Rising Stars: Astrocytes as a Therapeutic Target for ALS Disease

Michal Izrael[1]*, Shalom Guy Slutsky[1] and Michel Revel[1,2]

[1] Neurodegenerative Diseases Department at Kadimastem Ltd., Nes-Ziona, Israel, [2] Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Amyotrophic lateral sclerosis (ALS) is a multifactorial disease, characterized by a progressive loss of motor neurons that eventually leads to paralysis and death. The current ALS-approved drugs modestly change the clinical course of the disease. The mechanism by which motor neurons progressively degenerate remains unclear but entails a non-cell autonomous process. Astrocytes impaired biological functionality were implicated in multiple neurodegenerative diseases, including ALS, frontotemporal dementia (FTD), Parkinson's disease (PD), and Alzheimer disease (AD). In ALS disease patients, A1 reactive astrocytes were found to play a key role in the pathology of ALS disease and death of motor neurons, via loss or gain of function or acquired toxicity. The contribution of astrocytes to the maintenance of motor neurons by diverse mechanisms makes them a promising therapeutic candidate for the treatment of ALS. Therapeutic approaches targeting at modulating the function of endogenous astrocytes or replacing lost functionality by transplantation of healthy astrocytes, may contribute to the development of therapies which might slow down or even halt the progression ALS diseases. The proposed mechanisms by which astrocytes can potentially ameliorate ALS progression and the status of ALS clinical studies involving astrocytes are discussed.

Keywords: amyotrophic lateral sclerosis, astrocytes, TDP-43 aggregates, astrocyte cell-based therapy, A1 astrocyte, A2 astrocyte

INTRODUCTION

In Amyotrophic lateral sclerosis (ALS), selective degeneration of both upper and lower motor neurons (MNs) takes place in the central nervous system. Death of MNs leads to rapid and progressive paralysis of target muscles, which causes death within 3–5 years from disease onset, usually due to respiratory failure (Hardiman et al., 2011). The degeneration of MNs is associated with multiple pathophysiological processes including, mitochondrial dysfunction, protein aggregation and formation of inclusion bodies, impairment in RNA processing, elevation in reactive oxygen species (ROS) levels, lack of axonal transport, disruption of the neuromuscular junction and demyelination (Robberecht and Philips, 2013).

The causes for ALS disease are not well understood. The main pathological characteristic of ALS is the accumulation of misfolded proteins and cytoplasmic inclusions in MNs and glial cells, in both motor cortex and spinal cord (Rowland and Shneider, 2001). Around 10–15% of ALS cases are with family history (i.e., familial), and the other cases without family history but still might be genetic

(i.e., sporadic) (Kiernan et al., 2011). Familial ALS (fALS) includes mutations of Cu/Zn superoxide dismutase (Rosen, 1993), TAR-DNA-binding protein of 43 kDa (Neumann et al., 2006), fused in sarcoma (Fus) (Kwiatkowski et al., 2009; Vance et al., 2009) and amplification of hexanucleotide (GGGGCC) repeat expansions in the chromosome 9 open reading frame 72 (C9orf72) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). In these mutations, misfolded mutated proteins are spreading (i.e., TDP-43, hSOD1, and FUS), and their aggregation induces severe neuropathology (McAlary et al., 2019). Interestingly, some of these misfolded proteins are not confined to the familial form of the disease where the mutation is known, but also found in sporadic ALS (e.g., TDP-43 inclusions are found in 97% of sALS patients) (Prasad et al., 2019). The mechanism by which ALS mutated proteins become toxic to MNs may share some similarities with prion aggregation and propagation. For example, C9orf72 RNA can be translated into five different dipeptide repeat (DPR) proteins (Mori et al., 2013a,b) that can spread between cells, similar to TDP-43 misfolded protein (Westergard et al., 2016). Toxicity by C9orf72 mutation can also be facilitated by transcription into long repetitive RNA that forms foci of sense or antisense RNA, which segregate RNA Binding Proteins (RBPs) and interfere with their biological activities (Lagier-Tourenne et al., 2012; Fratta et al., 2013; Gendron et al., 2013; Mizielinska et al., 2013; Wen et al., 2017). In addition to ALS, the role of C9orf72 was also identified as the major genetic cause of frontotemporal dementia (FTD) and FTD-ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Vatsavayai et al., 2019). Furthermore, TDP-43 proteinopathy now also constitutes 45% of all FTD molecular pathologies (Arai et al., 2006; Ferrari et al., 2011; Hergesheimer et al., 2019). Misfolded protein inclusions are not restricted to ALS and FTD and were also reported in other neurological diseases such as Parkinson's disease (Trist et al., 2017, 2018), Alzheimer's disease (Josephs et al., 2014; Nag et al., 2015), and Huntington disease (Gao et al., 2018).

MNs are the main cells that die in ALS. MNs are substantially large cells with axon extensions that reach far distance locations (i.e., from motor cortex to spinal cord and target muscle) (Ragagnin et al., 2019). The size and function of these cells force them to be more active as compared to other cell types in the nervous system, in terms of cytoskeletal dynamics, energy consumption, RNA metabolism, and proteostasis (Vandoorne et al., 2018). Consequently, MNs are more vulnerable to changes in homeostasis, especially to proteinaceous aggregates (Weishaupt et al., 2016).

The key players in maintaining and supporting MN survival in the central nervous system are astrocytes. Astrocytes are the most common cells in the CNS and have multiple functions. In healthy conditions astrocytes regulate the concentration of different neurotransmitters and ions, supply various metabolites and energy, regulate osmolarity, modulate synaptic activity, secrete neurotrophic and neuroprotective factors, promote neurogenesis (Allen and Eroglu, 2017; Verkhratsky et al., 2017) and remyelination (Fasciani et al., 2018), play a role in immune-modulation (Liddelow and Barres, 2017) and blood-brain barrier

formation (Sweeney et al., 2019) as well as in the glymphatic system (Louveau et al., 2017).

ROLE AND THERAPEUTIC POTENTIAL OF ASTROCYTES IN ALS

Upon insult, stress, or injury in the CNS, astrocytes enter a reactive state, characterized by changes in their morphology and profile of gene expression. Depending on the signal, astrocytes can transform into reactive A1-type neurotoxic astrocytes, or neuroprotective A2-type astrocytes (Liddelow and Barres, 2015). For example, Neuroinflammatory stimuli, such as LPS, yield A1 reactive astrocytes that promote neurodegeneration and neurotoxicity. Formation of A2 is induced by ischemia, the reactive astrocytes which secrete neurotrophic factors promote neuroprotection and neural repair (Baldwin and Eroglu, 2017; Liddelow and Barres, 2017).

Astrocytes of ALS patients present A1 type characteristics and are actors in the non-autonomous cell disease dogma in ALS (Ilieva et al., 2009). The role of astrocytes in the progression of ALS pathology involves several mechanisms that can result in loss of homeostatic functions or gain of toxic functions. ALS Astrocytes isolated from both sporadic or familial post-mortem ALS patients were found to be toxic to healthy MNs in culture (Haidet-Phillips et al., 2011; Meyer et al., 2014). Toxicity to motor neurons was also demonstrated following coculture of direct conversion of SOD1 or C9orf72 mutated ALS patient's fibroblasts into induced neuronal progenitor cells (iNSC) and subsequent differentiation into astrocytes (i-astrocytes) (Meyer et al., 2014). This toxicity might be mediated by extracellular vesicles secreted by astrocytes containing miRNA such as miR-494-3p (Varcianna et al., 2019) or proteins such as SOD1, phospho-TDP-43, and FUS (Sproviero et al., 2018). Extracellular vesicles such as exosomes and ectosomes contain a specific composition of proteins, lipids, RNA, and DNA Cells (Gurunathan et al., 2019). Recent study demonstrated that mutated astrocytes derived from C9Orf72-iPSC were toxic to MNs via downregulation of antioxidant proteins secretion, the toxic effects of astrocytes were correlated with the length of astrocyte propagation in culture, consistent with the age-related nature of ALS (Birger et al., 2019). Other study showed that secretion of Tumor Necrosis Factor-Alpha (TNFα) by FUS mutated astrocytes was found to contribute MN-toxicity (Kia et al., 2018). Similar results were obtained with hSOD1G93A primary astrocytes co-cultured with either WT MNs or with MNs from ALS mice (Di Giorgio et al., 2007). The toxic effect on MNs was also demonstrated by the addition of astrocyte-conditioned-medium, indicating that the mechanism involves secretion of soluble molecules by mutated astrocytes (Marchetto et al., 2008).

In contrast, healthy astrocytes protect MNs. Recent study provides evidence for the beneficial role that astrocytes play in protecting MNs in ALS (Smethurst et al., 2020). In this study, the authors first demonstrated that iPSC-derived MNs are more vulnerable to seeded TDP-43 aggregation (extracted from sALS post-mortem spinal-cord) than iPSC-derived astrocytes, indicating a cell-type-specific difference in vulnerability. This

observation was further validated by the addition of proteasomal-inhibitors that enhanced the formation and propagation of TDP-43 aggregates. Under these conditions, the presence of seeded TDP-43 aggregation significantly increased MNs cell death, but to a much lesser extent in astrocytes. Next, it was shown that TDP-43 pathology spreads from MNs to astrocytes preferentially but could also be observed spreading from astrocytes to motor neurons. Interestingly, co-culture of healthy iPSC-derived astrocytes protects iPSC-derived MNs that were pre-exposed to TDP-43 aggregates for 3 days, by a significant reduction in TDP-43 aggregates and the apoptotic marker caspase-3 in MNs. This demonstrates that the presence of astrocytes protects MNs from seeded TDP-43 aggregation and its toxicity. Intriguingly, the addition of astrocyte-condition-media alone to iPSC-derived MNs, pre-exposed to TDP-43 aggregates, had similar effects on MN. Lastly, the authors demonstrated that highly purified recombinant TDP-43 oligomers reproduced the observed cell-type-specific toxicity (Smethurst et al., 2020).

Together, the data suggest that healthy astrocytes can protect MNs of ALS patients from a distance, through some secreted product. Among the most studied factors secreted by astrocytes are neurotrophins (Poyhonen et al., 2019). Neurotrophins are a family of proteins that induce the survival (Hempstead, 2006), development, and function of neurons (Reichardt, 2006). This includes brain-derived neurotrophic-factor (BDNF), Nerve-growth-factor (NGF) (Schwartz and Nishiyama, 1994), Vascular-Endothelial-Growth-Factor (VEGF) (Sondell et al., 2000), neurotrophin-3 (NT-3) (Thompson et al., 2014), ciliary-neurotrophic-factor (CNTF) (Thompson et al., 2014), Glial cell-derived neurotrophic factor (GDNF) (Rowitch, 2004) and neurturin (NRTN) (Thompson et al., 2014). Lower concentration of neurtrophins were found in the CSF of ALS patients (Ramamohan et al., 2007; Deepa et al., 2011; Mishra et al., 2016; Shruthi et al., 2017) and its supplementation was found to protect MNs (Storkebaum et al., 2005; Bogaert et al., 2010; Krakora et al., 2013; Shruthi et al., 2017). Astrocytes also release extracellular vesicles (Verkhratsky et al., 2016) that might target near or long-distance sites with a potential selectively to neurons (Venturini et al., 2019). For example astrocyte-derived extracellular vesicles were proven positive for neuroglobin, a protein functioning as neuroprotectant against cell insult (Venturini et al., 2019). Other groups of proteins secreted by astrocytes found to protect neurons are metalloproteases and their inhibitors (Gardner and Ghorpade, 2003) or immune-modulatory factors (Jha et al., 2019).

THERAPEUTIC APPROACHES TARGETING ASTROCYTES IN ALS

An interesting hypothesis is that in early-stages of ALS disease (pre-symptomatic stage), a period that may take years, there is a process of astrocyte transformation toward the A2-phenotype with neuroprotective properties. This would support the survival of MNs and delay disease onset. Then, upon disease onset and appearance of motor deficiency symptoms, probably after damage to the MN or astrocytes crosses metabolic threshold, the astrocytes acquire the A1 phenotype with neurotoxic properties. Transcriptomic data shows that astroglia in late stage of disease progression in ALS mouse model acquire A1-reactive astrocytic phenotype (Miller et al., 2017). In ALS patients' reactive astrocytes are observed in susceptible areas and the level of reactivity correlates with the neurodegeneration stage of ALS patients. These astrocytes are convoyed with numerous abnormalities of signaling pathways such as impaired lactate transport (Ferraiuolo et al., 2011), reduction of GLT-1 expression (Martorana et al., 2012), activation of p75-receptor signaling and elevation in pro-inflammatory signaling (Hashioka et al., 2009). In G93A-SOD1 mouse model, reducing mutant SOD1 in astrocytes was found to delay the disease progression, but not disease onset indicating supporting role of astrocytes toward disease onset (Yamanaka et al., 2008).

This hypothesis raises questions of (1) What is the astrocyte profile (A1 vs. A2) at different disease stages? What characterizes the specific threshold that changes the balance between A1 to A2 astrocytes? (2) Can A2 reactive astrocytes transform directly into A1 astrocytes and vice versa? Answers to these questions may provide tools to interfere at specific transformation checkpoints and exploit astrocytes neuroprotective properties to treat ALS. Thus, targeting astrocytes offers a promising approach to treat ALS and maybe also other neurological conditions.

ASTROCYTE-BASED CELL THERAPY

One therapeutic approach is to restore the functionality of endogenous malfunctioning astrocytes by transplantation of healthy human astrocytes. This might become a double-edged sword approach, in which astrocytes provide neurotrophic factors and neuroprotective support through the reduction of misfolded proteins such as TDP-43 to the diseased MNs from one hand, and from other hand might become malfunctioning or even toxic, A1 reactive astrocytes, once they will be introduced to hostile environment ALS patients CNS enriched with aggregations of mis-folded proteins. Comprehensive preclinical studies demonstrated that transplantation of glial-precursor-cells that were generated from iPSCs, or embryonic-stem-cells (ESC), had the potential to delay disease onset and ameliorate clinical symptoms in rodent models of ALS disease (Lepore et al., 2008; Kondo et al., 2014; Izrael et al., 2018) and shown to be safe (Izrael et al., 2018). However, other studies show that astrocytes acquire toxic neuroinflammatory role in response to the cerebrospinal-fluid from ALS patients (Mishra et al., 2016). The transplantation of human glial-restricted progenitors did not result in motor neuron protection or any therapeutic benefits on functional outcome measures (Lepore et al., 2011). Transplantation of genetically modified GDNF neural-stem cells presented efficient delivery of GDNF and preservation of motor neurons, however, MNs survival was not accompanied by continued innervation of muscle end-plates and thus resulted in no improvement in ipsilateral limb use (Suzuki et al., 2007). The difference observed between the therapeutic effect of transplanted cells might reside from difference in the population of astrocytes and their progenitors and should

be further investigated. These encouraging results led to the currently ongoing first-in-human phase I/IIa clinical study to evaluate the safety and efficacy of intrathecal transplantation of clinical-grade human astrocytes (AstroRx®) derived from human ESC in patients with ALS (ClinicalTrials.gov ID-NCT03482050). The advantage of intrathecal cell injection to cerebrospinal fluid is the distribution of human astrocytes throughout the neural axis, where the cells can reach and exert their effects on both upper and lower MNs. In addition, lumbar puncture is a standard clinical procedure and is generally safe. The mechanisms by which the transplanted astrocytes act still need to be fully elucidated. Most likely the astrocytes act by secreting neuroprotective factors that diffuse to the MNs (Gould and Oppenheim, 2011; Izrael et al., 2018; Thomsen et al., 2018). But many questions remain: Where they attach? Can astrocytes migrate from the CSF into the CNS-parenchyma and reach MNs? Will the astrocytes maintain their A2 characteristics in the hostile CNS environment of ALS or might transform to A1-phenotype further affecting disease course? How long will the transplanted astrocytes survive? Will they transform into the A1 state? The precise mechanisms of action that contribute to the astrocytes' effects *in vivo* still need to be fully understood, to optimize the potential benefit of these cells.

In another study, neural progenitor cells that were manipulated to overexpress glial cell-line derived neurotrophic factor (GDNF) enhanced the survival of MNs survival and attenuated the progression of the disease phenotype after their injection into the spinal cord (Klein et al., 2005) or motor cortex (Thomsen et al., 2018) of ALS rat model. These encouraging results led to a Phase I/IIa clinical trial (ClinicalTrials.gov ID-NCT02943850) aiming to assess the safety of transplantation of GDNF-producing human astrocyte-precursors into the spinal-cord lumbar segment of ALS patients. Finally, a phase-I/IIa clinical trial (ClinicalTrials.gov ID-NCT02478450) will explore the safety of transplanting Human Glial-Restricted-Progenitor Cells (Q-Cells®) into the cervical or lumbar region of the spinal cord in subjects with ALS (Lepore et al., 2011).

In these clinical trials, the cells are transplanted locally into the ventral horn (lumbar or cervical regions) in close vicinity to lower MNs. This might allow the cells to directly exert their therapeutic activity on a specific set of neurons as compared to the intrathecal approach.

CELL-BASED THERAPY USING MESENCHYMAL STEM CELLS

Another cell-based therapy approach is the use of mesenchymal-stem-cells (MSC). MSC are adult multipotent-precursors that can be derived from bone-marrow or placenta, with the potential to differentiate into osteocytes, chondrocytes, fibroblasts, and adipocytes (Pittenger et al., 1999). MSC are not natural residence of the CNS but can be induced to secrete some of the neurotrophic factors secreted by astrocytes (Bahat-Stroomza et al., 2009). Recently, single-dose transplantation of autologous MSC that were induced to secrete neurotrophic

factors (NurOwn) showed that single combined intramuscular and intrathecal transplantation of MSC-NTF cells demonstrated early promising signs of efficacy and shown to be safe (ClinicalTrials.gov ID NCT02017912) (Berry et al., 2019). These results lead to the ongoing multi-dose phase-III clinical trial in rapidly progressing ALS patients (ClinicalTrials.gov ID-NCT03280056). Preclinical studies in ALS animal model showed that transplantation of MSCs, have the potential to reduce MNs death, prolong animal survival and improved motor performance over sham-injected animals (Suzuki et al., 2008; Boucherie et al., 2009; Uccelli et al., 2012; Marconi et al., 2013). Intrathecal injection of autologous undifferentiated bone-marrow-derived MSCs (NEURONATA-R) to ALS patients (ClinicalTrials.gov ID-NCT01363401) resulted in stabilization of the ALSFRS-R score in all patients over 6 months after first cell injection. Also, levels of CSF immunomodulatory cytokines such as IL-10, TGF-β, and IL-6 were increased after MSC injection, this suggest that the effect of MSC treatment on ALS patients might be mediated by an immune response (Oh et al., 2015).

OTHER STRATEGIES TARGETING ASTROCYTES ACTIVITY

Compounds that can improve endogenous astrocyte functionality are also being tested. For example, a group of compounds that encompass astrocytic glutamate uptake. Excessive activation of glutamate receptor in MNs can result in cell death (Lapucci et al., 2017). Astrocytes uptake glutamate through EAAT2 (GLT-1) transporter, thus, increasing GLT-1 transporter expression in astrocytes may improve the survival of MNs. Compounds such as Class II HDAC inhibitor MC1568 (Lapucci et al., 2017), pyridazine derivative LDN/OSU-0212320 (Kong et al., 2014), b-lactam antibiotics (e.g., ceftriaxone) (Rothstein et al., 2005), neuroimmunophilin ligand (Ganel et al., 2006), and FDA-approved drug Riluzole (Liu et al., 2019) were found to enhance the expression of GLT-1 in astrocytes and delay symptoms of MN decline and in SOD1^{G93A} mice.

Compounds aiming at targeting endogenous-astrocytes to reduce oxidative-stress are of great therapeutic potential. Increasing availability of nicotinamide-adenine-dinucleotide (NAD+), an essential redox molecule (Belenky et al., 2007), leads to increased resistance to oxidative-stress and decreased mitochondrial reactive oxygen production (de Picciotto et al., 2016; Harlan et al., 2016). Activation of the transcription factor, erythroid-derived 2, like 2 (Nrf2) in astrocytes confers protection to neurons in culture and *in vivo* (Vargas et al., 2008; Chen et al., 2009). Treatment with nicotinamide-mononucleotide (NMN) or nicotinamide-riboside (NR) increases NAD+ availability in mutant hSOD1-expressing astrocytes, leading to increased resistance to oxidative-stress and reversion of their toxicity toward co-cultured MNs, through SIRT6 activity to Nrf2 activation (Harlan et al., 2019). Edaravon, the second FDA approved drug for ALS, is a free-radical-scavenger of peroxyl-radicals and peroxynitrite, has been shown to inhibit MNs death *in vivo* by reducing oxidative-stress (Ito et al., 2008). In a phase-III clinical trial (ClinicalTrials.gov, NCT01492686) this drug was

found safe and demonstrated safety and efficacy in ALSFRS-R (Writing and Edaravone, 2017).

Other mechanism to reduce astrocyte toxicity is by interfering with neuroinflammatory processes taking place in the progression of the disease. Pro-inflammatory cytokines and inflammatory-mediators are also linked to astrocyte-mediated toxicities. For example, an upregulation of interferon-α (IFNα) receptor in astrocytes was found in the spinal-cord of SOD1G93A mice and sALS, and reducing its expression extended survival in SOD1G93A mice (Wang et al., 2011). Additionally, mutant-SOD1 expressing astrocytes secrete IFN-γ, which induces degeneration in motor-neurons *in vitro* (Aebischer et al., 2011). The pro-inflammatory mediator, prostaglandin-D2 (PGD2), has also been linked to motor neuron death in *in vitro* experiments using co-culture of ES-cell derived human MNs and mutant SOD1 astrocytes (Di Giorgio et al., 2008). Transforming growth-factor β (TGF-β) is a multi-functional cytokine involved in many biological functions, including immune homeostasis, neurotrophic response, and microglial development (Butovsky et al., 2014). Alterations of TGF-β signaling have been implicated in ALS due to gene expression profiles (Phatnani et al., 2013). Next, it was demonstrated that astrocyte-derived TGF-β1 is a negative-regulator of the neuroprotective inflammatory response mediated by microglia and T-lymphocytes in ALS mice (Endo et al., 2015). In summary, several treatments have been tested on ALS animals with the aim of inhibiting or reducing the pro-inflammatory action of microglia and astrocytes (Geloso et al., 2017).

An additional way to interfere with endogenous astrocyte function is gene therapy. The challenges using these therapies are crossing blood-brain-barrier (BBB) and specifically targeting endogenous astrocytes cell population. Several clinical studies such as Tofersen (BIIB067) (Miller et al., 2013), miQure (targets down regelation of C9orf72) (Martier et al., 2019a,b), and VM2020 (Sufit et al., 2017) are already testing a gene therapy approach in the CNS. However, the exact mechanism and effect of these therapies on astrocytes and neurons crosstalk and functionality should be further investigated.

CONCLUSION AND OPEN QUESTIONS

In conclusion, astrocytes play a central role in ALS and other neurodegenerative diseases. Targeting astrocytes functionality using different therapeutic approaches might provide great benefit to ALS patients. Many questions are still left open, such as what defines a different subpopulation of astrocytes and their response to different pathological insults? What is the crosstalk between astrocytes and the immune system? What is the best site in the CNS for astrocyte transplantation? What is the optimal timing for transplantation during the progression of the disease as well as the dose of cells to be transplanted? Is immunosuppression required in the CNS to prevent graft rejection? Step by step, astrocytes become rising stars and show great promise in the treatment of ALS, based on preclinical studies and preliminary results from clinical trials targeting astrocytes in ALS.

AUTHOR CONTRIBUTIONS

REFERENCES

Aebischer, J., Cassina, P., Otsmane, B., Moumen, A., Seilhean, D., Meininger, V., et al. (2011). IFNgamma triggers a LIGHT-dependent selective death of motoneurons contributing to the non-cell-autonomous effects of mutant SOD1. *Cell Death Differ.* 18, 754–768. doi: 10.1038/cdd. 2010.143

Allen, N. J., and Eroglu, C. (2017). Cell biology of astrocyte-synapse interactions. *Neuron* 96, 697–708. doi: 10.1016/j.neuron.2017.09.056

Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., et al. (2006). TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.* 351, 602–611. doi: 10.1016/j.bbrc.2006.10.093

Bahat-Stroomza, M., Barhum, Y., Levy, Y. S., Karpov, O., Bulvik, S., Melamed, E., et al. (2009). Induction of adult human bone marrow mesenchymal stromal cells into functional astrocyte-like cells: potential for restorative treatment in Parkinson's disease. *J. Mol. Neurosci.* 39, 199–210. doi: 10.1007/s12031-008-9166-3

Baldwin, K. T., and Eroglu, C. (2017). Molecular mechanisms of astrocyte-induced synaptogenesis. *Curr. Opin. Neurobiol.* 45, 113–120. doi: 10.1016/j.conb.2017. 05.006

Belenky, P., Bogan, K. L., and Brenner, C. (2007). NAD+ metabolism in health and disease. *Trends Biochem. Sci.* 32, 12–19. doi: 10.1016/j.tibs.2006.11.006

Berry, J. D., Cudkowicz, M. E., Windebank, A. J., Staff, N. P., Owegi, M., and Nicholson, K. (2019). NurOwn, phase 2, randomized, clinical trial in patients with ALS: safety, clinical, and biomarker results. *Neurology* 93, e2294–e2305. doi: 10.1212/wnl.0000000000008620

Birger, A., Ben-Dor, I., Ottolenghi, M., Turetsky, T., Gil, Y., Sweetat, S., et al. (2019). Human iPSC-derived astrocytes from ALS patients with mutated C9ORF72 show increased oxidative stress and neurotoxicity. *EBioMedicine* 50, 274–289. doi: 10.1016/j.ebiom.2019.11.026

Bogaert, E., Van Damme, P., Poesen, K., Dhondt, J., Hersmus, N., Kiraly, D., et al. (2010). VEGF protects motor neurons against excitotoxicity by upregulation of GluR2. *Neurobiol. Aging* 31, 2185–2191. doi: 10.1016/j.neurobiolaging.2008.12. 007

Boucherie, C., Schafer, S., Lavand'homme, P., Maloteaux, J. M., and Hermans, E. (2009). Chimerization of astroglial population in the lumbar spinal cord after mesenchymal stem cell transplantation prolongs survival in a rat model of amyotrophic lateral sclerosis. *J. Neurosci. Res.* 87, 2034–2046. doi: 10.1002/jnr. 22038

Butovsky, O., Jedrychowski, M. P., Moore, C. S., Cialic, R., Lanser, A. J., and Gabriely, G. (2014). Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nat. Neurosci.* 17, 131–143. doi: 10.1038/ nn.3599

Chen, P. C., Vargas, M. R., Pani, A. K., Smeyne, R. J., Johnson, D. A., Kan, Y. W., et al. (2009). Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: critical role for the astrocyte. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2933–2938. doi: 10.1073/pnas.0813361106

de Picciotto, N. E., Gano, L. B., Johnson, L. C., Martens, C. R., Sindler, A. L., Mills, K. F., et al. (2016). Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* 15, 522–530. doi: 10.1111/acel.12461

Deepa, P., Shahani, N., Alladi, P. A., Vijayalakshmi, K., Sathyaprabha, T. N., Nalini, A., et al. (2011). Down regulation of trophic factors in neonatal rat spinal cord

after administration of cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients. *J. Neural Transm.* 118, 531–538. doi: 10.1007/s00702-010-0520-6

DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., and Rutherford, N. J. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256. doi: 10.1016/j.neuron.2011.09.011

Di Giorgio, F. P., Boulting, G. L., Bobrowicz, S., and Eggan, K. C. (2008). Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell* 3, 637–648. doi: 10.1016/j.stem.2008.09.017

Di Giorgio, F. P., Carrasco, M. A., Siao, M. C., Maniatis, T., and Eggan, K. (2007). Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat. Neurosci.* 10, 608–614. doi: 10.1038/nn1885

Endo, F., Komine, O., Fujimori-Tonou, N., Katsuno, M., Jin, S., Watanabe, S., et al. (2015). Astrocyte-derived TGF-beta1 accelerates disease progression in ALS mice by interfering with the neuroprotective functions of microglia and T cells. *Cell Rep.* 11, 592–604. doi: 10.1016/j.celrep.2015.03.053

Fasciani, I., Pluta, P., Gonzalez-Nieto, D., Martinez-Montero, P., Molano, J., Paino, C. L., et al. (2018). Directional coupling of oligodendrocyte connexin-47 and astrocyte connexin-43 gap junctions. *Glia* 66, 2340–2352. doi: 10.1002/glia.23471

Ferraiuolo, L., Higginbottom, A., Heath, P. R., Barber, S., Greenald, D., Kirby, J., et al. (2011). Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. *Brain* 134(Pt 9), 2627–2641. doi: 10.1093/brain/awr193

Ferrari, R., Kapogiannis, D., Huey, E. D., and Momeni, P. (2011). FTD and ALS: a tale of two diseases. *Curr. Alzheimer Res.* 8, 273–294. doi: 10.2174/156720511795563700

Fratta, P., Poulter, M., Lashley, T., Rohrer, J. D., Polke, J. M., Beck, J., et al. (2013). Homozygosity for the C9orf72 GGGGCC repeat expansion in frontotemporal dementia. *Acta Neuropathol.* 126, 401–409. doi: 10.1007/s00401-013-1147-0

Ganel, R., Ho, T., Maragakis, N. J., Jackson, M., Steiner, J. P., and Rothstein, J. D. (2006). Selective up-regulation of the glial Na+-dependent glutamate transporter GLT1 by a neuroimmunophilin ligand results in neuroprotection. *Neurobiol. Dis.* 21, 556–567. doi: 10.1016/j.nbd.2005.08.014

Gao, J., Wang, L., Huntley, M. L., Perry, G., and Wang, X. (2018). Pathomechanisms of TDP-43 in neurodegeneration. *J. Neurochem.* doi: 10.1111/jnc.14327 [Epub ahead of print].

Gardner, J., and Ghorpade, A. (2003). Tissue inhibitor of metalloproteinase (TIMP)-1: the TIMPed balance of matrix metalloproteinases in the central nervous system. *J. Neurosci. Res.* 74, 801–806. doi: 10.1002/jnr.10835

Geloso, M. C., Corvino, V., Marchese, E., Serrano, A., Michetti, F., and D'Ambrosi, N. (2017). The dual role of microglia in ALS: mechanisms and therapeutic approaches. *Front. Aging Neurosci.* 9:242. doi: 10.3389/fnagi.2017.00242

Gendron, T. F., Cosio, D. M., and Petrucelli, L. (2013). c9RAN translation: a potential therapeutic target for the treatment of amyotrophic lateral sclerosis and frontotemporal dementia. *Expert Opin. Ther. Targets* 17, 991–995. doi: 10.1517/14728222.2013.818659

Gould, T. W., and Oppenheim, R. W. (2011). Motor neuron trophic factors: therapeutic use in ALS? *Brain Res. Rev.* 67, 1–39. doi: 10.1016/j.brainresrev.2010.10.003

Gurunathan, S., Kang, M. H., Jeyaraj, M., Qasim, M., and Kim, J. H. (2019). Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. *Cells* 8:307. doi: 10.3390/cells8040307

Haidet-Phillips, A. M., Hester, M. E., Miranda, C. J., Meyer, K., and Braun, L. (2011). Frakes, Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nat. Biotechnol.* 29, 824–828. doi: 10.1038/nbt.1957

Hardiman, O., van den Berg, L. H., and Kiernan, M. C. (2011). Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat. Rev. Neurol.* 7, 639–649.

Harlan, B. A., Pehar, M., Killoy, K. M., and Vargas, M. R. (2019). Enhanced SIRT6 activity abrogates the neurotoxic phenotype of astrocytes expressing ALS-linked mutant SOD1. *FASEB J.* 33, 7084–7091. doi: 10.1096/fj.201802752r

Harlan, B. A., Pehar, M., Sharma, D. R., Beeson, G., Beeson, C. C., and Vargas, M. R. (2016). Enhancing NAD+ salvage pathway reverts the toxicity of primary astrocytes expressing amyotrophic lateral sclerosis-linked mutant superoxide dismutase 1 (SOD1). *J. Biol. Chem.* 291, 10836–10846. doi: 10.1074/jbc.m115.698779

Hashioka, S., Klegeris, A., Schwab, C., and McGeer, P. L. (2009). Interferon-gamma-dependent cytotoxic activation of human astrocytes and astrocytoma cells. *Neurobiol. Aging* 30, 1924–1935. doi: 10.1016/j.neurobiolaging.2008.02.019

Hempstead, B. L. (2006). Dissecting the diverse actions of pro- and mature neurotrophins. *Curr. Alzheimer Res.* 3, 19–24. doi: 10.2174/156720506775697061

Hergesheimer, R. C., Chami, A. A., de Assis, D. R., Vourc'h, P., Andres, C. R., Corcia, P., et al. (2019). The debated toxic role of aggregated TDP-43 in amyotrophic lateral sclerosis: a resolution in sight? *Brain* 142, 1176–1194. doi: 10.1093/brain/awz078

Ilieva, H., Polymenidou, M., and Cleveland, D. W. (2009). Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J. Cell Biol.* 187, 761–772. doi: 10.1083/jcb.200908164

Ito, H., Wate, R., Zhang, J., Ohnishi, S., Kaneko, S., Ito, H., et al. (2008). Treatment with edaravone, initiated at symptom onset, slows motor decline and decreases SOD1 deposition in ALS mice. *Exp. Neurol.* 213, 448–455. doi: 10.1016/j.expneurol.2008.07.017

Izrael, M., Slutsky, S. G., Admoni, T., Cohen, L., Granit, A., and Hasson, A. (2018). Safety and efficacy of human embryonic stem cell-derived astrocytes following intrathecal transplantation in SOD1(G93A) and NSG animal models. *Stem Cell Res. Ther.* 9:152.

Jha, M. K., Jo, M., Kim, J. H., and Suk, K. (2019). Microglia-astrocyte crosstalk: an intimate molecular conversation. *Neuroscientist* 25, 227–240. doi: 10.1177/1073858418783959

Josephs, K. A., Murray, M. E., Whitwell, J. L., Parisi, J. E., Petrucelli, L., Jack, C. R., et al. (2014). Staging TDP-43 pathology in Alzheimer's disease. *Acta Neuropathol.* 127, 441–450.

Kia, A., McAvoy, K., Krishnamurthy, K., Trotti, D., and Pasinelli, P. (2018). Astrocytes expressing ALS-linked mutant FUS induce motor neuron death through release of tumor necrosis factor-alpha. *Glia* 66, 1016–1033. doi: 10.1002/glia.23298

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., et al. (2011). Amyotrophic lateral sclerosis. *Lancet* 377, 942–955.

Klein, S. M., Behrstock, S., McHugh, J., Hoffmann, K., Wallace, K., Suzuki, M., et al. (2005). GDNF delivery using human neural progenitor cells in a rat model of ALS. *Hum. Gene Ther.* 16, 509–521. doi: 10.1089/hum.2005.16.509

Kondo, T., Funayama, M., Tsukita, K., Hotta, A., Yasuda, A., Nori, S., et al. (2014). Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. *Stem Cell Rep.* 3, 242–249. doi: 10.1016/j.stemcr.2014.05.017

Kong, Q., Chang, L. C., Takahashi, K., Liu, Q., Schulte, D. A., Lai, L., et al. (2014). Small-molecule activator of glutamate transporter EAAT2 translation provides neuroprotection. *J. Clin. Investig.* 124, 1255–1267. doi: 10.1172/jci66163

Krakora, D., Mulcrone, P., Meyer, M., Lewis, C., Bernau, K., Gowing, G., et al. (2013). Synergistic effects of GDNF and VEGF on lifespan and disease progression in a familial ALS rat model. *Mol. Ther.* 21, 1602–1610. doi: 10.1038/mt.2013.108

Kwiatkowski, T. J. Jr., Bosco, D. A., Leclerc, A. L., Tamrazian, E., Vanderburg, C. R., Russ, C., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323, 1205–1208.

Lagier-Tourenne, C., Polymenidou, M., Hutt, K. R., Vu, A. Q., Baughn, M., and Huelga, S. C. (2012). Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. *Nat. Neurosci.* 15, 1488–1497. doi: 10.1038/nn.3230

Lapucci, A., Cavone, L., Buonvicino, D., Felici, R., Gerace, E., Zwergel, C., et al. (2017). Effect of class II HDAC inhibition on glutamate transporter expression and survival in SOD1-ALS mice. *Neurosci. Lett.* 656, 120–125. doi: 10.1016/j.neulet.2017.07.033

Lepore, A. C., Dejea, C., Carmen, J., Rauck, B., Kerr, D. A., Sofroniew, M. V., et al. (2008). Selective ablation of proliferating astrocytes does not affect disease outcome in either acute or chronic models of motor neuron degeneration. *Exp. Neurol.* 211, 423–432. doi: 10.1016/j.expneurol.2008.02.020

Lepore, A. C., O'Donnell, J., Kim, A. S., Williams, T., Tuteja, A., Rao, M. S., et al. (2011). Human glial-restricted progenitor transplantation into cervical spinal cord of the SOD1 mouse model of ALS. *PLoS One* 6:e25968. doi: 10.1371/journal.pone.0025968

Liddelow, S., and Barres, B. (2015). SnapShot: astrocytes in health and disease. *Cell* 162, 1170–1170.e1. doi: 10.1016/j.cell.2015.08.029

Liddelow, S. A., and Barres, B. A. (2017). Reactive astrocytes: production, function, and therapeutic potential. *Immunity* 46, 957–967. doi: 10.1016/j.immuni.2017.06.006

Liu, Z., Xu, Y., Zhang, X., Miao, J., Han, J., and Zhu, Z. (2019). Riluzole blocks HU210-facilitated ventral tegmental long-term depression by enhancing glutamate uptake in astrocytes. *Neurosci. Lett.* 704, 201–207. doi: 10.1016/j.neulet.2019.04.021

Louveau, A., Plog, B. A., Antila, S., Alitalo, K., Nedergaard, M., and Kipnis, J. (2017). Understanding the functions and relationships of the glymphatic system and meningeal lymphatics. *J. Clin. Investig.* 127, 3210–3219. doi: 10.1172/jci90603

Marchetto, M. C., Muotri, A. R., Mu, Y., Smith, A. M., Cezar, G. G., and Gage, F. H. (2008). Non-cell-autonomous effect of human SOD1 G37R astrocytes on motor neurons derived from human embryonic stem cells. *Cell Stem Cell* 3, 649–657. doi: 10.1016/j.stem.2008.10.001

Marconi, S., Bonaconsa, M., Scambi, I., Squintani, G. M., Rui, W., and Turano, E. (2013). Systemic treatment with adipose-derived mesenchymal stem cells ameliorates clinical and pathological features in the amyotrophic lateral sclerosis murine model. *Neuroscience* 248, 333–343. doi: 10.1016/j.neuroscience.2013.05.034

Martier, R., Liefhebber, J. M., Garcia-Osta, A., Miniarikova, J., Cuadrado-Tejedor, M., Espelosin, M., et al. (2019a). Targeting RNA-mediated toxicity in C9orf72 ALS and/or FTD by RNAi-based gene therapy. *Mol. Ther. Nucleic Acids* 16, 26–37. doi: 10.1016/j.omtn.2019.02.001

Martier, R., Liefhebber, J. M., Miniarikova, J., van der Zon, T., Snapper, J., Kolder, I., et al. (2019b). Artificial MicroRNAs targeting C9orf72 can reduce accumulation of intra-nuclear transcripts in ALS and FTD patients. *Mol. Ther. Nucleic Acids* 14, 593–608. doi: 10.1016/j.omtn.2019.01.010

Martorana, F., Brambilla, L., Valori, C. F., Bergamaschi, C., Roncoroni, C., Aronica, E., et al. (2012). The BH4 domain of Bcl-X(L) rescues astrocyte degeneration in amyotrophic lateral sclerosis by modulating intracellular calcium signals. *Hum. Mol. Genet.* 21, 826–840. doi: 10.1093/hmg/ddr513

McAlary, L., Plotkin, S. S., Yerbury, J. J., and Cashman, N. R. (2019). Prion-like propagation of protein misfolding and aggregation in amyotrophic lateral sclerosis. *Front. Mol. Neurosci.* 12:262. doi: 10.3389/fnmol.2019.00262

Meyer, K., Ferraiuolo, L., Miranda, C. J., Likhite, S., McElroy, S., and Renusch, S. (2014). Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. *Proc. Natl. Acad. Sci. U.S.A.* 111, 829–832. doi: 10.1073/pnas.1314085111

Miller, S. J., Zhang, P. W., Glatzer, J., and Rothstein, J. D. (2017). Astroglial transcriptome dysregulation in early disease of an ALS mutant SOD1 mouse model. *J. Neurogenet.* 31, 37–48. doi: 10.1080/01677063.2016.1260128

Miller, T. M., Pestronk, A., David, W., Rothstein, J., Simpson, E., Appel, S. H., et al. (2013). An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. *Lancet Neurol.* 12, 435–442. doi: 10.1016/s1474-4422(13)70061-9

Mishra, P. S., Dhull, D. K., Nalini, A., Vijayalakshmi, K., Sathyaprabha, T. N., Alladi, P. A., et al. (2016). Astroglia acquires a toxic neuroinflammatory role in response to the cerebrospinal fluid from amyotrophic lateral sclerosis patients. *J. Neuroinflammation* 13:212.

Mizielinska, S., Lashley, T., Norona, F. E., Clayton, E. L., Ridler, C. E., Fratta, P., et al. (2013). C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. *Acta Neuropathol.* 126, 845–857. doi: 10.1007/s00401-013-1200-z

Mori, K., Arzberger, T., Grasser, F. A., Gijselinck, I., May, S., Rentzsch, K., et al. (2013a). Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. *Acta Neuropathol.* 126, 881–893. doi: 10.1007/s00401-013-1189-3

Mori, K., Weng, S. M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., et al. (2013b). The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science* 339, 1335–1338. doi: 10.1126/science.1232927

Nag, S., Yu, L., Capuano, A. W., Wilson, R. S., Leurgans, S. E., Bennett, D. A., et al. (2015). Hippocampal sclerosis and TDP-43 pathology in aging and Alzheimer disease. *Ann. Neurol.* 77, 942–952. doi: 10.1002/ana.24388

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., and Chou, T. T. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130–133.

Oh, K. W., Moon, C., Kim, H. Y., Oh, S. I., Park, J., Lee, J. H., et al. (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. doi: 10.5966/sctm.2014-0212

Phatnani, H. P., Guarnieri, P., Friedman, B. A., Carrasco, M. A., Muratet, M., O'Keeffe, S., et al. (2013). Intricate interplay between astrocytes and motor neurons in ALS. *Proc. Natl. Acad. Sci. U.S.A.* 110, E756–E765.

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147. doi: 10.1126/science.284.5411.143

Poyhonen, S., Er, S., Domanskyi, A., and Airavaara, M. (2019). Effects of neurotrophic factors in glial cells in the central nervous system: expression and properties in neurodegeneration and injury. *Front. Physiol.* 10:486. doi: 10.3389/fphys.2019.00486

Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., and Patel, B. K. (2019). Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. *Front. Mol. Neurosci.* 12:25. doi: 10.3389/fnmol.2019.00025

Ragagnin, A. M. G., Shadfar, S., Vidal, M., Jamali, M. S., and Atkin, J. D. (2019). Motor neuron susceptibility in ALS/FTD. *Front. Neurosci.* 13:532. doi: 10.3389/fnins.2019.00532

Ramamohan, P. Y., Gourie-Devi, M., Nalini, A., Shobha, K., Ramamohan, Y., Joshi, P., et al. (2007). Cerebrospinal fluid from amyotrophic lateral sclerosis patients causes fragmentation of the Golgi apparatus in the neonatal rat spinal cord. *Amyotroph. Lateral Scler.* 8, 79–82. doi: 10.1080/08037060601145489

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 361, 1545–1564. doi: 10.1098/rstb.2006.1894

Renton, A. E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J. R., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72, 257–268.

Robberecht, W., and Philips, T. (2013). The changing scene of amyotrophic lateral sclerosis. *Nat. Rev. Neurosci.* 14, 248–264. doi: 10.1038/nrn3430

Rosen, D. R. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362, 59–62.

Rothstein, J. D., Patel, S., Regan, M. R., Haenggeli, C., Huang, Y. H., Bergles, D. E., et al. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* 433, 73–77. doi: 10.1038/nature03180

Rowitch, D. H. (2004). Glial specification in the vertebrate neural tube. *Nat. Rev. Neurosci.* 5, 409–419. doi: 10.1038/nrn1389

Rowland, L. P., and Shneider, N. A. (2001). Amyotrophic lateral sclerosis. *N. Engl. J. Med.* 344, 1688–1700.

Schwartz, J. P., and Nishiyama, N. (1994). Neurotrophic factor gene expression in astrocytes during development and following injury. *Brain Res. Bull.* 35, 403–407. doi: 10.1016/0361-9230(94)90151-1

Shruthi, S., Sumitha, R., Varghese, A. M., Ashok, S., Chandrasekhar Sagar, B. K., Sathyaprabha, T. N., et al. (2017). Brain-derived neurotrophic factor facilitates functional recovery from ALS-cerebral spinal fluid-induced neurodegenerative changes in the NSC-34 motor neuron cell line. *Neurodegener. Dis.* 17, 44–58. doi: 10.1159/000447559

Smethurst, P., Risse, E., Tyzack, G. E., Mitchell, J. S., Taha, D. M., Chen, Y. R., et al. (2020). Distinct responses of neurons and astrocytes to TDP-43 proteinopathy in amyotrophic lateral sclerosis. *Brain* 143, 430–440. doi: 10.1093/brain/awz419

Sondell, M., Sundler, F., and Kanje, M. (2000). Vascular endothelial growth factor is a neurotrophic factor which stimulates axonal outgrowth through the flk-1 receptor. *Eur. J. Neurosci.* 12, 4243–4254. doi: 10.1046/j.0953-816x.2000.01326.x

Sproviero, D., La Salvia, S., Giannini, M., Crippa, V., Gagliardi, S., Bernuzzi, S., et al. (2018). Pathological proteins are transported by extracellular vesicles of sporadic amyotrophic lateral sclerosis patients. *Front. Neurosci.* 12:487. doi: 10.3389/fnins.2018.00487

Storkebaum, E., Lambrechts, D., Dewerchin, M., Moreno-Murciano, M. P., Appelmans, S., Oh, H., et al. (2005). Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nat. Neurosci.* 8, 85–92. doi: 10.1038/nn1360

Sufit, R. L., Ajroud-Driss, S., Casey, P., and Kessler, J. A. (2017). Open label study to assess the safety of VM202 in subjects with amyotrophic lateral sclerosis.

Amyotroph. Lateral Scler. Frontotemporal Degener. 18, 269–278. doi: 10.1080/21678421.2016.1259334

Suzuki, M., McHugh, J., Tork, C., Shelley, B., Hayes, A., Bellantuono, I., et al. (2008). Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. *Mol. Ther.* 16, 2002–2010. doi: 10.1038/mt.2008.197

Suzuki, M., McHugh, J., Tork, C., Shelley, B., Klein, S. M., Aebischer, P., et al. (2007). GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS One* 2:e689. doi: 10.1371/journal.pone.0000689

Sweeney, M. D., Zhao, Z., Montagne, A., Nelson, A. R., and Zlokovic, B. V. (2019). Blood-brain barrier: from physiology to disease and back. *Physiol. Rev.* 99, 21–78. doi: 10.1152/physrev.00050.2017

Thompson, C. L., Ng, L., Menon, V., Martinez, S., Lee, C. K., and Glattfelder, K. (2014). A high-resolution spatiotemporal atlas of gene expression of the developing mouse brain. *Neuron* 83, 309–323.

Thomsen, G. M., Avalos, P., Ma, A. A., Alkaslasi, M., Cho, N., Wyss, L., et al. (2018). Transplantation of neural progenitor cells expressing glial cell line-derived neurotrophic factor into the motor cortex as a strategy to treat amyotrophic lateral sclerosis. *Stem Cells* 36, 1122–1131. doi: 10.1002/stem.2825

Trist, B. G., Davies, K. M., Cottam, V., Genoud, S., Ortega, R., Roudeau, S., et al. (2017). Amyotrophic lateral sclerosis-like superoxide dismutase 1 proteinopathy is associated with neuronal loss in Parkinson's disease brain. *Acta Neuropathol.* 134, 113–127. doi: 10.1007/s00401-017-1726-6

Trist, B. G., Fifita, J. A., Freckleton, S. E., Hare, D. J., Lewis, S. J. G., Halliday, G. M., et al. (2018). Accumulation of dysfunctional SOD1 protein in Parkinson's disease is not associated with mutations in the SOD1 gene. *Acta Neuropathol.* 135, 155–156. doi: 10.1007/s00401-017-1779-6

Uccelli, A., Milanese, M., Principato, M. C., Morando, S., Bonifacino, T., Vergani, L., et al. (2012). Intravenous mesenchymal stem cells improve survival and motor function in experimental amyotrophic lateral sclerosis. *Mol. Med.* 18, 794–804. doi: 10.2119/molmed.2011.00498

Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K. J., Nishimura, A. L., and Sreedharan, J. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323, 1208–1211.

Vandoorne, T., De Bock, K., and Van Den Bosch, L. (2018). Energy metabolism in ALS: an underappreciated opportunity? *Acta Neuropathol.* 135, 489–509. doi: 10.1007/s00401-018-1835-x

Varcianna, A., Myszczynska, M. A., Castelli, L. M., O'Neill, B., Kim, Y., and Talbot, J. (2019). Micro-RNAs secreted through astrocyte-derived extracellular vesicles cause neuronal network degeneration in C9orf72 ALS. *EBioMedicine* 40, 626–635. doi: 10.1016/j.ebiom.2018.11.067

Vargas, M. R., Johnson, D. A., Sirkis, D. W., Messing, A., and Johnson, J. A. (2008). Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *J. Neurosci.* 28, 13574–13581. doi: 10.1523/jneurosci.4099-08.2008

Vatsavayai, S. C., Nana, A. L., Yokoyama, J. S., and Seeley, W. W. (2019). C9orf72-FTD/ALS pathogenesis: evidence from human neuropathological studies. *Acta Neuropathol.* 137, 1–26. doi: 10.1007/s00401-018-1921-0

Venturini, A., Passalacqua, M., Pelassa, S., Pastorino, F., Tedesco, M., Cortese, K., et al. (2019). Exosomes from astrocyte processes: signaling to neurons. *Front. Pharmacol.* 10:1452. doi: 10.3389/fphar.2019.01452

Verkhratsky, A., Matteoli, M., Parpura, V., Mothet, J. P., and Zorec, R. (2016). Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion. *EMBO J.* 35, 239–257. doi: 10.15252/embj.201592705

Verkhratsky, A., Zorec, R., and Parpura, V. (2017). Stratification of astrocytes in healthy and diseased brain. *Brain Pathol.* 27, 629–644. doi: 10.1111/bpa.12537

Wang, R., Yang, B., and Zhang, D. (2011). Activation of interferon signaling pathways in spinal cord astrocytes from an ALS mouse model. *Glia* 59, 946–958. doi: 10.1002/glia.21167

Weishaupt, J. H., Hyman, T., and Dikic, I. (2016). Common molecular pathways in amyotrophic lateral sclerosis and frontotemporal dementia. *Trends Mol. Med.* 22, 769–783. doi: 10.1016/j.molmed.2016.07.005

Wen, X., Westergard, T., Pasinelli, P., and Trotti, D. (2017). Pathogenic determinants and mechanisms of ALS/FTD linked to hexanucleotide repeat expansions in the C9orf72 gene. *Neurosci. Lett.* 636, 16–26. doi: 10.1016/j.neulet.2016.09.007

Westergard, T., Jensen, B. K., Wen, X., Cai, J., Kropf, E., Iacovitti, L., et al. (2016). Cell-to-cell transmission of dipeptide repeat proteins linked to C9orf72-ALS/FTD. *Cell Rep.* 17, 645–652. doi: 10.1016/j.celrep.2016.09.032

Writing, G., and Edaravone, A. L. S. S. G. (2017). Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* 16, 505–512.

Yamanaka, K., Chun, S. J., Boillee, S., Fujimori-Tonou, N., Yamashita, H., Gutmann, D. H., et al. (2008). Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nat. Neurosci.* 11, 251–253.