophic lateral sclerosis patients. J. Neuroinflammation13, 212. 10.1186/s12974-016-0698-0 [PubMed: 27578023]

Mitchell RM, Freeman WM, Randazzo WT, Stephens HE, Beard JL, Simmons Z, Connor JR, 2009. A CSF biomarker panel for identification of patients with amyotrophic lateral sclerosis. Neurology72, 14–19. 10.1212/01.wnl.0000333251.36681.a5 [PubMed: 18987350]

Monif M, Burnstock G, Williams DA, 2010. Microglia: proliferation and activation driven by the P2X7 receptor. Int. J. Biochem. Cell Biol. 42, 1753–1756. [PubMed: 20599520]

Mora JS, Genge A, Chio A, Estol CJ, Chaverri D, Hernández M, Marín S, Mascias J, Rodriguez GE, Povedano M, 2020. Masitinib as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a randomized clinical trial. Amyotroph. Lateral Scler. Front. Degener. 21, 5–14.

Mordes DA, Morrison BM, Ament XH, Cantrell C, Mok J, Eggan P, Xue C, Wang J-Y, Eggan K, Rothstein JD, 2020. Absence of Survival and Motor Deficits in 500 Repeat C9ORF72 BAC Mice. Neuron108, 775–783.e4. 10.1016/j.neuron.2020.08.009 [PubMed: 33022228]

Moreau C, Devos D, Brunaud-Danel V, Defebvre L, Perez T, Destée A, Tonnel AB, Lassalle P, Just N, 2005. Elevated IL-6 and TNF-alpha levels in patients with ALS: inflammation or hypoxia? Neurology65, 1958–1960. 10.1212/01.wnl.0000188907.97339.76 [PubMed: 16380619]

Morello G, Spampinato AG, Cavallaro S, 2017. Neuroinflammation and ALS: transcriptomic insights into molecular disease mechanisms and therapeutic targets. Mediators Inflamm. 2017.

Munitic I, Giardino Torchia ML, Meena NP, Zhu G, Li CC, Ashwell JD, 2013. Optineurin insufficiency impairs IRF3 but not NF-κB activation in immune cells. J. Immunol. 191, 6231–6240. 10.4049/jimmunol.1301696 [PubMed: 24244017]

Murdock BJ, Bender DE, Kashlan SR, Figueroa-Romero C, Backus C, Callaghan BC, Goutman SA, Feldman EL, 2016. Increased ratio of circulating neutrophils to monocytes in amyotrophic lateral sclerosis. Neurol. Neuroinflammation3.

Murdock BJ, Bender DE, Segal BM, Feldman EL, 2015. The dual roles of immunity in ALS: injury overrides protection. Neurobiol. Dis. 77, 1–12. [PubMed: 25726748]

Murdock BJ, Zhou T, Kashlan SR, Little RJ, Goutman SA, Feldman EL, 2017. Correlation of peripheral immunity with rapid amyotrophic lateral sclerosis progression. JAMA Neurol. 74, 1446–1454. [PubMed: 28973548]

Naor S, Keren Z, Bronshtein T, Goren E, Machluf M, Melamed D, 2009. Development of ALS-like disease in SOD-1 mice deficient of B lymphocytes. J. Neurol. 256, 1228–1235. [PubMed: 19280101]

Narayanaswami V, Dahl K, Bernard-Gauthier V, Josephson L, Cumming P, Vasdev N, 2018. Emerging PET radiotracers and targets for imaging of neuroinflammation in neurodegenerative diseases: outlook beyond TSPO. Mol. Imaging17, 1536012118792317. [PubMed: 30203712]

Nardo G, Trolese MC, de Vito G, Cecchi R, Riva N, Dina G, Heath PR, Quattrini A, Shaw PJ, Piazza V, Bendotti C, 2016. Immune response in peripheral axons delays disease progression in SOD1(G93A) mice. J. Neuroinflammation13, 261. 10.1186/s12974-016-0732-2 [PubMed: 27717377]

Nardo G, Trolese MC, Verderio M, Mariani A, de Paola M, Riva N, Dina G, Panini N, Erba E, Quattrini A, Bendotti C, 2018. Counteracting roles of MHCI and CD8(+) T cells in the peripheral and central nervous system of ALS SOD1(G93A) mice. Mol. Neurodegener. 13, 42. 10.1186/s13024-018-0271-7 [PubMed: 30092791]

Nelson LM, Topol B, Kaye W, Williamson D, Horton DK, Mehta P, Wagner T, 2018. Estimation of the prevalence of amyotrophic lateral sclerosis in the United States using national administrative healthcare data from 2002 to 2004 and capture-recapture methodology. Neuroepidemiology51, 149–157. [PubMed: 30092573]

Nicholson K, Bjornevik K, Abu-Ali G, Chan J, Cortese M, Dedi B, Jeon M, Xavier R, Huttenhower C, Ascherio A, 2020. The human gut microbiota in people with amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Front. Degener. 1–9.

Nimmerjahn A, Kirchhoff F, Helmchen F, 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science (80-.). 308, 1314–1318.

O'Rourke JG, Bogdanik L, Muhammad AKMG, Gendron TF, Kim KJ, Austin A, Cady J, Liu EY, Zarrow J, Grant S, Ho R, Bell S, Carmona S, Simpkinson M, Lall D, Wu K, Daughrity L, Dickson DW, Harms MB, Petrucelli L, Lee EB, Lutz CM, Baloh RH, 2015. C9orf72 BAC Transgenic Mice Display Typical Pathologic Features of ALS/FTD. Neuron88, 892–901. 10.1016/j.neuron.2015.10.027 [PubMed: 26637796]

O'Rourke JG, Bogdanik L, Yáñez A, Lall D, Wolf AJ, Muhammad AKMG, Ho R, Carmona S, Vit JP, Zarrow J, Kim KJ, Bell S, Harms MB, Miller TM, Dangler CA, Underhill DM, Goodridge HS, Lutz CM, Baloh RH, 2016. C9orf72 is required for proper macrophage and microglial function in mice. Science351, 1324–1329. 10.1126/science.aaf1064 [PubMed: 26989253]

Obál I, Klausz G, Mándi Y, Deli M, Siklós L, Engelhardt JI, 2016. Intraperitoneally administered IgG from patients with amyotrophic lateral sclerosis or from an immune-mediated goat model increase the levels of TNF-α, IL-6, and IL-10 in the spinal cord and serum of mice. J. Neuroinflammation13, 1–12. [PubMed: 26728181]

Oh K-W, Moon C, Kim HY, Oh S, Park J, Lee JH, Chang IY, Kim KS, Kim SH, 2015. Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. Stem Cells Transl. Med. 4, 590–597. [PubMed: 25934946]

Oh K, Noh M, Kwon M, Kim HY, Oh S, Park J, Kim H, Ki C, Kim SH, 2018. Repeated intrathecal mesenchymal stem cells for amyotrophic lateral sclerosis. Ann. Neurol. 84, 361–373. [PubMed: 30048006]

Orr MT, Lanier LL, 2010. Natural killer cell education and tolerance. Cell142, 847–856. [PubMed: 20850008]

Pagani MR, Gonzalez LE, Uchitel OD, 2011. Autoimmunity in amyotrophic lateral sclerosis: past and present. Neurol. Res. Int. 2011.

Paganoni S, Alshikho MJ, Luppino S, Chan J, Pothier L, Schoenfeld D, Andres PL, Babu S, Zürcher NR, Loggia ML, Barry RL, Luotti S, Nardo G, Trolese MC, Pantalone S, Bendotti C, Bonetto V, De Marchi F, Rosen B, Hooker J, Cudkowicz M, Atassi N, 2019. A pilot trial of RNS60 in amyotrophic lateral sclerosis. Muscle and Nerve59. 10.1002/mus.26385

Palma E, Reyes-Ruiz JM, Lopergolo D, Roseti C, Bertollini C, Ruffolo G, Cifelli P, Onesti E, Limatola C, Miledi R, Inghilleri M, 2016. Acetylcholine receptors from human muscle as pharmacological targets for ALS therapy. Proc. Natl. Acad. Sci. U. S. A. 113, 3060–3065. 10.1073/pnas.1600251113 [PubMed: 26929355]

Pehar M, Harlan BA, Killoy KM, Vargas MR, 2017. Role and therapeutic potential of astrocytes in amyotrophic lateral sclerosis. Curr. Pharm. Des. 23, 5010–5021. [PubMed: 28641533]

Peters OM, Cabrera GT, Tran H, Gendron TF, McKeon JE, Metterville J, Weiss A, Wightman N, Salameh J, Kim J, Sun H, Boylan KB, Dickson D, Kennedy Z, Lin Z, Zhang Y-J, Daughrity L, Jung C, Gao F-B, Sapp PC, Horvitz HR, Bosco DA, Brown SP, de Jong P, Petrucelli L, Mueller C, Brown RHJ, 2015. Human C9ORF72 Hexanucleotide Expansion Reproduces RNA Foci and Dipeptide Repeat Proteins but Not Neurodegeneration in BAC Transgenic Mice. Neuron88, 902–909. 10.1016/j.neuron.2015.11.018 [PubMed: 26637797]

Peters S, Zitzelsperger E, Kuespert S, Iberl S, Heydn R, Johannesen S, Petri S, Aigner L, Thal DR, Hermann A, Weishaupt JH, Bruun T-H, Bogdahn U, 2017. The TGF-β System As a Potential Pathogenic Player in Disease Modulation of Amyotrophic Lateral Sclerosis. Front. Neurol. 8, 669. 10.3389/fneur.2017.00669 [PubMed: 29326641]

Petrou P, Gothelf Y, Argov Z, Gotkine M, Levy YS, Kassis I, Vaknin-Dembinsky A, Ben-Hur T, Offen D, Abramsky O, 2016. Safety and clinical effects of mesenchymal stem cells secreting neurotrophic factor transplantation in patients with amyotrophic lateral sclerosis: results of phase 1/2 and 2a clinical trials. JAMA Neurol. 73, 337–344. [PubMed: 26751635]

Picher-Martel V, Valdmanis PN, Gould PV, Julien J-P, Dupré N, 2016. From animal models to human disease: a genetic approach for personalized medicine in ALS. Acta Neuropathol. Commun. 4, 70. 10.1186/s40478-016-0340-5 [PubMed: 27400686]

Prabhakar S, Marwaha N, Lal V, Sharma RR, Rajan R, Khandelwal N, 2012. Autologous bone marrow-derived stem cells in amyotrophic lateral sclerosis: a pilot study. Neurol. India60, 465. [PubMed: 23135021]

Pullen AH, Demestre M, Howard RS, Orrell RW, 2004. Passive transfer of purified IgG from patients with amyotrophic lateral sclerosis to mice results in degeneration of motor neurons accompanied by Ca 2+ enhancement. Acta Neuropathol. 107, 35–46. [PubMed: 14551798]

Ratai E-M, Alshikho MJ, Zürcher NR, Loggia ML, Cebulla CL, Cernasov P, Reynolds B, Fish J, Seth R, Babu S, 2018. Integrated imaging of [11C]-PBR28 PET, MR diffusion and magnetic resonance spectroscopy 1H-MRS in amyotrophic lateral sclerosis. NeuroImage Clin. 20, 357–364. [PubMed: 30112276]

Ravits JM, La Spada AR, 2009. ALS motor phenotype heterogeneity, focality, and spread: deconstructing motor neuron degeneration. Neurology73, 805–811. 10.1212/WNL.0b013e3181b6bbbd [PubMed: 19738176]

Redmond DE, Bjugstad KB, Teng YD, Ourednik V, Ourednik J, Wakeman DR, Parsons XH, Gonzalez R, Blanchard BC, Kim SU, 2007. Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. Proc. Natl. Acad. Sci. 104, 12175–12180. [PubMed: 17586681]

Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, Van Swieten JC, Myllykangas L, 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron72, 257–268. [PubMed: 21944779]

Roberts K, Zeineddine R, Corcoran L, Li W, Campbell IL, Yerbury JJ, 2013. Extracellular aggregated Cu/Zn superoxide dismutase activates microglia to give a cytotoxic phenotype. Glia61, 409–419. 10.1002/glia.22444 [PubMed: 23281114]

Rojas F, Cortes N, Abarzua S, Dyrda A, Van Zundert BAJ, 2014. Astrocytes expressing mutant SOD1 and TDP43 trigger motoneuron death that is mediated via sodium channels and nitroxidative stress. Front. Cell. Neurosci. 8, 24. [PubMed: 24570655]

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng H-X, 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature362, 59. [PubMed: 8446170]

Rossi C, Cusimano M, Zambito M, Finardi A, Capotondo A, Garcia-Manteiga JM, Comi G, Furlan R, Martino G, Muzio L, 2018. Interleukin 4 modulates microglia homeostasis and attenuates the early slowly progressive phase of amyotrophic lateral sclerosis. Cell Death Dis. 9, 250. 10.1038/s41419-018-0288-4 [PubMed: 29445154]

Rowin J, Xia Y, Jung B, Sun J, 2017. Gut inflammation and dysbiosis in human motor neuron disease. Physiol. Rep. 5.

Rusconi M, Gerardi F, Santus W, Lizio A, Sansone VA, Lunetta C, Zanoni I, Granucci F, 2017. Inflammatory role of dendritic cells in amyotrophic lateral sclerosis revealed by an analysis of patients' peripheral blood. Sci. Rep. 7, 1–9. [PubMed: 28127051]

Russo MV, McGavern DB, 2016. Inflammatory neuroprotection following traumatic brain injury. Science (80-.). 353, 783–785.

Sadan O, Bahat-Stromza M, Barhum Y, Levy YS, Pisnevsky A, Peretz H, Ilan AB, Bulvik S, Shemesh N, Krepel D, 2009. Protective effects of neurotrophic factor–secreting cells in a 6-OHDA rat model of Parkinson disease. Stem Cells Dev. 18, 1179–1190. [PubMed: 19243240]

Sandborn WJ, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, Danese S, Feagan BG, Reinisch W, Niezychowski W, 2017. Tofacitinib as induction and maintenance therapy for ulcerative colitis. N. Engl. J. Med. 376, 1723–1736. [PubMed: 28467869]

Saresella M, Piancone F, Tortorella P, Marventano I, Gatti A, Caputo D, Lunetta C, Corbo M, Rovaris M, Clerici M, 2013. T helper-17 activation dominates the immunologic milieu of both amyotrophic lateral sclerosis and progressive multiple sclerosis. Clin. Immunol. 148, 79–88. 10.1016/j.clim.2013.04.010 [PubMed: 23665549]

Sasaki S, 2011. Autophagy in spinal cord motor neurons in sporadic amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 70, 349–359. [PubMed: 21487309]

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B, 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron74, 691–705. [PubMed: 22632727]

Schiffer D, Cordera S, Cavalla P, Migheli A, 1996. Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. J. Neurol. Sci. 139, 27–33. [PubMed: 8899654]

Schreiber S, Schreiber F, Garz C, Debska-Vielhaber G, Assmann A, Perosa V, Petri S, Dengler R, Nestor P, Vielhaber S, 2019. Toward in vivo determination of peripheral nervous system immune activity in amyotrophic lateral sclerosis. Muscle Nerve59, 567–576. 10.1002/mus.26444 [PubMed: 30734322]

Schwartz M, Shechter R, 2010. Systemic inflammatory cells fight off neurodegenerative disease. Nat. Rev. Neurol. 6, 405. [PubMed: 20531383]

Sebba A, 2008. Tocilizumab: the first interleukin-6-receptor inhibitor. Am. J. Heal. Pharm. 65, 1413–1418.

Sheean RK, McKay FC, Cretney E, Bye CR, Perera ND, Tomas D, Weston RA, Scheller KJ, Djouma E, Menon P, Schibeci SD, Marmash N, Yerbury JJ, Nutt SL, Booth DR, Stewart GJ, Kiernan MC, Vucic S, Turner BJ, 2018. Association of Regulatory T-Cell Expansion With Progression of Amyotrophic Lateral Sclerosis: A Study of Humans and a Transgenic Mouse Model. JAMA Neurol. 75, 681–689. 10.1001/jamaneurol.2018.0035 [PubMed: 29507931]

Shi N, Kawano Y, Tateishi T, Kikuchi H, Osoegawa M, Ohyagi Y, Kira J, 2007. Increased IL-13-producing T cells in ALS: positive correlations with disease severity and progression rate. J. Neuroimmunol. 182, 232–235. 10.1016/j.jneuroim.2006.10.001 [PubMed: 17097743]

Si Y, Kim S, Cui X, Zheng L, Oh SJ, Anderson T, AlSharabati M, Kazamel M, Volpicelli-Daley L, Bamman MM, Yu S, King PH, 2015. Transforming Growth Factor Beta (TGF-β) Is a Muscle Biomarker of Disease Progression in ALS and Correlates with Smad Expression. PLoS One10, e0138425. 10.1371/journal.pone.0138425 [PubMed: 26375954]

Sironi F, Vallarola A, Violatto MB, Talamini L, Freschi M, De Gioia R, Capelli C, Agostini A, Moscatelli D, Tortarolo M, Bigini P, Introna M, Bendotti C, 2017. Multiple intracerebroventricular injections of human umbilical cord mesenchymal stem cells delay motor neurons loss but not disease progression of SOD1G93A mice. Stem Cell Res. 25, 166–178. 10.1016/j.scr.2017.11.005 [PubMed: 29154076]

Skaper SD, Facci L, Giusti P, 2013. Glia and mast cells as targets for palmitoylethanolamide, an anti-inflammatory and neuroprotective lipid mediator. Mol. Neurobiol. 48, 340–352. 10.1007/s12035-013-8487-6 [PubMed: 23813098]

Slowicka K, Vereecke L, Mc Guire C, Sze M, Maelfait J, Kolpe A, Saelens X, Beyaert R, van Loo G, 2016. Optineurin deficiency in mice is associated with increased sensitivity to Salmonella but does not affect proinflammatory NF-κB signaling. Eur. J. Immunol. 46, 971–980. 10.1002/eji.201545863 [PubMed: 26677802]

Smitt PAES, Blaauwgeers HGT, Troost D, de Jong JMBV, 1992. Metallothionein immunoreactivity is increased in the spinal cord of patients with amyotrophic lateral sclerosis. Neurosci. Lett. 144, 107–110. [PubMed: 1436688]

Sofroniew MV, 2015. Astrocyte barriers to neurotoxic inflammation. Nat. Rev. Neurosci. 16, 249–263. [PubMed: 25891508]

Sonomoto K, Yamaoka K, Kubo S, Hirata S, Fukuyo S, Maeshima K, Suzuki K, Saito K, Tanaka Y, 2014. Effects of tofacitinib on lymphocytes in rheumatoid arthritis: relation to efficacy and infectious adverse events. Rheumatology53, 914–918. [PubMed: 24441153]

Spiller KJ, Restrepo CR, Khan T, Dominique MA, Fang TC, Canter RG, Roberts CJ, Miller KR, Ransohoff RM, Trojanowski JQ, Lee VM-Y, 2018. Microglia-mediated recovery from ALS-relevant motor neuron degeneration in a mouse model of TDP-43 proteinopathy. Nat. Neurosci. 21, 329–340. 10.1038/s41593-018-0083-7 [PubMed: 29463850]

Sta M, Sylva-Steenland RMR, Casula M, de Jong J, Troost D, Aronica E, Baas F, 2011. Innate and adaptive immunity in amyotrophic lateral sclerosis: evidence of complement activation. Neurobiol. Dis. 42, 211–220. [PubMed: 21220013]

Staff NP, Madigan NN, Morris J, Jentoft M, Sorenson EJ, Butler G, Gastineau D, Dietz A, Windebank AJ, 2016. Safety of intrathecal autologous adipose-derived mesenchymal stromal cells in patients with ALS. Neurology87, 2230–2234. [PubMed: 27784774]

Steinacker P, Hendrich C, Sperfeld AD, Jesse S, von Arnim CAF, Lehnert S, Pabst A, Uttner I, Tumani H, Lee VM-Y, Trojanowski JQ, Kretzschmar HA, Ludolph A, Neumann M, Otto M, 2008. TDP-43 in cerebrospinal fluid of patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Arch. Neurol. 65, 1481–1487. 10.1001/archneur.65.11.1481 [PubMed: 19001167]

Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, 2007. The classical complement cascade mediates CNS synapse elimination. Cell131, 1164–1178. [PubMed: 18083105]

Sun H, Bénardais K, Stanslowsky N, Thau-Habermann N, Hensel N, Huang D, Claus P, Dengler R, Stangel M, Petri S, 2013. Therapeutic potential of mesenchymal stromal cells and MSC conditioned medium in amyotrophic lateral sclerosis (ALS)-in vitro evidence from primary motor neuron cultures, NSC-34 cells, astrocytes and microglia. PLoS One8, e72926. [PubMed: 24069165]

Swarup V, Phaneuf D, Bareil C, Robertson J, Rouleau GA, Kriz J, Julien J-P, 2011. Pathological hallmarks of amyotrophic lateral sclerosis/frontotemporal lobar degeneration in transgenic mice produced with TDP-43 genomic fragments. Brain134, 2610–2626. [PubMed: 21752789]

Swash M, 2020. Chitinases, neuroinflammation and biomarkers in ALS. J. Neurol. Neurosurg. Psychiatry. 10.1136/jnnp-2019-322520

Tadesse T, Gearing M, Senitzer D, Saxe D, Brat DJ, Bray R, Gebel H, Hill C, Boulis N, Riley J, 2014. Analysis of graft survival in a trial of stem cell transplant in ALS. Ann. Clin. Transl. Neurol. 1, 900–908. [PubMed: 25540804]

Tam OH, Rozhkov NV, Shaw R, Kim D, Hubbard I, Fennessey S, Propp N, Phatnani H, Kwan J, Sareen D, 2019. Postmortem cortex samples identify distinct molecular subtypes of als: retrotransposon activation, oxidative stress, and activated glia. Cell Rep. 29, 1164–1177. [PubMed: 31665631]

Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, Piccirillo CA, Salomon BL, Bluestone JA, 2008. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity28, 687–697. [PubMed: 18468463]

Tateishi T, Yamasaki R, Tanaka M, Matsushita T, Kikuchi H, Isobe N, Ohyagi Y, Kira J, 2010. CSF chemokine alterations related to the clinical course of amyotrophic lateral sclerosis. J. Neuroimmunol. 222, 76–81. 10.1016/j.jneuroim.2010.03.004 [PubMed: 20381883]

Taylor JP, Brown RH Jr, Cleveland DW, 2016. Decoding ALS: from genes to mechanism. Nature539, 197–206. [PubMed: 27830784]

Teng YD, Benn SC, Kalkanis SN, Shefner JM, Onario RC, Cheng B, Lachyankar MB, Marconi M, Li J, Yu D, 2012. Multimodal actions of neural stem cells in a mouse model of ALS: a meta-analysis. Sci. Transl. Med. 4, 165ra164–165ra164.

Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D, Langer R, Snyder EY, 2002. Functional recovery following traumatic spinal cord injury mediated by a unique polymer

scaffold seeded with neural stem cells. Proc. Natl. Acad. Sci. 99, 3024–3029. [PubMed: 11867737]

Thangavelu SR, Tripathi PP, Arya U, Mishra HK, Subramaniam JR, 2011. ALS associated mutant SOD1 impairs the motor neurons and astrocytes and wild type astrocyte secreted-factors reverse the impaired motor neurons. Ann. Neurosci. 18, 48. [PubMed: 25205921]

Thompson AG, Gray E, Bampton A, Raciborska D, Talbot K, Turner MR, 2019. CSF chitinase proteins in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry90, 1215–1220. 10.1136/jnnp-2019-320442 [PubMed: 31123140]

Thonhoff JR, Beers DR, Zhao W, Pleitez M, Simpson EP, Berry JD, Cudkowicz ME, Appel SH, 2018a. Expanded autologous regulatory T-lymphocyte infusions in ALS: A phase I, first-in-human study. Neurol. Neuroinflammation5.

Thonhoff JR, Simpson EP, Appel SH, 2018b. Neuroinflammatory mechanisms in amyotrophic lateral sclerosis pathogenesis. Curr. Opin. Neurol. 31, 635–639. 10.1097/WCO.0000000000000599 [PubMed: 30048339]

Thorarensen A, Dowty ME, Banker ME, Juba B, Jussif J, Lin T, Vincent F, Czerwinski RM, Casimiro-Garcia A, Unwalla R, 2017. Design of a Janus Kinase 3 (JAK3) Specific Inhibitor 1-((2 S, 5 R)-5-((7 H-Pyrrolo [2, 3-d] pyrimidin-4-yl) amino)-2-methylpiperidin-1-yl) prop-2-en-1-one (PF-06651600) Allowing for the Interrogation of JAK3 Signaling in Humans. J. Med. Chem. 60, 1971–1993. [PubMed: 28139931]

Tondo G, Iaccarino L, Cerami C, Vanoli GE, Presotto L, Masiello V, Coliva A, Salvi F, Bartolomei I, Mosca L, n.d.11C-PK11195 PET–based molecular study of microglia activation in SOD1 amyotrophic lateral sclerosis. Ann. Clin. Transl. Neurol.

Tortarolo M, Lo Coco D, Veglianese P, Vallarola A, Giordana MT, Marcon G, Beghi E, Poloni M, Strong MJ, Iyer AM, 2017. Amyotrophic lateral sclerosis, a multisystem pathology: insights into the role of TNFα. Mediators Inflamm. 2017.

Trias E, Ibarburu S, Barreto-Núñez R, Varela V, Moura IC, Dubreuil P, Hermine O, Beckman JS, Barbeito L, 2017. Evidence for mast cells contributing to neuromuscular pathology in an inherited model of ALS. JCI insight2. 10.1172/jci.insight.95934

Trias E, King PH, Si Y, Kwon Y, Varela V, Ibarburu S, Kovacs M, Moura IC, Beckman JS, Hermine O, 2018. Mast cells and neutrophils mediate peripheral motor pathway degeneration in ALS. JCI insight3.

Troost D, Van den Oord JJ, JONG JMBVDE, 1990. Immunohistochemical characterization of the inflammatory infiltrate in amyotrophic lateral sclerosis. Neuropathol. Appl. Neurobiol. 16, 401–410. [PubMed: 2263315]

Turner MR, Cagnin A, Turkheimer FE, Miller CCJ, Shaw CE, Brooks DJ, Leigh PN, Banati RB, 2004. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiol. Dis. 15, 601–609. [PubMed: 15056468]

Urushitani M, Sik A, Sakurai T, Nukina N, Takahashi R, Julien J-P, 2006. Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. Nat. Neurosci. 9, 108–118. 10.1038/nn1603 [PubMed: 16369483]

Vainchtein ID, Molofsky AV, 2020. Astrocytes and microglia: in sickness and in health. Trends Neurosci. 43, 144–154. [PubMed: 32044129]

Vallarola A, Sironi F, Tortarolo M, Gatto N, De Gioia R, Pasetto L, De Paola M, Mariani A, Ghosh S, Watson R, 2018. RNS60 exerts therapeutic effects in the SOD1 ALS mouse model through protective glia and peripheral nerve rescue. J. Neuroinflammation15, 65. [PubMed: 29495962]

Van Gurp E, Weimar W, Gaston R, Brennan D, Mendez R, Pirsch J, Swan S, Pescovitz MD, Ni G, Wang C, 2008. Phase 1 dose-escalation study of CP-690 550 in stable renal allograft recipients: preliminary findings of safety, tolerability, effects on lymphocyte subsets and pharmacokinetics. Am. J. Transplant. 8, 1711–1718. [PubMed: 18557720]

Van Weehaeghe D, Babu S, De Vocht J, Zurcher NR, Chew S, Tseng C-EJ, Loggia M, Koole M, Rezaei A, Schramm G, 2020a. Moving towards multicenter therapeutic trials in ALS: feasibility of data pooling using different TSPO positron emission tomography (PET) radioligands. J. Nucl. Med. jnumed-119.

Van Weehaeghe D, Devrome M, Schramm G, De Vocht J, Deckers W, Baete K, Van Damme P, Koole M, Van Laere K, 2020b. Combined brain and spinal FDG PET allows differentiation between ALS and ALS mimics. Eur. J. Nucl. Med. Mol. Imaging1–10.

Van Weehaeghe D, Van Schoor E, De Vocht J, Koole M, Attili B, Celen S, Declercq L, Thal DR, Van Damme P, Bormans G, 2020c. TSPO Versus P2X7 as a Target for Neuroinflammation: An In Vitro and In Vivo Study. J. Nucl. Med. 61, 604–607. [PubMed: 31562223]

Varghese AM, Sharma A, Mishra P, Vijayalakshmi K, Harsha HC, Sathyaprabha TN, Bharath SM, Nalini A, Alladi PA, Raju TR, 2013. Chitotriosidase - a putative biomarker for sporadic amyotrophic lateral sclerosis. Clin. Proteomics10, 19. 10.1186/1559-0275-10-19 [PubMed: 24295388]

Velebit J, Horvat A, Smolič T, Mihevc SP, Rogelj B, Zorec R, Vardjan N, 2020. Astrocytes with TDP-43 inclusions exhibit reduced noradrenergic cAMP and Ca 2+ signaling and dysregulated cell metabolism. Sci. Rep. 10, 1–18. [PubMed: 31913322]

Volonté C, Amadio S, Cavaliere F, D'Ambrosi N, Vacca F, Bernardi G, 2003. Extracellular ATP and neurodegeneration. Curr. Drug Targets. CNS Neurol. Disord. 2, 403–412. 10.2174/1568007033482643 [PubMed: 14683468]

Wallis N, Lau CL, Farg MA, Atkin JD, Beart PM, O'Shea RD, 2018. SOD1 mutations causing familial amyotrophic lateral sclerosis induce toxicity in astrocytes: evidence for bystander effects in a continuum of astrogliosis. Neurochem. Res. 43, 166–179. [PubMed: 28861673]

Wang HA, Lee JD, Lee KM, Woodruff TM, Noakes PG, 2017. Complement C5a-C5aR1 signalling drives skeletal muscle macrophage recruitment in the hSOD1(G93A) mouse model of amyotrophic lateral sclerosis. Skelet. Muscle7, 10. 10.1186/s13395-017-0128-8 [PubMed: 28571586]

Watanabe Hazuki, Atsuta N, Nakamura R, Hirakawa A, Watanabe Hirohisa, Ito M, Senda J, Katsuno M, Izumi Y, Morita M, Tomiyama H, Taniguchi A, Aiba I, Abe K, Mizoguchi K, Oda M, Kano O, Okamoto K, Kuwabara S, Hasegawa K, Imai T, Aoki M, Tsuji S, Nakano I, Kaji R, Sobue G, 2015. Factors affecting longitudinal functional decline and survival in amyotrophic lateral sclerosis patients. Amyotroph. Lateral Scler. Frontotemporal Degener. 16, 230–236. 10.3109/21678421.2014.990036 [PubMed: 25548957]

Weimer RM, Easley-Neal C, Foreman O, Sharma N, Zarrin AA, 2019. CSF1R ligands IL-34 and CSF1 are differentially required for microglia development and maintenance in white and gray matter brain regions. Front. Immunol. 10, 2199. [PubMed: 31616414]

Werry EL, Bright FM, Piguet O, Ittner LM, Halliday GM, Hodges JR, Kiernan MC, Loy CT, Kril JJ, Kassiou M, 2019. Recent developments in TSPO PET imaging as a biomarker of neuroinflammation in neurodegenerative disorders. Int. J. Mol. Sci. 20, 3161.

Wies Mancini VSB, Pasquini JM, Correale JD, Pasquini LA, 2019. Microglial modulation through colony-stimulating factor-1 receptor inhibition attenuates demyelination. Glia67, 291–308. [PubMed: 30456797]

Wilke C, Pujol-Calderón F, Barro C, Stransky E, Blennow K, Michalak Z, Deuschle C, Jeromin A, Zetterberg H, Schüle R, Höglund K, Kuhle J, Synofzik M, 2019. Correlations between serum and CSF pNfH levels in ALS, FTD and controls: a comparison of three analytical approaches. Clin. Chem. Lab. Med. 57, 1556–1564. 10.1515/cclm-2019-0015 [PubMed: 31251725]

Wosiski-Kuhn M, Caress JB, Cartwright MS, Hawkins GA, Milligan C, 2021. Interleukin 6 (IL6) level is a biomarker for functional disease progression within IL6R(358)Ala variant groups in amyotrophic lateral sclerosis patients. Amyotroph. Lateral Scler. Frontotemporal Degener. 22, 248–259. 10.1080/21678421.2020.1813310 [PubMed: 32924606]

Wosiski-Kuhn M, Robinson M, Strupe J, Arounleut P, Martin M, Caress J, Cartwright M, Bowser R, Cudkowicz M, Langefeld C, Hawkins GA, Milligan C, 2019. IL6 receptor(358)Ala variant and trans-signaling are disease modifiers in amyotrophic lateral sclerosis. Neurol. Neuroimmunol. neuroinflammation6, e631. 10.1212/NXI.0000000000000631

Wu S, Yi J, Zhang Y, Zhou J, Sun J, 2015. Leaky intestine and impaired microbiome in an amyotrophic lateral sclerosis mouse model. Physiol. Rep. 3.

Yang B, Wu Y, Wang Y, Yang H, Du B, Di W, Xu X, Shi X, 2020. Cerebrospinal fluid MFG-E8 as a promising biomarker of amyotrophic lateral sclerosis. Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol. 41, 2915–2920. 10.1007/s10072-020-04416-3

Yu C-H, Davidson S, Harapas CR, Hilton JB, Mlodzianoski MJ, Laohamonthonkul P, Louis C, Low RRJ, Moecking J, De Nardo D, 2020. TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. Cell183, 636–649. [PubMed: 33031745]

Zanotti-Fregonara P, Pascual B, Rizzo G, Yu M, Pal N, Beers D, Carter R, Appel SH, Atassi N, Masdeu JC, 2018. Head-to-Head Comparison of 11C-PBR28 and 18F-GE180 for Quantification of the Translocator Protein in the Human Brain. J. Nucl. Med. 59, 1260–1266. [PubMed: 29348317]

Zanotti-Fregonara P, Pascual B, Veronese M, Yu M, Beers D, Appel SH, Masdeu JC, 2019. Head-to-head comparison of 11 C-PBR28 and 11 C-ER176 for quantification of the translocator protein in the human brain. Eur. J. Nucl. Med. Mol. Imaging46, 1822–1829. [PubMed: 31152207]

Zeng Q, Shen J, Chen K, Zhou J, Liao Q, Lu K, Yuan J, Bi F, 2020. The alteration of gut microbiome and metabolism in amyotrophic lateral sclerosis patients. Sci. Rep. 10. [PubMed: 32001736]

Zhai C-D, Zheng J-J, An B-C, Huang H-F, Tan Z-C, 2019. Intestinal microbiota composition in patients with amyotrophic lateral sclerosis: establishment of bacterial and archaeal communities analyses. Chin. Med. J. (Engl). 132, 1815. [PubMed: 31306225]

Zhang J, 2015. Mapping neuroinflammation in frontotemporal dementia with molecular PET imaging. J. Neuroinflammation12, 108. [PubMed: 26022249]

Zhang M, Zhou Q, Dorfman RG, Huang X, Fan T, Zhang H, Zhang J, Yu C, 2016. Butyrate inhibits interleukin-17 and generates Tregs to ameliorate colorectal colitis in rats. Bmc Gastroenterol. 16, 1–9. [PubMed: 26796772]

Zhang R, Gascon R, Miller RG, Gelinas DF, Mass J, Hadlock K, Jin X, Reis J, Narvaez A, McGrath MS, 2005. Evidence for systemic immune system alterations in sporadic amyotrophic lateral sclerosis (sALS). J. Neuroimmunol. 159, 215–224. 10.1016/j.jneuroim.2004.10.009 [PubMed: 15652422]

Zhang Y, Wu S, Yi J, Xia Y, Jin D, Zhou J, Sun J, 2017. Target intestinal microbiota to alleviate disease progression in amyotrophic lateral sclerosis. Clin. Ther. 39, 322–336. [PubMed: 28129947]

Zhao W, Beers DR, Appel SH, 2013. Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J. Neuroimmune Pharmacol. 8, 888–899. [PubMed: 23881705]

Zhao W, Beers DR, Henkel JS, Zhang W, Urushitani M, Julien J, Appel SH, 2010. Extracellular mutant SOD1 induces microglial-mediated motoneuron injury. Glia58, 231–243. [PubMed: 19672969]

Zhao W, Beers DR, Hooten KG, Sieglaff DH, Zhang A, Kalyana-Sundaram S, Traini CM, Halsey WS, Hughes AM, Sathe GM, 2017. Characterization of gene expression phenotype in amyotrophic lateral sclerosis monocytes. JAMA Neurol. 74, 677–685. [PubMed: 28437540]

Zhao W, Xie W, Le W, Beers DR, He Y, Henkel JS, Simpson EP, Yen AA, Xiao Q, Appel SH, 2004. Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism. J. Neuropathol. Exp. Neurol. 63, 964–977. [PubMed: 15453095]

Zhong Z, Deane R, Ali Z, Parisi M, Shapovalov Y, O'Banion MK, Stojanovic K, Sagare A, Boillee S, Cleveland DW, 2008. ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. Nat. Neurosci. 11, 420–422. [PubMed: 18344992]

Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D, Bellucci R, Raderschall E, Canning C, Soiffer RJ, 2006. IL-2 regulates FOXP3 expression in human CD4+ CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood108, 1571–1579. [PubMed: 16645171]

Zubiri I, Lombardi V, Bremang M, Mitra V, Nardo G, Adiutori R, Lu C-H, Leoni E, Yip P, Yildiz O, Ward M, Greensmith L, Bendotti C, Pike I, Malaspina A, 2018. Tissue-enhanced plasma proteomic analysis for disease stratification in amyotrophic lateral sclerosis. Mol. Neurodegener. 13, 60. 10.1186/s13024-018-0292-2 [PubMed: 30404656]

Zürcher NR, Loggia ML, Lawson R, Chonde DB, Izquierdo-Garcia D, Yasek JE, Akeju O, Catana C, Rosen BR, Cudkowicz ME, 2015. Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: assessed with [11C]-PBR28. NeuroImage Clin. 7, 409–414. [PubMed: 25685708]

Highlights

- Evidence regarding the role of the immune system in ALS is rapidly expanding
- ALS involves a complex interplay between innate and adaptive immunity
- Animal model and patient data reveal a multi-phased immune response
- Several cell types contribute to immune mechanisms in ALS pathogenesis
- PET provides neuroinflammatory response monitoring and possible ALS biomarkers
- Clinical trials of emerging immune-targeted therapies provide hope in ALS

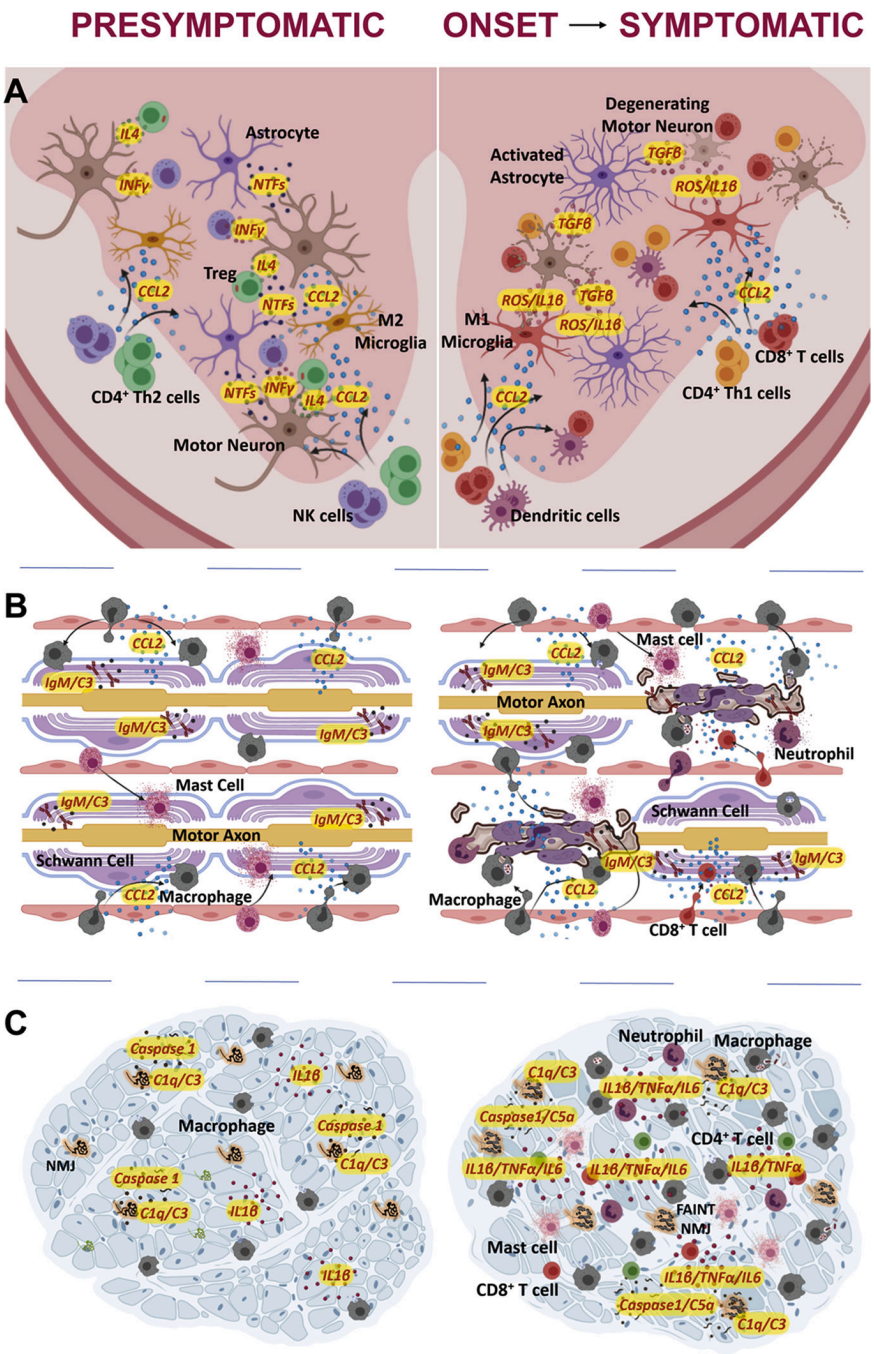

Figure 1. The immune cell response in the CNS and periphery of SOD1^{G93A} rodent models during disease progression.

(A) Spinal cord: At the presymptomatic stage, CCL2 is activated by motor neurons and microglia to recruit neuroprotective CD4^{+} T cells (Th2 and Tregs), which together with microglia in an M2-activate state and with mildly activated astrocytes release neurotrophic (NTFs) and anti-inflammatory factors to preserve motor neurons. However, this phenomenon appears to be counterbalanced by infiltrating NK cells that injure motor neurons while inhibiting Tregs and M2 microglia through the expression of IFN-γ. During the disease course, a massive release of CCL2 enhances the recruitment of DCs alongside

with $CD8^+$ and $CD4^+$ T cells (Th1). Proinflammatory T cells influence the activation status of astrocytes, which release pro-inflammatory factors and TGF-β1, which polarize microglia to a M1 phenotype. M1 microglia in turn release ROS and pro-inflammatory factors (IL-1β, TNF-α) detrimental to motor neurons. Peripheral monocyte infiltration in the spinal cord of $SOD1^{G93A}$ rodent models is still controversial. (B) Sciatic nerve: At the presymptomatic stage, early production of CCL2 along with the complement-mediated opsonization of motor axons and IgM, IgG deposition elicit the infiltration of macrophages and mast cells. At onset, increased permeability of the blood-nerve barrier together with CCL2 overexpression and a massive IgM/IgG deposition promotes a further infiltration of mast cells, neutrophils, macrophages, and $CD8^+$ T cells, which contribute to the massive degradation of peripheral motor axons and myelinating Schwann cells. (C) Skeletal muscle: At the presymptomatic stage, peripheral macrophages infiltrate the skeletal muscle of transgenic rodents alongside the increased expression of proinflammatory response initiator (Caspase1; C1q/C3) and byproducts (IL-1β). This likely is the response to neuromuscular junction (NMJ) alterations/denervation which precedes the motor neuron death according to the "dying back" hypothesis. Whether this response is an attempt to protect the myofibers or has a pathogenic role is still unknown. During disease progression, macrophages massively infiltrate the skeletal muscle together with mast cells, neutrophils, and T cells. This event is accompanied by increased inflammation due to IL-6, TNF-α, TGF-β1, TGF-β3, and C5a expression.

Abbreviations: C-C Motif Chemokine Ligand 2 (CCL2), T helper (Th), regulatory T cell (Treg), neurotrophic factors (NTFs), natural killer (NK), interferon gamma (IFN-γ), dendritic cell (DC), transforming growth factor (TGF), reactive oxygen species (ROS), Interleukin (IL), tumor necrosis factor-α (TNF-α), immunoglobulin (Ig). *Image created in* Biorender.com

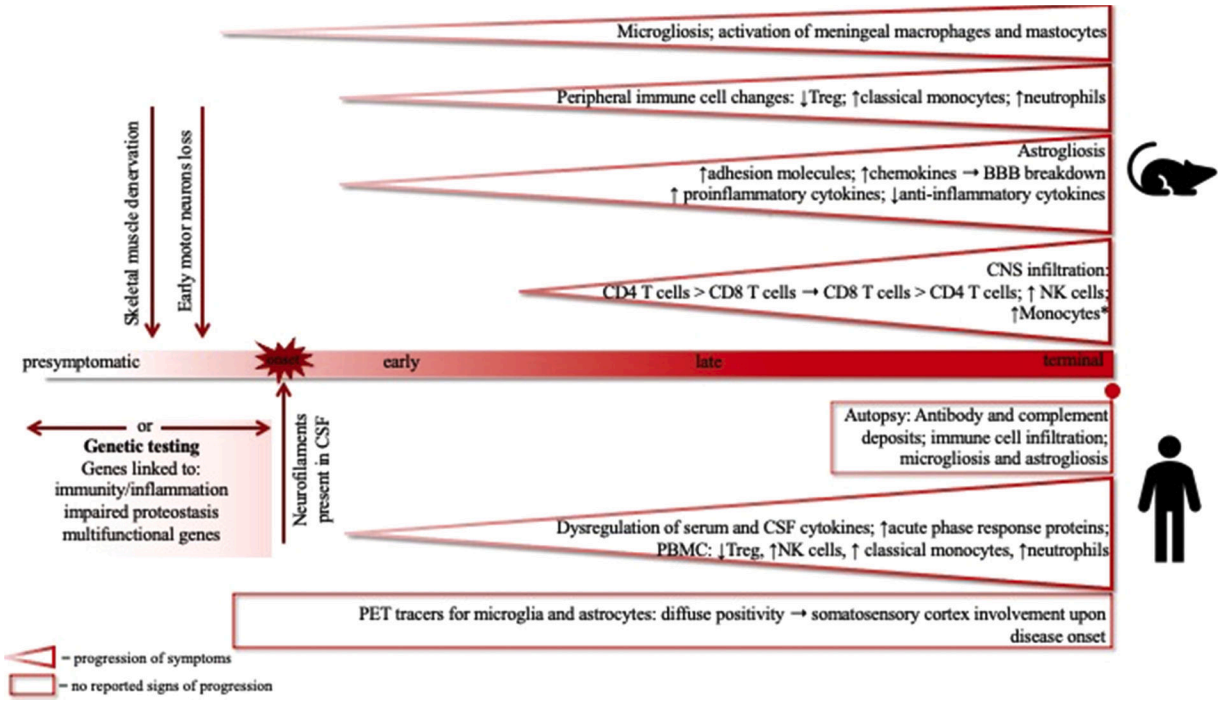

Figure 2. The timeline of immune system involvement in ALS: evidence from animal models and patients.

The progression of the immune response in animal models (top) and human ALS patients (bottom) is depicted over time. Horizontal arrows mark the conversion of immune biomarkers when applicable, while vertical arrows mark non-immune milestones of disease. Monocyte infiltration is still controversial (*). Current studies reported no progression in PET tracing of microgliosis and astrogliosis but studies need to be expanded to include more patients. Abbreviations: cerebrospinal fluid (CSF), regulatory T cell (Treg), blood–brain barrier (BBB), central nervous system (CNS), natural killer (NK), peripheral blood mononuclear cell (PBMC), positron emission tomography (PET).