

Expert Opinion

1. Introduction
2. Principles of stem cell transplantation in ALS
3. Stem cell types
4. Stem-cell-derived motor neurons from the patient as a diagnostic tool and a source of cells to transplant
5. Stem cell therapy for ALS
6. Mesenchymal stem cells and ALS
7. Administration routes
8. Association of stem cell therapy to delivery of trophic factors
9. Clinical trials
10. Expert opinion

Stem cells in amyotrophic lateral sclerosis: state of the art

Letizia Mazzini[†], Alessandro Vercelli, Ivana Ferrero, Katia Mareschi, Marina Boido, Serena Servo, Gaia Donata Oggioni, Lucia Testa, Francesco Monaco & Franca Fagioli

[†]*Eastern Piedmont University, "Maggiore della Carità" Hospital, ALS Centre, Department of Neurology, Corso Mazzini 18, 28100, Novara, Italy*

Amyotrophic lateral sclerosis (ALS) is a devastating incurable neurodegenerative disease that targets motor neurons, manifesting as a linear decline in muscular function and leading to death within 2 – 5 years of diagnosis. The vast majority of ALS cases are sporadic, the aetiopathology of which is incompletely understood. Recent data have implicated the microenvironment of the motor neuron as a primary target of the pathophysiology. Any experimental therapeutic approach to ALS is very difficult because of some peculiarities of the disease, such as the unknown origin, the spatial diffusion of motor neuron loss and the paucity of animal models. Despite such daunting challenges, in experimental models a number of potential benefits of stem cells in ALS therapy have been demonstrated: by providing non-compromised supporting cells such as astrocytes, microglia or growth factor-excreting cells, onset can be delayed and survival increased. Moreover, in animal models of acute or chronic motor neuron injury, neural stem cells implanted into the spinal cord have been shown to differentiate into motor neurons, with some evidence of axonal sprouting and formation of neuromuscular junctions with host muscle. Here we summarise and discuss current preclinical and clinical evidence regarding stem cells application in ALS, particularly focusing on methodological issues.

Keywords: amyotrophic lateral sclerosis, animal models, cell therapy, stem cells

Expert Opin. Biol. Ther. [Early Online]

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating incurable neurodegenerative disease that targets motor neurons (MNs) and their connections to muscle. The decline in the number of MNs in ALS is very rapid compared with other neurodegenerative diseases [1] leading to death due to respiratory failure within 2 – 5 years from the clinical onset. Notwithstanding the improved knowledge about the mechanisms of cell death in the disease, ALS still represents one of the most frustrating diagnoses for a neurologist and one of the most dramatic diseases for a patient: it also is considered one of the great conundrums of clinical neuroscience [2]. ALS aetiology is unknown and the pathogenesis remains elusive. Recent data have implicated the microenvironment of the MN, rather than the MN itself, as a primary target of the pathophysiology.

Putative mechanisms of toxicity targeting MNs include oxidative damage, accumulation of intracellular aggregates, mitochondrial dysfunction, defects in axonal transport, impairment of growth factor trophic support, altered glial function, aberrant RNA metabolism and glutamate excitotoxicity. The convergence of these events is likely to promote the onset and the progression of the disease: the question remains on which is the cause and which is the epiphenomenon [3]. All these

informa
healthcare

mechanisms represent a potential therapeutic target and many clinical trials have been developed even though currently none of the candidate compounds has been demonstrated to be effective [4].

Stem cells represent a promising therapeutic strategy since they potentially target several of these putative mechanisms. Moreover, stem cells can be a good means for studying disease-specific cellular pathways and can represent a model system to test new therapeutics, and finally to achieve direct cell-based therapy.

2. Principles of stem cell transplantation in ALS

ALS is characterised by the extensive degeneration of MNs of spinal cord, brain stem and cerebral cortex. Therefore, due to the diffuse distribution of affected MNs, a relevant aspect in cellular therapy is to select the best implantation site, in order to rescue and eventually replace the greatest number of host MNs.

There are two main strategies for transplantation: the systemic and the local. The rationale for the systemic injection consists of the possibility for stem cells to extravasate and cross the blood brain barrier (BBB), especially in the pathological nervous system. In addition, it has been suggested that the pathological brain can attract stem cells. And finally, there is evidence that systemic injection of stem cells can be effective even though they do not enter the CNS [5]. Systemic injections require larger amounts of stem cells to transplant, and can require immunosuppression, even though stem cells can be immunomodulatory themselves [6,7].

Local injections of stem cells, close to the anterior horn of the spinal cord, have the advantage that the operator can place the cells close to their target. CNS is an immune-privileged tissue, and there is evidence that transplant of stem cells does not require immunosuppression. The adverse effects consist of the need of multiple injections due to the length of the spinal cord and in the risk of lesion of the spinal cord. Intramuscular injections of stem cells can also be performed either as an alternative

more they can proliferate and give rise to different cell types. On the other hand, adult stem cells display a lower potential for developing into tumours following transplantation than highly pluripotent cells such as embryonic stem (ES) and induced pluripotent stem (iPS) cells that can give rise to teratoma [8]. An alternative strategy consists of orienting and differentiating stem cells *in vitro*, before transplantation. In the CNS, the most obvious cell type to be transplanted are neural stem cells (NSCs); in addition, immortalised cell lines, mesenchymal stem cells and genetically engineered stem cells can be used.

In order to assess the efficacy of treatment, it is necessary to obtain a general consensus on the outcome to be achieved. Fundamentally, the outcomes should be positive on two sides: anatomical and functional. The anatomical and morphological outcome consists first of the higher number of MNs compared with vehicle-treated diseased animals. A future, very ambitious, goal will be to increase the number of MNs by differentiating stem cells. Relative to the functional outcome, it will consist of a less rapid decrease in motor performance as assessed with a battery of behavioural tests, such as the rotarod test, paw grip endurance test and hanging wire test in animal models. Similarly, a battery of motor and behavioural tests must be used in patients to investigate the functional outcome of stem cell transplantation. Very sensitive and reliable electrophysiological protocols must be developed to detect changes in the progression of the disease related to collateral reinnervation or preservation of MNs such as Motor Units Number Estimations (MUNEs). Moreover, objective standardised measures of muscular strength [9,10] could help in monitoring the functional changes in the transplanted areas.

Two major objectives can be aimed at in applying stem cells for ALS therapy: replacement and neural protection. Replacement consists of differentiating MNs either *in vitro* or *in vivo* from embryonic or adult stem cells: these newly generated MNs should send their axons through the anterior branch of the spinal nerve to their appropriate target muscle. MNs have been obtained from embryonic neural stem cells [11], and several authors have claimed that neurons

by the axon to the cell body and systemic injections, stem subarachnoideal space or the administration allows them to the risk of lesion of the CNS. through the ventricular system is not trivial, since there are continuity of these cavities and stem cells can cross the pia over the nervous tissue.

is the type of stem cells to be differentiated stem cells are, the

differentiation of MSCs has been questioned [13,14]. Therefore, as yet, the neurotrophic and immunomodulatory roles seem more relevant than the potential for cell replacement. In fact, as discussed in the following sections, stem cells express immunomodulatory and neuroprotective substances, which can play a fundamental role in protecting MNs in ALS.

3. Stem cell types

Stem cells can be defined as cells able to self-renew giving rise to additional undifferentiated stem cells and to differentiate into committed mature cells. According to their developmental

to be retrogradely transported of the MN.

Somewhat in between local cells can be injected into the lateral ventricles; this way of bypass the BBB, reducing the However, the diffusion of cells and the subarachnoideal space frequent interruptions in the since it is unclear to what extent or the ependymal layer to enter

Another fundamental issue transplanted. The more undifferentiated

status, stem cells can be divided into embryonic and adult cells: the former are isolated from the inner layer of the blastocyst, formed 4 – 5 days after fertilisation; the latter are specialised cells found within many tissues of the body (brain, bone marrow, liver, skin, gastrointestinal tract, cornea, retina and dental pulp, for example), that are in a dynamic state even in absence of injury and are able to differentiate into several different cell types [15].

According to their differentiation potential, stem cells can be further classified as totipotent, pluripotent, multipotent and unipotent.

Totipotent stem cells derive from early (1 – 3 days) embryos and can give rise to all differentiated cell types in an organism; pluripotent cells derive from the 5 – 14-day-old blastocyst and can originate almost any cell type: fetal tissue, cord blood, peripheral blood and bone marrow for example contain multipotent stem cells, that can differentiate into cell types characteristic of only one specific tissue; finally, unipotent cells can self-renew and originate only one cell type [16,17].

For their potential to originate new cells of any type, stem cells are ideal candidates for regenerative medicine, tissue engineering, gene therapy and cancer therapies.

In fact, *in vitro* studies have demonstrated that embryonic stem cells can grow indefinitely depending on culture conditions, and can differentiate into somatic and somatic-like cells, as neurons, cardiomyocytes, hepatocytes and others [17]. Moreover such cells have been employed in numerous studies in animal models, of conditions such as Parkinson's disease, diabetes, spinal cord injury, Puntkinje cell degeneration, Duchenne muscular dystrophy, liver and heart failure, and osteogenesis imperfecta [18].

Also adult stem cells can differentiate *in vitro* into several cell types, such as osteoblasts, chondrocytes, endothelial cells, glia, neurons, skeletal and cardiac myocytes, according to their origin [17]. However, the main source from which adult stem cells can be isolated remains bone marrow, containing hematopoietic stem cells and stromal mesenchymal cells. Hematopoietic stem cells can produce cell types and blood cells, and for this reason are successfully used in the treatment of blood cancers and disorders; on the other hand, mesenchymal cells can give rise to bone, cartilage, adipose tissue and muscle cells, and represent a promising tool for the treatment of bone defects, ischemic heart and liver diseases [16].

Ethical issues have been raised relative to the use of embryonic cells, which are not relevant to adult stem cell research. However, recently it has been shown that adult differentiated cells, such as mouse fibroblasts, can be reprogrammed to an embryonic-like state by introducing four factors, Oct3/4, Sox2, c-Myc and Klf4 [19], even if following studies demonstrated development of tumours correlated with the presence of these oncogenes.

Stem cells can be used for both *in vitro* disease modelling and therapeutic applications.

4. Stem-cell-derived motor neurons from the patient as a diagnostic tool and a source of cells to transplant

Human cell culture is an essential complement to research with animal models of disease. Murine models of ALS, in fact, do not fully mimic human disease. Stem cells can provide human samples from the patients themselves, which are not transformed or genetically modified, allowing creation of reliable disease models for laboratory studies and drug research [20].

Several groups have generated MNs from human embryonic stem cell (hESC) lines [21] ESCs can be used effectively to identify factors involved in motor neuron degeneration as well as small neuroprotective molecules. Genetically defined ESCs from animal models of amyotrophic lateral sclerosis can give important insights into the pathophysiology of MN deterioration [22]. Human ESC-derived MNs genetically manipulated to express SOD1 mutants exhibit typical signs of MN degeneration linked to ALS, such as reduced cell survival and shortened axonal processes [23].

Moreover, in co-culture experiments, human MNs were selectively sensitive to the toxic effects of glial cells harbouring a mutant allele of the SOD1 gene, while interneuron populations produced from embryonic stem cells were unaffected, in association with several significant changes in glial gene expression. These cultures were used to screen several candidate molecules, possibly involved in the toxic effect of mutant glia, identifying prostaglandin D2 as a toxic molecule for motor neurons [24].

Another considerably interesting approach is to generate iPS lines from patients affected by ALS. iPS cells are the product of somatic cell reprogramming to an embryonic-like state. This occurs by the introduction of a defined and limited set of transcription factors and by culturing these cells under ESC conditions. The method was first described by Shinya Yamanaka and colleagues [25] and recently MNs have been derived from an old patient bearing a familial form of ALS [26]. Human iPS cells might represent an ideal cell source for cell therapy given that iPS cells can be derived from the patients themselves thus preventing immune rejection. However, human iPS cells have not yet been directed to differentiate into a specific functional tissue [27].

5. Stem cell therapy for ALS

Any experimental therapeutic approach to ALS is complicated by the unknown origin, the spatial diffusion of the MN loss and the paucity of animal models. Despite such daunting challenges, a number of the potential benefits of stem cells in ALS therapy have been demonstrated in experimental models.

First, transplanted stem cells of both neural and bone marrow lineages improve survival and function of endogenous glial and neural precursors by producing neurotrophic and

growth factors [28,29]. The efficacy of this approach could be improved by genetically modifying the stem cells to secrete molecules that promote motor neuron survival (reviewed in [30]).

Second, both neural and non neural stem cells can differentiate into glial lineages [31,32] replacing the surrounding cells, which nurse and protect neurons. Moreover neural stem cells seem to be neuroprotective against glutamate-induced spinal MN neurotoxicity [33], which represent a well established pathogenetic mechanism in ALS. More recently Lepore and colleagues [34] reported an improvement of survival and disease duration in SOD1^{G93A} transgenic mice engrafted with astrocyte precursors, mediated by the primary astrocyte glutamate transporter GLT1.

Third, both neural precursor cells [35] and mesenchymal stem cells [23,36] promote 'bystander' immunomodulation, as they can release soluble molecules and express immuno-relevant receptors that are able to modify the inflammatory environment. Although ALS aetiology is not yet well understood, an inflammatory reaction characterised by increase of immunoglobulins and lymphocytes has been demonstrated [37]. Therefore inflammation could represent a possible therapeutic target, eventually exploiting stem cells ability. When transplanted intravenously [38,39], stem cells were also found in the spleen parenchyma and improved disease outcome, probably through immune modulation. Similar results were obtained in a model of intracerebral haemorrhage followed by intravenous graft of neural stem cells: NSCs reduced cerebral inflammation, modulating the splenic inflammatory pathway [5]. Therefore these cells seem able to promote a bystander modulation, changing inflammatory environment both by release of cytokines and chemokines, and by expression of immune-relevant receptors [35].

Differentiation toward a neural-like morphology has been demonstrated for both non-neuronal stem cells and neural progenitors [32,40,41], with some evidence of axonal sprouting in the second ones [42,43]. Xu *et al.* recently demonstrated that fetal neural stem cells grafted into the spinal cords of normal and SOD1-mutated rats differentiate into interneurons and form structurally mature synapses [43]. When induced to differentiate, neural stem cells can give rise both *in vitro* and *in vivo* to cholinergic motoneurons, even though in a small percentage [44].

In light of all these stem cells properties, future stem cell therapies for MN diseases could include a synergic combination of strategies aimed at both neuroprotection of host MNs and cellular replacement of neurons and glia. Many experiments have been performed in animal models of the MN diseases [45], however no conclusive data can be drawn because of the heterogeneity of the approaches (Table 1). The translation of preclinical studies in mutant SOD1 rodent models to humans is limited by different sources of stem cells, routes of delivery, timing of therapeutic intervention and relevance of the animal model of familial ALS to human patients.

The mobilisation of endogenous precursors from germinal niche could be an attractive technique, but proliferation of endogenous progenitors in the adult spinal cord in response to ALS neurodegeneration cannot compensate for the pathogenic loss of MNs [46]. Moreover, the NSC/neuroprogenitor niche in the adult forebrain of ALS mice displays altered proliferation and phenotypic characteristics [47].

Candidate cell types for stem cell therapy in ALS must be able to survive and influence the pathological tissue environment, including inflammatory and immune reactions, and migrate into the sites of diffuse neurodegeneration. Moreover, it is fundamental for clinical application that stem cells are safe, and can be easily isolated and expanded.

ESCs display a great plasticity [48]. In models of acute MN death, ESC-derived MNs implanted into the spinal cord can extend axonal processes to innervate muscle targets [49]. Nevertheless, the differentiation *in vivo* of uncommitted embryonic and fetal stem cells is conditioned by environmental cues, and how such differentiation can be directed to generate specific subtypes of MNs still remains to be elucidated [45]. Moreover, in addition to ethical considerations concerning the source, the clinical use of ESCs in humans is limited by some key issues, such as their unlimited *in vitro* proliferation and *in vivo* teratocarcinoma formation [50].

Neural stem cells might represent a ready-to-use cell source for cell-based therapies, because, when placed in culture, they can be grown and extensively expanded for months, allowing the generation of stem cell lines which maintain stable and constant functional properties, and they have been used *in vivo* without tumour formation or overt toxic or other side effects [51].

Their derivatives, human neural progenitor cells (NPs), can be expanded in culture for long periods; they survive and continue to proliferate after transplantation into the adult rodent central nervous system [52]. NSCs constitutively produce neurotrophic factors, in particular GDNF, that can exert a potent protective and neurite outgrowth-promoting effect on motor neurons [33]. Moreover, NSCs are neuroprotective against glutamate-induced spinal MN neurotoxicity in a model of MN apoptosis induced by facial nerve axotomy [28]. Similarly, NSCs can be neuroprotective in SOD1 mice, delaying onset and progression of disease, and extending survival [44]. Neural precursors can also be genetically modified [53] to release GDNF, and following unilateral transplantation into the spinal cord of SOD1-G93A rats there was robust cellular migration into degenerating areas, efficient delivery of GDNF and remarkable preservation of MNs at early and end stages of the disease within chimerical regions [54,55]. Interestingly, this robust MN survival was not accompanied by continued innervation of muscle end plates and thus resulted in no improvement in ipsilateral limb use. Behavioural effects after grafting neuron-like hNT cells (human teratocarcinoma cell-line cultured with retinoic acid) at level of the L4 – L5 spinal segment of transgenic mice have been observed [56].

Table 1. Preclinical transplantation studies of stem cells in animal models of ALS.

Cell source	Disease model	Route of delivery	Number of cells	Proposed therapeutic mechanism	Outcomes	Ref.
Human UCBs (pooled donors)	Presymptomatic, irradiated sod1 (g93a) mice	Intravenous (retro-ocular)	34.2 – 35 × 10 ⁶	Immunomodulation/ providing non mutant (functional) sod1 enzyme	Delay in disease onset (22 days) and increased lifespan (21 days)	[38]
Wild-type mice BMCs	Presymptomatic, irradiated sod1 (g93a) mice	Intravenous (retro-ocular)	5 × 10 ⁶	Immunomodulation/ providing non mutant (functional) sod1 enzyme	Delay in disease onset (7 days) and increased lifespan (12 – 13 days)	[38]
Human UCBs	Presymptomatic, SOD1 (G93A) mice	Intravenous	10 ⁶	Neuroprotection by modulation of autoimmune processes	Delayed disease progression (at least 2 – 3 weeks) and modestly increased lifespan	[39]
Human UCBs	Presymptomatic, SOD1 (G93A) mice	Intravenous	10 × 10 ⁶ 25 × 10 ⁶ 50 × 10 ⁶	Modulating the host immune inflammatory system response	Dose of 25 × 10 ⁶ cells increased lifespan by 20 – 25% and delayed disease progression by 15%	[97]
Wild-type mice versus SOD1 (G93A) BMCs (mesenchymal)	Presymptomatic, irradiated SOD1 (G93A) mice	Intraperitoneal	3 × 10 ⁶	Positive 'non-neuronal environmental' effects	Delay in onset (14 days) and increased lifespan (12 – 13 days) of wild-type BMCs, no effect of SOD1 mice BMCs	[98]
Human bone marrow MSCs from adult donors	Presymptomatic, SOD1 (G93A) mice	Bilateral lumbar spinal cord injection, different levels	Total amount 10 ⁵	Increasing neuron survival and preventing astrogliosis and microglia activation	Increased motor neuron count, decreased astrogliosis and microglia activation, increased lifespan in males, amelioration of motor performances	[29]
NSCs from spinal cord of human embryo (8 weeks old)	Presymptomatic, immunosuppressed SOD1 (G93A) mice	Bilateral lumbar spinal cord injections	Four sites, 5 × 10 ⁴ cells/site	Differentiation of NSCs into neurons, initial networks with host nerve cells, release of growth factors	Delay in onset (7 days) and increased average lifespan (11 days)	[116]
Wild-type mice embryonic stem cells	Adult rats with chronic diffused motor neuron deficiency (sindbis virus)	Bilateral lumbar spinal cord injections, one site	6 × 10 ⁴	Motor neurons differentiation, forming junctions with host muscle	Partial recovery from paralysis	[49]
Wild-type adult mice NSCs (purified from adult brain and primed into a motor neuron phenotype)	Presymptomatic, immunosuppressed SOD1 (G93A) mice	Bilateral lumbar spinal cord injections, one site	10 ⁴	Neuronal and glial differentiation, release of growth factors (trophic support)	Delay in onset (21 days) and increased average lifespan (22 – 23 days) (unchanged progression). Delayed loss of lumbar motor neurons	[44]
Wild-type Mice NSCs from embryonic spinal cord	Presymptomatic nmd mice (animal model of SMARD1)	Intrathecal delivery	2 × 10 ⁴	Neuronal and glial differentiation, release of growth factors (trophic support)	Delayed onset and increased average lifespan (18 – 19 days) Decreased loss of motor neurons	[99]

BMC: Bone marrow cell; GRP: Glial-restricted precursor; h: Human; MSC: Mesenchymal stem cell; MSC (GDNF): MSC engineered to secrete glial cell line-derived neurotrophic factor; NSC: Neural stem cell; NSC(GDNF): NSC genetically modified to release GDNF; SMARD1: Spinal muscular atrophy with respiratory distress type 1; UCB cell: Umbilical cord blood cell.

Table 1. Preclinical transplantation studies of stem cells in animal models of ALS (continued).

Cell source	Disease model	Route of delivery	Number of cells	Proposed therapeutic mechanism	Outcomes	Ref.
Human NSCs (GDNF)	Presymptomatic, immunosuppressed SOD1 (G93A) rat	Unilateral lumbar subcutaneous injections, one site	12 – 18 × 10 ⁴	Trophic support	Efficient delivery of GDNF, motor neuron preservation, no improvement in ipsilateral limb use [55]	[55]
Human MSCs (GDNF) from neonatal bone marrow	Presymptomatic, immunosuppressed SOD1 (G93A) rat	Bilateral injection into three skeletal muscle groups	12 × 10 ⁵	Trophic support	Increased number of neuromuscular connections and motor neuron cell bodies in the spinal cord. Increased overall lifespan by up to 28 days [92]	[92]
Glial-restricted precursors (GRPs)	SOD1 (G93A) rat	Transplantation around cervical spinal cord	9 × 10 ⁵	GRPs, efficiently differentiated into astrocytes and reduced microgliosis	Extended survival and disease duration, attenuated motor neuron loss and slowed declines in forelimb motor and respiratory physiological functions [34]	[34]
Wild-type rat mesenchymal stem cells (MSCs)	14 weeks transgenic SOD1-Intrathecal Leu126delTT mice	transplantation via the fourth cerebral ventricle	3 – 4 × 10 ⁵	Neuroprotection, modulation of the neural environment	Females, but not males, showed a statistically longer disease duration [100]	[100]
Mouse olfactory ensheathing cells (OECs)	14 weeks transgenic SOD1-Intrathecal Leu126delTT mice	transplantation via the fourth cerebral ventricle	3 – 4 × 10 ⁵	No benefits	No significant differences in clinical evaluation [101]	[101]
Human bone marrow-derived mesodermal stromal cells (hMSCs)	Pre-symptomatic ALS mouse model overexpressing G93A	Intrathecal transplantation (via cisterna magna)	10 ⁵	No benefits	Negative outcome [100]	[100]
Umbilical cord blood cells (hUBCs)	Pre-symptomatic ALS mouse model overexpressing G93A	Intrathecal transplantation (via cisterna magna)	10 ⁵	No benefits	Negative outcome [100]	[100]
Wild-type rats MSCs	Symptomatic hSOD1G93A	Intrathecal delivery (lumbar level)	2 × 10 ⁶	MSCs substantial infiltration into the ventral horn; massive differentiation into astrocytes; decreased motor neuron loss	In treated rats the first signs of paralysis were detected 14 days later compared with sham animals; the life expectancy was increased by 16 days [117]	[117]

BMC: Bone marrow cell; GRP: Glial-restricted precursor; h: Human; MSC: Mesenchymal stem cell; MSC (GDNF): MSC engineered to secrete glial cell line-derived neurotrophic factor; NSC: Neural stem cell; NSC(GDNF): NSC genetically modified to release GDNF; SMARD1: Spinal muscular atrophy with respiratory distress type 1; UCB cell: Umbilical cord blood cell.

Physiologically MNs and glial cells mutually affect their function and survival, but in a degenerative disease like ALS a disturbance of this balance between glial cells and neurons occurs, influencing disease onset and leading to the death of MNs. In the light of this evidence, therapeutic strategies involving glial cells could represent a feasible approach for developing new ALS treatments [57,58].

Olfactory epithelium represents another easily accessible source of stem-like progenitors, that can differentiate both into supporting cells or neurons [59]. Experimental trials in various different models of acute or chronic rodent spinal cord injury have suggested the ability of olfactory ensheathing cells (OECs), taken from olfactory bulb or mucosa, to stimulate tissue sparing and neuroprotection, to enhance outgrowth of both intact and lesioned axons, activate angiogenesis, change the response status of endogenous glia after lesion and remyelinate axons after demyelinating insults. Their ability to stimulate regeneration in specific tracts appears, however, limited (reviewed in [60]).

6. Mesenchymal stem cells and ALS

Numerous reports demonstrate the plasticity of non-neural cells, such as bone marrow stromal and cord blood adult stem cells (reviewed in [61]). Pluripotent hematopoietic stem cells (HSCs) from adult bone marrow may give rise to neurons, oligodendrocytes and astrocytes after transplantation into newborn brains [62]. When grafted into the spinal cord, HSCs express GDNF (glial cell line-derived neurotrophic factor) and induce its production by the host cells [63]. Healthy bone marrow transplantation in mutant SOD mice demonstrated that functional CD4⁺ T cells, either directly or indirectly, can modulate microglial and astroglial activation, attending trophic/cytotoxic balance of glia and, in this way, assuring neuroprotection and prolonging survival [64]. Autologous transplantation of bone marrow stem cells (BMSCs) fully circumvents the problem of immune rejection, does not cause the formation of teratomas and bears no ethical or political concerns. Pre-clinical studies have shown the effectiveness and positive safety profile of treatment with autologous bone marrow cells [65].

Mesenchymal stem cells (MSCs) are very attractive multipotent stem cells for ALS cell therapy because of their great plasticity [66] and their ability to provide the host tissue with growth factors or to modulate the host immune system [67]. They can be easily isolated from bone marrow (BM) and expanded in culture. Although MSCs lack unique cell markers, minimal criteria for their characterisation, including immunophenotype and differentiating potential, have been established [68]. Despite evidence that MSCs can transdifferentiate into multiple cell types *in vitro* and *in vivo*, their real contribution to tissue repair is still unclear [69].

Actually, human MSCs (hMSCs) under specific culture conditions can express neural markers, such as GFAP, Nestin, Tuj-1, tyrosine hydroxylase and MAP2 [70-73] and display at

electrophysiology K⁺ channels usually expressed in cerebral cortex [40]. Our experiments *in vivo* [29], in agreement with others [12,62,74-75], seem to support a neural differentiation of hMSCs. On the other hand, neural differentiation has been questioned [4,13]: the morphological changes in culture might be consequent to cellular toxicity and related cytoskeletal changes [76-78] and even undifferentiated MSCs express markers for different cell lines at very low levels which can be increased [79,80]. Therefore, as for neural stem cells, the neurotrophic and immunomodulatory roles seem more relevant than the potential for cell replacement. The production of trophic factors might support the survival, migration and differentiation of endogenous precursors [81]. Moreover, the immunomodulatory properties of stem cells, rather than transdifferentiation potential, could be relevant for experimental results.

MSCs have been tested with success in rodent models to treat diseases such as multiple sclerosis and diabetes where immunomodulation is thought to be the main operative mechanism [82,83]. MSC transplantation increases neuron survival and prevents astrogliosis and microglia activation [29]. Astrocytes are both the target and cause of neuroinflammation, since when stimulated by mediators released from microglia they downregulate the expression of neurotrophic factors and release additional inflammatory mediators, which, in turn, further activate microglia [84]. Reactive astrocytosis is present in the presymptomatic stage and gradually increases to end-stage ALS [85]. Several studies demonstrated that the expression of proinflammatory mediators is an early event in murine ALS, even preceding the development of clinical signs. A role in preventing astrogliosis and microglial activation has been suggested also for neural stem cells (reviewed in [86]).

MSCs can rescue neurons and oligodendrocytes from apoptosis through the release of trophic and anti-apoptotic molecules, resulting in the induction of a neuroprotective microenvironment. In addition, MSCs can promote the proliferation and maturation of local neural precursor cells, leading to their differentiation into mature neurons and oligodendrocytes [6,36]. MSCs can be considered as trophic mediators [87] via the production of an assortment of cytokines, of the angiogenic VEGF and of the prosurvival gene Akt1, and can be genetically modified to produce and deliver neurotrophic factors [88,89] or angiogenic factor [90] respectively to protect neurons and favour revascularisation in neurodegenerative diseases [91]. Subclones of MSCs already produce brain-derived neurotrophic factor and β -nerve growth factor [28]. Trophic factors produced by MSCs such as VEGF [87] or BDNF [28] can support motoneuron survival both diffusing at a distance and by local interaction with motoneurons. In addition, transplanted hMSCs can provide motoneurons with wild type SOD. Similar results were reported by authors using neural stem cells (reviewed in [45]) or hMSCs [91,92] that secrete GDNF.

In addition to their potential therapeutic effects, BM-derived MSCs are almost free from significant adverse

effects. When put in culture, they do not display malignant transformation [93] and maintain a stable profile of mRNA expression of tumour suppressor genes (p53, p16 and RB) and oncogenes (H-RAS) [94]. Most importantly, *in vivo* transplantation of long-term cultured hMSCs in nude mice did not result in tumour formation [78].

Since they originate from a different lineage from MNs, MSCs are potentially less vulnerable to the pathological mechanisms underlying ALS. We have demonstrated that MSCs isolated from the bone marrow of ALS patients maintain all their peculiar characteristics and when expanded *in vitro* do not display chromosomal alterations or cellular senescence. Moreover they acquire, under specific culture conditions, new morphological characteristics and neural markers which are suggestive of neural differentiation as well as those obtained in healthy donors [41].

Umbilical cord blood samples, collected from placentas and umbilical cord blood (UCB), are a source of stem and progenitor cells as well. Interestingly, CD34⁺ and CD45⁻ mesenchymal-like stem cells could be isolated and propagated through adherent cluster from both cord blood and the cord stroma and exhibited a potential to differentiate into neuron-like cells in culture [74,75]. Moreover, Garbuzova-Davis *et al.* [39], showed that a single intravenous administration of mononuclear cells from hUCB into pre-symptomatic G93ASOD1 mice delayed disease symptom progression and extended lifespan.

7. Administration routes

In view of the different approaches to stem cell therapy, it is mandatory to develop feasible and reliable methods of delivery. Proposed approaches include direct cell transplantation into the CNS parenchima, intravenous or intrathecal delivery and combined strategies. The route of cell administration, which represents another constraint for stem cell therapy in neurological diseases, is very much dependent on the CNS lesion sites. Given the widespread cell loss in ALS a 'systemic' route of administration could be the most effective therapeutic approach.

Intravenous delivery represents the less invasive approach, but is it uncertain whether the infused stem cells can successfully cross the BBB. In SOD1 mice BBB is damaged [95], even though it has been reported that the tight junctions of the capillary endothelial cells, that prevent the passive diffusion of circulating cells from blood, appear intact [96]. Nevertheless, intravenous administration of mononuclear cells from human UCB into pre-symptomatic SOD1^{G93A} mice delayed the progression of the disease and extended lifespan [39,97]. Similar positive results were obtained after injection of hMSC into retro-ocular space or into murine tail vein [38] or intraperitoneally [98]: in fact, in this last case bone marrow cells were found in brain, cerebellum, spinal cord, heart and skeletal muscles, contributing to ameliorating the disease phenotype of SOD1 mice. Although intravenous and intraperitoneal transplantations are feasible, not invasive and

without side-effects, they require a large number of grafted cells (up to 35×10^6 cells in mice) and immunosuppression in the case of non-autologous cells. The therapeutic effect is probably related to decreased pro-inflammatory cytokines in the brain and spinal cord, reduced microglia density in the spinal cord, and restored leukocyte profiles in the blood of treated mice, even in absence of a significant cell penetration in the CNS.

Intrathecal delivery is characterised by the low invasiveness and allows multiple engraftments at required intervals. This administration route is already used for effective delivery of other substances such as neuropeptides and neurotrophic molecules [such as, ciliary neurotrophic factor (CNTF); brain-derived neurotrophic factor (BDNF), IGF-1] or drugs.

Intrathecal transplantation of MSCs in SOD1 rats with a catheter directed from the cisterna magna towards the lumbar enlargement prolonged survival, decreased inflammation and showed neuroprotective effects on MNs. This administration route was used also in other neurodegenerative pathologies, such spinal muscular atrophy with respiratory distress type 1 (SMARD1), delaying disease progression and increasing lifespan [99]. On the contrary, no or poor effect were obtained with intrathecal transplantation of human bone marrow-stromal cells (hMSCs), umbilical cord blood cells (hUBCs) and their neuroectodermal derivatives [100], olfactory ensheathing cells (OECs) and BM-MSCs [101]: grafted cells often remain on the surface, without invading the spinal cord parenchima. As yet, no clinical effects have been observed in ALS patients following intrathecal or peripheral blood stem cells [102].

Positive outcomes have been achieved in ALS animal models with both neuronal and non neuronal stem cells by spinal intraparenchymal implantation [29,56,103-105]. It has been suggested that the proximity of grafted cells favours the diffusion of trophic and immunomodulatory factors to MNs and surrounding glia. This holds true especially when targeting specific neuromers of the spinal cord, even though in some cases multiple injections do not seem to provide advantages relative to single-site transplant [29,56,103-105].

Recently local injections produced a neuroprotective effect, using neural progenitors [55] and glial restricted precursors [34]. Reactive astrocytes can affect the survival of healthy MNs; in fact, astrocytes carrying the SOD1 mutation release toxic factors to motor neurons [105]. Thus, replacement of glial cells via stem cell transplantation could dilute the toxic effects of host astrocytes and release neuroprotective factors [30]. Astrocyte precursors, transplanted into the cervical spinal cord close to respiratory MN pools of transgenic SOD1 rodents, survive, differentiate into astrocytes and reduce microgliosis. Moreover, they extend survival, attenuate MN loss and slow the decline in forelimb motor and respiratory functions [34].

All these experimental data seem to suggest that targeting multisegmental cell delivery to the cervical spinal cord might

be a promising therapeutic strategy for slowing focal MN loss associated with ALS.

Intraparenchymal delivery in patients requires a technology capable of a safe targeted, localised administration: a stabilised platform can be used to achieve accurate targeting of infused cells to the ventral horn with the use of MER and motor evoked potentials as guidance tools [106].

Finally, while ALS is characterised by progressive loss of neurons and their connections to muscles, and moreover while recent studies have demonstrated that skeletal muscle is a primary target of SOD1^{G93A}-mediated toxicity [107], intramuscular transplantation could represent another helpful therapeutic approach: in particular, grafting of stem cells (myoblasts or MSCs) genetically modified to produce GDNF significantly delayed progression of disease, probably acting as 'mini-pumps' delivering growth factors directly into affected muscles [92]. These data referred to transplantation in gastrocnemius, tibialis anterior muscle, forelimb triceps brachii and long muscles of dorsal trunk, but in the future the aim could be targeting muscles of the diaphragm in order to protect respiratory MNs.

8. Association of stem cell therapy to delivery of trophic factors

Furthermore, treatment might combine cell transplantation and administration of growth factors [45]. We can, in fact, suggest that there is a synergy between stem cells and the growth factors release when administered together. Some studies where expression of GDNF in the lumbar spinal cord was achieved using direct lentiviral expression had no effects on motor neuron survival [108] while in other studies using wild-type hNPC, those secreting GDNF had a highly significant protective effect on motor neuron cell death in chimaeric regions of the SOD1G93A rat spinal cord [55]. One report showed that myoblasts modified to secrete GDNF can prevent motor neuron loss in a model of ALS [109] while hMSCs engineered to secrete glial-cell-line-derived neurotrophic factor (hMSC-GDNF) and transplanted into the skeletal muscles of SOD1G93A rats, significantly increases the number of neuromuscular connections and motor neuron cell bodies in the spinal cord at mid-stages of the disease and delays disease progression, increasing overall lifespan [92].

9. Clinical trials

Questions related to the source and optimal number of cells to engraft and the ideal way of delivery to guarantee stem cells availability in the affected regions of the central nervous system are still open. However, given the absence of a primate model and the lack of any effective treatment, it is mandatory to undergo clinical trials when the major requirements for cell therapy in animal model are satisfied and successful. This position is also reported in the Guidelines for the

Clinical Translation of Stem Cells, by the International Society for Stem Cell Research [110].

Strict cooperation between clinicians and basic science researchers is mandatory to develop reliable protocols. Clinics where harvesting, culture, purification and storage of the human stem cells are performed in authorised and highly specialised laboratories and the patients are recruited, treated and monitored in a tertiary ALS centre are the ideal sites for well managed clinical trials.

Over the past few years stem cell research has greatly expanded in clinics in order to develop innovative therapies for treating incurable neurodegenerative diseases. Some clinical trials have been developed for ALS (Table 2).

We tested the feasibility of mesenchymal stem cells transplantation in ALS patients in two Phase I clinical trials. MSCs were isolated, expanded and analyzed as described elsewhere in detail [111], according to the GMP conditions (European Medicines Agency, 1999). Surgery was uneventful in all patients. Mild (OMS grade I – II) and reversible symptoms were reported by patients: pain in the trunk, light-touch sensory impairment and tingling sensation in one lower limb, sensory light-touch impairment in the sacral region, in the absence of structural parenchymal changes and syringomyelia at the MRI both at short and long term.

Recently, another trial was conducted by collecting and re-infusing G-CSF-mobilised peripheral blood stem cells (PBSC) in ALS patients [112], without adverse effects, but with no significant changes in disease progression. However it was concluded that the results paved the way for a properly powered therapeutic trial with an optimised regimen of G-CSF.

Allogenic transplantation of hematopoietic stem cells (HSC) from human leukocyte antigen identically matched sibling donors in sporadic ALS [113] following total body irradiation show that HSC enter the human CNS primarily at pathological sites acting as immunomodulatory cells. Even though the transplant did not extend survival of patients, such cells might provide a cellular vehicle for future CNS gene therapy.

Bone marrow-derived hematopoietic progenitor stem cells, transplanted into the caudal brain stem and the cranial spinal cord (C1 – C2) in patients affected by sporadic ALS, have been reported to improve symptoms in half patients [114]. In another clinical trial, autologous blood CD133⁺ stem cells transplanted into the frontal motor cortex were reported to be safe and well-tolerated (at the one-year follow-up) and to delay disease progression, improving quality of life [115].

To sum up, stem cell therapy from different cell sources and employing different routes of administration is safe and well tolerated, even though the follow up in several studies is probably too short to exclude possible delayed complications, such as tumour formation. On the other hand, only some studies report clinical benefits: anyway, in our opinion, given the small number of clinical trials and some methodological issues, the results do not allow clear cut conclusions.

Table 2. Clinical trials of stem cells in ALS.

Stem cells	Route of delivery	Number of patients	Patients characteristics	Outcome	Ref.
Autologous MSC (from bone marrow)	Injection into the central part of thoracic SC after laminectomy and mielotomy	9	Spinal onset, FVC > 50%, normal polisomnography, ambulation with assistance or wheelchair bound Age 32 – 75. Months from diagnosis 8 – 60	Safe and well-tolerated even in long-term (4 years)	[111]
Peripheral blood stem cells (PBSC)	Mobilisation of autologous PBSC with GCSF	8	Seven patients had limb onset. Time interval from onset: 3 months to 4 years. Three patients wheelchair-bound and five ambulatory. Pre-treatment FVC range \pm 50 – 150%	Safe and well tolerated. No significant changes in disease progression	[112]
Allogenic hematopoietic stem cell (HSCT)	Intravenous infusion following total body irradiation; immuno-suppression	6	Spinal cord or bulbar onset, FVC > 60%, Age 35 – 59; Months from diagnosis 5 – 30	Tolerated (three chronic GVHD). No clinical benefits. Autopsies: spinal cord engrafted with immune cells, probably donor-derived	[113]
Autologous Bone marrow (BM)-derived hematopoietic progenitors	Laminectomy; cells injected to the anterior part of the spinal at the C1 – C2 level. (free hand?)	13	2 – 5 years from disease onset; age 34 – 71; 'moderate or severe' symptoms, three patients ventilation bounded	Nine patients 'became much better' (improved neck and limbs MRC; EMG findings of 'regeneration'). One patient was stable. Three patients died (1.5, 2 and 9 months after), of lung infection or myocardial infarction	[114]
Autologous blood purified CD133 ⁺ stem cells	Bilateral implantation in frontal motor cortex, with stereotactic or navigation guidance	10	Age 38 – 62; 18 – 42 months from diagnosis; no patients with severe bulbar involvement or malnutrition; occurrence of FVC values	Safe and well-tolerated (1 year follow-up). Patients survival significantly higher than control group (10 non-operated ALS patients)	[115]

ALS-FRS: ALS-functional rating scale; FVC: Forced vital capacity, GVHD: Graft-versus-host disease; MSC: Mesenchymal stem cell; SC: Spinal cord.

Stem cell clinical trials represents a new scenario in ALS clinical research. Our and other author's studies demonstrate that a surgical approach in ALS is feasible. The concerns that the surgical procedure may be harmful to the spinal cord or that general anaesthesia may cause severe complications in ALS patients are, apparently, not legitimate. Efforts should be directed to designing brief clinical trials that may provide the most meaningful information. It is important for the application and development of stem cell therapeutic approaches to develop non-invasive methods to monitor the modifications of cerebral and spinal cord tissue that lead to functional outcome. A better monitoring of transplanted MSCs and of their morphological outcomes could be obtained from ongoing neuroimaging studies (i.e., diffusion tensor imaging). Moreover, patient selection represents a fundamental issue, taking into account the location, severity, and clinical form of the disease and the adequacy of the site of injection. Delivery of stem cells to the key motor neuron

pools innervating respiratory muscles and ultimately affecting survival in ALS patients might represent a helpful short-term clinical approach.

10. Expert opinion

From the study of pharmacological clinical trials we know the extremely limited results in human ALS of several agents successfully tested in animal models and that a drug can have distinct pharmacological effects in different patients. It may lend us to suggest that similar problems will occur for stem cells. Despite many rodent studies showing that cell transplantation can change the disease course the variables responsible for the success of these therapies are largely unknown. Researchers have used different cell types, rodent models, transplant target locations, time of administration, and behaviour tests to assess the transplant's efficacy. The types of cells that may prove to be safer or better remain

purely speculative and await comparison studies. Recent technologies that reprogram adult dermal cells into MNs should be extensively studied in the near future in the attempt to develop an autologous stem cell-based intervention. The route of delivery represents another main point that is under debate. A direct injection might be the most reliable to ensure cells are near the MNs but a less invasive route such as intravenous administration could lead to a clinical efficacy. Laboratory studies on cell therapy for ALS should consider the standardisation of treatment protocols in symptomatic animals and of the outcome measures and safety indices. A primary goal could be to harness a collaboration across laboratories that have extensive experience in conducting studies in animal models using a standardised set of behavioural and histological outcome measures, and demonstrating mechanisms of action for testing the potential of cell therapies in ALS. Investigation of the effect of age and sex on cell transplantation therapy should also be considered as such parameters are generally overlooked in current rodent studies but are of critical clinical significance. The fact that more laboratories will carry out identical studies will help confirm the results for each parameter, which lends a high degree of veracity that is essential for translational studies.

Although ALS is a complex condition to consider for cell therapy, moving into the clinic will be warranted when the main points of the requirements of cell therapy clinical trials are successfully met, because of its severity and the lack of any effective therapy. In order to successfully and accurately

translate laboratory research to clinical practice we look forward to new clinical studies ahead to answer some main questions. What kind of patients should be recruited? When should cellular injection be contemplated during the course of the disease? Which cells should be tested in clinical trials? Do we need comparison studies? What kind of motor and behavioural tests must be used in patients to investigate the functional outcome of stem cell transplantation? How long should patients be followed in initial safety studies?

The future for cell therapy is exciting and opens a new scenario in the organisation of clinical trials in ALS. Many efforts should be addressed by clinicians expert in ALS to develop new small meaningful Phase I clinical trial. The physicians facing ALS patients in their clinical practice know very well that many of them are queuing to be treated with stem cells in countries where provision of such treatments is not strictly regulated. Rigorously conducted and well managed and designed controlled clinical trials might represent a hope and an answer for these patients, avoiding therapeutic misconception resulting from the patient's desire for a miracle cure.

Declaration of interest

This work was supported by grants from Compagnia di San Paolo to F Fagioli and A Vercelli, the Italian Ministry of Health to L Mazzini, and by Regione Piemonte to A Vercelli and the Fondazione Vialli e Mauro per la Ricerca e lo Sport.

Bibliography

- Kanazawa I. How do neurons die in neurodegenerative diseases? *Trends Mol Med* 2001;7(8):339-44
- Bradley WG. Updates on amyotrophic lateral sclerosis: improving patient care. *Ann Neurol* 2009;65(1):S1-2
- Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann Neurol* 2009;65(1):S3-9
- Brooks BR. Managing amyotrophic lateral sclerosis: slowing disease progression and improving patient quality of life. *Ann Neurol* 2009;65(1):S17-23
- Lee ST, Chu K, Jung KH, et al. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain* 2008;131(Pt 3):616-29
- Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;8:726-36
- Ben-Hur T. Immunomodulation by neural stem cells. *J Neurol Sci* 2008;265:102-4
- Knoepfler PS. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. *Stem Cells* 2009;27:1050-6
- Thonhoff JR, Jordan PM, Karam JR, et al. Identification of early disease progression in an ALS rat model. *Neurosci Lett* 2007;415(3):264-8
- De Carvalho M, Costa J, Swash M. Clinical trials in ALS: a review of the role of clinical and neurophysiological measurements. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2005;6(4):202-12
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;282:1145-7
- Brazelton TR, Rossi FMV, Keshet GI, et al. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 2000;290:1775-9
- Vallières L, Sawchenko PE. Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. *J Neurosci* 2003;23:5197-207
- Castro RF, Jackson KA, Goodell MA, et al. Failure of bone marrow cells to transdifferentiate into neural cells in vivo. *Science* 2002;297:1299
- Fuchs E, Segre JA. Stem cells: a new lease on life. *Cell* 2000;100:143-55
- Choumerianou DM, Dimitriou H, Kalmanti M. Stem cells: promises versus limitations. *Tissue Eng Part B Rev* 2008;14:53-60
- Aejaz HM, Aleem AK, Parveen N, et al. Stem cell therapy-present status. *Transplant Proc* 2007;39:694-9
- Henningson CT Jr, Stanislaus MA, Gewirtz AM, et al. Embryonic and adult stem cells therapy. *J Allergy Clin Immunol* 2003;111:S745-53
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-76

20. Chandran S. What are the prospects of stem cell therapy for neurology? *BMJ* 2008;337:a1934
21. Li XJ, Du ZW, Zarnowska ED, et al. Specification of motoneurons from human embryonic stem cells. *Nat Biotechnol* 2005;23:215-21
22. Di Giorgio FP, Carrasco MA, Siao MC, et al. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci* 2007;10:608-14
23. Karumbayaram S, Kelly TK, Paucar AA, et al. Human embryonic stem cell-derived motor neurons expressing SOD1 mutants exhibit typical signs of motor neuron degeneration linked to ALS. *Dis Model Mech* 2009;2(3-4):189-95
24. Di Giorgio FP, Boulting GL, Bobrowicz S, Eggan KC. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell* 2008;3(6):637-48
25. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* 2007;1:39-49
26. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can generate motor neurons. *Science* 2008;321:1218-21
27. Nishikawa S, Goldstein RA, Nierras CR. The promise of human induced pluripotent stem cells for research and therapy. *Nat Rev Mol Cell Biol* 2008;9(9):725-29
28. Crigler L, Robey RC, Asawachaicharn A, et al. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neurogenesis. *Exp Neurol* 2006;198:54-64
29. Vercelli A, Mereuta OM, Garbossa D, et al. Human mesenchymal stem cell transplantation extends survival, improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 2008;31(3):395-05
30. Suzuki M, Svendsen CN. Combining growth factor and stem cell therapy for amyotrophic lateral sclerosis. *Trends Neurosci* 2008;31(4):192-8
31. Gordon D, Scolding NJ. Human mesenchymal stem cell culture for neural transplantation. *Methods Mol Biol* 2009;549:103-18
32. Aiba K, Sharov A, Carter M, et al. Mouse embryonic stem cells and adult neural stem/progenitor cells defining a developmental path to neural fate by global expression profiling of mouse embryonic stem cells and adult neural stem/progenitor cells. *Stem Cells* 2006;24:889-95
33. Lladó J, Haenggeli C, Maragakis NJ, et al. Neural stem cells protect against glutamate-induced excitotoxicity and promote survival of injured motor neurons through the secretion of neurotrophic factors. *Mol Cell Neurosci* 2004;27(3):322-31
34. Lepore AC, Rauck B, Dejea C, et al. Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. *Nat Neurosci* 2008;11(11):1294-01
35. Martino G, Pluchino S. The therapeutic potential of neural stem cells. *Nat Rev Neurosci* 2006;7(5):395-06
36. Tsyb AE, Yuzhakov VV, Roshal' LM, et al. Morphofunctional study of the therapeutic efficacy of human mesenchymal and neural stem cells in rats with diffuse brain injury. *Bull Exp Biol Med* 2009;147(1):132-46
37. Kawamata T, Akiyama H, Yamada T, McGeer PL. Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am J Pathol* 1992;140(3):691-07
38. Ende N, Weinstein E, Chen R, Ende M. Human umbilical cord blood effect on sod mice (amyotrophic lateral sclerosis). *Life Sci* 2000;67(1):53-9
39. Garbuzova-Davis S, Willing AE, Zigova T, et al. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *Hematother Stem Cell Res* 2003;12(3):255-70
40. Mareschi K, Novara M, Rustichelli D, et al. Neural differentiation of human mesenchymal stem cells: evidence for expression of neural markers and eag K⁺ channel types. *Exp Hematol* 2006;34:1563-72
41. Ferrero I, Mazzini L, Rustichelli D, et al. Bone marrow mesenchymal stem cells from healthy donors and sporadic amyotrophic lateral sclerosis patients. *Cell Transplant* 2008;17(3):255-66
42. Yan J, Xu L, Welsh A, et al. Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in amyotrophic lateral sclerosis transgenic mice. *Stem Cells* 2006;24:1976-85
43. Xu L, Ryugo DK, Pongstaporn T, et al. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. *J Comp Neurol* 2009;514:297-9
44. Corti S, Locatelli F, Papadimitriou D, et al. Neural stem cells LewisX⁺ CXCR4⁺ modify disease progression in an amyotrophic lateral sclerosis model. *Brain* 2007;130(Pt 5):1289-305
45. Hedlund E, Hefferan MP, Marsala M, Isacson O. Cell therapy and stem cells in animal models of motor neuron disorders. *Eur J Neurosci* 2007;26:1721-37
46. De Hemptinne I, Boucherie C, Pochet R, et al. Unilateral induction of progenitors in the spinal cord of hSOD1G93A transgenic rats correlates with an asymmetrical hind limb paralysis. *Neurosci Lett* 2006;401:25-9
47. Liu Z, Martin LJ. The adult neural stem and progenitor cell niche is altered in amyotrophic lateral sclerosis mouse brain. *J Comp Neurol* 2006;497:468-88
48. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev* 2005;19:1129-55
49. Deshpande DM, Kim YS, Martinez T, et al. Recovery from paralysis in adult rats using embryonic stem cells. *Ann Neurol* 2006;60:32-44
50. Vogel G. Cell biology. Ready or not? Human ES cells head toward the clinic. *Science* 2005;308:1534-38
51. Galli R, Gritti A, Bonfanti L, Vescovi L. Neural stem cells: an overview. *Circ Res* 2003;92:598-08
52. Emgård M, Holmberg L, Samuelsson EB, et al. Human neural precursor cells continue to proliferate and exhibit low cell death after transplantation to the injured rat spinal cord. *Brain Res* 2009;30(1278):15-26
53. Capowski EE, Schneider BL, Ebert AD, et al. Lentiviral vector-mediated genetic modification of human neural progenitor cells for ex vivo gene therapy. *J Neurosci Methods* 2007;30:163(2):338-49

54. Klein SM, Behrstock S, McHugh J, et al. GDNF delivery using human neural progenitor cells in a rat model of ALS. *Hum Gene Ther* 2005;16(4):509-21
55. Suzuki M, McHugh J, Tork C, et al. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS ONE* 2007;2(1):e689, published online 1 August 2007 doi:10.1371/journal.pone.0000689
56. Garbuzova-Davis S, Willing AE, Milliken M, et al. Positive effect of transplantation of hNT neurons (NTERA 2/D1 cell-line) in a model of familial amyotrophic lateral sclerosis. *Exp Neurol* 2002;174(2):169-80
57. Neusch C, Bähr M, Schneider-Gold C. Glia cells in amyotrophic lateral sclerosis: new clues to understanding an old disease? *Muscle Nerve* 2007;35(6):712-24
58. Van Den Bosch L, Robberecht W. Crosstalk between astrocytes and motor neurons: what is the message? *Exp Neurol* 2008;211(1):1-6
59. Huard J, Youngento B, Goldstein B, et al. Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells. *J Comp Neurol* 1998;400:469-86
60. Richter MW, Roskams AJ. Olfactory ensheathing cell transplantation following spinal cord injury: hype or hope? *Exp Neurol* 2008;209:353-67
61. Garbuzova-Davis S, Sanberg PR. Feasibility of cell therapy for amyotrophic lateral sclerosis. *Exp Neurol* 2009;216(1):3-6
62. Bonilla S, Silva A, Geijo E, et al. Functional neural stem cells derived from adult bone marrow. *Neuroscience* 2005;133:85-95
63. Moraleda JM, Blanquer M, Bleda P, et al. Adult stem cell therapy: dream or reality? *Transpl Immunol* 2006;17:74-7
64. Beers DR, Henkel JS, Zhao W, et al. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc Natl Acad Sci USA* 2008;105(40):1558-63
65. Rice CM, Scolding NJ. Adult stem cells for the treatment of neurological disease. *Methods Mol Biol* 2009;549:17-32
66. Chen Y, Shao JZ, Xiang LX, et al. Mesenchymal stem cells: a promising candidate in regenerative medicine. *Int J Biochem Cell Biol* 2008;40(5):815-20
67. Garbuzova-Davis S, Willing AE, Saporta S, et al. Novel cell therapy approaches for brain repair. *Prog Brain Res* 2006;157:207-22
68. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-17
69. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. *Stem Cells* 2007;25(11):2896-902
70. Woodbury D, Schwarz EJ, Prockop DJ, et al. Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 2000;61:364-70
71. Deng W, Obrocka M, Fischer I, et al. In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. *Biochem Biophys Res Commun* 2001;282:148-52
72. Kohyama J, Abe H, Shimazaki T. Brain from bone: efficient metadifferentiation of marrow stroma-derived mature osteoblasts to neurons with Noggin or a demethylating agent. *Differentiation* 2001;68:235-44
73. Kim BJ, Seo JH, Bubien JK, et al. Differentiation of adult bone marrow stem cells into neuroprogenitor cells in vitro. *Neuroreport* 2002;13:1185-8
74. Muñoz-Elías G, Woodbury D, Black IB. Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor functions. *Stem Cells* 2003;21:437-48
75. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol* 2000;164:247-56
76. Lu P, Blesch A, Tuszynski MH. Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? *J Neurosci Res* 2004;77:174-91
77. Bertani N, Malatesta P, Volpi G, et al. Neurogenic potential of human mesenchymal stem cells revisited: analysis by immunostaining, time-lapse video and microarray. *J Cell Sci* 2005;118:3925-36
78. Kim J, Kang JW, Park JH, et al. Biological characterization of long-term cultured human mesenchymal stem cells. *Arch Pharm Res* 2009;32(1):117-26
79. Minguell JJ, Fierro FA, Epanan MJ, et al. Nonstimulated human uncommitted mesenchymal stem cells express cell markers of mesenchymal and neural lineages. *Stem Cells Dev* 2005;14:408-14
80. Blondheim NR, Levy YS, Ben-Zur T, et al. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. *Stem Cells Dev* 2006;15:141-64
81. Rice CM, Scolding NJ. Adult stem cells—reprogramming neurological repair? *Lancet* 2004;364(9429):193-9
82. Abdi R, Fiorina P, Adra CN, et al. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes* 2008;57(7):1759-67
83. Uccelli A, Zappia E, Benvenuto F, et al. Stem cells in inflammatory demyelinating disorders: a dual role for immunosuppression and neuroprotection. *Expert Opin Biol Ther* 2006;6:17-22
84. Borchelt DR. Amyotrophic lateral sclerosis—are microglia killing motor neurons? *N Engl J Med* 2005;55:1611-13
85. Feeney SJ, McKelvie PA, Austin L, et al. Presymptomatic motor neuron loss and reactive astrocytosis in the SOD1 mouse model of amyotrophic lateral sclerosis. *Muscle Nerve* 2001;24:1510-9
86. Christou YA, Moore HD, Shaw PJ, et al. Embryonic stem cells and prospects for their use in regenerative medicine approaches to motor neuron disease. *Neuropathol Appl Neurobiol* 2007;33:485-98
87. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006;98:1076-84
88. Hamada H, Kobune M, Nakamura K, et al. Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy. *Cancer Sci* 2005;96:149-56
89. Zwart I, Hill AJ, Al-Allaf F, et al. Umbilical cord blood mesenchymal stromal cells are neuroprotective and promote regeneration in a rat optic tract model. *Exp Neurol* 2009;216(2):439-48
90. Liu H, Honmou O, Harada K, et al. Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. *Brain* 2006;129:2734-45

91. McMahon JM, Conroy S, Lyons M, et al. Gene transfer into rat mesenchymal stem cells: a comparative study of viral and nonviral vectors. *Stem Cells Dev* 2006;15:87-96
92. Suzuki M, McHugh J, Tork C, et al. Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. *Mol Ther* 2008;16(12):2002-10
93. Bernardo ME, Zaffaroni N, Novara F, et al. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 2007;67(19):9142-49
94. Choumerianou DM, Dimitriou H, Perdikogianni C, et al. Study of oncogenic transformation in ex vivo expanded mesenchymal cells, from paediatric bone marrow. *Cell Prolif* 2008;41(6):909-22
95. Zhong Z, Deane R, Ali Z, et al. ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. *Nat Neurosci* 2008;11:420-2
96. Garbuzova-Davis S, Haller E, Saporta S, et al. Ultrastructure of blood-brain barrier and blood-spinal cord barrier in SOD1 mice modeling ALS. *Brain Res* 2007;1157:126-37
97. Garbuzova-Davis S, Sanberg CD, Kuzmin-Nichols N, et al. Human umbilical cord blood treatment in a mouse model of ALS: optimization of cell dose. *PLoS ONE* 2008;3(6):e2494, published online 25 June 2008, doi:10.1371/journal.pone.0002494
98. Corti S, Locatelli F, Donadoni C, et al. Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. *Brain* 2004;127:2518-32
99. Corti S, Locatelli F, Papadimitriou D, et al. Transplanted ALDHhiSSC neural stem cells generate motor neurons and delay disease progression of nmd mice, an animal model of SMARD1. *Hum Mol Genet* 2006;15(2):167-87
100. Habisch HJ, Janowski M, Binder D, et al. Intrathecal application of neuroectodermally converted stem cells into a mouse model of ALS: limited intraparenchymal migration and survival narrows therapeutic effects. *J Neural Transm* 2007;114(11):1395-06
101. Morita E, Watanabe Y, Ishimoto M, et al. A novel cell transplantation protocol and its application to an ALS mouse model. *Exp Neurol* 2008;213(2):431-38
102. Janson CG, Ramesh TM, Doring MJ, et al. Human intrathecal transplantation of peripheral blood stem cells in amyotrophic Lateral Sclerosis. *J Hemat Stem Cell Res* 2001;10:913-15
103. Garbuzova-Davis S, Willing AE, Milliken M, et al. Intraspinal implantation of hNT neurons into SOD1 mice with apparent motor deficit. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2001;2:175-80
104. Hemendinger R, Wang J, Malik S, et al. Sertoli cells improve survival of motor neurons in SOD1 transgenic mice, a model of amyotrophic lateral sclerosis. *Exp Neurol* 2005;196:235-43
105. Nagai M, Re DB, Nagata T, et al. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci* 2007;10:615-22
106. Riley J, Butler J, Baker KB, et al. Targeted spinal cord therapeutics delivery: stabilized platform and microelectrode recording guidance validation. *Stereotact Funct Neurosurg* 2008;86(2):67-74
107. Dobrowolny G, Aucello M, Rizzuto E, et al. Skeletal muscle is a primary target of SOD1G93A-mediated toxicity. *Cell Metab* 2008;8(5):425-36
108. Guillot S, Azzouz M, Déglon N, et al. Local GDNF expression mediated by lentiviral vector protects facial nerve motoneurons but not spinal motoneurons in SOD1G93A transgenic mice. *Neurobiol Dis* 2004;16:139-49
109. Mohajeri MH, Figlewicz DA, Bohn MC. Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and disease progression in a mouse model of familial amyotrophic lateral sclerosis. *Hum Gene Ther* 1999;10(11):1853-66
110. Guidelines for the Clinical Translation of Stem Cells. Deerfield, Illinois, International Society for Stem Cell Research, 2008. Available from: http://www.isscr.org/clinical_trans/. [Last accessed 31 July 2009]
111. Mazzini L, Mareschi K, Ferrero I, et al. Stem cell treatment in amyotrophic lateral sclerosis. *J Neurol Sci* 2008;265:78-83
112. Cashman N, Tan LY, Krieger C, et al. Pilot study of granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells in amyotrophic lateral sclerosis (ALS). *Muscle Nerve* 2008;37:620-25
113. Appel SH, Engelhardt JI, Henkel JS. Hematopoietic stem cell transplantation in patients with sporadic amyotrophic lateral sclerosis. *Neurology* 2008;71:1326-34
114. Deda H, Inci MC, Kürekçi AE, et al. Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-year follow-up. *Cytotherapy* 2009;11(1):18-2
115. Martinez HR, Gonzalez-Garza MT, Moreno-Cuevas JE, et al. Stem-cell transplantation into the frontal motor cortex in amyotrophic lateral sclerosis patients. *Cytotherapy* 2009;11(1):26-34
116. Xu L, Yan J, Chen D, et al. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. *Transplantation* 2006;82:865-75
117. Boucherie C, Schäfer S, Lavand'homme P, et al. Chimerization of astroglial population in the lumbar spinal cord after mesenchymal stem cell transplantation prolongs survival in a rat model of amyotrophic lateral sclerosis. *J Neurosci Res* 2009;87(9):2034-46

Affiliation

Letizia Mazzini¹, Alessandro Vercelli², Ivana Ferrero⁴, Katia Mareschi⁴, Marina Boido², Serena Servo¹, Gaia Donata Oggioni¹, Lucia Testa¹, Francesco Monaco¹ & Franca Fagioli³

[†]Author for correspondence
¹Eastern Piedmont University, "Maggiore della Carità" Hospital, ALS Centre, Department of Neurology, Corso Mazzini 18, 28100, Novara, Italy
 Tel: +39 0321 3733834; Fax: +39 0321 3733298; E-mail: mazzini.l@libero.it

²University of Torino, Neuroscience Institute in Torino, Italy

³Regina Margherita Children's Hospital, Stem Cell Transplantation and Cellular Therapy Unit, Pediatric Onco-Hematology Department, Torino, Italy

⁴University of Torino, Regina Margherita Children's Hospital, Department of Pediatrics, Torino, Italy