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# Stem Cell Application for Amyotrophic Lateral Sclerosis: Growth Factor Delivery and Cell Therapy

Masatoshi Suzuki, Chak Foon Tso and Michael G. Meyer
*University of Wisconsin-Madison*
*U. S. A.*

## 1. Introduction

### 1.1 ALS and the SOD1 rodent models

Amyotrophic lateral sclerosis (ALS) is a progressive disorder that leads to degeneration of upper and lower motor neurons, muscular atrophy, and (ultimately) death. A clinical diagnosis of ALS requires signs of progressive degeneration in both upper and lower motor neurons, with no evidence that suggest that the signs can be explained by other disease processes (Brooks et al., 1994, 2000). The incidence rate of the disease is around 2 in 100,000 people (Hirtz et al., 2007). The onset age of sporadic and most familial form of ALS is between 50-60 years, and is generally fatal within 1-5 years of onset (Cleveland & Rothstein, 2001). Riluzile is the only drug that demonstrates a beneficial effect on ALS patients, but only increases survival by a matter of months (Zoccolella et al., 2009).

Motor neuron cell death in ALS probably involves multiple pathways. Most ALS cases are sporadic in nature, while ~10% arise from a dominantly inherited trait (familial ALS or FALS) (Brown, 1995). The cause for sporadic ALS remains unclear, while 20% of FALS patients have a point mutation in the cytosolic $Cu^{2+}/Zn^{2+}$ superoxide dismutase 1 (SOD1) gene (Rosen et al., 1993). Recent reports suggested that other causes of FALS also include mutations in TDP-43 (the 43-KDa TAR DNA binding protein) and FUS (Fused in sarcoma/translocated in liposarcoma) genes (Ticozzi et al, 2011). From various lines of transgenic mice, we can observe that motor neuron disease is developed in mutants with elevated SOD1 levels (ex. hSOD1-G93A line), while no symptoms are observed in SOD1 knockout mice. The combined effect shows that SOD1 acts through a toxic gain of function rather than loss of dismutase activity (Julien et al., 2001). Both mouse and rat models over-expressing SOD1 genes show similar disease phenotypes and disease progression to those observed in human ALS patients (Gurney, 1994; Nagai et al., 2001; Howland et al., 2002).

The mechanism underlying motor neuron death in ALS is still unknown. However, SOD1 mutant induces non-cell-autonomous motor neuron killing by an unknown gain of toxicity, which means the gain of toxicity arises from damage to cells other than motor neurons (Boillée et al., 2006a). Multiple mechanisms account for the selective vulnerability of motor neurons including protein misfolding, mitochondrial dysfunction, oxidative damage, defective axonal transport, excitoxicity, insufficient growth factor signaling, and inflammation (Boillée et al., 2006a). Of course there are a lot of shortcomings for using

G93A and other SOD1 transgenic rodent models as SOD1 mutation is only found in a small proportion of human ALS patients. However, it is still an excellent tool for ALS researchers as transgenic mice have proven to be one of the most useful tools to understand the complexity of neurodegenerative diseases because of their usefulness to unveil underlying mechanisms of the disease and evaluating potential treatments (Rothstein, 2004). In this review we will overview the extensive use of SOD1 transgenic rodent models in ALS research and how those findings can be transferred to treat human ALS patients.

### 1.2 Chapter overview

Topics covered in this chapter include growth factor therapy and stem cell therapy for ALS. For growth factor therapy, we will introduce different delivery methods and injection sites. As for stem cell transplantation therapy, we will look into strategies that aim to replace or protect motor neurons. After that, we will summarize studies that utilize stem cells as a tool to deliver growth factors. We will conclude the chapter by looking forward to future development in the field.

## 2. Growth factors and gene therapy in ALS

### 2.1 Growth factors and the nervous system

Growth factors are a class of naturally occurring proteins that are capable of stimulating cell growth, proliferation, and differentiation. In development of the nervous system, they are crucial because they are essential for neuronal survival and differentiation. For adults, they are also required in some cases to maintain normal function of the nervous system, but only at very low levels. However, the presence of low levels of growth factors in adult tissues is critical because motor neurons rely on them for survival and repair upon stress and injury. Experiments have been performed to investigate the effect of growth factors on alleviating the symptoms of ALS. Those growth factors includes glial cell line-derived neurotrophic factor (GDNF), insulin growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), and brain derived neurotrophic factor (BDNF). For each of the growth factors listed above, there are studies on hSOD1-G93A transgenic rodent models that show some degree of improvement, which includes some or all of the following: delay onset, slow disease progression, decrease motor neuron loss, preserve neuromuscular junction and prolong survival.

### 2.2 Strategies of growth factor delivery

#### 2.2.1 Methods of delivery

Currently, three different methods of have been used to deliver the growth factor into the motor nervous system to ALS patients or rodent models. The first is subcutaneous injection of the growth factor protein. The obvious advantage of this method is the ease and simplicity to administrate. Some growth factors are pharmaceutically available to treat other neurodevelopmental diseases, such as IGF-1 to treat IGF deficiency in children. This is the reason why it is the only method of delivery that has been tested on human ALS patients. However, a statistically significant result has not been observed in this method of delivery. The only successful case is the North American study on IGF-1 in 1997 (Lai et al., 1997), but was immediately challenged by an almost identical study in Europe in 1998 (Borasio et al., 1998) and other later studies. The failure of this classical method of delivery to alleviate ALS symptoms includes (i) inability of some of the chemical of interest to pass the blood-brain

barrier; (ii) unwanted side effects in non-targeted sites, and (iii) a relative short half-life of the protein. The significance of these issues is amplified in the human nervous system because of greater cross-sectional area when compared to rodents. Further penetration is needed for the injected growth factor to reach the deep structure in the brain or spinal cord to give its desired effect. Similar issues are found in clinical trials for patients with Parkinson's disease using the same strategy to deliver growth factors.

The second method is to deliver the chemical of interest by implanting a catheter directly into the site of the brain that needs the growth factor, as seen in a couple Parkinson's disease studies (Gill et al., 2003; Slevin et al., 2005). It is better than the previous method as it overcomes the distance problem seen in large animals. However, there are a couple of drawbacks if this is applied to ALS patients to deliver the growth factor into the spinal cord instead of the brain for Parkinson's disease. The implanted catheter might interrupt the ascending and/or descending white matter track, and the natural movement of the spinal cord in patients increase the shearing forces may cause further damage. Therefore catheter delivery would not be a desirable method of ALS growth factor delivery.

The last approach uses viral vectors to circumvent all those issues. Those viruses include lentivirus (Cisterni et al., 2000; Hottinger et al., 2000; Azzouz et al., 2004), adenovirus (Acsadi et al., 2002; Hasse et al., 2007), and adeno-associated virus (AAV) (Kasper et al., 2003; Wang et al., 2002). They are used because of the ability to deliver genes to non-dividing cells, which includes mature neurons. Thus they are ready to be engineered to encode the therapeutic protein. Extensive studies of AAV delivery of potential drugs to specific brain regions have been published, suggesting viral vector delivery is a practical method.

### 2.2.2 Sites of delivery

Studies have been done to inject vectors encoding the growth factor of interest into two distinctive types of tissues: (i) limb/respiratory muscles and (ii) the connecting motor neurons. In most ALS studies the vectors are injected in the muscle. Although positive results are shown in studies with GDNF and IGF-1, researchers believes that motor neurons may detach from the muscle at early stages of the disease (Fischer et al., 2004), or the cellular transport mechanism is heavily impaired (Williamson & Cleveland, 1999; De Vos et al., 2007). Again due to their large cross-sectional area, retrograde transport is more severely affected in larger mammals when compared to mice, and thus requires a longer distance of transport. This factor may slow the translation of this successful strategy to clinical trials.

To overcome the potential problems of retrograde transport that may be encountered in muscle injections in humans, studies that inject vectors directly to motor neurons within spinal cord has been performed. Surprisingly, only a few studies have been published on this approach and the effect is less significant than the muscle injection studies. In a GDNF study on ALS mice, neuroprotection is only seen on facial but not lumbar motor neurons (Guillot, 2004). Another study supports the above idea by showing that GDNF is neuroprotective when it is overexpressed in skeletal muscles, but has no effect when the growth factor is overexpressed in motor neurons (Li et al., 2007). Disease progression is only slowed when GDNF is expressed in skeletal muscles, but not when it is expressed in the motor neurons.

## 2.3 Insights from growth factor studies to understand ALS disease progression

Although the ultimate goal of growth factor therapy for ALS is to alleviate symptoms, prolong survival, delay onset, and slow disease progression, during the course of

investigation several interesting findings have been observed and may provide insights to better understand the underlying mechanism of the disease. For example, finding growth factors' targets may help us find how the disease is initiated. Currently, the growth factors' targets are not fully known. It could be the degenerating motor neuron itself, the neighboring neuron, or surrounding glial cells. But a recent report about wild type non-neuronal cells extending survival of SOD1 mutant motor neurons in chimeric ALS mice (Clement et al., 2003) may provide adequate evidence showing that the growth factor's target is the supporting glia instead of neurons.

Another point of interest is the similarity of the growth factors that have been used. All GDNF, IGF-1, VEGF, and BDNF interact with receptor tyrosine kinases to produce downstream effects. Experiments have shown that those growth factors indeed work in a similar pathway and mechanism as there is no additional improvement observed when they work in combination (IGF-1 and VEGF) as compared to working individually (Dodge et al. 2010). Another article reports that VEGF promotes motor neuron survival by blocking Caspase through Phosphoinositide 3-kinase/ protein kinase B (PI3K/Akt) pathway (Lunn et al., 2009). Further investigation on the PI3K/Akt pathway may provide clues on how motor neuron death is triggered in ALS.

## 3. Stem cell therapy for ALS

### 3.1 The motor neuron replacement strategy

As motor neuron loss is the key diagnostic feature of ALS, the most straightforward strategy is to derive motor neurons from various types of stem cells and try to use them to replace the dead motor neurons in patients. For adult stem cells, cells expressing neuron and glial lineage markers were successfully derived from trans-differentiation of human umbilical cord blood cells (McGuckin et al., 2004) and mouse bone marrow stem cells (Croft et al. 2006). However, those cells' electrophysiological properties, survival, differentiation, and efficacy of integration to functional neurons and glial cells either *in vitro* or *in vivo* were not tested. Neural stem cells are the only type of adult stem cells which have successfully derived motor neurons that are functional *in vivo* (Gao et al., 2005). Human neural stem cells, which are scarce in the human body, are usually derived from embryonic stem cells or fetal brain tissues (Tai & Svendsen, 2004).

More promising results were shown in experiments using pluripotent stem cells. From mouse embryonic stem (ES) cells, motor neurons were successfully generated by induction of developmentally relevant signaling factors. The derived cells survive when transplanted into chick embryonic spinal cord, extend axons, and exhibit signs of presynaptic specialization when reaching targeted muscles (Wichterle et al., 2002). Another study shows that those cells possess immunohistochemical and electrophysiological features of normal motor neurons (Miles et al., 2004). Similar to mouse ES cells, human ES cells have been reported to form functional neurons (Li et al., 2005; Lee et al., 2007).

Functional motor neurons can also be derived from human induced pluripotent stem (iPS) cells, a possible alternative that may avoid the ethical concerns for the use of human ES cells (Karumbayaram et al., 2009). iPS cells are somatic cells that are reprogrammed into pluripotent stem cells (Yu et al., 2007; Takahashi, 2007), with great similarity to embryonic stem cells. They are capable of deriving patient-specific differentiated cells like neurons and glia, which allows them to potentially be used for autologous cell replacement in ALS patients. iPS cells have been generated from ALS patients and the cells are capable of differentiating into motor neurons

(Dimos, 2008). However, introduction of new genes during the production of iPS cells may give rise to additional technical concerns when translating to clinical studies.

Mouse ES-derived motor neurons reportedly grow around the ventral horn when transplanted into the spinal cord of rats with impaired motor neurons (Harper et al., 2004). In combination with chemicals that overcome myelin-mediated repulsion and GDNF that stimulates axon guidance towards skeletal muscles, further improvement in survival and engraftment of the transplanted cells was observed. Improvement in motor function of the paralyzed rats was also observed (Despande et al., 2006).

Despite the excitement that these transplantation studies brings to the field, the fact that these studies were performed on static models of motor neuron loss does not guarantee success in progressive motor neuron diseases like ALS. In addition, in order for the motor neuron replacement strategy to be successful, the transplanted motor neuron will first need to receive synaptic input from the presynaptic neurons and extend it's axon all the way to the targeted muscle at a rate of 1-3 mm/day, which takes months to years in humans, before innervation to the targeted muscle can be possible (Papadeas & Maragakis, 2009). Therefore motor neuron replacement may not be a legitimate treatment at this moment.

### 3.2 The neuroprotection strategy

#### 3.2.1 Non-cell autonomous nature of motor neuron death in ALS

Previously, little attention has been paid to the function of glial cells in the nervous system. However, we now know that glial cells modulate neuronal functions such as glutamate uptake, synaptic plasticity, trophic factor support, and even neuronal transmission (Kirchhoff et al., 2001). Studies also show that motor neuron death in ALS is non-cell autonomous, or mediated by astrocytes and microglia (Hall et al., 1998; Barbeito et al., 2004). Researchers also hypothesize that astrocytes and/or microglia form a positive feedback loop with motor neurons that leads to further propagation of the disease (Rao & Weiss, 2004). Moreover, chimeric mice with increased proportion of healthy, wild type glial cells increase survival of nearby human SOD1 mutant neurons *in vivo* (Clement et al., 2003). Using a CRE-lox system, selective reduction of the mutant gene in microglia and astrocytes in SOD1 transgenic mice slows disease progression, but has no effect on disease onset (Boillée et al., 2006b; Yamanaka et al., 2008).

Additional evidence is provided by stem cell–derived motor neurons/astrocytes co-culture. A study in 2007 shows that primary and ES cell-derived motor neurons are complementary in an *in vitro* motor neuron/astrocytes study for ALS (Nagai et al., 2007). From then on, studies using the following combinations have been performed: hES cell derived motor neurons with primary hSOD1-G93A or wild type mouse primary astrocytes (Di Giorgio et al. 2008); hSOD1-G93A mouse ES derived motor neuron with hSOD1-G93A derived mouse primary astrocytes (Di Giorgio 2007); and hES cells derived motor neuron with primary human astrocytes transfected with hSOD1-G47R genes (Marchetto, 2008). The Marchetto paper also uses that approach to verify a potential drug that has been beneficial in ALS rodent models. The success in this approach provides an easily accessible *in vitro* testing platform for cell-cell interactions in ALS and underlying disease mechanisms. Drug discovery will also accelerate as high throughput drug screening can be performed on the cultures.

#### 3.2.2 Astrocyte replacement

Based on non-cell autonomous nature of motor neuron death in ALS, astrocyte replacement is another feasible strategy for ALS stem cell therapy. Researchers transplant

glial restricted precursor (GRP) cells (lineage-restricted as derived from developing spinal cord) focally to cervical spinal cord that controls respiratory function in SOD1 rats (Lepore et al., 2008). The effect of the GRP transplant is significant: GRP cells survive and differentiated into mature astrocytes *in vivo*. The treatment also reduces microgliosis, prolongs survival, ameliorates motor neuron loss, and slows motor function decline. The group also found that the ALS rats with grafted GRP cells maintain normal level of glutamate transporter (GLT-1), an astrocyte-specific protein that has reduced expression in both ALS model rats and human patients (Howland et al., 2002; Rothstein et al., 1995). This may provide further evidence that astrocyte replacement is a sound strategy for ALS cell therapy.

#### 3.2.3 Immunomodulation

Other than replacement strategies, some stem cell therapies modulate the immunological environment around the degenerating motor neurons to prevent them from dying. Bone marrow cells provide a rich source of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). HSCs can give rise to a great variety of blood cells and cells in the immune system, but will particularly differentiate into microglia when introduced to the nervous system (Vitry et al., 2003). MSCs do not have the ability to differentiate into cells in the nervous system, but contribute to improved locomotion by differentiating into cells in the skeletal muscle lineage (Corti et al., 2004). Bone marrow transplanted into irradiated $SOD1^{G93A}/PU1^{-/-}$ double mutants (born without microglia and peripheral immune cells) prolonged survival and slowed disease progression (Beers et al., 2006). Another similar experiment confirms the result (Corti et al., 2004). This led to clinical trials of MSC and HSC transplants to sporadic ALS patients (Appel et al., 2008; Mazzini et al., 2008). Some of these studies show promising results **(Table 1)**.

### 3.3 Protective effect of neural stem cell and other cells in the neural lineage

Although most transplantations involving cells in the neural lineage were aimed at replacement of motor neurons, researchers now find that neuro-protection was instead the main effect. Various cell transplantations have been performed on hSOD1-G93A rodent models. They include: i) human embryonic germ cell delivered to cerebral spinal fluid (Kerr et al., 2003); ii) human neural stem cells grafted into the spinal cord (Yan et al., 2006); iii) hNT neurons derived from a human teratocarcinoma cell line grafted into spinal cord (Garbuzova-Davis et al., 2002); mouse Sertoli cells into parenchyma (Hemendinger et al., 2005); and human umbilical cord blood cells transfused into the systemic circulation (Habisch et al., 2007). In each of the cases, there was some degree of positive effect on motor neuron survival and life span of the animals. In addition, in most cases the positive effect is related to growth factor release (Suzuki & Svendsen, 2008). However, these studies do not specify which cell types are eventually exerting the protective effect or releasing the growth factors, though they are expected to be astrocytes (See Section 3.2 of this chapter). However, one human neural stem cell (NSC) transplant study suggests that the neuroprotective effect of host motor neurons stems from the ability of NSCs to differentiate into neuronal subtypes other than motor neurons such as GABAergic neurons that forms synaptic connection between grafted and host motor neurons (Xu et al., 2009). These neurons may provide additional benefits other than that from glial cells.

| Cell type | Subject | Injection Site | Effect | Paper |
|---|---|---|---|---|
| Mouse GRP | hSOD1-G93A rats | bilateral cervival spinal cord injection | cells survive and differentiated into mature astrocytes; reduces microgliosis; prolongs survival, ameliorates motor neuron loss and slows down motor function decline; normal GLT-1 level | Lepore et al. 2008 |
| Mouse bone marrow cell | hSOD1-G93A /PU1$^{-/-}$ double mutant mice | i.p. injection | cells effectively differentiated into microglia cells; prolongs survival; suppressed cytotoxicity; restore glial activation | Beers et al. 2006 |
| Mouse Bone marrow transplant | hSOD1-G93A mice | i.p. injection | delayed onset, increase life span | Corti et al. 2004 |
| Human embryonic germ cell | rats with diffused motor neuron injury | i.c.v injection (CSF) | cells distributed extensively over the rostrocaudal length of the spinal cord and migrated into the spinal cord parenchymal partially recovered motor function 12 and 24 weeks after transplantation | Kerr et al. 2003 |
| hNT cell | hSOD1-G93A mice | L4-L5 segments of the ventral horn spinal cord | delay onset, prolong survival, | Garbuzova-Davis et al. 2002 |
| Mouse Sertoli cell | hSOD1-G93A | unilateral spinal injection into the L4-L5 ventral horn | significant increase in motor neuron survival; no effect on disease onset and progression | Hemendiner et al. 2005 |
| Neuroectodermal derivatives of hUBS (hUBS-NSCs) | hSOD1-G93A | direct injection into the CSF (the cisterna magna). | No effect | Habisch et al. 2007 |

| Cell type | Subject | Injection Site | Effect | Paper |
|---|---|---|---|---|
| hUBC | hSOD1-G93A mice | i.v. injection | reduce microgliosis; increased lifespan; delayed disease progrssion | Garbuzova-Davis et al. 2008 |
| hNPC-GDNF | hSOD1-G93A rats | Unilateral lumbar spinal cord injection | Robust migration of the transplanted cells into the degenerating region; efficient delivery of GDNF as well as preservation of a large proportion of motor neurons; no continued innervations of motor neuron to the skeletal muscle end plates, no effect on ipsilateral hind limb function. | Suzuki et al. 2007 |
| hMSC-GDNF | hSOD1-G93A rats | Skeletal muscles | Transplanted cells survive within host skeletal muscles and release GDNF; significant increase in neuromuscular junctions; improves motor neuron survival | Suzuki et al. 2008 |
| CD34+ HSCs, HLA-matched sibling donors | ALS patients | i.v. injection | No clinical benefits | Appel et al. 2008 |
| Autologous bone marrow derived MSCs | ALS patients | multiple thoracic spinal cord injection | Decelerated linear decline of the forced vital capacity and of the ALS-FRS score in some patients | Mazzini et al. 2010 |
| Autologous CD133+ cells | ALS patients | bilateral injection into frontal motor cortex | lives 47 months more than the control group | Martinez et al. 2009 |

Table 1. Stem Cell Trials for ALS GRP. Glial restricted precursor; hUBC: human umbilical cord blood cells; NSCs: neural stem cells; hNPC: human neural progenitor cell; hMSC: human mesenchymal stem cell; HSCs: hematopoietic stem cells.

## 4. Working in combination: Genetically engineered stem cells as a tool of growth factor delivery for ALS

We have introduced two successful strategies for slowing ALS disease progression in the previous sections of this chapter. Although both of them in some degree involve the release of neuroprotective growth factors, both strategies have their shortcomings. In viral delivery of growth factors, the cells still carry the mutant SOD1 gene or has the disease phenotype. Therefore the cells that are delivering the treatment are indeed still doing harm on the surrounding cells at the same time. On the other hand, neuroprotective strategy of stem cell transplants, though increases the proportion of wild type (normal) cells around the injection site(s), the transplanted cells may not naturally produce the desired neuroprotective growth factors in a pharmaceutically adequate amount (Gonzalez, 2009). Therefore, it is reasonable for us to combine the two strategies and see if they can complement each other and produce a great synergic effect.

### 4.1 hNPC-GDNF injection to spinal cord

Based on the logic above, our group genetically engineered human neural progenitor cells (hNPC) that express and secrete GDNF through lentiviral infection (Klein et al., 2005; Suzuki et al., 2007). hNPC are comprised of multiple classes of neural stem cells and lineage-restricted precursors. They are isolated from fetal brain cortical tissue (Svendsen et al., 1996; Keyoung et al., 2001; Tamaki et al., 2006; Suslov, 2002) and can be maintained for over 50 weeks in the presence of mitogen while retaining the ability to differentiate into astrocytes (Wright et al, 2003). With their special properties, hNPC can thus serve as "mini-pumps" to provide glial replacement and deliver trophic factors through transplantation into specific sites in the brain and spinal cord of diseased animals and patients. hNPC-GDNF were transplanted to the lumbar region of the spinal cord of hSOD1-G93A rats. We observed robust migration of the transplanted cells into the degenerating region, efficient delivery of GDNF, as well as preservation of a large proportion of motor neurons at both early and late stages of the disease within chimeric regions (Suzuki et al. 2007). However, the preservation of motor neurons does not accompany with continued innervations of motor neuron to the skeletal muscle end plates, thus had no effect on ipsilateral hind limb function.

### 4.2 hMSC-GDNF injection to skeletal muscles

Skeletal muscles clearly play an important role in guiding and attracting the developing neurons; and provide trophic support to maintain motor neuron function (Dobrowolny et al., 2005). A previous study showed that transplants of genetically engineered myoblasts (a kind of skeletal muscle precursor which has the ability to fuse with mature myofibers) secreting GDNF ameliorates motor neuron loss in ALS mice (Mohajeri et al., 1999). Thus we genetically engineered human MSCs (hMSCs) that express and secrete GDNF and transplanted them to three muscle groups in hSOD1-G93A rats (Suzuki et al., 2008). MSCs can be easily obtained from bone marrow from donations and have the ability to differentiate into the skeletal muscle lineage (Caplan & Arnold, 2009). The transplanted cells survives in the host skeletal muscle and releases GDNF. Moreover, it significantly increases the number of functional neuromuscular junctions and improves motor neuron survival in spinal cord at the mid-stage of disease. Furthermore, intramuscular hMSC-GDNF transplantation remarkably prolongs disease progression, increasing overall life span up to 28 days, which is one of the greatest improvements ever observed in familial ALS model rats.

### 4.3 Future research directions

From the two sets of experiments described in this section, we can conclude that stem cell delivery of growth factors is an effective strategy for ALS treatment. We also know that different sets of delivery tools are needed for the motor neuron cell bodies in the spinal cord and their synaptic connections to the skeletal muscles. Our current knowledge leads us to an initial thought for future development of the field of ALS growth factor/stem cell therapy. Motor neuron cell body protection will be provided by stem cell derived wild type astrocytes and microglia (from hNPC for example); while synaptic/axonal protection will be provided by stem cell derived myoblasts (from hMSC for example). Those cells will be genetically modified to enhance delivery of neurotrophic factors. Lastly, GDNF is only one of the many neurotrophic factors that showed to have beneficial effect on ALS rodent models as mentioned in Section 2 of this chapter. We expect there will soon be tests on the other neurotrophic factors.

## 5. Clinical translation

Despite the exciting breakthroughs in stem cell research aiming to treat ALS, there is still a long way to go to translate those successes to the clinic and help patients. Since we are still uncertain about the fate of stem cells after transplantation, thorough safety tests are needed. Then, optimal cell dose, source of cells, stage of cells, route of delivery, injection sites, and immunosuppressive regimen (to ensure grafted cell survival in host) will need to be determined as well (Papadeas and Margaskis, 2009).

Clinical trials that involve stem cells on ALS patients are in the initial stage. In 2010 the phrase I clinical trial of hMSC transplantation performed in Italy was reported. (Mazzini et. al., 2010) Autologous MSC isolated from bone marrow derived cells were transplanted to the thoracic region of 9 ALS patients. Neither adverse effect nor significant improvement was found. However, it provides initial evidence that MSC injection is safe. Large volume (1 mL) of cells can be infused to the spinal cord without causing observable defects.

Neuralstem and Emory ALS center have begun the phase I trial of spinal cord derived stem cells for patients with ALS. The advantages of using neural stem cells derived from human fetal spinal cord are no tumor formation and minimal HLA (human leukocyte antigen) expression, thus, resulting in a low overall antigenicity of the cells. The first surgery of the trial took place a year ago, and the 9th surgery was performed earlier in 2011, without the need for patients to be on ventilators or to be taken to intensive care post-operation. The trial was staged, first enrolling non-ambulatory patients, and the first ambulatory patient was enrolled early 2011.

## 6. Conclusion

In this chapter, we introduced the current application of stem cells in ALS (summarized in Figure 1). There are three points we should keep in mind about this topic. First, stem cell therapy design should be aimed at neuroprotection rather than motor neuron replacement. Motor neuron replacement is technically difficult to achieve. Also, in theory it will not bring much improvement to the patients because the evidence shows that glial cells are the actual determinant of ALS disease progression. Secondly, combining stem cell transplantation and growth factor delivery provides the best result in slowing disease progression and

prolonging survival, as the two greatly complement each other. Finally, we are now convinced that injections of stem cells in multiple sites are needed in order to alleviate symptoms of ALS. There should be at least one injection that focuses on protecting cell bodies of motor neurons and another that aims to maintain neuromuscular connections. To sum up, stem cell applications have made a lot of contributions to ALS research and have great potential to bring breakthroughs to the field in the near future.

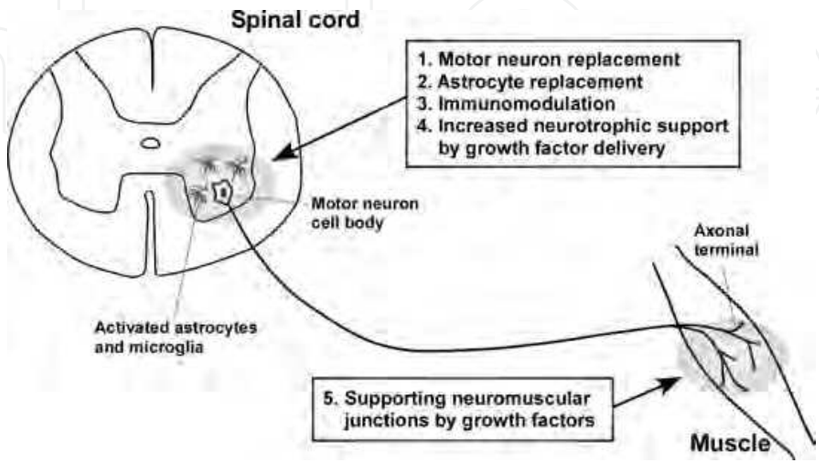


Fig. 1. Schematic illustration of possible stem cell interventions for ALS therapies. These could include: (1) Motor neuron replacement, differentiation of neural progenitor cells to motor neurons and projection to the periphery; (2) Differentiation and replacement of dysfunctional astrocytes; (3) Modulation of immunological environment around the degenerating motor neuron; (4) Trophic/growth factor delivery via stem cells to provide neuroprotective support for the endogenous populations; (5) Local delivery of growth factors to support neuromuscular junctions and axon integrity.

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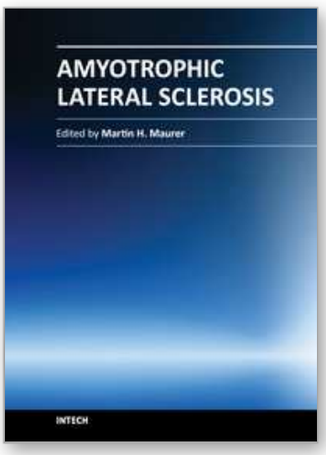


**Amyotrophic Lateral Sclerosis**

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Though considerable amount of research, both pre-clinical and clinical, has been conducted during recent years, Amyotrophic Lateral Sclerosis (ALS) remains one of the mysterious diseases of the 21st century. Great efforts have been made to develop pathophysiological models and to clarify the underlying pathology, and with novel instruments in genetics and transgenic techniques, the aim for finding a durable cure comes into scope. On the other hand, most pharmacological trials failed to show a benefit for ALS patients. In this book, the reader will find a compilation of state-of-the-art reviews about the etiology, epidemiology, and pathophysiology of ALS, the molecular basis of disease progression and clinical manifestations, the genetics familial ALS, as well as novel diagnostic criteria in the field of electrophysiology. An overview over all relevant pharmacological trials in ALS patients is also included, while the book concludes with a discussion on current advances and future trends in ALS research.

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