Motor neuron replacement therapy for amyotrophic lateral sclerosis

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Abstract
Amyotrophic lateral sclerosis is a motor neuron degenerative disease that is also known as Lou Gehrig’s disease in the United States, Charcot’s disease in France, and motor neuron disease in the UK. The loss of motor neurons causes muscle wasting, paralysis, and eventually death, which is commonly related to respiratory failure, within 3–5 years after onset of the disease. Although there are a limited number of drugs approved for amyotrophic lateral sclerosis, they have had little success at treating the associated symptoms, and they cannot reverse the course of motor neuron degeneration. Thus, there is still a lack of effective treatment for this debilitating neurodegenerative disorder. Stem cell therapy for amyotrophic lateral sclerosis is a very attractive strategy for both basic and clinical researchers, particularly as transplanted stem cells and stem cell-derived neural progenitor/precursor cells can protect endogenous motor neurons and directly replace the lost or dying motor neurons. Stem cell therapies may also be able to re-establish the motor control of voluntary muscles. Here, we review the recent progress in the use of neural stem cells and neural progenitor cells for the treatment of amyotrophic lateral sclerosis. We focus on MN progenitor cells derived from fetal central nervous system tissue, embryonic stem cells, and induced pluripotent stem cells. In our recent studies, we found that transplanted human induced pluripotent stem cell-derived motor neuron progenitors survive well, differentiate into motor neurons, and extend axons into the host white matter, not only in the rostrocaudal direction, but also along motor axon tracts towards the ventral roots in the immunodeficient rat spinal cord. Furthermore, the significant motor axonal extension after neural progenitor cell transplantation in amyotrophic lateral sclerosis models demonstrates that motor neuron replacement therapy could be a promising therapeutic strategy for amyotrophic lateral sclerosis, particularly as a variety of stem cell derivatives, including induced pluripotent stem cells, are being considered for clinical trials for various diseases.

Key Words: amyotrophic lateral sclerosis; motor neuron replacement; neural progenitor/precursor cells; neural stem cells; stem cells

Introduction
Amyotrophic lateral sclerosis (ALS), one of the most common progressive neurodegenerative diseases, was first identified by Sir Charles Bell in 1830, and was fully characterized by Jean-Martin Charcot in 1874. The disease is also known as Lou Gehrig’s disease in the USA, Charcot’s disease in France, and motor neuron (MN) disease in the UK. The average incidence rate of ALS worldwide is about 1/50,000 per year, which equates to more than 100,000 new diagnoses per year (Norris et al., 2020). The loss of MNs can occur within both the lower spinal cord and upper cortical circuits. Because these neurons transmit signals between the brain and the voluntary muscles, the loss of MNs can lead to deterioration of motor control. Symptoms include progressive muscle wasting, paralysis, eating and breathing difficulties, and muscle atrophy, all of which eventually lead to death related to respiratory failure within 3–5 years of disease onset (Atassi et al., 2016; Chen et al., 2016). A small fraction (~5–10%) of ALS cases are familial, and are associated with pathogenic gene mutations in C9ORF72, Cu/Zn superoxide dismutase 1 (SOD1), TAR DNA-binding protein, FUS and ubiquilin 2. However, the vast majority (~over 90%) of ALS cases are considered sporadic, and are the result of genetic load, aging and environmental exposure in susceptible individuals (Hardiman et al., 2017; van Es et al., 2017).

At present, no treatment is available to reverse the pathological progression of ALS. Two drugs with very modest effect on survival

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have been approved by the US Food and Drug Administration (FDA): Rilutek (riluzole) and Radicava (edaravone). Rilutek was approved by the FDA in 1995 and has been listed in Canada, Australia, Europe and other countries. Rilutek is thought to interfere with the activity of glutamate, one of the chemical messengers that transmit signals between nerve cells. Excess glutamate can be toxic in the brain and spinal cord, as in ALS (Mignani et al., 2020; Calabrese et al., 2021). Radicava was approved by the FDA in 2017 and by Health Canada in 2018, based on findings from Study 19 (2011-NCT01492686), a double-blind phase 3 clinical trial in Japan that assessed the safety and efficacy of Radicava in people with ALS. In this clinical trial, participants on Radicava experienced a significantly slower decline (by 33%) in their ability to perform everyday activities, compared with those given placebo. Currently, the treatment is only available in the United States, Canada, Japan and South Korea, although the European drug administration is also considering its use. Radicava is thought to counteract the excessive oxidative stress in ALS (Jackson et al., 2019).

Stem cell therapy for ALS continues to be a hot topic in both basic and clinical research. No current drug or non-drug treatment for ALS has stimulated as much anticipation as stem cell therapies. Neuroprotection is one of the major objectives of stem cell therapy in ALS clinical trials. Stem cells can provide immunomodulation, secrete growth factors, and produce supporting cells, such as astrocytes and oligodendrocytes, or interneurons, which may provide a supportive environment for remnant damaged MNs (Atassi et al., 2016; Berry et al., 2019). Neuronal replacement is another promising therapeutic strategy for ALS. Here, the transplanted stem cells or stem cell-derived neural progenitor cells can directly replace the dying or dead MNs in the host and re-establish the reciprocal connections to restore the motor control of voluntary muscles in ALS (Abati et al., 2019b; Forostyak et al., 2020). However, for ALS, there are still no approved stem cell therapies, although there are a few ongoing stem cell clinical trials (2021-NCT02478450, 2020-NCT03296501, 2018-NCT03482050). In this review, we will cover the progress and results of these trials using stem/progenitor cells derived from a variety of sources. We will also review our own work and provide a historical perspective on how and why stem cells are considered a potential treatment option for patients with this incurable neurodegenerative disease.

The challenges in this approach revolve around the delivery and source of stem cells. Obviously, the location of stem cell delivery will affect the therapeutic effect. Several preclinical studies have reported transplantation of glial cell line-derived neurotrophic factor (GDNF)-secreting neural progenitor cells into SOD1G93A rats. In one study, neural progenitor cells were injected into four unilateral sites in the lumbar L1/L2 spinal cord in rats at the pre-symptomatic stage (70 days of age). Surprisingly, despite the survival of some MNs 2–6 weeks after transplantation, neither MN–muscle contact nor improvements in ipsilateral hindlimb function could be observed (Suzuki et al., 2007). In contrast, transplantation protects MNs and restores function when GDNF-secreting neural progenitor cells are injected into four unilateral sites at the cervical C3 and C6 spinal cord levels in the early to mid-symptomatic stage at 110–120 days of age (Nichols et al., 2013; Zalfa et al., 2019). In addition to spinal cord transplantation, human cortical-derived GDNF-secreting neural progenitor cells injected into 20 sites in the motor cortex of SOD1G93A rats at the pre-symptomatic stage at ~80 days of age prevents degeneration of spin al MNs, delays disease pathology, improves function, and increases life span (Thomsen et al., 2018). With the development of new stereotactic devices, it is feasible to transplant neural precursor cells into the brain parenchyma. Nonetheless, the benchmarks transplant routes for ALS treatment need to be carefully examined. In future cell therapy studies, the impact of the delivery route should be carefully assessed.

Over the past few decades, various cell sources such as neural stem cells (NSCs) isolated from embryonic central nervous system tissue (brain and spinal cord), mesenchymal stem cells (MSCs), hematopoietic stem cells and embryonic stem cells (ESCs) have been employed to treat ALS patients (Figure 1). Recently, induced pluripotent stem cells (iPSCs) derived from adult human tissues have been employed to treat FOG1- or induced pluripotent stem cells (iPSCs, reprogrammed from peripheral blood mononuclear cells (PBMCs)). The iPSCs can be directly used for transplantation or following one more step that specifies the cells to lineage-specific precursor cells, such as gial-restricted precursors (GRPs) and neuronal precursor cells.

Fetal Central Nervous System-Derived Neural Stem/Progenitor Cells

Fetal human CNS tissue contains a population of neural stem/progenitor cells primed to differentiate into neurons, astrocytes and oligodendrocytes (Lyman et al., 1991; Mazzini et al., 2015; Ferrari et al., 2018). In 1977, the first evidence was presented that grafts of fetal brain tissue into the adult CNS could counteract an experimentally-induced neurological deficit (Nygren et al., 1977). Then, in 1983, the first successful transplantation of fetal spinal cord into the adult spinal cord was reported (Patel and Bernstein, 1983).

After the success of fetal CNS tissue-based transplantation, NSCs were isolated from fetal CNS tissues and cultured in vitro. By using growth factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), fetal CNS-derived neural stem/progenitor cells could be maintained and passaged long-term without losing stemness (Shihabuddin et al., 1999). In animal studies, these cells were shown to differentiate mostly into interneurons and less frequently into astrocytes that release numerous growth factors (Suzuki et al., 2007; Nichols et al., 2013). It was also reported that fetal-derived stem/progenitor cells originally isolated from neurogenic and non-neurogenic zones could be delivered into the spinal cord to protect MNs in animal models (Baloh et al., 2018).

The generation of cholinergic MNs in MN replacement therapy is considered of fundamental importance in ALS treatment. In an early study published in 2002, Wu et al. reported a new in vitro priming procedure that involves treatment of NSCs (K048) derived from the cortex of an 8-week legally-aborted human fetus with numerous chemical compounds and growth factors, including bFGF, EGF, leukemia inhibitory factor, mouse sonic hedgehog amino-terminal peptide, all-trans retinoic acid, NGF, BDNF, NT-3, NT-4, natural mouse laminin and heparin (Wu et al., 2002). With this manipulation, almost all fetal brain-derived NSCs differentiate into a nearly pure population of neurons after grafting into the adult rat CNS. Furthermore, the transplanted cells differentiate into cholinergic MNs in a region-specific manner, particularly in the spinal cord ventral horn, an area where MNs are lost in ALS patients. These studies have brought neuronal replacement therapy one step closer to being a potential candidate to treat ALS in upcoming clinical trials.

In another study, human gial-restricted progenitor cells (hGRPs) were tested in both transgenic rat and mouse ALS models carrying the hSOD1G93A mutation for MN protection (Howland et al., 2002; Lepore et al., 2011). In this case, specific growth factors such as bFGF...
and platelet-derived growth factor-AA were used in the priming procedure. With this approach, fetal brain-derived cells lineage-specifically differentiate into GRPs that can further differentiate into astrocytes and oligodendrocytes. After transplantation of hGRPs into the cervical spinal cord of hSOD1G93A mice, the cells survived and migrated in both gray and white matter, and differentiated into astrocytes. However, the transplantation neither protected MNs nor improved motor function (Lepore et al., 2011). In contrast, in a paper previously published by the same group, several therapeutic effects, including MN protection, delay in respiratory failure and slowed disease progression, were reported for rat-derived GRP-based therapy in the hSOD1G93A rat model (Lepore et al., 2008). Notably, these two GRP-based strategies share common features, including common cell types, delivery routes and intervention times (6–4 bilateral injection sites at C4–C6 or C4–C5 ventral horn in the hSOD1G93A rat or the hSOD1G93A mouse at 90 or 50–60 days of age). This suggests that the immune response may play a critical role in the effectiveness of these therapeutic approaches, particularly as one is a xenograft and the other is an allograft. Nevertheless, the therapeutic potential of GRPs is significant, as is evidenced by the key role of glial cells in disease and neuronal survival in SOD1 ALS animal models.

Several trials using expanded human fetal-derived stem/progenitor cells to treat ALS have been completed in Europe (Mazzini et al., 2015; Zalfa et al., 2019) and the United States (Glass et al., 2012, 2013; Lepore et al., 2015). Importantly, one of these trials was intended to replace lost MNs, which would be impractical because the new MNs would need to connect to distant target muscles. Instead, the investigators aimed to replace the interneurons and astrocytes surrounding the dying MNs, release potent growth factors into degenerated or degenerating areas, and/or provide immunomodulatory effects to reduce toxic inflammation or some other detrimental process. In addition to modulating the microenvironment, another rationale of neuronal cell transplantation is that once new synaptic connections are established between engrafted neurons and host degenerating MNs, the enhanced electrophysiological activity afforded by the newly-formed synapses may slow down the pathological degeneration of MNs in ALS. The largest among these trials was a phase 1 and 2 trial of intraspinal transplantation of cells derived from human fetal spinal cord tissue, with 15 participants in three centers, and was supported by Neuralstem Inc. and the National Institute of Neurological Disorders and Stroke in the USA (Glass et al., 2016).

In one study, NSCs/progenitor cells were extracted from the brain of an 8-week-old fetus, amplified in culture, and frozen in varying doses. MNs were successfully induced from these CNS-derived progenitor cells (Guo et al., 2010). Another source is the fetal cortex, which generates protective astrocytes when transplanted into the spinal cord of rodents. These cells have been genetically engineered to express GDNF, which has a protective effect on MNs in animal models of hSOD1G93A (Kapur et al., 2007; Nichols et al., 2013). This combination therapy strategy is currently being evaluated in a single-center phase 1 and 2a dose-escalation clinical trial in California (2017-NCT02943850). In this trial, Baloh et al. (2018) transplanted cells into the lumbar spine in 18 patients (as of October, 2019). The primary outcome measures are safety and tolerability.

A phase 3 study (2017-NCT03288056) was recently completed on October 30, 2020. In this trial, 261 participants were recruited at six U.S. sites to evaluate the efficacy and safety of BrainStorm Cell Therapeutics’ candidate NurO Wrong, which is an autologous neurotrophic factor-secreting mesenchymal stromal cell. In February, 2021, the FDA concluded in a preliminary review that the data were insufficient to support approval of the treatment. Nevertheless, NurO Wrong is still considered one of the most promising therapeutic candidates for clinical application for ALS.

Despite the substantial progress over the past few years, cell therapies based on fetal CNS-derived NSCs/progenitor cells must still overcome a number of limitations before clinical application. These include relatively high immunogenicity, low engraftment success and low survival rate after transplantation. In addition, it remains a challenge to generate phenotype-specific neuronal progenitor cells, such as MNs and MN-associated interneurons, for MN replacement therapy. Moreover, ethical concerns and the limited supply of human fetal tissue are additional hurdles to clinical translation.

### Embryonic Stem Cell-Derived Neuronal Progenitor Cells

ESCs show unlimited capacity for self-renewal and can be expanded indefinitely in culture. Notably, ESCs have the potential to differentiate into any cell type of the three germ layers, including NSCs with neuronal or glial fates. Therefore, they have great therapeutic potential for degenerative diseases, such as in regenerative medicine or replacement of MNs in ALS. However, it remains controversial whether MNs derived from ESCs can exert beneficial effects in animal models of ALS (Deshpande et al., 2006; Lopez-Gonzalez et al., 2009). Because of the ethical concerns as well as difficulties in generating high-purity lineage-fate-specific cell lines with tolerable risk of tumorigenesis, the clinical application of ESCs is still limited.

In 2004, Harper et al. generated spinal MNs from mouse ESCs to evaluate the developmental potential of these cells in vitro and they further examined their capacity to replace MNs in the adult mammalian spinal cord. In this study, 5–7-week-old male Lewis rats were given intracranial injection of rat-adapted neuroadapted Sindbis virus (NSV) to damage the resident MNs. These rats then served as hosts for transplant of MN-fated mouse ESCs into the spinal cord. One month later, over 3000 mouse ESC-derived MNs (~25% of input) survived (Harper et al., 2004). However, transplant-derived neurons extend very few axons into the host white matter. An inhibitory effect of white matter myelin on ESC-derived neuronal axonal growth has been reported. This result, however, is not consistent with our own observations. In our recent studies, we reported that ESC-derived motor neurons survived in adult host white matter (Lu et al., 2012, 2014, 2017; Poplawski et al., 2018). Nevertheless, the inhibitory effect of myelin can be overcome by treatment with dibutyryl cAMP (dbcAMP) or Y27632 (a Rho kinase inhibitor) both in vitro and in vivo. Importantly, in the dbcAMP-treated group, but not in the non-dbcAMP-treated group, ESC-derived motor axons extended into the ventral roots of the spinal cord (Harper et al., 2004). These results suggest that ESC-derived neural stem/progenitor cells have the capacity to replace lost or dying MNs in ALS and other MN degeneration-associated diseases.

To investigate whether transplanted MNs exert a beneficial effect in MN degenerative diseases by replacing host MNs or preventing their degeneration, MNs differentiated from mouse ESCs were transplanted into the cervical spinal cord of wild-type (WT) and hSOD1G93A rats at 10 weeks, i.e., during the pre-symptomatic stage. MNs were identified by expression of a green fluorescent protein (GFP) marker under control of the promoter for hB9, a MN-specific gene (Lopez-Gonzalez et al., 2009). These motor neuronal-like grafted cells can survive for at least 1 week in hSOD1G93A animals. However, neither grafted GFP neurons nor the endogenous choline acetyl transferase (ChAT)/Hoechst/GFP MNs survived in either sham or grafted SOD1G93A rats in the long-term. In contrast, in WT rat spinal cords of the same age, grafted GFP MNs were detected. The loss of both host and transplanted MNs is correlated with a sudden decrease in motor performance from week 16 onwards. These results indicate that the environment in transgenic hSOD1G93A animals is antagonistic to both endogenous and grafted MNs, raising the concern of whether the direct replacement of lost MNs can reverse the pathological progression of ALS (Lopez-Gonzalez et al., 2009).

### Application of Induced Pluripotent Stem Cells Derivatives in the Treatment of Amyotrophic Lateral Sclerosis

Cell therapy for ALS works mainly through cell replacement or supraphysiological effects to delay the progression of ALS. As described above, numerous animal and clinical studies of ALS have been carried out. Cell intervention therapy seems to have positive effects for ALS, but obtaining a stable and sufficient cell source is still a prerequisite for cell therapy in clinical trials. NPCs derived from embryonic neural progenitors or differentiated ESCs are the only cell type that can avoid the limitation of cell numbers or avoid ethical concerns. Obtaining enough cells for transplantation is still a key challenge in the field. The generation of iPSCs reprogrammed from somatic cells (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008) has afforded an opportunity for the development of autologous cell replacement therapy. Stem and Daley, 2012; Yamanaka, 2012; Okano et al., 2013; Glicksman, 2018). With the development of transdifferentiation manipulation, the starter cell types and reprogramming methods have
progressed (Loh et al., 2009; Malik and Rao, 2013; Isogai et al., 2018), the differentiation efficiency has been improved, and the generation of iPSCs has been made simpler. iPSCs are essentially infinitely proliferative and can be stored and differentiated into any type of cell. This feature solves the cell source problem and provides an unprecedented opportunity for autologous transplantation. However, obtaining autogenous iPSCs from patients takes a long time and is costly. To overcome these difficulties, several groups are currently trying to bank clinical-grade iPSC lines from human leukocyte antigen (HLA)-compatible donors (Taylor et al., 2005; Azuma and Yamanaka, 2016), and clinical trials are also being carried out (Morziane, 2019).

Although iPSCs are a promising cell type for cell replacement therapy, they carry an enormous risk of tumor formation. The risk of tumorigenesis is associated with three key factors. The first is that cell differentiation may not be complete. After the undifferentiated/immature cells are transplanted, they may undergo tumor MNs. Second, because the reprogramming factors may remain active in iPSCs, they may promote the occurrence of tumor. Third, tumorigenesis may be caused by genetic mutations that occur during the reprogramming or culture processes (Yamanaka, 2020). Accordingly, numerous studies have been carried out to improve iPSC reprogramming technology, and the emergence of new non-integrated methods, animal component-free culture and innovative virus-free strategies have greatly reduced the risk of tumorigenesis and enhanced the therapeutic applicability of iPSCs (Stadtfeld et al., 2008; Fusiaki et al., 2009; Ban et al., 2011; Malik and Rao, 2013; Hamada et al., 2020). Mandai et al. (2017) conducted the first clinical trial with iPSC-derived MNs that were generated using a commercially available permissive microenvironment. This may account for why ESC and iPSCs are widely used as cell replacements for LD (Lunn et al., 2014; Goutman et al., 2019). In studies in which human iPSC-derived MNs or their progenitors can be used not only for neurological disease modeling and toxicology studies, but also have potential for MN replacement therapy for ALS. These MNs have greater than 85% purity as measured by immunostaining for the motor neuronal markers Isl1, Hb9 and ChAT (Figure 2A). In addition, iXCells™ hiPSC-derived MNs are functionally verified by neuromuscular junction assay (Figure 2B) and electrophysiological assays.

In a joint effort, iXCells Biotechnologies is collaborating with the Lu lab and the Chen lab to test these cells in ALS animal models. We have obtained MNs derived from hPSCs such as SODG93A transgenic and WT monkeys, and transplanted hPSCs-MN progenitor cells into the spinal cord ventral horn in immunodeficient rats. Nine rats received 0.25 μL of cells at a concentration of 100,000 cells/μL per site for a total of eight sites spanning 0.5 mm apart in both the right and left hemispheres at the C5 spinal cord using a pulled glass needle connected to a PicoSpritzer II (Lu et al., 2012). Animals survived 3 or 6 months post transplantation. Transplanted iXCells™ hPSC-derived MN progenitors labeled with GFP survived well in the host spinal cord, as verified by both GFP and human nuclei marker immunolabeling in a pilot study (Figure 3A). The engrafted cells could differentiate into mature ChAT™ MNs (Figure 3B), indicating preservation of MN fate and continuous maturation of MNs in vivo. Furthermore, transplanted MNs extended into a large number of nerve fibers following the motor axon tracts along the white matter of the spinal cord (Figure 3C). Notably, some of these nerve fibers were ChAT™, indicating that they are transplanted-derived motor axons. In addition, some of these fibers reached the spinal cord ventral border and even extended into the ventral roots. In addition, transplanted hPSC-derived MNs extended through the spinal nerve fibers following the motor axon tracts along the white matter of the spinal cord (Figure 3C). Notably, some of these nerve fibers were ChAT™, indicating that they are transplanted-derived motor axons. In addition, some of these fibers reached the spinal cord ventral border and even extended into the ventral roots. In addition, transplanted hPSC-derived MNs extended.
direct reprogramming of human blood mononuclear cells. These transplanted cells, researchers are now developing stable induced after transplant (Abati et al., 2019a; Wang et al., 2020). To overcome biomaterials to improve durability and performance in the host tissue gene engineering. Recently, researchers have encased graft cells in factors (VEGF, EPOR, GDNF and NT-3) can be upregulated by hypoxia, and Bcl-2), antioxidants (SOD2, catalase and Nrf2) and trophic microenvironment. For example, some anti-apoptotic genes (HIF1a by modifying the transplanted cells or improving the challenge is enhancing the survival of transplanted MN precursors. The survival rate of the transplanted cells can be improved negatively impacts transplanted grafts as well. Therefore, a major exacerbates the degeneration of endogenous MNs and inevitably or dying MNs, but also improve the ALS microenvironment to delay 2014). Thus, stem cell therapy may not only need to replace dead or even reverse disease progression. The hostile niche significantly involved in the progression of ALS (Zhao et al., 2013; Rodrigues et al., (Arbour et al., 2017; Rostalski et al., 2019; Izrael et al., 2020). Recent evidence suggests that both innate and adaptive immunity are also 6 months after transplantation, indicating good safety. We have also established ALS disease models of mice and monkeys. In the future, we will transplant hiPSC-MNs into these ALS animals to study the potential of MN replacement therapy for ALS.

Future Perspectives

The pathogenesis of ALS is not fully understood, and there are many pathogenic mechanisms that may affect the occurrence and development of the disease. Rodent studies have demonstrated that ALS may include a non-cell autonomous mechanism, with glial cells playing an important role in the onset and progression of ALS (Arbour et al., 2017; Rostalski et al., 2019; Izrael et al., 2020). Recent evidence suggests that both innate and adaptive immunity are also involved in the progression of ALS (Zhao et al., 2013; Rodrigues et al., 2014). Thus, stem cell therapy may not only need to replace dead or dying MNs, but also improve the ALS microenvironment to delay or even reverse disease progression. The hostile niche significantly exacerbates the degeneration of endogenous MNs and inevitably negatively impacts transplanted grafts as well. Therefore, a major challenge is enhancing the survival of transplanted MN precursors.

The survival rate of the transplanted cells can be improved by modifying the transplanted cells or improving the in vivo microenvironment. For example, some anti-apoptotic genes (HIF1a and Bcl-2), antioxidants (SOD2, catalase and Nrf2) and trophic factors (VEGF, EPOR, GDNF and NT-3) can be upregulated by hypoxia, neurotrophic factors or small molecule drug preconditioning or gene engineering. Recently, researchers have encased graft cells in biomaterials to improve durability and performance in the host tissue after transplant (Abati et al., 2019a; Wang et al., 2020). To overcome the strong immunogenicity that potentially hinders the survival of transplanted cells, researchers are now developing stable induced NSCs (iNSCs) as well as MNPs cells derived from iPSCs or through direct reprogramming of human blood mononuclear cells. These cells can be used to treat neurological diseases such as ALS (Rosati et al., 2018; Yuan et al., 2018). To overcome the adverse environment, combination therapy of MSCs and NSCs could also be a good choice for future clinical trials (Petrou et al., 2016; Berry et al., 2019). NSCs and MSCs are two promising cell types for potential clinical applications. The biological characteristics of MSCs and the results of clinical trials indicate that MSCs can be used as immunomodulators for ALS treatment, either through intravenous or intrathecal infusion (Bonafe and Mariotti, 2017; Tang, 2017; Gugliandolo et al., 2019). Furthermore, NSCs can be directly transplanted intraspinally to target degenerating MNs in the ventral horn. Implanted NSCs mainly differentiate into neurons and glia, such as astrocytes, which do not only ameliorate the toxic microenvironment surrounding MNs by releasing growth factors and immunomodulatory molecules, but may also replace degenerated MNs for the reconstruction of neural circuits (Abati et al., 2019a). For the application of MNP cells, the addition of niche-modulating cells, such as MSCs and astrocytes (Chandrasekaran et al., 2016; Izrael et al., 2018; Filippi et al., 2020), or co-transplantation of biomaterials carrying growth factors and neurotrophic factors (Moshayedi et al., 2016) might improve the efficacy of the treatment of ALS.

The second challenge is evaluating the engraftment of transplanted cells in clinical trials. The majority of published techniques to evaluate engraftment, such as immunofluorescent staining and fluorescent protein labeling, are difficult to implement in the clinical setting. In contrast to the lack of safe, non-invasive and time efficient technique(s) to monitor and assess the therapeutic efficacy of transplanted cells in ALS patients, molecular imaging techniques have been used for the tracking and evaluation of therapeutic outcomes in stem cell-based therapies (Klontzas et al., 2021) in cardiac diseases (Li and Hacker, 2017), Parkinson’s disease (Jang et al., 2020) and Alzheimer’s disease (Alipour et al., 2019; Klontzas et al., 2021). Thus, noninvasive imaging techniques such as magnetic resonance imaging, positron emission tomography and single photon emission computed tomography also have great potential to track the fate of transplanted cells in ALS patients.

The third challenge is establishing connectivity between transplanted MNs and remote downstream targets, such as muscles, glands and various organs, which requires extensive long-distance growth of motor axons. This is in contrast to development, where axons travel only for a short distance to reach their targets. Transplantation of MNs in the peripheral nerve close to their targets could be a shortcut for connectivity. Transplantation of interneurons in the peripheral nerve to relay the motor signals to their targets could be another strategy worthy of investigation.

The last challenge is selecting appropriate patients for enrollment. Ultimately, MN replacement therapy for ALS aims to protect endogenous MNs or even directly replace the lost or dying MNs. However, re-establishing these connections may require several months or even years. Thus, only patients with a medium or slow progression of disease should be included. In addition, because of the strong placebo effect, diverse progression rates and subtypes, double-blind design is crucial for ALS trials. An extreme example is contributed by the antibiotic minocycline. Animal studies and open-label human trials have shown that minocycline is beneficial for ALS. However, a larger placebo-controlled trial suggests that it is not, and may even be harmful (Lou et al., 2010). As intraspinal transplantation is an invasive treatment, persuading patients to be included in a double-blind trial is always a challenge.

Figure 3 | Transplanted MNPs differentiate into motor neurons and extend their axons along motor axon tracts.

Figure 4 | Induced pluripotent stem cells-derived motor neurons (MNs) survive and extend axons in the host.

(A) A diagram showing human induced pluripotent stem cell-derived MNs transplanted into 10 sites bilaterally between the C6–T2 vertebrae (red dots, transplant sites; T1 vertebra was removed by laminectomy). (B) MNs survived well in the spinal cord of adult immunodeficient rats 6 months after transplantation. Moreover, a large number of axons had projected to the caudal host spinal cord. (C) An enlarged view of the inset in B (red channel only) to show graft-derived axons in the caudal region. d: Dorsal; r: rostral; red: Stem121-labeled transplanted human cells; blue: 4′,6-diamidino-2-phenylindole (DAPI); scale bars: 250 μm in B, and 100 μm in C. Unpublished data.
As of the writing of this manuscript, the FDA granted investigational new drug trial approval for the use of ESC-derived dopaminergic neuronal precursor cells for the treatment of Parkinson's disease (Piao et al., 2021; Takahashi, 2021) after the investigators showed rigorous safety and efficacy data in preclinical studies. This may herald the advent of a new era of cellular therapy for neurological disorders. ESCs, iPSCs and their derivatives can be differentiated into MNs and non-supporting cells. After robust safety and efficacy testing, it is possible to see these cells being tested in a well-designed clinical trial in the near future.

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Abati E, Bresolin N, Comi G, Corti S (2019a) Advances, challenges, and perspectives in translational stem cell therapy for amyotrophic lateral sclerosis. Mo Neurobiol 56:6703-6715.

Abati E, Bresolin N, Comi G, Corti S (2019b) Preconditioning and cellular engineering of supporting cells. After robust safety and efficacy testing, it is possible to see these cells being tested in a well-designed clinical trial in the near future.

Author contributions: PL, CZ, and LZ designed the manuscript and finalized this review. BL and ML wrote the manuscript. All authors approved the final manuscript.

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