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Stem cell treatments for amyotrophic lateral sclerosis (ALS): A critical overview of early phase trials

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Abstract

Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of cortical, brainstem, and spinal motor neurons; it causes progressive muscle weakness and atrophy, respiratory failure and death. No currently available treatment either stops or reverses this disease. Therapeutics to slow, stop and reverse ALS are needed. Stem cells may be a viable solution to sustain and nurture diseased motor neurons. Several early stage clinical trials have been launched to assess the potential of stem cells for ALS treatment.

Areas covered: This review covers the key advances from early phase clinical trials of stem cell therapy for ALS and identifies promising avenues and key challenges.

Expert opinion: Clinical trials in humans are still in the nascent stages of development. It will be critical to ensure that powered, well-controlled trials are conducted, that optimal treatment windows are identified, and that the ideal cell type, cell dose, and delivery site and method are determined. Several trials have used more invasive procedures, and ethical concerns of sham procedures on patients in the control arm and on their safety should to be considered.

Keywords

Amyotrophic lateral sclerosis; ALS; clinical trial; preclinical; stem cells; therapy

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of cortical, brainstem, and spinal motor neurons (MNs) that leads to skeletal muscle weakness and atrophy, with death typically resulting from respiratory failure 2 to 4 years after disease diagnosis [1]. Changes in executive function are common and occur in up to 50% of individuals, and up to 15% of persons with ALS manifest frontotemporal dementia due to

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Declaration of interest

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neuronal dysfunction in the prefrontal and temporal cortex [2]. The incidence of ALS ranges from 1 to 4 cases per 100,000, although some subpopulations exhibit above average incidence [3, 4]. The disease exhibits clinical and pathophysiologic heterogeneity; both familial and sporadic cases occur, and the underlying genetic cause also varies, although mutations to *C9ORF72*, *SOD1*, *TARDBP*, and *FUS* are the most common [3, 5]. There is no cure for ALS; the Food and Drug Administration (FDA) has approved riluzole [6], a putative glutamate receptor antagonist, and edaravone [7], a possible free radical scavenger, which produce modest benefits. The heterogeneity of ALS, both from a molecular standpoint and clinical phenotype, suggests that a pharmacological one-solution-fits-all approach may be challenging, which has been borne out by the lack of successful trials to date [8]. Stem cell therapy is considered an attractive route that directly addresses the loss of MNs through multiple potential mechanisms, and encouraging preclinical studies have justified early phase clinical trials in humans.

2. Stem cell therapy for ALS

Stem cells were originally proposed as an ALS treatment to replenish the populations of progressively lost MNs. Stem cells possess the ability to self-renew and maintain an undifferentiated state. When they divide, the parent cell retains stemness while the daughter cell can differentiate [9]. Embryonic stem cells (ESCs) are totipotent and can differentiate into any cell type, while pluripotent stem cells (PSCs) can differentiate into more limited, specific cell types [9]. Neural progenitor cells (NPCs) are PSCs that can differentiate into neuronal or glial cells [9, 10]. The original notion for ALS stem cell therapy was to employ ESCs or PSCs to generate MNs for transplantation into ALS patients. Unfortunately, in practice, the concept proved harder to implement [11, 12, 13]. In order to integrate seamlessly with preexisting neural circuits, transplanted stem cell-derived MNs need to project axons, frequently over significant distances, and synapse with endogenous neurons and muscle, all the while enduring a diseased microenvironment [11, 13].

For these reasons, the field has reformulated the approach into a “neighborhood theory” of stem cell therapy, where transplanted stem cells adopt a supportive role by providing a nurturing, neuroprotective microenvironment that ameliorates detrimental conditions for diseased MNs, thereby slowing neurodegeneration and neuronal death. Transplanted stem cells in this capacity secrete neurotrophic factors, differentiate into non-diseased, non-neuronal cells, such as astrocytes and microglia, or into modulatory neurons that synapse with diseased MNs [14]. Preclinical studies in this vein in animal models were encouraging and demonstrated the potential applicability of stem cells to treat ALS. They also revealed mechanisms by which stem cells could improve the MN microenvironment in ALS by the “neighborhood” approach, identifying platforms from which to launch more promising approaches in human clinical trials.

3. Preclinical animal studies of stem cell therapy for ALS

While a comprehensive analysis of preclinical studies is outside the scope of this review and has been previously addressed [15], it is worth mentioning the pivotal preclinical studies that demonstrated the potential benefit of stem cell therapy for ALS, since they served as the

stimulus for human trials. The majority of preclinical stem cell studies have been performed in mouse and rat ALS models expressing mutant superoxide dismutase 1 (SOD1) [15]. SOD1 was the first identified ALS gene and remains among the more prevalent ALS mutations [3]. Mutant SOD1 animals capture the salient features of ALS, including selective MN degeneration concurrent with muscle atrophy and motor dysfunction. In an early seminal report, human umbilical cord blood (hUCB) was administered intravenously to irradiated SOD1^{G93A} mice, which delayed the onset of motor symptoms and extended overall survival [16, 17]. Histological analysis of treated mice indicated that hUCBs had infiltrated the brain and spinal cord parenchyma in regions with MN degeneration and were expressing neural biomarkers, including glial fibrillary acidic protein (GFAP), nestin, and neuron-specific class III β -tubulin (TUJ1) [18]. Additionally, pro-inflammatory cytokine expression was lowered in the brain and spinal cord [19].

Another early study directly engrafted neurons derived from mouse ESCs into SOD1^{G93A} rats [12]. In this study, mouse ESCs were differentiated into MNs, which were then grafted into the lumbar spinal cord of SOD1^{G93A} and wild-type (WT) rats prior to symptom onset. Grafted SOD1^{G93A} rats displayed improved motor function in weeks 16 and 17 compared to sham operated mutant rats, but eventually succumbed to paralysis by week 19. Histological analysis revealed loss of mouse-derived MNs in SOD1^{G93A} but not in WT rats indicating that engrafted MNs failed to integrate into host neural circuits in the diseased microenvironment. Nevertheless, the temporary amelioration in motor function suggested that the transiently engrafted MNs conferred some advantage, via what is presently considered the “neighborhood” mechanism.

The “neighborhood” theory underlines an important aspect. ALS is not necessarily a cell autonomous disorder that is restricted to neurons, at least in mutant SOD1 cases [20]. Toxicity to neighboring, supportive cells, such as astrocytes and microglia, is also possible and produces a microenvironment with dysregulation in numerous cellular compartments. However, it also suggests that neurodegeneration in ALS may be mitigated by cells other than MNs, and this avenue has been widely pursued in animal studies. Rather than correcting defects in MNs, a range of diverse stem cells and stem cell-derived cells have been implanted into ALS animal models.

Engrafted adult hematopoietic stem cells [21] and glial-restricted precursors (*i.e.*, differentiate into astrocytes) [22] improved the disease phenotype in ALS mice and rats, respectively. Likewise, orthotopic bone marrow (BM) transplants from WT donors into SOD1^{G93A} recipient mice slowed disease progression and prolonged survival [23], while intravascular injection of BM-derived c-kit(+) stem/progenitor cells into SOD1^{G93A} mice lengthened lifespan [24]. Engraftment of olfactory ensheathing cells (OECs) into mutant SOD1 ALS mice delayed symptom onset, improved lifespan, and resulted in the differentiation of OECs into cells expressing neuron, oligodendrocyte, and astrocyte markers [25], suggesting that diverse cell types can mitigate ALS pathogenesis. Additional beneficial effects have also been noted with human mesenchymal stem cells (MSCs) engineered to secrete glial cell-derived neurotrophic factor (GDNF) [26] or vascular endothelial growth factor (VEGF) [27]; injection of these cells into skeletal muscle of ALS rat models demonstrated a neuroprotective effect on MN survival. Spinal cord-implanted NPCs

expressing CD15 and CD184 also improved the disease phenotype in SOD1^{G93A} mice in a VEGF- and (insulin-like growth factor-1) IGF-1-dependent manner, further implicating a neuroprotective effect of neurotrophins [28]. Implantation of human NPCs engineered to express VEGF, GDNF, IGF-1, and brain-derived neurotrophic factor (BDNF) have demonstrated varying levels of success [29, 30, 31]. Induced pluripotent stem cells (iPSCs) also represent a ready source of autologous cells that can be reprogrammed into diverse cell types and hold great promise for treating degenerative diseases, including ALS [32]. They circumvent ethical dilemmas and graft rejection, and preclinical research utilizing human fibroblast cell lines that were reprogrammed into iPSCs and then differentiated into NPCs revealed the engrafted cells could integrate into rat spinal cord [33]. The technology is still in the nascent stages of development, however, and no clinical trials of ALS using iPSCs have been launched to date. Other nontransplant animal models [15] have further demonstrated the same overriding message: transplantation of cells other than MNs can produce a therapeutic gain in preclinical animal models.

Cumulatively, the overall body of evidence from preclinical studies indicated that stem cell therapy for ALS could work if the correct conditions, *i.e.*, stem cell source, dose, and delivery methods, could be achieved in humans. A number of early phase trials in humans have since been launched, primarily focused on safety assessment in a relatively small number of patients (Table 1).

4. Critical overview of stem cell clinical trials for ALS

Initial and ongoing clinical trials of stem cell therapies for ALS have evaluated a variety of parameters, including the source of stem cells, stem cell delivery mechanism, and methods to determine clinical outcomes of the therapeutic. While preclinical animals models demonstrated that a number of stem cells types could be viable options for ALS therapy – including ESCs, fetal or adult tissue-derived NPCs, and non-neural progenitor cells (non-NPCs) that can modulate the MN microenvironment – they did not narrow the focus of which stem cell types should be tested in humans [14]. Therefore, the safety and potential efficacy of an array of stem cell transplantations have been tested in early phase clinical trials of ALS, among them autologous BM- and adipose- derived MSCs, OECs, granulocyte colony-stimulating factor (G-CSF)-stimulated peripheral blood stem cells (PBSCs), and, most recently, the NSI-566RSC NPC line and human fetal cortex-derived NPCs engineered to release GDNF.

4.1 Stem cell sources in human ALS clinical trials

The spectrum of possible stem cell sources merits important consideration as advantages and disadvantages are weighed (Table 2). The underlying mechanism of G-CSF induction is that it mobilizes BM CD34+ hematopoietic stem cells into the peripheral blood stream to generate PBSCs. In mice with brain injury, the G-CSF-stimulated PBSCs infiltrated the central nervous system (CNS) and integrated into injured cerebral tissue where they transdifferentiated into neural cells [34]. G-CSF administration by subcutaneous (s.c.) injection is noninvasive and does not require immunosuppression of the patient, eliminating possible complications from lowered immunity. Additionally, G-CSF may possess

neuroprotective properties [35]; therefore, G-CSF-mobilized PBSCs have been considered a possible strategy for ALS treatment. On the other hand, although CNS penetration was observed in mouse model, translation to humans is uncertain. Injection of G-CSF-induced PBSCs directly into the CNS can circumvent low brain penetrance, but that is offset by greater risk from a more invasive procedure.

Another approach directly isolates BM-MSCs by BM aspiration. BM-MSCs are multipotent stromal cells that retain the ability to differentiate into several cell types and possess a multitude of useful properties for ALS. BM-MSCs: (i) have demonstrated preliminary potential [36], (ii) generate a neuroprotective environment by releasing neurotrophic, anti-inflammatory, and anti-apoptotic factors, and by inducing neighboring cells to adopt a protective role [37], (iii) are a readily available source and amenable to *ex vivo* expansion, (iv) are autologous and do not necessitate immunosuppression to prevent rejection, and (v) lower the chance of malignant transformation [38]. There is also evidence to support that MSCs may differentiate into neuron- or glia-like lineages [39, 40, 41], though this ability is disputed [42]. However, both BM-MSCs harvesting and then most administration routes are moderately to highly invasive, subjecting the patient to risk. In future advanced randomized, controlled trials, this will especially translate to multiple risky sham procedures for patients in the control arm.

An alternative to BM-MSCs are adipose-derived MSCs (AD-MSCs) from subcutaneous or visceral white adipose tissue (WAT) [43]. Harvesting AD-MSCs by liposuction or lipectomy is a less invasive procedure than collecting BM-MSCs by BM aspiration. Furthermore, AD-MSCs comprise approximately 1% of total WAT cells, whereas BM-MSCs encompass only 0.001-0.002% of total BM cells. AD-MSCs are phenotypically similar to BM-MSCs, and, although they display some variation in CD marker expression, still adopt a fibroblast-like morphology, express MSC markers, and retain the capacity to differentiate into osteoblasts, chondrocytes, and adipocytes under specific conditions. Preclinical studies in SOD1^{G93A} mice have shown the potential efficacy of AD-MSCs [44]. AD-MSCs were intravenously injected into SOD1^{G93A} mice at symptom onset, which slowed deterioration in motor function for 4 to 6 weeks. Histological analysis of spinal cord tissue demonstrated a greater number of lumbar MNs in AD-MSC-treated mice versus vehicle. AD-MSCs are autologous, thus sparing the ALS patient from immunosuppressants, and are a readily available source. Although the harvesting procedure is relatively noninvasive, the transplant procedure suffers the same less of invasiveness as BM-MSCs.

OECs are CNS glial cells that ensheath unmyelinated olfactory neuron axons, possess axonal protective and regenerative properties [45], and secrete neurotrophic factors [46]. Preclinical mouse studies reported a delay of disease onset and increase in survival in SOD1^{G93A} mice that received OECs [25]. In human trials, they are derived from human fetal olfactory bulb tissue and are therefore not a readily available stem cell source and may face ethical dilemmas. Furthermore, implantation of primary OECs would ideally require human leukocyte antigen (HLA) matching or immune suppression; yet, in one trial conducted to date [47], neither was performed, while in another trial [48], HLA matching was conducted, but no discussion of immune suppression was reported.

NPCs are pluripotent stem cells specifically capable of differentiating into neural or glial cells, and as such, could have an especially well-matched role in enriching the environment in degenerating cortical or spinal cord tissue in ALS patients. They are an alternative source to ESCs and pose a lower risk of teratoma. NSI-566RSC is an NPC cell line originally derived from the spinal cord of a human fetus in accordance with guidelines from the FDA, National Institute of Health (NIH), and an independent ethics review board, which can be differentiated into functional motor neurons [49]. Indeed, its engraftment into SOD1^{G93A} rat spinal cord delayed disease onset and progression, improved survival, and generated differentiated neurons that formed synapses with host neurons [50, 51, 52]. The NSI-566RSC cell line can be readily propagated and is therefore an accessible stem cell source; however, recipients must also receive immunosuppressants to prevent graft rejection.

In addition to the ideal source, early phase clinical trials have also tested diverse delivery methods, ranging from noninvasive procedures, such as intravenous and intra-arterial injections, to more invasive ones, including intrathecal, intraspinal, and intracerebral delivery. Furthermore, clinical presentation of ALS is heterogenous, with variability in the region of onset, pattern of spread, and the relative involvement of upper motor neuron (UMN), lower motor neuron (LMN), and cognitive pathology. Consequently, there is uncertainty about the ideal location for stem cell transplantation, which is a consideration moving forward.

Finally, successful clinical trials of stem cell therapy in ALS also require reliable and reproducible outcome measures. To date, there is no universally accepted *in vivo* test to prove successful stem cell transplantation, incorporation, and neurotrophic factor secretion. Thus, these trials require the use of standard ALS clinical trial outcome measures including the ALS Functional Rating Scale-Revised (ALSFRS-R), respiratory function, and survival. In small, underpowered trials lacking placebo arms—such as the case for the majority of stem cell ALS trials which have focused on safety rather than efficacy—these measures are often insufficient to prove efficacy in the absence of a remarkable treatment response. Additionally, it is important to recognize that many of the trials enrolled participants in various stages of their disease. More invasive therapies tended to start with more severely affected individuals to lessen the outcome of an adverse event, while other studies enrolled individuals more typical of ALS drug trials, such as those earlier in disease course with preserved respiratory function. These clinical population factors should be considered when evaluating study outcomes.

Despite the variability and lack of statistically significant evidence from the trials to date, the clinical insights acquired do serve as the foundation for future trials. In this review, we will cover clinical trials by stem cell type/source, and provide a critical overview of each study, highlighting key findings that strengthen the foundation for future studies.

4.2 G-CSF-induced PBSCs

The relative ease of G-CSF administration to patients and the lack of need for immunosuppressants has generated interest and several clinical trials of PBSC induction. In an open label study of 13 participants, G-CSF provided s.c. at 2 g/kg daily for five days reduced the rate of decline in the ALSFRS-R and compound muscle action potential

(CMAP, unspecified muscle(s)) in the 6 months after treatment relative to the rate over the 3 months prior to therapy [53]. In this study, however, the pre-therapy decline in ALSFRS-R was 3.23 points per month, well above the typical average of 1.02 [54], which could have accounted for reported changes. Another study incorporated a placebo arm (n=18 with 10 drop outs) in addition to the G-CSF treatment arm (n=17 with 12 drop outs) of 5 µg/kg/day s.c. for 4 days repeated at months 0, 3, 6, and 9. Increased blood levels of CD34+ PBSCs were found at day 5, showing cell mobilization; however, no statistically significant differences in the treatment compared to placebo arm were seen in the study outcomes, including declines in ALSFRS-R, respiratory function (forced vital capacity), and muscle strength testing [55]. Two participants experienced bone and muscle pain. Attempts to increase CNS penetration of PBSCs motivated the STEMALS study, which included co-administration of mannitol in the days following G-CSF injections to enhance blood-brain barrier (BBB) permeability in 24 participants [56, 57]. These participants received 5 µg/kg s.c. G-CSF every 12 hours for 4 days repeated at months 0, 3, 6, and 9. Bone pain and flu-like symptoms were reported, and one subject developed a leukocytosis. Circulating CD34+ PBSCs were identified as well as lowered interleukin-17 (IL-17) and monocyte chemoattractant protein-1 (MCP-1) in cerebrospinal fluid (CSF). An alteration in ALS disease course, however, was not observed. Another approach focused on the enrichment of PBSCs following G-CSF therapy [58]; G-CSF 300-600 µg s.c. daily for 5-6 days was administered to 8 patients and their PBSCs were collected with leukapheresis for a target of greater than 2.0×10^6 CD34+ cells/kg. These cells were reinfused on day 7. This study did not disclose significant adverse events and did not yield an improvement in disease progression, but also was not designed to do so. A more aggressive approach utilized human leukocyte antigen-matched donor-mobilized CD34+ peripheral blood cell infusion following full body radiation to 6 ALS participants [59]. Despite co-administration of immunosuppression, at least half developed graft-versus-host disease, and none showed any slowing in clinical decline. Nevertheless, engraftment occurred in 4 patients, and in 2, donor DNA was detected at sites of MN pathology.

G-CSF offers the advantage of noninvasive administration, but it relies on the eventual penetration of PBSCs into the CNS. To overcome this barrier, alternative trials have adopted a more invasive approach by delivering PBSCs directly into the CNS. One small study (n=3) isolated G-CSF-induced CD34+ PBSCs and transplanted them intrathecally at various levels and with various techniques, which was overall well tolerated [60]. Two studies by another group employed stereotactic injection into the frontal motor cortex to deliver G-CSF-stimulated CD133+ cells [61, 62]. The first trial enrolled 10 participants into the treatment arm and compared them to 10 control participants who did not undergo the procedure [61]. The authors reported a survival benefit in the treatment group, but this is likely offset by the difference in disease duration between the treatment group (30.1 months) and the control group (14.3 months) which biased the study towards the treatment group. The follow up study by the same group applied the same technique to 67 participants in an open label fashion, with the aim of reporting safety [62]. They noted two deaths, one of which was attributed to the procedure, and the authors concluded that further studies were needed to ascertain the procedure's efficacy.

In general, procedures from pilot G-CSF trials are well tolerated and have an acceptable safety profile. However, the less invasive method of subcutaneous G-CSF administration may lead to low levels of PBSC CNS penetration, while the more invasive injection of G-CSF-stimulated PBSCs into the CNS poses greater risks. Preclinical research suggests potential therapeutic gain from G-CSF. Although the human trials hint at some possible benefit, they were underpowered and not designed to confirm efficacy, so the question in humans remains open. If subcutaneous G-CSF does not produce clinical benefit in participants, one challenge will be to determine whether that arises from lack of PBSC CNS penetration or intrinsic lack of therapeutic benefit from G-CSF-induced PBSCs. Therefore, consensus on a delivery method will be a critical aspect for evaluating the efficacy of G-CSF.

4.3 BM-derived MSCs (BM-MSCs)

As with G-CSF stimulation, autologous BM-MSCs don't require immunosuppression, thus eliminating any possible complications from administration of immunosuppressants. Consequently, several clinical trials have selected MSCs as their cell source for ALS therapy. One phase I/II study, which focused on enumerating adverse effects, harvested MSCs by bone marrow aspiration and then administered the MSCs by intrathecal injection (n=10) or by both intrathecal and intravenous injection (n=9) [38]. The investigators did not note any significant adverse reactions, and the disease was stable for 6 months following BM-MS administration. The safety of MSCs was further evaluated in another 10 participants who received intrathecal BM-MSCs [63]. There were no reported acute adverse effects, and ALSFRS-R scores were stable in some participants, but declined in others, so the efficacy was uncertain. Safety was further assessed in an additional 14 ALS participants [64]. Deceleration in ALSFRS-R was suggested in a study of 26 participants receiving intrathecal BM-MSCs; however, 3 participants were excluded from the analysis, one of whom died 2 months after the procedure [65]. Another study focused on lineage-negative hematopoietic stem cells (Lin⁻ cells) due to their expression of neurotrophins [66]. The 12 participants in the study were divided into a slower progressing group (Group I; n=6) and a faster progressing group (Group II; n=6) following transplantation. Group I was younger, had a greater duration of symptoms, and a higher mean baseline ALSFRS-R (26.3 versus 15.5), and while demographic differences could account for treatment differences, the authors also reported greater uptake of Lin⁻ cells in Group I.

In another phase I study, 7 participants received two intrathecal injections of autologous BM-MSCs. Although there did not appear to be any improvement to ALSFRS-R scores, the procedure was well tolerated and there were no acute adverse reactions [67]. These findings supported a randomized controlled phase II trial by the same group, which enrolled 64 participants [68]. The treatment arm (n=33) received two intrathecal injections of autologous BM-MSCs, while the control arm (n = 31) received only riluzole; they did not undergo sham procedures due to ethical concerns. No differences in treatment-related adverse events were noted across the treatment and control arms. Encouragingly, the mean change in ALSFRS-R performance from baseline to 4- and 6-months post-BM-MS injection were smaller, and statistically significant, in the treatment versus control arm; however, no long-term change in survival was detected. Additionally, BM-MS injections also lowered the levels of

neuroinflammatory biomarkers. The investigators concluded that a future randomized, double-blind, large-scale phase III clinical trial was warranted to evaluate long-term safety and efficacy. Additionally, they considered whether ongoing repeated intrathecal BM-MSCs would be more efficacious and could account for the detection of short-term benefits without a long-term survival benefit.

MSCs have also been delivered into the CNS via the more invasive route of intraspinal injection to ensure crossing of the BBB. Nineteen participants in total received BM-MSCs by direct surgical implantation into the dorsal spinal cord as part of two consecutive phase I trials [36, 69, 70]. The procedure was safe, and participants were monitored long-term [71]; progression was slowed in 6 participants, of which 4 were the youngest and of which 2 had a lower motor neuron-predominant disease phenotype. Therefore, the authors concluded that the decelerated progression may have been due to the disease phenotype and not the treatment. Another 11 participants underwent autologous BM mononuclear cell injection into the spinal cord, which was deemed safe but did not slow progression [72]. Histologically, the extent of neurodegeneration appeared to have been attenuated near the injection site. The group later reported the impact autologous BM-MSCs injections may have had on breathing pattern during sleep, concluding there were no adverse effects to corticomedullary diaphragmatic pathways [73]. A more radical approach was taken by a study that performed a C1-C2 laminectomy and injected autologous BM-MSCs into the anterior part of the spinal cord of 13 ALS participants with severe respiratory and/or bulbar disease [74]. The authors concluded the procedure was safe and that the observed 3 deaths were attributable to lung infection and myocardial infarction, though additional safety tests would be needed to conclude this with certainty due to the small participant sample size. Furthermore, an improvement was observed in 9 of the participants according to electromyography and functional scales. An Ommaya reservoir was implanted into one participant to infuse BM-MSCs intraventricularly, which was well tolerated; more systematic evaluations would be needed to determine if this method should be further pursued [75].

A number of studies have tested adjuvant treatments or modified the BM-MSCs prior to injection. Chronic activation of the immune system is a hallmark of several neurodegenerative diseases, including ALS [76, 77, 78]. In light of this, one study combined BM-MSCs with T-cell vaccination to treat 7 ALS participants [79]. The technique was deemed safe and demonstrated some temporary improvement in ALSFRS-R scores. Another modified approach was adopted in a phase I/II and IIa clinical trial, which isolated BM-MSCs, expanded them *ex vivo*, and then induced them to express neurotrophic factors (MSC-NTFs) by stimulation with dibutyrylcyclic adenosine monophosphate, human basic fibroblast growth factor (bFGF), human platelet-derived growth factor (PDGF), and human heregulin $\beta 1$ [80]. MSC-NTFs were intramuscularly (n=6) or intrathecally (n=6) injected into participants (part of the phase I/II trial), and then both intramuscularly and intrathecally injected into 14 participants (phase IIa) with a dose escalation. The therapy was deemed safe, with only minor, temporary adverse events. Slowing of disease progression was suggested based on disease slopes 6 months after intervention compared to 3 months before the intervention. Confirmation in randomized controlled trials (RCTs) with longer follow up is needed, and MSC-NTFs are the subject of an ongoing phase III randomized, double-blind,

placebo-controlled multicenter clinical trial (). This trial aims to enroll 200 participants that will be randomized to the treatment arm, which will receive three intrathecal injections of autologous, *ex vivo* expanded MSC-NTFs, versus a control arm, which will receive placebo injections. The primary trial endpoints are safety and efficacy, while secondary endpoints are CSF evaluation of neurotrophic factors, inflammatory factors, and cytokines.

Overall, BM-MSCs have proved to be an accessible source of stem cells amenable to *ex vivo* expansion with an acceptable safety profile. Delivery of BM-MSCs have tended to be more invasive than subcutaneous G-CSF injection; however, this may increase the number of cells delivered to the site of neurodegeneration. Delivery method aside, comparison in the efficacy of G-CSF-induced PBSCs versus BM-MSCs remains to be evaluated.

4.4 Adipose-derived MSCs (AD-MSCs)

AD-MSCs benefit from being autologous, like BM-MSCs, but without the need for an invasive BM aspiration procedure. A phase I safety study of intrathecal autologous AD-MSC injection into 27 participants was well tolerated, with side effects related to increased protein and nucleated cell CSF levels and a thickening of lumbosacral nerve roots according to magnetic resonance imaging (MRI) in some participants [81]. Four participants died 31 days to 54 weeks post AD-MSC injection, of which 3 had displayed anomalies in their spinal cord MRI. Autopsy did not reveal any gross abnormalities or tumors. In terms of efficacy, ALSFRS-R scores continued to decline from the time of injection, although some subjective improvement in clinical symptoms were reported by participants. The investigators advocated a phase II trial to more objectively evaluate the efficacy of AD-MSCs, which is ongoing (NCT03268603).

4.5 OECs

Preclinical work provided the incentive to test OECs in human trials [25]. An initial nonrandomized, nonblinded pilot (n=15 treatment arm; n=20 placebo arm) injected participants with fetal OECs into the bilateral corona radiata and reported some clinical benefit [47]. This study had limitations however: treatments were performed in China although all participants were international; the efficacy outcome was the ALSFRS and not the ALSFRS-R; and patients and caregivers performed the outcome assessment as opposed to the research staff. The authors later reported their experience with 507 patients whom underwent OEC transplantation into the spinal cord and brain, some of which (at least 42) received several injections [48]. The study reported that participants benefited, with a statistically significant rise in ALSFRS scores and a trend towards better pulmonary function, and no side effects were detected. However, notable exceptions occurred in several instances. Symptoms rapidly worsened for one participant who was evaluated in her local ALS clinic and her brain MRI showed evidence of vasogenic edema and hemosiderin deposits [82], and another seven participants evaluated at their local institution did not demonstrate improvement [83]. Histological examination of autopsy tissue from participants that underwent OEC injection revealed an encased graft of undifferentiated cells and elevated neuroinflammation along the delivery tract [84].

Although preclinical studies do support the potential of OECs, clinical trials to date have failed to implement good trial practices. Therefore, results cannot not be interpreted objectively. Further, based on discrepancies between the reporting of safety from the research site compared to the institutions that followed these patients clinically, the ethical use of this therapy has been questioned [82].

4.6 NPCs

Promising preclinical studies utilizing the NSI-566RSC NPC line served as the impetus for trials in ALS participants [49, 50, 51]. A FDA-approved phase I clinical trial was initiated to test the safety of intraspinal NSI-566RSC cell transplants in 15 participants [85, 86, 87, 88]. Delivery among the participants was: (i) 6 received unilateral lumbar injections, (ii) 3 received bilateral lumbar injections, (iii) 3 received unilateral cervical injection,s and (iv) 3 received both unilateral cervical and bilateral lumbar injections. Injections were performed utilizing a custom injection platform with a floating cannula design to decrease the risk of spinal cord injury from cardiorespiratory motion (Figure 1) [87, 88]. Accurate injection into the ventral horn was achieved by pre-surgery MRI. The procedure was safe, and, although not set up to test treatment effectiveness, the study did indicate improvement in the rate of change in ALSFRS-R scores, particularly in participants who were treated with the highest number of NPCs [88].

Detailed graphical analysis was performed for the participants who received the highest dose (both lumbar and cervical injections) as a rate of change in ALSFRS-R scores per year versus time [88]. The evolution in scores demonstrates an improvement in the rate of decline (positive slope) post lumbar and cervical injections, but with intervening periods of worsening in the rate of decline (negative slope) between the injections. The analysis implies that there is a therapeutic window following each injection within which maximal benefit occurs before the effectiveness begins to taper. Also important is the time between symptom onset and the start of stem cell treatment; participants who received NPCs within 2 years of disease onset and had not begun to exhibit bulbar symptoms benefited the most [88]. Histological and molecular examination at autopsy from 6 participants observed nests of stem cells and detected stem cell DNA in the spinal cord close to the injection site [89].

The favorable safety profile of the phase I trial motivated a multicenter phase II trial aimed at assessing the safety and tolerability of escalating doses of NSI-566RSC transplantation via initially cervical and then combined cervical and lumbar injections. Fifteen participants were equally distributed across 5 treatment arms: (i) 2 million cells delivered by 5 bilateral cervical injections (10 total), (ii) 4 million cells delivered by 10 bilateral cervical injections (20 total), (iii) 6 million cells by 20 cervical injections, (iv) 8 million cells by 20 cervical injections, and (v) the highest dose of 16 million cells, delivering 8 million by 20 injections into the lumbar and cervical regions, respectively [90]. The procedure was generally safe with escalated cell doses, with some minor effects, although one participant suffered acute neurological deterioration and one participant exhibited central pain syndrome, both of whom had received the highest dose. This may advocate caution for this particular cell dose, though it was tolerated in other participants. In comparison to three historical groups, there

was no acceleration in decline, as measured by ALSFRS-R, forced vital capacity, and quantitative measures of strength.

A long-term follow-up report of all combined participants from the phase I [87] and phase II [90] trials further compared trial outcomes to historical controls [91]. The comparison was made for survival, ALSFRS-R, and a composite score (ALS/SURV) to the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) historical control. Promisingly, the analysis demonstrated a significant improvement in combined phase I and II ALS/SURV versus PRO-ACT controls. Comparison to historical controls is not without several limitations; nevertheless, the analysis advocates for a powered, randomized, sham surgery-controlled phase III trial of NSI-566RSCs.

An Italian group, which has run phase I trials of intraspinal BM-MSC injections [36, 69, 70, 71], has more recently tested intraspinal delivery of NPCs derived from human fetuses that experienced a natural *in utero* death [92]. Six participants were enrolled in this uncontrolled phase I trial and received either 3 unilateral lumbar microinjections or a larger cell dose with 3 bilateral lumbar (total 6) microinjections utilizing the same surgical procedure utilized for the NSI-566RSC trials. The procedure was safe, and no acceleration in decline was seen for up to 18 months. In fact, the investigators reported some evidence of improvement, but controlled trials will be necessary to validate those findings.

An additional group is currently evaluating lumbar intraspinal injections of NPCs engineered to produce GDNF [93] to promote formation of astrocytes (NCT02943850). This clinical study, however, is still ongoing and results are not yet published.

5. Ethical considerations

The possibility of treating neurodegenerative diseases, including ALS, with stem cells has generated intense public interest, in particular in hopeful patients and their families. This has also led to “stem cell tourism.” “Stem cell tourism” is an unethical industry that offers patients untested and potentially unsafe procedures [94, 95]. The procedures are conducted outside of regulatory oversight, without consideration for patient safety [94]. These industries are motivated by profit and therefore exploit patients financially through a false hope of a treatment response. The treatments are not part of any approved clinical trials, are untested products that lack oversight, do not conform to research or clinical rigor, and patients—who are not properly made aware of the risks—therefore cannot provide informed consent [96]. In addition to placing patients at risk, they harm the legitimacy of genuine stem cell research, which could undermine public trust [94, 95]. Therefore, guidelines for stem cell research (*e.g.*, regulatory oversight, ethical approval, procedures conducted within versus outside of a clinical trial, data sharing, possible conflicts of interest) and for ALS patients (*e.g.*, genuine informed consent, explanation of risk-benefit ratios, safety measures) are advocated [96].

6. Conclusions

Preclinical studies have laid the foundation for human trials, classifying stem cells with therapeutic potential and revealing mechanisms that might ameliorate toxicity within the

disease microenvironment to slow neurodegeneration. Continued preclinical research would keep the pipeline for alternative, potential stem cells, for example iPSCs, open to discovery. In humans, although still in development, early clinical trials of stem cell therapy for ALS have made great strides towards delineating safety. Of critical importance now is determining treatment efficacy, while in parallel identifying the most effective stem cell source, cell dose, and delivery site and method among those that have proven safe. Additionally, the therapeutic window of opportunity will need to be clearly defined, and this area of research may need to liaise with methods of early ALS diagnosis, if therapy will be most beneficial early in the course of disease. The early indicators are that stem cell therapy for ALS holds great promise. Throughout its development from bench to bedside, it will be essential to safeguard its legitimacy and patients' safety, advocating good clinical trial design to truly assess treatment efficacy.

7. Expert opinion

Though still in the nascent phase of development, stem cell therapy to treat ALS is making clear, definable progress in both the preclinical and early phase clinical arenas. Preclinical studies are helping elucidate the underlying mechanism of neuroprotection (*e.g.*, neighborhood theory), while identifying potential stem cell sources that may provide therapeutic benefit. Early phase clinical studies are making headway in the safety evaluation of various stem cells and their delivery methods. We anticipate that the key areas of focus discussed below will keep research moving forward.

7.1 Preclinical research:

Since clinical trials to date have not identified an ideal stem cell source, it will be essential to keep the pipeline from preclinical studies open, as novel stem cell candidates may prove more promising in humans than current ones. Animal models of ALS, particularly SOD1^{G93A} mice and rats, have served this purpose well; however, the model does have an important drawback since mutations to *C9ORF72*, *TARDBP*, and *FUS* better reflect the pathology of a majority of ALS patients [3, 5]. The underlying pathogenesis of ALS is still not completely understood, and treatment outcomes may be dependent on genomics. Patient-derived iPSCs could potentially offer a way forward by generating patient specific models of ALS that could enable mechanistic studies stratified by genetic mutations [97, 98]. It may also eventually lead to studies on how stem cells act as disease modifiers in microenvironments from diverse genetic backgrounds, which could impact the success or failure of stem cell therapies.

7.2 Clinical research:

The safety of certain types of stem cells (*e.g.*, G-CSF-induced PBSCs, BM-MSCs, NPCs) via assorted delivery methods have proven safe in many cases, and across studies by different groups in various countries. Safety trials remain necessary for less studied stem cell sources. For treatments deemed safe and well-tolerated, long-term phase II and III studies are necessary to test efficacy (stem cell type, dose, delivery site and method) and assess for late-onset adverse events. In order to maintain rigor, and draw firm conclusions, ideally upcoming trials should adopt well powered, randomized/double-blinded designs, although

ethical considerations must be weighed. Methods, in addition to typical ALS clinical trial outcomes, to assess the extent of MN neurodegeneration, graft survival, and migration of stem cells to the site of degeneration would be ideal but are not easily performed in living patients. Some studies have attempted to trace tagged injected stem cells, but this does not necessarily reflect effectiveness, merely their location. Autopsy will enable appraisal of graft survival, possible stem cell differentiation, and the presence of synapses on endogenous MNs. However, it is not possible by this method to discern which native MNs would have survived or degenerated without the treatment. The neighborhood theory suggests that secretion of neurotrophic factors can ameliorate the diseased microenvironment, so quantification of these factors could serve as a surrogate for successful engraftment. Patient survival and ALSFRS-R scores are feasible endpoints but are not specific to engrafted stem cell function or the condition of the MN microenvironment, though they serve as good clinical outcome measures, and are currently widely adopted. Research into alternative more accurate or reproducible outcome biomarkers of ALS progression have been considered in order to shorten trial duration and lower the number of participants required to power trials [99, 100]. As stem cell trials for ALS progress into the more advanced stages, with clinical efficacy as the primary endpoint, alternative outcome biomarkers could streamline evaluation of stem cell efficacy.

7.3 Technological advances:

Improved imaging techniques and stem cell delivery methods will minimize risk to patients by ensuring injections are guided to the correct location with minimal disruption to the surrounding tissue. This will also improve reproducibility, enabling better evaluation of outcome measures. In the phase I and II trials of NSI-566RSCs, the customized injection platform with a floating cannula reduced the possibility of spinal cord damage from cardiorespiratory motion during the injection, while the use of pre-surgical spinal cord MRI enabled delivery of stem cells with great accuracy [87, 88]. Such technological advances will minimize patient risk and reduce variability, leading to fewer adverse effects and a more accurate interpretation of treatment efficacy.

7.4 Early ALS diagnosis:

Evidence suggests that a therapeutic window of opportunity exists and that patients treated with stem cells in the earlier stages of the disease show the greatest response [88]. Unfortunately, diagnosis of ALS can be delayed by as much as a year [101], at which stage the microenvironment has already suffered extensive degradation. Therefore, earlier methods of ALS diagnosis will broaden the therapeutic window for patients and help enroll them to trials sooner. Robust and specific biomarkers [102] and improved imaging modalities [103] will aid both diagnosis as well as the evaluation of treatment efficacy.

7.5 Ethical considerations:

The selection of delivery route, and hence the level of invasiveness, has ramifications for patients in the trial's control arm, who will undergo sham procedures with possible safety complications. Although problematic, placebo arms improve trial integrity. Therefore, technological progress (see point above) will be essential to minimize risks to participants randomized to the control arm. Stem cell therapy guidelines will also need to be

implemented, with regulatory oversight, reporting procedures, and patient safety measures, to preserve the field's legitimacy and restrict the tide of proliferating "stem cell tourism" clinics.

7.6 Concluding remarks:

Breakthroughs in basic research (*e.g.*, elucidation of molecular mechanism of pathogenesis), bioengineering (*e.g.*, improved methods of stem cell production, development of iPSCs and other types of stem cells), instrument technology (*e.g.*, delivery and imaging modalities), and medical expertise could help transport stem cell therapy for ALS into the clinic. Continuous progress is bringing the vision closer to reality, which would bring much needed therapeutic options to persons with ALS.

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Article highlights

- There is no cure for ALS despite numerous clinical trials; current therapies are palliative and only extend survival a few months.
- Stem cell therapy is considered an attractive approach for ALS that addresses the complex disease pathogenesis through multiple potential mechanisms.
- The premise of stem cell therapy for ALS is based on improving the diseased microenvironment. While stem cells are unable to directly replace diseased motor neurons, transplanted stem cells secrete neurotrophic factors and differentiate into supportive cells, such as astrocytes and microglia, generating a neuroprotective milieu that can slow degeneration of motor neurons.
- Encouraging results in preclinical animal models advocated human clinical trials.
- Several stem cell sources, cell doses, and methods of delivery are presently being evaluated in early phase clinical trials. Determination of an optimal window of treatment will also aid endeavors to identify promising treatment options.
- Most current clinical trials are assessing safety as their primary objective. However, the determination of whether stem cells offer a viable treatment strategy for ALS rests on well-designed and appropriately powered future clinical trials. Randomized, double-blinded, and sham-controlled studies would be valuable, but ethical implications should be considered.

Figure 1: Spinal cord stabilization and injection systems for intraspinal stem cell transplantation.

a | Platform anchored to patient's spine consists of two bridge rails (blue), one of which is scored at 2-mm intervals to aid regular positioning of injections. Gondola (green) compensates for slight movements in the platform application. Mechanical Z drive (orange) allows precise raising and lowering of a floating cannula. **b** | Cannula tip is positioned 1 mm medial to dorsal root entry zone. **c** | Needle penetrates into spinal cord ~4 mm from pial surface. **d** | Once needle tip is positioned at the target, metal outer sleeve is pulled up, leaving flexible tubing exposed. Reproduced with permission from: *Boulis NM, Federici T,*

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Table 1.

Summary of Clinical Stem Cell Trials in ALS

Stem Cell Type	Clinical Trial Phase	Delivery Method (target)	Dose	Patient Eligibility	Patients (Controls)	Planned Outcomes	Results	Reference(s)
PBSC	Pilot		G-CSF 2 µg/kg/day SC x 5 days	Not provided	13 (0)	Primary: ALSFRS-R Secondary: CMAP amplitude (muscle(s) not specified)	Reduction in slope of decline of ALSFRS-R and CMAP amplitude post procedure	Zhang (2009)[53]
	Pilot		G-CSF 5 µg/kg/day SC x 4 days repeated at months 0, 3, 6, 9	Age: 18-85 EEC: D, Pr, Symptom duration: < 6 years FVC: > 50% FALS: Excluded Cognition: Normal	19 (20), but only those completing 6 months of study included in analysis Patients assessed at each month 0: 19 (20) 3: 17 (18) 6: 12 (16) 9: 9 (12)	Primary: ALSFRS-R Secondary: FVC, MMT megascor, CMAP megascor, NI, QoL; tracheostomy, death	No difference in clinical measures between treatment and placebo group No safety concerns G-CSF resulted in increased WBC count & circulating CD34+ cells	Nefussy (2010) [55]
	Phase I/II		G-CSF 5 µg/kg/day SC every 12 hours x 4 days at months 0, 3, 6, 9 with 125 mL 18% mannitol IV four times per day x 5 days starting on day 3 of G-CSF	Age: 40-65 EEC: D, Pr, Pr-LS Symptom duration: < 12 months FVC: > 80% FALS: Excluded Cognition: FTD excluded	24 (0) Patients assessed at each month 0: 24 3: 24 6: 21 9: 20	Primary: safety & tolerability, clinical progression, and changes in chemokine & cytokine levels	No safety concerns No change in disease progression ↑ WBC & CD34+ cells in blood ↓MCP-1 in CSF/serum ↑IL-17 in CSF ↑ IP-10 in serum	Tarella (2010)[56] Chio (2011)[57]
	Pilot	IV	G-CSF 300-600 µg/day SC x 5-6 days Leukapheresis for target of 2.0×10^6 CD34+ cells/kg	Not clearly specified	8 (0)	Not clearly specified	No safety concerns No chance in disease progression No change in MRS NAA/Cr ratio	Cashman (2008) [58]
	Pilot	Intrathecal	G-CSF administration followed by leukopheresis to isolate CD34+ cells	Case series	3 (0) Patient 1: 100 million cells via lumbar intrathecal catheter over 2 days Patient 2: 20 million cells over minutes intrathecal at L3/4 and cisterna magna Patient 3: 100 million cells intrathecal at C1/2 and lumbar region		Patient 1: 2 hour loss of sensation in lower limbs, subjective speech improvements Patient 2: no change in disease Patient 3: gain in leg and neck strength	Janson (2001)[60]
	Pilot	IV	Donor-mobilized CD34+ cells generated by G-CSF following total body radiation, fludarabine, and horse ATG Tacrolimus and methotrexate for GVHD prophylaxis	Age: 20-65 EEC: D FVC: > 60% HLA-identical related donor	6 (matched historical controls)	Primary: Donor engraftment, clinical measures	Engraftment successful Cases of cutaneous and limited GVHD	Appel (2008)[59]
	Pilot	Intracortical	G-CSF 300 µg/daily SC x 3 days followed by isolation of CD133 cells by leukapheresis	EEC: any FVC: any Severe bulbar involvement excluded	10 (13) Control patients were those that did not accept treatment but met inclusion criteria, or those that applied after study completed recruitment	Primary: survival rate Secondary: ALSFRS-R	No safety concerns. Improved survival in treatment group (disease duration 30.1 months in treatment group vs 14.3 in controls)	Martinez (2009) [61]

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Stem Cell Type	Clinical Trial Phase	Delivery Method (target)	Dose	Patient Eligibility	Patients (Controls)	Planned Outcomes	Results	Reference(s)
	Unspecified	Intracortical	G-CSF 300 µg/daily SC x 3 days followed by isolation of CD133+ cells by leukapheresis Bilateral injections into frontal motor cortex 3-4 cm from midline	EEC: any FVC: > 30%	67 (0) Appears to have included patients previously reported in Martinez 2009[61]	Primary: Safety (not clearly specified)	2 subject deaths in post-operative period (respiratory failure, MI/SDH); otherwise procedure was well tolerated	Martinez (2012) [62]
Bone marrow derived MSCs	Phase I/II	Intrathecal (10 patients) Intrathecal + Intravenous (9 patients)		EEC: D Age: 25-65 At least 5-point decline in ALSFRS in one year	19 (0)	Primary: Safety analysis	No safety concerns	Karussis (2010) [38]
	Pilot	Intrathecal	Intrathecal administration at L2-3 or L3-4	EED: D, Pr, Pr-LS Age: > 18	10 (0)	Primary: ALSFRS-R at day 90, 180, 270, 365 Secondary: ALSFRS-R subscores, time to 4-point worsening, survival	Trend towards stabilization of ALSFRS-R (individual patient characteristics are not reported such as disease duration which could impact interpretation) No safety concerns	Prabhakar (2012) [63]
	Phase I	Intrathecal (8 patients) Intravenous (6 patients)	2×10^6 cells/kg	EEC: D ALSFRS-R 24 FVC 40%	14 (0)	Primary: Safety Secondary: AALSFRS-R, FVC, MRI	1 subject death; 1 lost to follow up per group No safety concerns	Nabavi (2019)[64]
	Phase I/IIa	Intrathecal	$15 \pm 4.5 \times 10^6$	EEC: D Age: 18-65 Riluzole naïve or stable dose for 2 mo Life expectancy 2 yr	26 (0)	Primary: Safety Secondary: ALSFRS-R, FVC, weakness scales	No safety concerns Reduction in ALSFRS-R decline at 3 and up to 6 mo; FVC stable of above 70% for 9 mo in 80% subjects; stable weakness scales at 3 mo in 75% patients	Syokva (2017)[65]
	Phase I	Intrathecal	$7.89 \pm 5.77 \times 10^6$ Lin- cells administered at L3/4 or L4/5	EEC: D Age: <65 Survival prognosis 12 mo	12 (0)	Primary: Safety Secondary: CSF/plasma microRNAs, Norris scale, ALSFRS-R	No safety concerns Decreased inflammatory proteins Effects on progression correlated with higher Lin- doses	Sobus (2017)[66]
	Phase I	Intrathecal	1×10^6 cells/kg x 2 injections 26 days apart	EED: D, Pr Age: 25-75 ALSFRS-R 31-46 Stable Riluzole dose >3 mo Disease duration 5 yr FVC >40%	8 enrolled but only 7 completed treatment (0)	Primary: Safety Secondary: ALSFRS-R, Appel score, FVC	1 subject death before treatment; no acceleration in ALSFRS-R decline rates No safety concerns	Oh (2015)[67]
	Phase II	Intrathecal	1×10^6 cells/kg x 2 injections 26 days apart	EED: D, Pr Age: 25-75 ALSFRS-R 31-46 Stable Riluzole dose >3 mo Disease duration 5 yr FVC >40%	33 (31)	Primary: Safety, ALSFRS-R Secondary: CSF biomarkers, survival	No safety concerns Reduced ALSFRS-R rate changes from baseline Reduced proinflammatory and increased antiinflammatory cytokines; TGFβ1 inversely correlated with MCP-1 in good responders No survival difference	Oh (2018)[68]
	Phase III	Intrathecal	3 bimonthly injections of MSCs induced to secrete NTFs or placebo		100 estimated (100)	Primary: Safety, efficacy Secondary: CSF/serum neurotrophic factors, inflammatory factors, cytokines	Study in process, results not yet reported	Clinical Trial

Stem Cell Type	Clinical Trial Phase	Delivery Method (target)	Dose	Patient Eligibility	Patients (Controls)	Planned Outcomes	Results	Reference(s)
	Phase II	Intramuscular, Intrathecal	MSCs induced to secrete NTFs	EEC: D, Pr; Pr<LS, Po Age: 18-75 Disease duration < 24 months ALSFRS-R > 30 SVC > 65%	48 estimated (0)	Primary: Safety Secondary: change in ALSFRS-R, change in SVC	Study in process, results not yet reported	Clinical Trial
	Phase I/II, Phase IIa	Intramuscular or Intrathecal (Phase 1/2) Intramuscular +Intrathecal (Phase 2a)	MSCs induced to secrete NTFs 24 × 10 ⁶ cells intramuscular or 1 × 10 ⁶ cells/kg intrathecal Intramuscular/Intrathecal Low: 24×10 ⁶ /1×10 ⁶ /kg Mid: 36×10 ⁶ /1.5×10 ⁶ /kg High: 48×10 ⁶ /2×10 ⁶ /kg	EEC: D, Pr; Disease duration < 24 months Phase 1/2: ALSFRS-R >30 for intramuscular; between 15-30 and FVC > 50% intrathecal Phase 2a: ALSFRS-R >30; FVC > 50%	26 (0) 6 patients: Intramuscular 6 patients, Intrathecal 14 patients: Intramuscular +Intrathecal	Primary: Safety Secondary: ALSFRS-R, FVC	No safety concerns Progression declines in ALSFRS-R and FVC reduced in intrathecal and in intramuscular + intrathecal groups	Petrou (2016)[80]
	Case report	Intraventricular	1 × 10 ⁷ cells/kg	Case report of 63-year-old with definite ALS by EEC	1 (0)	Authors state ALS was too advanced to assess efficacy	No safety concerns.	Baek (2012)[75]
	Phase I	Intraspinal	Cells injected into thoracic cord (central part of spinal cord) 1 mm apart in 3 rows spaced by 3 mm	EEC: D Ages: 21-75 Disease duration: 6-96 months Mild to severe functional impairment at spinal level No or mild bulbar involvement No respiratory failure	7 (0) (+ 2 patients under compassionate use)	Primary: ALSFRS-R, Norris score, FVC every 3 months following 6-month lead in period	4 patients showed a reduction in the ALSFRS-R and FVC decline No safety concerns	Mazzini (2006) [70] Mazzini (2008)[36] Mazzini (2012) [71]
	Phase I	Intraspinal	Same methods as 2006 study[70]	EEC: D, Pr Ages: 20-65 Disease Duration: < 3 years FVC: > 50% FALS; excluded Onset: spinal	10 (0)	Primary: ALSFRS-R, MRC, respiratory assessment, MUNE, neurophysiological index, MRI, DTL, safety	No change in the rate of decline of clinical measures No safety concerns	Mazzini (2010) [69] Mazzini (2012)[71]
	Phase I	Intraspinal	T3-4 injections 1-2.5 mm from midline at depth of 6 mm	EEC: D Ages: 20-65 Disease duration: 6-36 months FVC: > 50% Onset: Spinal	11 (0)	Primary: Safety Secondary: FVC, ALSRFS-R, MRC, Norris scale	No safety concerns No changes in disease progression	Blanquer (2010) [104] Blanquer (2012)[72]
	Phase II	Intraspinal	C1-2 laminectomy and multiple injections at these levels	Disease duration: > 6 months FALS; excluded Rapid decline, FVC in terminal period (on mechanical ventilator or unable to speak)	13 (0)	Primary: not specified	No safety concerns Authors report 7 of 13 patients improved post procedure (no clear criteria for assessment)	Deda (2009)[74]
	Pilot	Intraarterial	T-cell vaccination every 28 days for 10 doses Bone marrow harvest, following purification, one aliquot given to patient on same day “by selective intralesion infusion into the feeding artery” MSCs differentiated into NSCs and given intraarterial	Disease duration 3-5 years	7 (0), only 5 completed full regimen	Primary: ALSFRS-R	No safety concerns Results not well reported, no apparent change in disease progression	Moviglia (2012) [79]
Adipose derived MSCs	Phase I	Intrathecal	Dose escalation over 5 groups receiving between 1 × 10 ⁷ cells in 1 dose up to 1 × 10 ⁸ cells in 2 monthly doses	EEC: D FVC >65% Disease duration between 1-2 years	27 (0)	Primary: Safety Secondary: ALSFRS-R, MRI, CSF/blood	Positive safety profile Side effects related to increased protein and nucleated cell levels in CSF and thickened lumbosacral nerve roots 4 subject deaths; autopsy revealed no tumors Efficacy testing in progress (see)	Staff (2016)[81]

Stem Cell Type	Clinical Trial Phase	Delivery Method (target)	Dose	Patient Eligibility	Patients (Controls)	Planned Outcomes	Results	Reference(s)
	Phase II	Intrathecal	1 × 10 ⁸ cells every 3 mo for 12 mo Dose reductions may occur for subsequent doses per Dose Modification Rules	EEC: any Age: 18 Disease duration <2 y Stable or no Riluzole 30 d	60 estimated (0)	Primary: Safety Secondary: ALSFRS-R	Study in process, results not yet reported	Clinical Trial
OECs	Pilot	Intracortical	OECs extracted from human fetal olfactory bulb tissue 2 million OECs injected into bilateral corona radiata	EEC: D, Pr Age: 20-70 ALSFRS-R 15	15 (20) Controls not randomized, rather first 15 patients served as cases and next 20 as controls; no formal matching No patients share nationality of the study (China)	Primary: ALSFRS-R at four months provided by patient, caregiver, and family member	Rate of decline between month 3 and 4 was slower for the treatment group compared to control Authors do not report presence/absence of adverse events, however, additional safety and efficacy outcomes reported in references [82, 83, 84](see text)	Huang (2008)[47]
	Unspecified	Intracortical, Intraspinal	intraspinal injections not standardized, reported to occur at impaired segments Authors suggest all patients received intraspinal injections, although the 2007 report suggest some patients only received intracortical injections	EEC: D, Pr Age: > 18	507 (0) Intracortical only: 35 patients second injections; 5 patients 3 injections; 1 patient 4 injections; 1 patient 5 injections	ALSFRS-R, Norris scale, video recordings of patients, EMG, PFT	Authors report improved ALSFRS-R and respiratory measures after each treatment, although a progressive decline in ALSFRS-R continued	Chen (2007)[105] Chen (2012)[48]
NPCs	Phase I	Intraspinal	Dose escalation Patients immunosuppressed with basiliximab mycophenolate mofetil, and tacrolimus and received tapering steroid dose following injections	Disease severity changed during trial to enroll less severely affected patients in later groups	15 (0)	Primary: Safety	No safety concerns Possible slowing of disease progression in patients without bulbar symptoms early in disease course, however, number of subjects fulfilling this criteria is small	Riley (2012)[85] Glass (2012)[86] Riley (2014)[87] Tadesse (2014)[89] Feldman (2014) [88]
	Phase II	Intraspinal	Dose escalation Same immunosuppression regimen as phase I	EEC: D, Pr, Pr-LS Age: > 18 FVC: > 60% seated, > 50% supine SALS or FALS	15 (0)	Primary: Safety Secondary: attenuation of motor function loss, maintenance of respiratory capacity, stabilization of ALSFRS-R, reduction of spasticity/rigidity, graft survival	No safety concerns 1 post-op deterioration in neurological function, 1 central pain syndrome No effect on progression rates versus historical controls Improved functional and survival outcomes using ALS/SURV measure relative to historical controls after longer-term follow up	Glass (2016)[90] Goutman (2018) [91]
	Phase I	Intraspinal	3 unilateral lumbar injections (3 patients) 3+3 bilateral lumbar injections (3 patients) NPC source from natural miscarriages	EEC: D, Pr Age: 20-75 Non-ambulatory Evidence of progression in last 6 mo No apnea; O ₂ sat 90%FVC >60%	6 (0)	Primary: Safety Secondary: ALSFRS-R, FVC	No safety concerns No increase in disease progression rates through 18 mo Three patient deaths	Mazinni (2015) [92]
	Phase I	Intraspinal	NPCs modified to express GDNF Two doses delivered unilaterally to the lumbar region	EEC: D, Pr, Pr-LS Disease duration 36 mo Lower extremity weakness FVC >60% Age: 18 Stable or no Riluzole 30 d	18 estimated (0)	Primary: Safety Secondary: CMAP, ATLAS, quantitative muscle MRI, EIM; CSF GDNF levels	Study in process, results not yet reported	Clinical trial

ALSFRS-R, ALS Functional Rating Scale Revised; ATLAS, Accurate Test of Limb Isometric Strength; CMAP, compound muscle action potential; Cr, creatine; D, Definite; EEC, El Escorial Criteria; EIM, electrical impedance myography; FALS, familial ALS; FVC, forced vital capacity; GDNF, glial-derived neurotrophic factor; HLA, human leukocyte antigen; IV, intravenous; MMT, manual muscle strength; MRS, magnetic resonance spectroscopy; MSC, mesenchymal stromal cells; NAA, N-acetylaspate; NI, neurophysiological index (NI = CMAP amplitude x F-wave persistence / distal motor latency in ulnar nerves); NPC, neural progenitor cell; NSC, neural stem cells; NTF, neurotrophic factors; OECs, olfactory ensheathing cells; Po, Possible; Pr, Probable; Pr-LS, Probable-Laboratory Supported; QoL, quality of life; SALS, sporadic ALS; SVC, slow vital capacity

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Table 2.

Summary of Stem Cell Sources for ALS Clinical Trials

Stem cell type	Source	Advantages	Disadvantages
G-CSF induced PBSCs	G-CSF injection mobilizes BM CD34+ HSCs into the peripheral blood stream to generate PBSCs	<ul style="list-style-type: none"> • Noninvasive s.c. G-CSF administration • Readily available source • Does not require immunosuppression • G-CSF may be neuroprotective • Demonstrated preclinical efficacy 	<ul style="list-style-type: none"> • CNS penetration uncertain • PBSC administration into the CNS is invasive
BM-MSCs	BM aspirate followed by MSC enrichment and ex vivo expansion	<ul style="list-style-type: none"> • Readily available source • Autologous and do not require immunosuppression • Neuroprotective by secretion of neurotrophic, anti-inflammatory, and anti-apoptotic factors • Lower risk of malignant transformation • Demonstrated preclinical efficacy 	<ul style="list-style-type: none"> • BM aspiration is relatively invasive • BM-MSC administration by most common intrathecal or intraspinal routes is relatively invasive
AD-MSCs	Liposuction or lipectomy followed by MSC enrichment and ex vivo expansion	<ul style="list-style-type: none"> • Readily available source • Relatively noninvasive harvesting procedure • Autologous and do not require immunosuppression • More abundant in WAT than MSCs in BM • Demonstrated preclinical efficacy 	<ul style="list-style-type: none"> • AD-MSC administration by only route reported to date, intrathecal, is relatively invasive
OECs	Primary isolation from human fetus OB with/without HLA matching	<ul style="list-style-type: none"> • Possess axonal protective and regenerative properties, and secrete neurotrophic factors • Demonstrated preclinical efficacy 	<ul style="list-style-type: none"> • Not a readily available source • Non-autologous and requires immunosuppression • OEC administration by intracortical or intraspinal routes is invasive • Ethical dilemma present
NPCs, NSI-566RSC cell line	Cell line derived from the umbilical cord of human fetus in accordance with guidelines from the FDA, NIH, and an independent ethics review board	<ul style="list-style-type: none"> • Readily available source • Possess capacity to differentiate into neural or glial cells • Lower risk of malignant transformation • Demonstrated preclinical efficacy 	<ul style="list-style-type: none"> • Non-autologous and requires immunosuppression • NSI-566RSC administration by intraspinal route is invasive

AD-MSCs, adipose-derived mesenchymal stem cells; BM, bone marrow; BM-MSCs, bone marrow-derived mesenchymal stem cells; CNS, central nervous system; FDA, Food and Drug Administration; G-CSF, granulocyte colony-stimulating factor; HLA, human leukocyte antigen; HSCs, hematopoietic stem cells; NIH, National Institute of Health; NPCs, neural progenitor cells; OB, olfactory bulb; OECs, olfactory ensheathing cells; PBSCs, peripheral blood stem cells; s.c., subcutaneous; WAT, white adipose tissue