Histol Histopathol (2011) 26: 1093-1109

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Neural stem cell-mediated therapy for rare brain diseases: perspectives in the near future for LSDs and MNDs

Lidia de Filippis

Department of Biotechnologies and Bioscience, Università Milano Bicocca, Milan, Italy

Summary. Lysosomal storage diseases (LSDs) are genetically inherited disorders affecting most patients in pediatric age and progressively lead to severe, even lethal, multiorgan dysfunction and brain neurodegeneration. Motor neuron diseases (MNDs) or Amyotrophic Lateral Sclerosis (ALS)-related syndromes are neurodegenerative disorders occurring in the majority of cases sporadically and affect adult middle-aged patients. Despite being divergent in most pathological and physiological hallmarks, both MNDs and LSDs are characterized by tremendous clinical heterogeneity due to poor prognosis and variable onset of the symptoms. Moreover, both LSDs and MNDs are characterized by the concurrence of multiple pathogenetic processes, such as the development of inflammatory and excitotoxic environments. Furthermore, pharmacological, enzyme or genetic therapies have proven to be ineffective and no cure is currently available for the neurodegeneration in either LSD or ALS affected patients. Recent studies have identified non-neuronal cell types, such as astrocytes and microglia, as being involved in non cell-autonomous effects on MND or LSD progression. These findings have prompted the use of neural stem cells for the replacement of non-neuronal cells rather than neuronal cells, which may result in neuroprotection and immunomodulation. The choice of an appropriate tissue source and the establishment of standardized paradigms to culture human neural stem cells (hNSC) will allow their use for future clinical trials on both ALS and LSD affected patients and parallel drug screening studies with novel breakthroughs in the knowledge of neurodegenerative diseases.

Offprint requests to: Lidia De Filippis, Department of Biotechnologies and Bioscience, University Milan Bicocca, Piazza della Scienza 2, 20126 Milan, Italy. e-mail: lidia.defilippis@unimib.it

Key words: Neural stem cells, Lysosomal storage diseases, Motor neuron diseases, Amyotrophic lateral sclerosis, Clinical trials

Introduction

The wide spectrum of neurological pathologies with poor prognosis and severe, often lethal diagnosis, has raised a great interest in the study of cures able to at least delay or, at best, arrest the disease progression. The three main strategies currently promoted as therapies for neurological disorders encompass pharmacological drugs, therapeutic gene delivery systems and transplantation of stem cells. In particular, rare metabolic and motor neuron diseases are multisystemic disorders with a low life expectancy, and no treatment able to address all their clinical manifestations is available so far. In the last few years, scientific and pharmacological interest has been particularly focused on lysosomal storage diseases (LSDs), which are autosomal recessive disorders characterized by the deficiency of specific lysosomal enzymes. In parallel, an increasing number of patients affected by ALS or ALS-related disorders has raised the awareness of motor neuron pathologies, for the majority of which the causes are still under investigation. For LSDs, available therapeutic tools mainly including enzyme targeted drugs or pharmacological chaperones (enzyme enhancement), substrate targeted drugs (substrate deprivation) (Mehta et al., 2010; Elstein, 2011), and the recently developed Enzyme replacement therapy (ERT), are currently in use or being tested and have proven to be useful in correcting non-neurological symptoms and pain, but the general inability of the different compounds to traverse the blood brain barrier strongly limits their diffusion throughout the central nervous system (CNS). Genetic therapy has been approved for clinical applications in

LSDs such as Canavan's disease (Janson et al., 2006), but in most cases it has been shown to be only a palliative cure and a major drawback still remains, namely, that of secondary effects due to genetic manipulation (Kohn et al., 2003; Raper et al., 2003). On the other hand, MNDs and, in particular, ALS, are multifactorial disorders with a mostly idiopathic occurrence. Hence, gene delivery does not represent a valuable therapeutic option, and pharmacological compounds such as riluzole have been proven to be ineffective or able to prolong lifespan by a short time. Despite developing through very different physiopathological processes, both LSDs and MNDs share a tremendous clinical heterogeneity (the degree of severity of the signs, the appearance of diagnostic markers) and pathological hallmarks, such as the degeneration of neural and non-neural cells and the development of a diffuse inflammatory environment. Transplantation of neural stem cells in animal models of LSDs or ALS has shown the ability of exogenous NSCs to modulate both cell-autonomous and non-cell-autonomous components of the pathology and, eventually, to exert immunomodulatory effects, thus supporting the rationale to exploit NSC-mediated therapy for both neural and non-neural cell replacement. In this review we will discuss several neurological findings which have emerged in pre-clinical experimental studies. Finally, we will evaluate innovative and potentially efficacious therapeutic approaches to use neural stem cell transplantation for the cure of neurodegenerative diseases such as LSDs and MNDs (Gieselmann and Krageloh-Mann, 2010).

Neural stem cells (NSCs)

Somatic adult NSCs are tissue-specific cells endowed with fundamental properties, such as self-renewal capacity and the ability to differentiate into neurons, oligodendrocytes and astrocytes. Therefore they maintain the functional and structural integrity of the brain in physiological conditions. Reynolds and Weiss (Reynolds and Weiss, 1992) first demonstrated a stem cell niche in the CNS. In particular, the finding of adult neurogenesis in the SVZ, which leads to the generation of neural progenitors migrating to the olfactory bulbs and to the cortex, has favoured the idea that newborn neurons might subserve cognitive functions and contribute to the homeostasis of the telencephalic-diencephalic area. However, given the inherent resilience of the post-natal, and particularly of the adult mammalian brain, the addition of new cells to pre-existing circuitry and the repair of damaged brain tissue by endogenous cell replacement are very limited. For this reason, many studies have been focused on NSCs as a therapeutic tool for a wide array of neurodegenerative disorders, including both genetic diseases like metachromatic Leukodystrophy (MLD), Huntington's Disease HD, Alzheimer's Disease (AD) (sporadic) and idiopathic diseases like Parkinson's Disease (PD), AD, Multiple Sclerosis (MS), Amyotrophyc Lateral Sclerosis (ALS), stroke, etc. Thus far NSC lines have been derived from germinative zones of the brain such as the hippocampal dentate gyrus, the olfactory bulb, the SVZ surrounding the ventricles, the subcallosal zone underlying the corpus callosum and from the spinal cord of the embryonic, neonatal, and adult rodent CNS, and they have been propagated *in vitro* both by using mitogens and propagating genes such as v-myc or large T-antigen (T-Ag) (Bottai et al., 2003; Jandial et al., 2008).

Our lab has established *in vitro* conditions to isolate and propagate NSCs from adult rodent and fetal human brain (Gritti et al., 1995, 1996, 1999, 2002; Vescovi et al., 1999) and from brain tumors (Galli et al., 2004). We have developed a methodology that allows the isolation and expansion of NSCs by means of growth factors (EGF and FGF2) without genetic modification and without serum: the neurosphere assay. Following this paradigm, NSCs fulfill the cardinal requirements for "stemness" throughout extensive culturing: self-renewal capacity, functional stability and multipotentiality (Gritti et al., 1995, 1996, 1999). Upon growth factor removal, NSCs cease proliferation and terminally differentiate into the three major neural lineages, i.e. astrocytes, oligodendrocytes and functional neurons, the latter able to elicit action potentials *in vitro*, as demonstrated by molecular biology, biochemistry, immunocytochemistry, fluorescence microscopy and electrophysiological assays (Vescovi et al., 1999). We performed a detailed study of the propensity of NSCs to develop potentially tumorigenic alterations and we conclusively demonstrated that NSCs can be extensively propagated *in vitro* (over 100 passages) maintaining a strikingly stable profile with regard to self-renewal, differentiation, growth factor dependence, karyotype, and molecular profiling. Most importantly, the long-term culturing of NSCs did not result in the formation of tumors *in vivo*, even when NSCs were transduced with Myc and Ras oncogenes (Foroni et al., 2007). These *in vitro* results showed that we had established a standardized paradigm for NSC culture, which is a key point to translate NSC application from the bench to the bedside, and paved the way to investigate the clinical therapeutic potential of NSCs *in vivo*.

In this view, an extensive characterization of the biological properties of NSCs has been performed *in vivo* by transplantation into the CNS of different animal models of neurodegenerative diseases (Givogri et al., 2008; Pluchino et al., 2009; Neri et al., 2010; , Rota Nodari et al., 2010). NSCs are able to integrate within the brain parenchyma without signs of tumorigenity or overgrowth, to migrate inside the CNS into damaged regions and to contribute to tissue repair by replacement of degenerated cells or by neurotrophic support to actively degenerating cells (Neri et al., 2010; Rota Nodari et al., 2010). In recent studies (Pluchino et al., 2003; Pluchino et al., 2009), we have also shown that following intravenous or intracerebral injection in mice

affected by an experimental form of MS (EAE), NSCs can selectively reach brain and spinal cord areas affected by the demyelinating-inflammatory process and contribute to myelin restoration and reduction of astrogliosis in those damaged areas, with maximal efficacy if injected in the early phase of the disease (Pluchino et al., 2003). Given their multimodal therapeutic potential, NSCs could be further exploited as a delivery route of therapeutic genes through either inherent or ectopically induced expression. To this aim, lentiviral vectors were used to transduce NSCs *in vitro* and *in vivo* (Consiglio et al., 2004), and no alteration of the basic stem properties of NSCs was observed. Indeed, when a lentiviral vector carrying the gfp gene was injected into the SVZ of adult mice, endogenous stem cells were monitored along the RMS up to the olfactory bulb (OB), and within 6 months of the injection a chimeric mosaicism of transduced and non-transduced cells was detectable in the OB, demonstrating that the neurogenic ability of NSCs is not affected by lentiviral transduction and that the expression of a therapeutic gene product by NSCs can be used as a valid approach to perform genetic trans-correction. Indeed, GDNF-overexpressing NSC were shown to be able to increase the survival of neuronal cells in the striatum of HD rodents and to delay the degeneration of motor neurons in the spinal cord of ALS rats (Suzuki et al., 2007; Ebert et al., 2010), while IGF-overexpressing NSC displayed a protective effect on dopamine neurons in a rat model of PD (Ebert et al., 2008). Similarly, genetic modification of NSCs with NT-3 has been reported to promote myelination and to reduce astroglial scarring after transplantation in rodents lesioned by spinal cord injury or ischemic brain injury (Park et al., 2006; Kusano et al., 2010). Also, the genetic induction of the expression of immunomodulatory cytokines, such as IL-12 and IL-10, has been exploited to modulate the inflammatory environments associated to glioma (Yang et al., 2004) or to MS (Yang et al., 2009) respectively.

The results obtained with rodent NSCs demonstrated that NSCs possess a wide range of potentially therapeutic effects, amongst which the replacement of functional neuronal cells (Imitola et al., 2004b) seems to have the smallest impact on the neuroregeneration process. This effect appears to be likely complemented by or, to some extent, eclipsed by other actions, such as the above cited delivery of therapeutic gene products (Martinez-Serrano et al., 1995a; Snyder et al., 1995; Flax et al., 1998; Park and Teng et al., 2002; Givogri et al., 2006), scavenging of toxic molecules present in the microenvironment (Park and Teng et al., 2002; Kondo et al., 2005; Givogri et al., 2006) or the replacement of multiple elements characterizing a specific CNS area (Trujillo et al., 2009; Xuan et al., 2009; Neri et al., 2010). It is also important to consider that from a wider point of view, stem cell-mediated therapy may synergize and complement other therapies, such as pharmacological or genetic therapies. The success in a wide spectrum of neurodegeneration models using rodent NSCs has thus prompted an intensive effort to develop procedures for a safe generation of equivalent human NSC (hNSC) lines. Several clonal, genetically homogeneous hNSC cell lines have been obtained by genetic perpetuation methods (Flax et al., 1998; Villa et al., 2000). Taking advantage of their non-transformed nature, human origin, multipotentiality, fast but conditional growth, unlimited availability and suitability for molecular manipulation, these cell lines have provided important breakthroughs for the development of cell replacement and/or gene transfer-based therapies. Hence, cell therapy in the CNS is reaching the stage of clinical application, with the first few clinical trials already underway in some post-traumatic, post-ischemic or neurodegenerative disorders. However, a critical element in this endeavor is represented by the cells to be used in neural transplantation, and both the advantages and the limitations involved in their application must be taken into careful consideration. Embryonic stem cells (ESCs) are found in the blastocyst (inner cell mass), display unlimited growth in culture and are able to originate all the tissues of the organism (pluripotency), but the availability of the tissue is strongly limited by ethical issues, and the risk of these cells forming teratomas is a major concern (Aleckovic and Simon, 2008). Unlike ESCs, fetal brain NSCs do not form tumors, although they can be similarly expanded long term *in vitro* and exhibit a multiple differentiation potential, that is to originate neurons, astrocytes and oligodendrocytes. In contrast to exogenous ESCs or fetal NSCs, adult stem cells can be exploited without ethical and immunological constraints and are located in several tissue compartments of the body, such as the bone marrow, but they are only capable of generating a restricted range of cells and are harder to cultivate in the lab. Induced pluripotent stem cells (iPS) have been recently proposed for autologous transplantation, but a major drawback of these genetically manipulated cells is the high risk of cancer formation, mainly due to the uncontrolled integration of retroviral vectors and recombination events. Notwithstanding several clinical trials harnessing various sources of neural stem cells are currently ongoing (Table 1). The first phase I clinical trial in the world involving NSCs was authorized by the FDA in 2005 for the cure of Batten's Disease in the USA. The researchers selected fetal NSCs as the most suitable candidates for transplantation into humans because they are unlikely to develop into any other phenotype than neural differentiated cells (Taupin, 2006a-c). The cells were provided by Stem Cells Inc., which procures tissue from aborted fetuses with the consent of the mothers, thus overriding ethical limitations. In 2009, two clinical trials were approved by the FDA in the USA for the cure of ALS patients by transplantation of neural stem cells into the spinal cord (Raore et al., 2011). One trial was promoted by the company Neuralstem, which cultured neural stem cells derived from the spinal cord of a single eight-week-old foetus, whereas the second one was commenced by

Geron and intended to treat spinal cord injury using ESCs pre-differentiated into precursors of neuron-support cells (Alper, 2009).

In 2008, Aboody and her colleagues from City of Hope's Department of Neurosciences demonstrated the inherent propensity, also known as tropism, of neural stem cells to home in on invasive tumor cells (Aboody et al., 2008), even migrating from the opposite side of the brain or across the blood-brain barrier, and harnessed the tumor-tropism of neural stem cells to deliver therapeutic agents to invasive tumor sites. Ten years later, in 2010, the FDA has approved a phase I clinical trial for NSC-mediated therapy of high-grade gliomas, further supporting the use of NSCs as an efficacious and safe therapeutic tool for the cure of a wide array of neurodegenerative disorders. The therapy uses a genetically modified human NSC line, generated by Seung U. Kim, M.D., Ph.D. (Division of Neurology at the University of British Columbia), to deliver a prodrug-activating enzyme (cytosine deaminase) to brain tumor sites.

Alongside the running US clinical study for spinal cord injury, in November 2010 ReNeuron launched clinical trials injecting human embryonic stem cells into the brain of stroke victims in Glasgow (Stroemer et al., 2009). They use a clonal stem cell line, CTX (Stroemer et al., 2008), derived from a single donor of embryonic stem cells which showed targeted migration and neuronogenesis when injected into rats with stroke damage (Kelly et al., 2004). These important breakthroughs in the clinical application of NSCs demonstrate that a continuous and standardized clinical grade source of normal human CNS cells (hNSCs), combining the plasticity of fetal tissue with extensive proliferative capacity and functional stability, is of paramount importance.

In Italy, the Stem Cell Factory (Ospedale S. Maria Terni) (Vescovi et al., 1999) is currently establishing continuous NSC lines from fetal human CNS, with an emphasis on cells from spontaneous miscarriages, which provide a plentiful and consistent source of non-transformed human neural cells. These hNSCs are generated in a good manufacturing practice (GMP) cell factory and will be compatible with the development of standardized clinical trials in neurodegenerative disorders. Our hNSCs have now been serially expanded under chemically defined conditions, resulting in a 10^7-10^8 fold increase in cell number, and are being cryopreserved, establishing a GMP-grade, hNSCs bank. In order to certify these cells by the GMP standard, a panel of cellular, functional and biochemical criteria must be met prior to cell release, which include, but are not limited to, karyotype analysis, stable differentiation and growth capacity, and lack of biological contamination by adventitious agents. We already obtained some evidence of their efficacy and immunogenic tolerance upon transplantation into animal models of neurological disorders (Rota Nodari et al., 2010) such as transient global ischemia, which is a model of vascular dementia and resembles several pathological features of AD. 3 days after global ischemic injury, hNSC immortalized with v-myc (IhNSC) were unilaterally implantated into the corpus callosum or the hippocampal fissure of adult rat brains. After 1 month, IhNSCs were detected to migrate through the corpus callosum, into the cortex or throughout the dentate gyrus of the hippocampus, and by the fourth month, to reach the ipsilateral subventricular zone, the CA1-3 hippocampal layers and the controlateral hemisphere, proving to be non-tumorigenic and to undergo a proper regional differentiation into GABAergic and GLUTAmatergic neurons (Rota Nodari et al., 2010). Electron microscopy analysis pointed to the formation of mature synaptic contacts between host and donor-derived neurons, showing the full maturation of the IhNSC-derived neurons and their likely functional integration into the host tissue. Notably, these results could be accomplished using transient immuno-

Table 1. Clinical trials in progress with human neural stem cells (www.clinicaltrials.org).

STEM CELL SOURCE	DISEASE	DELIVERY	YEAR	REFERENCE
Fetal neural stem cells (Stem Cells Inc.)	Batten's Disease or neuronal ceroid lipofuscinosis (NCL)	Brain neurosurgery single dose	2006	R. Steiner et al., Oregon Health and Science University (Taupin 2006c)
Embryonic stem cells-derived oligodendrocyte progenitors (GRNOPC1 from Geron)	Spinal cord injury	Spinal cord injection	2009	G.K. Steinberg, Stanford University, california; and R.G. Fessler, Northwestern University, Illinois (Alper, 2009)
Fetal neural stem cells (8 weeks-old foetus)(Neuralstem)	Amyotrophic Lateral Sclerosis (ALS)	Multi-injection into the spinal cord	2010	K.Johe, N. Boulis, Emory University in Atlanta (USA) (Raore et al., 2011)
Human fetal stem cells (CTX0E03 from Reneuron)	stroke	Brain neurosurgery (stereotaxic injection in the putamen region)	2010	K. Muir, Glasgow Southern General Hospital, UK (Stroemer et al., 2009)
Genetically modified Human neural stem cells to produce cytosine deaminase enzyme (Seung U. Kim, university of British Columbia)	glioma	Intravenous delivery	2010	K.S. Aboody and J. Portnow, City of Hope (Aboody et al., 2008)

suppression, i.e administering cyclosporine for 15 days following the ischemic event. A wide array of studies have shown that NSCs are not susceptible to immunological rejection (Bjorklund et al., 2003; Wennersten et al., 2006; Olstorn et al., 2007; Mendez et al., 2008) even when transplanted in animal models like EAE, characterized by a constitutively activated immunological response (Pluchino et al., 2003, 2005). Similar results were also obtained in a different context: after transplantation into the adult rat brain lesioned by focal demyelination (Ferrari et al., submitted), hNSCs have demonstrated to integrate into the NSC host niche and to migrate toward the lesioned corpus callosum, where they properly differentiated into myelinating oligodendrocytes. No sign of tumorigenicity was ever detected upon transplantation of hNSCs (unpublished observation) nor of hNSC immortalized with proliferating genes such as c-myc, c-myc T58A and v-myc (De Filippis et al., 2007, 2008). These results confirm that hNSC are scarcely immunogenic.

Besides neurodegeneration per se, one of the hallmarks characterizing most neurodegenerative disorders like stroke, AD, PD, ALS, MLD, is the development of an inflammatory environment (Fig. 1), which can contribute to tissue damage(Glass et al., 2010). Recent studies have shown that NSCs may also exert their therapeutic potential through an immunomodulatory action (Pluchino et al., 2005, 2009; Bacigaluppi et al., 2009). Both in the globally ischemic and focally demyelinated rat brains, we observed that transplantation of hNSCs can effectively decrease reactive astrogliosis and dampen microglial activation in the injuried areas (Rota Nodari et al., 2010); unpublished results). This effect exclusively occurred in the transplanted regions and was most prominent at 15 days from transplantation, when the inflammatory reaction appeared to reach its nadir. There was an obvious effect on the state of activation of microglia, whose cells shifted from the activated, macrophagic-amoeboid phenotype to the resting, stellate one, with a concomitant shift of astrocytes from fibrotic and globular to star-shaped and long-branching in the transplanted areas. This phenomenon may, in fact, participate in the low immunogenic response that these cells seem to elicit in the CNS, together with the lack of expression of Molecular Histocompatibility Complex class II components (MHCII) (Imitola and Comabella et al., 2004; Imitola and Raddassi et al., 2004). Notwithstanding, it is also true that some level of immune surveillance is maintained in the adult brain upon NSC engraftment, which explains the widespread need to use immune suppression (Wennersten et al., 2006) in experimental and clinical intracerebral transplantation (Bjorklund et al., 2003; Olstorn et al., 2007). The succesful use of transient immuno-suppression proposes a suitable milder approach to immunosuppression for the prospective use of hNSCs for clinical purposes. The fact that discontinuous treatment with cyclosporine does not affect integration of transplanted cells in most of the brain regions, which to all effects emerge as immunoprivileged when considering hNSCs, is in good accordance with most recent findings (Wennersten et al., 2006). This view led to the idea of using hNSCs as a suitable tool to model transplantation in pre-clinical settings and further support the exploitation of GMP-grade hNSC for stem cell-mediated therapy of neurodegenerative disorders in human patients.

Lysosomal storage disases (LSDs)

Most neurodegenerative diseases are symptomatic in adulthood, but several rare pathologies occur in children, leading to severe mental retardation and premature death with devastating consequences on the psychological profile of their relatives and dramatic impact on the sanitary social costs (http://ec.europa.eu/health-eu/health_problems/rare_diseases/index_it.htm). Pediatric neurodegenerative diseases often derive from a wide range of genetic metabolic disorders, including lysosomal storage diseases (LSDs). LSDs encompass at least 40 distinct diseases with a global incidence ranging from 1:5000 to 1:8000 and are caused by the lack of specific lysosomal enzymes or components essential for the complete degradation of macromolecules and the recycling of resting metabolites (Wraith, 2002). The accumulation of intermediate degradation products affects the appropriate activity of the lysosomes and other cell organelles and this metabolic impairment results in the progressive disregulation of tissue and organ functions (Meikle et al., 1999). The alteration of the mechanisms involved in the degradation of metabolites like glycosfingolipids (GSL) in Sandhoff's disease or heparan sulfate oligosaccharide (HSO) chains in muchoplolysaccharidosis (MPS) I, II and III, primarily affects the CNS. Hence, at the neonatal stage, the accumulation of catabolites primes the progression of the disease, leading to mental retardation, progressive neurodegeneration and to the precocious death of the patients.

The cascade of events triggered by the deficit of an enzyme or its aberrant production also leads to secondary effects like brain inflammation (Fig. 1A-D) and global alteration of macromolecule degradation systems, which result in the abnormal storage of inefficiently degraded materials in cell vacuoles and the formation of lysosomal aggregates (Fig. 2A,B). Both phenomena are likely to contribute to a reduction of the inherent plasticity of the CNS with progressive deleterious consequences on cognition and behaviour. Affected neurons die through apoptosis or necrosis and the massive neuronal loss usually becomes evident at advanced stages of the disease.

Several breakthroughs in the study of the mechanisms involved in the targeting of enzymes to the lysosomal compartment led to the identification of the pathway driven by the mannose-6P receptor (Kornfeld and Mellman, 1989). Since then, the identification and

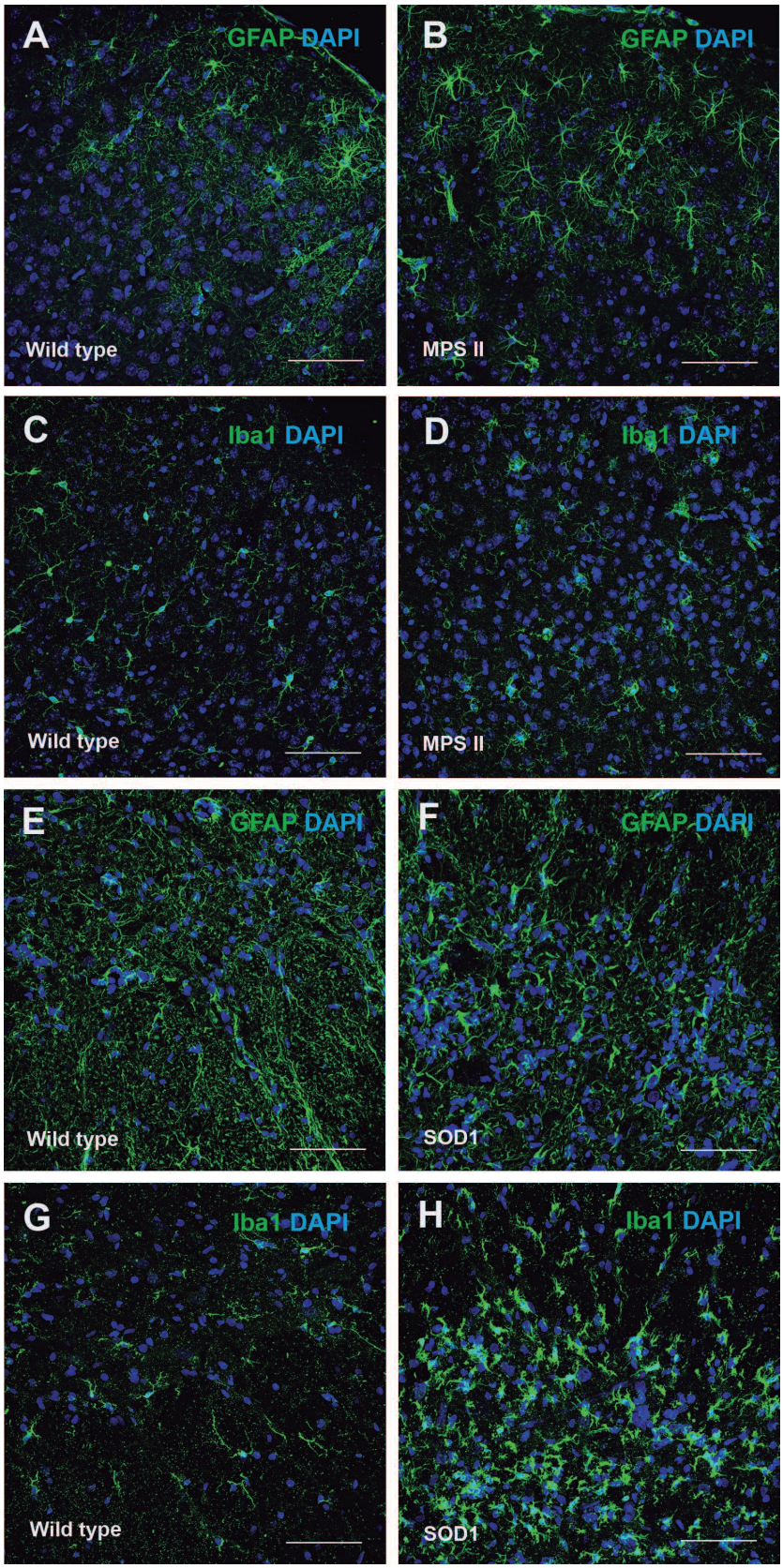

Fig. 1. Confocal microscopy analysis showing astrogliosis and microgliosis in the brain of MPSII (LSD) and in the spinal cord of SOD1 (ALS) rodents. **A-B**, **E-F**) Astroglial cells (GFAP+, green) appear more abundant and with stretched, fibroblast-shaped morphology in the MPSII brain **(B)** (striatum, p224) and SOD1 (p120) spinal cord **(F)** (ventral horns) than in matching wild-type controls **(A, E)**. **C-D, G-H.** Increase of microglial cells (Iba1+, green) displaying an amoeboid, globular morphology in the MPSII brain **(D)** (striatum) and SOD1 spinal cord **(H)**(ventral horns) compared to stellate resident microglial cells in matched wild-type controls **(C, G)**. Total nuclei are indicated by dapi staining (blue). Scale bar: 75 μm.

cloning of the genes encoding for all the known lysosomal enzymes has been achieved, enabling the generation of reliable animal models and recombinant forms of the involved, defective lysosomal enzymes. The study of LSDs is greatly facilitated by the availability of genetic murine models allowing us to recapitulate the pathological steps of the disease and to validate several strategies that could be promoted for their cure and therapy (Haskins et al., 2006). Currently available drugs include substances able to favour the correct folding of the mutant enzyme, such as pharmacological chaperone proteins (enzyme enhancement) (Elstein, 2011; Pan, 2011), or substrate targeted drugs which inhibit synthesis or accumulation of the substrate (substrate deprivation) by modifying its structure (Trujillo et al., 2009; Xuan et al., 2009; Mehta et al., 2010; Elstein, 2011).

Further achievements allowed the development of Enzyme Replacement Therapy (ERT), currently used for the treatment of some LSDs. In ERT, the wild-type isoform of the missing lysosomal enzyme is intravenously infused to compensate for the primary defect. The recombinant enzyme is uptaken by endogenous cells and transported to the lysosomes, where it catalyses the missing step of the degradation pathway, thus eliminating the partially degraded catabolites that have toxic effects on the cell. ERT has revolutionised the treatment of several LSDs, such as Gaucher's, Fabry's, Pompe's and Hunter's diseases (Van den Hout et al., 2000; Eng et al., 2001, Kakkis et al., 2001; Muenzer et al., 2006, 2007; Wraith, 2008). Still, a major drawback is that the large glycoproteins administered by ERT are unable to cross the BBB in order to exert persistent, effective therapeutic levels of enzymatic activity in the affected areas (Sly and Vogler, 2002). Nonetheless, it has been shown that the CNS benefits from the delivery of functional enzymes in animal models of LSDs (Lee et al., 2007). These results have been achieved through (i) gene therapy based on the injection into the brain parenchima of adeno-associated viral vectors (AAV) containing DNA sequences coding for the missing lysosomal enzyme (Cardone et al., 2006), or (ii) intrathecal infusion of the recombinant enzyme in the cerebro-spinal fluid (CSF). In particular, the success obtained with repeated intrathecal injections of recombinant sulfamidase in MPSIIIA mice provided a proof-of-concept for feasibility and efficacy (Fraldi et al., 2007).

Considering that ERT is limited by the scarce BBB permeability to the enzyme, and that genetic therapy with AAV requires a periodical administration accompanied by quite invasive surgery, a feasible alternative could be to exploit the integration and migration ability of NSCs throughout the brain parenchyma for the delivery of the correct enzyme, either intrinsically produced by the cells or overexpressed through a safe and vigorously tested lentiviral manipulation. Thus, findings from the studies on LSDs may provide useful insights into the natural history of CNS involvement, neurodegeneration pathophysiology and the regenerative capacity of the brain (Wada et al., 2000; Myerowitz et al., 2002; Jeyakumar et al., 2003; Glass et al., 2010) and, importantly, into the physiopathology of widespread neurodegenerative diseases in adults, such as AD and PD.

NSC-mediated therapy of LSDs

Previous experiments in animal models of LSDs showed that therapeutic levels of enzymes could be achieved in the brain by direct inoculation of genetically engineered mouse NPCs (Snyder et al., 1995; Flax et al., 1998), fibroblasts (Taylor and Wolfe, 1997), or amniotic epithelial cells (Kosuga et al., 2001). When transplanted in a mouse model of Tay Sachs Disease, characterized by the total absence of hexosaminidase enzyme activity, human immortalized NSCs were able to clear the GM2 ganglioside accumulation from the neuronal cytoplasm (Flax et al., 1998). In a mouse model of MPSVII, an LSD caused by a defective activity of the enzyme ß-glucoronidase (ß-gluc), genetically manipulated mouse NSCs overexpressing ß-gluc were transplanted into the cerebral ventricles and led to the clearance of lysosomal storage through a widespread delivery of the missing enzyme over diffuse areas of the brain, thanks to their extensive migratory capacity (Snyder et al., 1995; Meng et al., 2003). Recent findings showed that presymptomatic MLD pups showed an improved ASA activity with a significant amelioration of neuro-degeneration and motor-learning/memory deficits upon intra-cerebroventricular transplantation of dissociated neurospheres from syngenic wild-type animals into the brain of MLD mice (Givogri et al., 2008). Most transplanted NSCs differentiated spontaneously into the astroglial phenotype, able to intrinsically produce ASA enzyme. Indeed, the effect of neural stem cells *in vivo* was not due to a replacement of degenerated oligodendrocytes as expected, but to a cross-correction of the host cells by the donor cells. This result is in line with the advancing trend of studies suggesting NSCs as a source of trophic factors supporting regenerative and neurogenetic processes, besides beeing a pool for cell replacement therapy. The mechanisms that influence the decision of multipotential stem cells to undergo a particular lineage are still largely unclear, but the local environmental cues present in a specific neuro-degenerative context could play a major role into addressing NSCs towards a specific phenotype (Ma et al., 2010).

The previous results showed that NSCs are able to integrate, migrate and cross correct a genetic defect *in vivo* by intrinsic expression of the target protein (Givogri et al., 2008).

Experiments performed on animal models of MPS syndromes have shown that enzyme-ko mice quite faithfully reproduce the clinical features of the human disorder, from a biochemical, hystological and

morphological point of view (Hess et al., 1996; Muenzer et al., 2002; Garcia et al., 2007). The undetectable activity of the target enzyme both in the plasma and in the tissues, allows an easy identification of induced activity, even at low levels (Friso et al., 2005). In MPSII mice, the consistent GAG accumulation, together with an accompanying storage of glycosphingolipids, mainly GM2 and GM3 ganglyosides, and cholesterol (McGlynn et al., 2004) detected in the tissues, permits an evaluation of the reduction due to the treatment and, thus, a facilitated readout of the therapeutic efficacy.

Considering that administration of NSCs in the early phase of the disease has been shown to be much more effective than at the full-blown symptomatic stage, the presymptomatic treatment of LSD mice with NSCs appears to be the most appropriate therapeutic strategy, in order to prevent the progression of neurological impairment (Jeyakumar et al., 2003; Pluchino et al., 2003; Fukuhara et al., 2006; Lee et al., 2007; Givogri et al., 2008). With respect to the use of vector systems also used for clinical therapies, the employment of NSCs presents the advantage of exploiting the physiological ability of these cells to follow neurogenetic pathways and to provide a widespread delivery of the therapeutic protein. Indeed, neural progenitors have been shown to be capable of extensive migration, particularly in the young brain of the murine model of Batten's Disease, and to increase the diffusion of the therapeutic gene and enzyme, thus setting the basis for the only human clinical trial so far performed using NSCs (Taupin, 2006a). The integration of large numbers of new, wild-type or genetically engineered neural cells in the diseased brain of LSD mice represents the main goal of NSC-mediated therapy. In this sense, two alternative strategies appear as feasible candidates:

Transplantation of exogenous wild-type NSCs

Several studies have already shown that intra-cerebroventricular transplantation (Pluchino et al., 2003; Lee et al., 2007; Givogri et al., 2008) of NSCs succeeds in NSC integration and migration throughout the CNS parenchyma in models for chronic neurodegenerative diseases, as the chronic inflammatory condition generates a permissive environment for NSC passage through the ependymal layer. Similarly, most LSDs are characterized by a prominent injurious inflammatory signature (Wada et al., 2000; Myerowitz et al., 2002; Jeyakumar et al., 2003) and injection of NSCs in presymptomatic pups of LSD animal models results in a delayed disease onset, reduced pathology and prolonged survival. In particular, NSCs have been shown to modulate the inflammatory environment in LSD murine models (Lee et al., 2007). Although donor-derived cells were integrated within chimeric regions, the small degree of degenerating cell replacement alone could not account for the improvement of the pathology. NSCs partially restored the level of the defective enzyme in the brain, reduced the storage of aberrant metabolites and diminished activated microgliosis. Indeed, the delivery of therapeutic gene products synthesized inherently by the stem cell (Martinez-Serrano et al., 1995b; Snyder et al., 1995; Flax et al., 1998; Park and Ourednik et al., 2002; Givogri et al., 2006, 2008)) such as the blunting of toxic components of the microenvironment (Park and Ourednik et al., 2002; Kondo et al., 2005; Givogri et al., 2006) or the secretion of neurotrophic factors ("bystander effect") (Pluchino et al., 2003) are included in the cohort of NSC-mediated therapeutic effects.

In vivo mobilization by recruitment of endogenous NSCs following in situ infection with lentiviral vectors

Some evidence from previous studies has shown that NSC niches like the SVZ and the hippocampal dentate gyrus are activated to proliferate after acute injury (Lichtenwalner and Parent, 2006) even if the physiological response from stem cell niches is often unable to guarantee the rescue of the lesion; moreover, *in vivo* injection of mitogens like EGF and/or FGF2 (Craig et al., 1996; Kuhn et al., 1997; Nakatomi et al., 2002; Gonzalez-Perez et al., 2009) has been shown to induce the mobilization of endogenous stem cells to specific migration neurogenetic pathways. This represents a strategy to enforce the endogenous response.

To date, no reliable data are available on the NSC compartment in LSD affected patients. The validity of NSC cell lines established from Tay Sachs and Sandhoff animal models has been recently shown as an *in vitro* model for the study of LSDs (Martino et al., 2009). The inability of ERT to block neurodegeneration in the LSD brain has been correlated to the difficulty of effectively reaching all districts of the brain parenchyma, although we can not completely rule out the possibility that factors other than accumulation of unprocessed metabolites might contribute to brain damage. Knowing the molecular mechanisms leading to or concurring with cell damage would be of outstanding value in tailoring a more efficacious therapy for LSD neurological features. In the near future, an *in vitro* characterization of NSCs from LSD mice would be helpful to elucidate the impact of the mutated gene on NSC basal stem properties, as well as the possible gain of enzymatic function deriving from the viral transduction of NSCs with the wild type copy of the enzyme.

In combination with the mobilization from the SVZ, an intraventricular injection of lentiviral therapeutic vectors carrying the correct gene would lead to the recruitment of genetically corrected endogenous stem cells from the SVZ niche and to the correct enzyme delivery to widespread areas of the brain, including striatal parenchyma, cortex and olfactory bulbs. This strategy could override all the limits imposed by a transplantation procedure, such as rejection or

inflammatory reaction related to the graft.

Motor neuron diseases (MNDs)

Motor neuron diseases (MNDs) are a group of neurological disorders that selectively affect motor neurons. Their incidence seems to be increasing, but this is probably due to significant advances in early diagnosis, an improved characterization of the disease and an aging population (Leigh and Ray-Chaudhuri, 1994). The incidence is higher after the age of 40 (Logroscino et al., 2010) and the disease progressively leads to death within five years from the diagnosis, usually from respiratory failure (Rowland and Shneider, 2001; Bruijn et al., 2004). 5-10% of the cases are familial, and the rest are sporadic (Sathasivam, 2010).

The clinical spectrum in MND results from a degeneration of upper motor neurons in the motor cortex, lower motor neurons of the brainstem and spinal cord, or both. At present, the most common variant is amyotrophic lateral sclerosis (ALS), which is characterized by the combinatory loss of both spinal and upper motor neurons.

Primary lateral sclerosis (PLS) and Progressive muscular atrophy (PMA), are two other main variants of MND, which include 2% to 4% of MND cases (Norris et al., 1993) and in most cases converge to ALS pathophysiology (Gordon et al., 2006; Tartaglia et al., 2007; Visser et al., 2008).

PLS is a syndrome that specifically exhibits a progressive upper motor neuron degeneration with limb and bulbar dysfunction, with no loss of function of the lower motor neurons. PLS symptoms manifest five to ten years earlier than in ALS patients and the progression of the disease is slower, together with a longer survival of the patients (Gordon et al., 2006). Both PLS, commonly referring to the bulbar onset of MNDs, and PMA, which is associated with a degeneration of the lower motor neurons of the spinal cord in the absence of upper motor neuron and bulbar features, are currently the object of controversial discussions about their definition as distinct disorders from ALS (Norris et al., 1975; Visser et al., 2008).

Given the severity of the disease and the general impairment of voluntary and, partially, of unvoluntary activities, MNDs is so far considered as a multisystem neurodegenerative disease. To note, cognitive impairment is correlated to MNDs pathology too (Lomen-Hoerth et al., 2003; Ringholz et al., 2005). Unfortunately, the pathological hallmarks of MNDs are shared by a series of other unrelated neurological disorders, such as multiple sclerosis or multifocal motor neuropathy, and the diagnosis often occurs very late in the course of disease progression (Belsh and Schiffman, 1990). Although a number of studies have been done looking to provide important breakthroughs in the elucidation of MNDs pathogenesis, there are still no cures or efficacious treatments for these diseases. Many therapeutic molecules with anti-inflammatory, anti-apoptotic and anti-oxidant activities have been exploited in human clinical trials and have been proven to be ineffective (Dietrich-Neto et al., 2000), with the exception of riluzole for ALS, which is an anti-excitotoxicity agent, thus succeeding in prolonging the lifespan for 2-3 months. In the last years significant developments in stem cell research have been applied to MNDs, particularly regarding neuroprotection and cell replacement. Human embryonic stem cells (hESCs) could provide an inexhaustible supply of differentiated cell types, including motor neurons that could be used for MND therapies. Up to 50% efficiency of motor neuron differentiation has been recently accomplished by treating human ESCs with purmorphamine, a molecule able to activate the Shh pathway (Li et al., 2008) and ESC derived motor neurons have also been shown to be functionally mature (Li et al., 2005). As well as a correct commitment of ESCs, appropriate differentiation of NSCs, derived from spinal cord (Weiss et al., 1996; Corti et al., 2006) or from the brain (Wu et al., 2002) have also been shown to efficiently generate motor neurons *in vitro* (Jordan et al., 2009) and *in vivo* (Wu et al., 2002; Gao et al., 2005; Corti et al., 2007; Martin and Liu, 2007).

Recently, it has been demonstrated that induced pluripotent stem cells (iPS) may serve as an alternative source of motor neurons, since they share ES characteristics, i.e. self-renewal, and the potential to differentiate into any somatic cell type, and could provide an escape from the risk of immunorejection or from the hazardously teratogenic ESCs. Reprogramming of fibroblasts from two elderly ALS patients has allowed the generation of motor neurons potentially available for autologous transplantation (Singh Roy et al., 2005; Dimos et al., 2008), but, even in this case, as discussed above, the genetic manipulation implied by the procedure can drive the iPSs to final tumorigenic modifications, so current studies are still underway to assess novel protocols for a higher grade of safety (Okita et al., 2008). Moreover, it has still to be determined to what extent the original ALS-affected microenvironment of iPSs is able to condition the novel –regenerated- motor neurons and whether these are particularly sensitive when implanted in the ALS spinal cord. Hence, iPS- together with hESC-derived motor neurons are currently exploited for *in vitro* modelling of MND (Nizzardo et al., 2010), thus providing a preliminary platform for drug screening and discovery. Taking these considerations into due account, NSCs from fetal and adult human brain actually appear to be the safest and most efficacious solution proximal to therapeutic application.

NSC-mediated therapy of ALS

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disease characterized by the progressive degeneration of corticospinal and spinal motor neurons. In about 1-2% of the patients the

disorder is caused by mutations in the gene encoding for the Cu-Zn superoxide dismutase (SOD1), with lethal neurological involvement. In most non-familial cases, ALS is characterized by motor neuron cell death in the brain and spinal cord, probably induced by multiple causes, such as formation of protein aggregates, axonal transport defects, oxidative damage, mitochondrial defects, glutamate toxicity and alterations in calcium homeostasis (Thonhoff, 2009). Given the multi-factorial nature of ALS, much remains to be learned about the genetics and mechanisms of cell death in ALS, making the design, testing and translation of novel therapies in sporadic patients difficult. Therapies are currently validated in the rodent models representing FALS, but the only true test of a new therapy for ALS would be a clinical trial in humans and this is the direction towards which current efforts have been focused. One approach for overcoming the generally limited capacity of the mature CNS to regenerate axons or new neurons in response to cell loss is the transplantation of neural stem cells. More recent advances in neuroscience strongly support NSCs as "bystander" regulators of tissue homeostasis by the secretion of neurotrophic factors, cytokines and small molecules, or by a scavenging activity leading to the detoxification of a pathological environment, thus delaying or even arresting a progressive neurodegenerative process (Lindvall and Bjorklund, 2004; Lindvall et al., 2004). In this view, stem cell transplantation might be aimed to replace motor neurons or protect endogenous motor neurons by replacement of non-neuronal glial cells such as astrocytes and microglia. Broadly, either extrinsic or inherent expression of neurotrophic factors or antinflammatory cytokines by NSC represents an attractive and safe therapeutic strategy for the near future (Nayak et al., 2006; Hedlund et al., 2007; Suzuki and Svendsen, 2008). Many types of stem cells have been shown to be efficacious in ALS rodent models, from peripheral blood stem cells (Cashman et al., 2008), mesenchimal stem cells (Mazzini et al., 2008), umbilical cord blood stem cells (Chen and Ende, 2000; Ende et al., 2000) to fetal neural stem cells and progenitors (Yan et al., 2006; Suzuki et al., 2007; Thonhoff et al., 2007, 2009).

In ALS onset and progression, the cascade of events implicated in motor neuron degeneration is complex and mostly obscure, but some hints have been provided by the landmark observation that non-cell-autonomous processes involving the interaction with the neighbouring non-neuronal cells, particularly microglia and astrocytes, contributes to the death of the motor cells (Barbeito et al., 2004; Sargsyan et al., 2005; Weydt and Moller, 2005; Thonhoff et al., 2009). There is evidence that prior to disease onset, mutant SOD1 causes a partial disruption of the spinal cord blood barrier together with macrophage infiltration and microglial activation (Alexianu et al., 2001; Hensley et al., 2002). Consistently, recent findings from Graber et al. (2010) have elucidated that accumulation of both microglial cells and macrophages peaks before clinical onset of the disease in SOD rats, irrespective of the development of hypertrophic astrocytes, which becomes detectable only after clinical onset (Fig. 1E-H). This massive activation of microglia/macrophages occurs both in the spinal cord and in the peripheral nerve. In particular, activated microglial cells appear to cluster in the ventral horn of the spinal cord while macrophages coil round the axonal terminals of peripheral nerves, as a hallmark of progressive motor neuron degeneration (Fig. 2C-D) (Thonhoff et al., 2009). Hence, although immunological activity further increases after clinical onset up to the end-stage, a feasible hypothesis is that inflammation occurring in the pre-clinical phase is mostly involved in the induction of motor neuron degeneration. On the contrary, later, the persistence of an inflammatory reaction is likely related to other processes, such as scavenging of toxic molecules and clearing of debris deriving from the degeneration of neuronal cells or the dysfunction of non-neuronal cells such as the same astroglial (Fig. 1E-F) and microglial cells (Fig. 1 G-H). The main consequences of microglial activation in ALS are the production of inflammatory citokynes such as IL-1ß, IL-6, TNFα and TGFß (Xie et al., 2004), an excitotoxic enhancement of glutamate release (Rossi et al., 2008), oxidation of proteins, lipids and DNA accompanied by an increased production of ROS and RNS (Andrus et al., 1998; Simpson et al., 2004; Casoni et al., 2005). Although the complex network of initiating event/s has still to be unraveled, it is well established that microglia from ALS transgenic animals are particularly sensitive to cytokine stimuli with respect to wild-type littermates, and release superoxide under pro-inflammatory conditions (Xiao et al., 2007). In the physiological endeavour to protect neurons from oxidative stress, healthy astrocytes play a fundamental role by releasing reduced gluthathione in the extracellular fluid; conversely, ALS astroglia appears hypertrophic and becomes toxic rather than protective to motor neurons (Barbeito et al., 2004; Weydt and Moller, 2005).

This view opens new perspectives for a therapeutic approach where dysfunctional non-neuronal glial cells, such as microglia or astrocytes, can be either replaced by stem cell transplantation or specifically targeted with pharmacological treatments aiming at preserving their function and/or survival. Previous studies have shown that neural stem/precursor cells display an optimal survival rate, integration into the host tissue and therapeutic plasticity *in vivo*, where they are able to differentiate into neurons, astrocytes and oligodendrocytes (Neri et al., 2010; Rota Nodari et al., 2010). NSCs from the adult brain are able to integrate into the host spinal cord and to delay motor neuron degeneration after injection into the EAE multiple sclerosis mice model (Pluchino et al., 2003), likely acting as deliverers of trophic factors. Parallel studies showed that they were able to delay metachromatic leucodystrophy (MLD) progression when transplanted into MLD mice by cross-correcting the missing enzymatic activity (Givogri et al., 2008) and to efficiently integrating and migrating into

the adult brain after transduction with lentiviral vectors (Consiglio et al., 2004). These results provided exhaustive evidence to promote NSC as a therapeutic strategy, with or without genetic manipulation, to express enzymes, transporters or specific growth factors as well as to replace degenerating cells.

From perspective of clinical application, the availability of a heterogeneous cohort of stem cell lineages as putative candidates for NSC-mediated therapy imposes a rigorous characterization of the cell

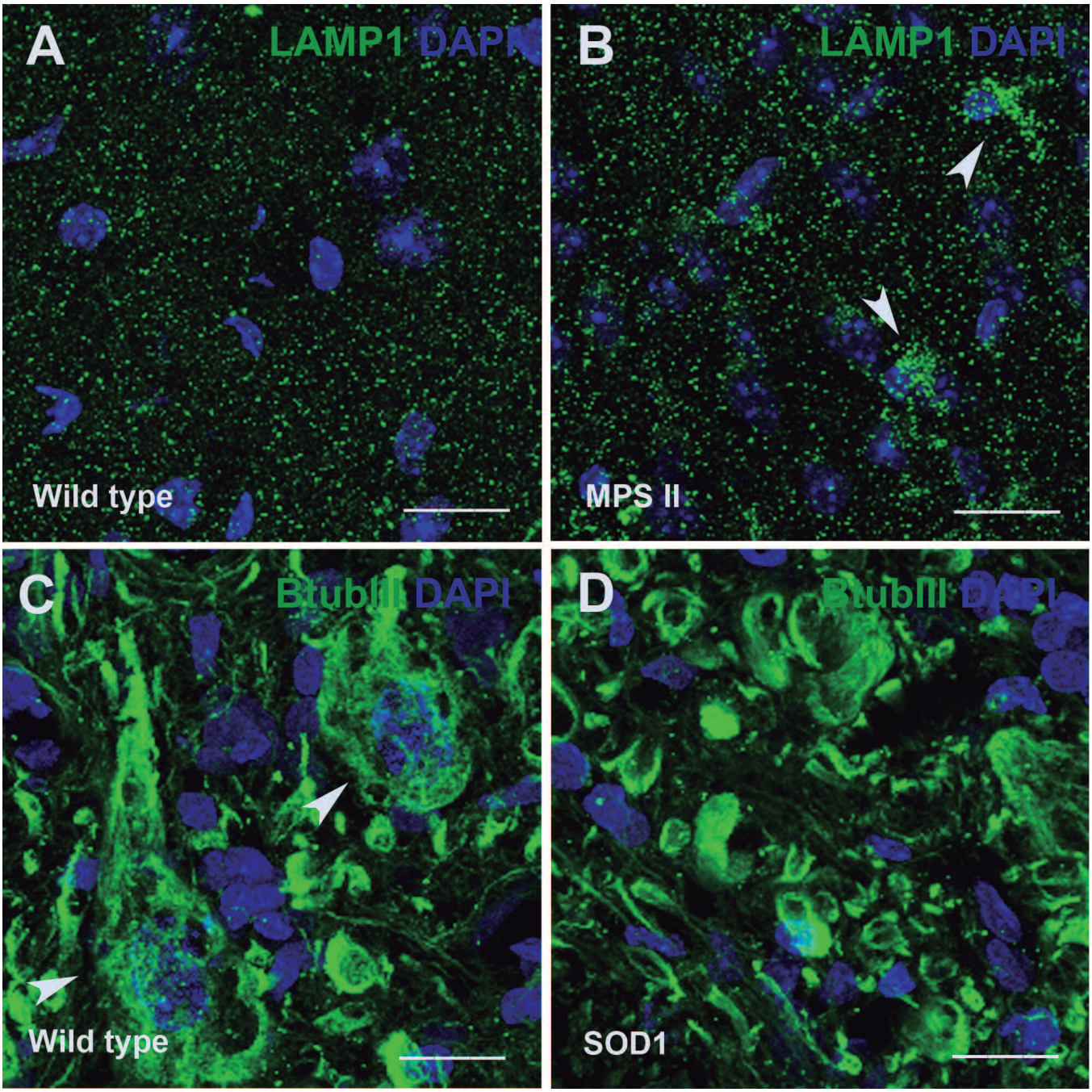

Fig. 2. Confocal microscopy analysis of two pathological hallmarks in MPSII (LSD) and SOD1 (ALS) rodents. **A, B.** A specific immunostaining of lysosome organelles in the brain cortex of wild-type **(A)** and MPSII **(B)** mice at p224 shows the presence of abnormal lysosomal aggregates (Lamp1+, green) in MPSII mice (white arrowhead in **B**). **C, D.** A specific immunostaining of neuronal cells in the spinal cord of wild-type **(C)** and SOD1 **(D)** rats at p120 shows the lack of motor neurons (ß-tubulinIII +, white arrowhead in **C**) in ALS rats. Total nuclei are indicated by dapi staining (blue). Scale bar: 20 *µ*m.

source, together with an accurate selection of standardized protocols for cell culture and neurosurgery. The surgical procedure approved for clinical trials on ALS patients and shown as the most effective in transplantation studies is multiinjection of SC into the ventral horns of the spinal cord by different segments. This procedure was initially devised in order to allow motor neuron replacement by transplanted SCs, although this is currently considered unfeasible. Indeed, both the regional specification of motor neuron subtypes along the rostro-caudal axis of the spinal cord, as well as the motor neuron columnar and pool identity, are established through the segmental expression of different Hox regulatory networks (Dasen et al., 2003, 2005; Hedlund et al., 2007) and it still remains to be elucidated how the differentiation of SC can be directed to generate specific subtypes of motor neurons. Moreover, assuming that a transplanted SC could differentiate into the proper phenotype *in vivo* and thereafter regenerate a motor neuron, it would take too long to reach the muscle targets in the patient. From the reviewed studies, the exploitation of NSCs as vectors for the delivery of trophic factors or antinflammatory citokynes, or for the replacement of interneurons and astroglial cells, seems the most reliable approach. Current studies are mostly addressed to identify the epigenetic factors mediating the SC "bystander" effects and environmental concerns, such as a regional patterning of the astroglial cells in the spinal cord (complementary to motor neuron organization) which could further contribute to the etiological heterogeneity and high variability of ALS symptomatology and condition the diverse efficacy of NSC-therapy in different regions of the spinal cord.

Thus, a translational approach is currently aimed at testing the clinical efficacy of a cohort of subpopulations of neural cells derived from hNSCs, according to specific lineage differentiation protocols. Hence, the comprehension of the mechanism(s) that underpin the therapeutic functions of NSCs and of the subpopulations included in the progeny of NSC is of paramount importance for the development of a cell based therapy for neurodegenerative disorders.

The use of hNSC therapy could finally lead to their application for the cure of ALS patients through two complementary approaches, each addressing specific issues concerning NSC manipulation and transplantation.

A first approach should be likely concerned with a characterization *in vitro* of hNSC lines established from different areas and/or stages of development of the CNS in order to compare their basal stem properties, like self-renewal and multipotency, together with their intrinsic ability to release specific factors, previously shown as efficacious in ALS animal studies (Acsadi et al., 2002; Kaspar et al., 2003; Azzouz et al., 2004; Zheng et al., 2004; Storkebaum et al., 2005). Furthermore, a highly qualifying aspect of these studies should include the exploitation of stable human NSC lines, established and cultured according to Good Manufacturing Practices (GMP) as pivotal to their clinical application.

A second approach should be addressed to the *in vivo* validation of the best candidate hNSC lines (from the studies above) by transplantation into the spinal cord of SOD mutated animal models. The therapeutic efficacy of hNSC-derived subpopulations can be evaluated by their ability to induce a reduction or even a partial recovery of neurological impairments in SOD rats after transplantation. A delay or the arrest of ALS progression is a feasible expectancy considering the several possible NSC-mediated effects: the replacement of impaired astrocytes, the buffering of the noxious milieu, the scavenging of toxic molecules or trophic neuroprotection. The therapeutic effect has to be elucidated together with safety issues concerning the non-toxicity and non-tumorigenicity of hNSC, both essential requirements to develop a cell therapy for ALS patients. These advances would provide scientific knowledge supporting the employment of GMP grade hNSCs in clinical trials for patients affected by ALS and further insights into the different mechanisms of the disease.

Concluding remarks

Considering that the endogenous reservoir of NSCs in the adult brain is limited, as well as its contribution to tissue regeneration, one of the current, most valued therapeutic hypotheses is to accomplish neuro-regeneration by transplantation of exogenous cells. Although it has now emerged that transplanted cells may actually replace and partially repopulate damaged areas, one of the most promising approaches in cell therapy is to exploit the ability of new and healthy NSCs to exert a plethora of "healing actions" on the damaged brain tissue. Indeed, NSCs may act as a reservoir, providing trophic support to surviving cells and synapses at various levels, perhaps by scavenging toxic compounds in genetic, metabolic disorders such as Tay-Sachs, Sandoff's, Canavan's and Batten's disease or by releasing trophic factors in post-traumatic or ischemic injury and neurodegenerative diseases like ALS or by modulating the inflammatory environment shared by the majority of neurodegenerative disorders (Lindvall et al., 2004).

Acknowledgements. I thank my collaborators Laura Rota Nodari, Daniela Ferrari, Cristina Zalfa, Elena Fusar Poli and Maurizio Gelati for their precious technical and scientific support. Prof. Angelo Vescovi supported me with important suggestions and critical discussions. A special thanks to MD Maurizio Scarpa and PhD Rosella Tomanin, who provided an update of the current available therapies for LSDs. I thank the no-profit Foundations Neurothon, Cellule Staminali (Terni, Italy) and Borgonovo, the research Institute Casa Sollievo della Sofferenza (S.Giovanni Rotondo, Italy), Stem Cell Factory (Ospedale S.Maria, Terni), which are funding and providing the necessary support to start up the clinical trial for ALS patients in Italy (information available at the site: www.adottaunacellula.org).

References

Aboody K.S., Najbauer J. and Danks M.K. (2008). Stem and progenitor cell-mediated tumor selective gene therapy. Gene Ther. 15, 739-752.

Acsadi G., Anguelov R.A., Yang H., Toth G., Thomas R., Jani A., Wang Y., Ianakova E., Mohammad S., Lewis R.A. and Shy M.E. (2002). Increased survival and function of sod1 mice after glial cell-derived neurotrophic factor gene therapy. Hum. Gene Ther. 13, 1047-1059.

Aleckovic M. and Simon C. (2008). Is teratoma formation in stem cell research a characterization tool or a window to developmental biology? Reprod. Biomed. Online 17, 270-280.

Alexianu M.E., Kozovska M. and Appel S.H. (2001). Immune reactivity in a mouse model of familial als correlates with disease progression. Neurology 57, 1282-1289.

Alper J. (2009). Geron gets green light for human trial of es cell-derived product. Nat. Biotechnol. 27, 213-214.

Andrus P.K., Fleck T.J., Gurney M.E. and Hall E.D. (1998). Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. J. Neurochem. 71, 2041-2048.

Azzouz M., Ralph G.S., Storkebaum E., Walmsley L.E., Mitrophanous K.A., Kingsman S. M., Carmeliet P. and Mazarakis N. D. (2004). Vegf delivery with retrogradely transported lentivector prolongs survival in a mouse als model. Nature 429, 413-417.

Bacigaluppi M., Pluchino S., Peruzzotti Jametti L., Kilic E., Kilic U., Salani G., Brambilla E., West M.J., Comi G., Martino G. and Hermann D.M. (2009). Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. Brain 132, 2239-2251.

Barbeito L.H., Pehar M., Cassina P., Vargas M.R., Peluffo H., Viera L., Estevez A.G. and Beckman J.S. (2004). A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis. Brain Res. Brain Res. Rev. 47, 263-274.

Belsh J.M. and Schiffman P.L. (1990). Misdiagnosis in patients with amyotrophic lateral sclerosis. Arch. Intern. Med. 150, 2301-2305.

Bjorklund A., Dunnett S.B., Brundin P., Stoessl A.J., Freed C.R., Breeze R.E., Levivier M., Peschanski M., Studer L. and Barker R. (2003). Neural transplantation for the treatment of Parkinson's disease. Lancet Neurol. 2, 437-445.

Bottai D., Fiocco R., Gelain F., De Filippis L., Galli R., Gritti A. and Vescovi L.A. (2003). Neural stem cells in the adult nervous system. J. Hematother. Stem Cell Res. 12, 655-670.

Bruijn L.I., Miller T.M. and Cleveland D.W. (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu. Rev. Neurosci. 27, 723-749.

Cardone M., Polito V.A., Pepe S., Mann L., D'Azzo A., Auricchio A., Ballabio A. and Cosma M.P. (2006). Correction of hunter syndrome in the mpsii mouse model by aav2/8-mediated gene delivery. Hum. Mol. Genet. 15, 1225-1236.

Cashman N., Tan L.Y., Krieger C., Madler B., Mackay A., Mackenzie I., Benny B., Nantel S., Fabros M., Shinobu L., Yousefi M. and Eisen A. (2008). Pilot study of granulocyte colony stimulating factor (g-csf)-mobilized peripheral blood stem cells in amyotrophic lateral sclerosis (als). Muscle Nerve 37, 620-625.

Casoni F., Basso M., Massignan T., Gianazza E., Cheroni C., Salmona M., Bendotti C. and Bonetto V. (2005). Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: Possible multifunctional role in the pathogenesis. J. Biol. Chem. 280, 16295-16304.

Chen R. and Ende N. (2000). The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in sod1 mice. J. Med. 31, 21-30.

Consiglio A., Gritti A., Dolcetta D., Follenzi A., Bordignon C., Gage F.H., Vescovi A.L. and Naldini L. (2004). Robust *in vivo* gene transfer into adult mammalian neural stem cells by lentiviral vectors. Proc. Natl. Acad. Sci. USA 101, 14835-14840.

Corti S., Locatelli F., Papadimitriou D., Del Bo R., Nizzardo M., Nardini M., Donadoni C., Salani S., Fortunato F., Strazzer S., Bresolin N. and Comi G.P. (2007). Neural stem cells lewisx+ cxcr4+ modify disease progression in an amyotrophic lateral sclerosis model. Brain 130, 1289-1305.

Corti S., Locatelli F., Papadimitriou D., Donadoni C., Del Bo R., Crimi M., Bordoni A., Fortunato F., Strazzer S., Menozzi G., Salani S., Bresolin N. and Comi G.P. (2006). Transplanted aldhhissclo neural stem cells generate motor neurons and delay disease progression of nmd mice, an animal model of smard1. Hum. Mol. Genet. 15, 167-187.

Craig C.G., Tropepe V., Morshead C.M., Reynolds B.A., Weiss S. and van der Kooy D. (1996). In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. J. Neurosci. 16, 2649-2658.

Dasen J.S., Liu J.P. and Jessell T.M. (2003). Motor neuron columnar fate imposed by sequential phases of hox-c activity. Nature 425, 926-933.

Dasen J.S., Tice B.C., Brenner-Morton S. and Jessell T.M. (2005). A hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. Cell 123, 477-491.

De Filippis L., Lamorte G., Snyder E.Y., Malgaroli A. and Vescovi A.L. (2007). A novel, immortal, and multipotent human neural stem cell line generating functional neurons and oligodendrocytes. Stem cells (Dayton, Ohio) 25, 2312-2321.

De Filippis L., Ferrari D., Rota Nodari L., Amati B., Snyder E. and Vescovi A.L. (2008). Immortalization of human neural stem cells with the c-myc mutant T58A. PloS one 3, e3310.

Dietrich-Neto F., Callegaro D., Dias-Tosta E., Silva H.A., Ferraz M.E., Lima J.M. and Oliveira A.S. (2000). Amyotrophic lateral sclerosis in brazil: 1998 national survey. Arq. Neuropsiquiatr. 58, 607-615.

Dimos J.T., Rodolfa K.T., Niakan K.K., Weisenthal L.M., Mitsumoto H., Chung W., Croft G.F., Saphier G., Leibel R., Goland R., Wichterle H., Henderson C.E. and Eggan K. (2008). Induced pluripotent stem cells generated from patients with als can be differentiated into motor neurons. Science 321, 1218-1221.

Ebert A.D., Beres A.J., Barber A.E. and Svendsen C.N. (2008). Human neural progenitor cells over-expressing igf-1 protect dopamine neurons and restore function in a rat model of parkinson's disease. Exp. Neurol. 209, 213-223.

Ebert A.D., Barber A.E., Heins B.M. and Svendsen C.N. (2010). Ex vivo delivery of gdnf maintains motor function and prevents neuronal loss in a transgenic mouse model of huntington's disease. Exp. Neurol. 224, 155-162.

Elstein D. (2011). Recent advances in treatment approaches to gaucher disease. Curr. Pharm. Biotechnol.

Ende N., Weinstein F., Chen R. and Ende M. (2000). Human umbilical cord blood effect on sod mice (amyotrophic lateral sclerosis). Life Sci. 67, 53-59.

Eng C.M., Guffon N., Wilcox W.R., Germain D.P., Lee P., Waldek S., Caplan L., Linthorst G.E. and Desnick R.J. (2001). Safety and efficacy of recombinant human alpha-galactosidase a--replacement

therapy in fabry's disease. N. Engl. J. Med. 345, 9-16.

Flax J.D., Aurora S., Yang C., Simonin C., Wills A.M., Billinghurst L.L., Jendoubi M., Sidman R.L., Wolfe J.H., Kim S.U. and Snyder E.Y. (1998). Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. Nat. biotechnol. 16, 1033-1039.

Foroni C., Galli R., Cipelletti B., Caumo A., Alberti S., Fiocco R. and Vescovi A. (2007). Resilience to transformation and inherent genetic and functional stability of adult neural stem cells *ex vivo.* Cancer Res. 67, 3725-3733.

Fraldi A., Hemsley K., Crawley A., Lombardi A., Lau A., Sutherland L., Auricchio A., Ballabio A. and Hopwood J.J. (2007). Functional correction of cns lesions in an mps-iiia mouse model by intracerebral aav-mediated delivery of sulfamidase and sumf1 genes. Human Mol. Genet. 16, 2693-2702.

Friso A., Tomanin R., Alba S., Gasparotto N., Puicher E.P., Fusco M., Hortelano G., Muenzer J., Marin O., Zacchello F. and Scarpa M. (2005). Reduction of gag storage in mps ii mouse model following implantation of encapsulated recombinant myoblasts. J. Gene Med. 7, 1482-1491.

Fukuhara Y., Li X.K., Kitazawa Y., Inagaki M., Matsuoka K., Kosuga M., Kosaki R., Shimazaki T., Endo H., Umezawa A., Okano H., Takahashi T. and Okuyama T. (2006). Histopathological and behavioral improvement of murine mucopolysaccharidosis type vii by intracerebral transplantation of neural stem cells. Mol. Ther. 13, 548-555.

Galli R., Binda E., Orfanelli U., Cipelletti B., Gritti A., De Vitis S., Fiocco R., Foroni C., Dimeco F. and Vescovi A. (2004). Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 64, 7011-7021.

Gao J., Coggeshall R.E., Tarasenko Y.I. and Wu P. (2005). Human neural stem cell-derived cholinergic neurons innervate muscle in motoneuron deficient adult rats. Neuroscience 131, 257-262.

Garcia A.R., Pan J., Lamsa J.C. and Muenzer J. (2007). The characterization of a murine model of mucopolysaccharidosis ii (hunter syndrome). J. Inherit. Metab. Dis. 30, 924-934.

Gieselmann V. and Krageloh-Mann I. (2010). Metachromatic leukodystrophy--an update. Neuropediatrics 41, 1-6.

Givogri M.I., Bottai D., Zhu H.L., Fasano S., Lamorte G., Brambilla R., Vescovi A., Wrabetz L. and Bongarzone E. (2008). Multipotential neural precursors transplanted into the metachromatic leukodystrophy brain fail to generate oligodendrocytes but contribute to limit brain dysfunction. Dev. Neurosci. 30, 340-357.

Givogri M.I., Galbiati F., Fasano S., Amadio S., Perani L., Superchi D., Morana P., Del Carro U., Marchesini S., Brambilla R., Wrabetz L. and Bongarzone E. (2006). Oligodendroglial progenitor cell therapy limits central neurological deficits in mice with metachromatic leukodystrophy. J. Neurosci. 26, 3109-3119.

Glass C.K., Saijo K., Winner B., Marchetto M.C. and Gage F.H. (2010). Mechanisms underlying inflammation in neurodegeneration. Cell 140, 918-934.

Gonzalez-Perez O., Romero-Rodriguez R., Soriano-Navarro M., Garcia-Verdugo J.M. and Alvarez-Buylla A. (2009). Epidermal growth factor induces the progeny of subventricular zone type b cells to migrate and differentiate into oligodendrocytes. Stem cells (Dayton, Ohio) 27, 2032-2043.

Gordon P.H., Cheng B., Katz I.B., Pinto M., Hays A.P., Mitsumoto H. and Rowland L.P. (2006). The natural history of primary lateral sclerosis. Neurology 66, 647-653.

Graber D.J., Hickey W.F. and Harris B.T. (2010). Progressive changes in microglia and macrophages in spinal cord and peripheral nerve in the transgenic rat model of amyotrophic lateral sclerosis. J. Neuroinflammation 7, 8.

Gritti A., Cova L., Parati E.A., Galli R. and Vescovi A.L. (1995). Basic fibroblast growth factor supports the proliferation of epidermal growth factor-generated neuronal precursor cells of the adult mouse cns. Neurosci. Lett. 185, 151-154.

Gritti A., Frolichsthal-Schoeller P., Galli R., Parati E.A., Cova L., Pagano S.F., Bjornson C.R. and Vescovi A.L. (1999). Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. J. Neurosci. 19, 3287-3297.

Gritti A., Bonfanti L., Doetsch F., Caille I., Alvarez-Buylla A., Lim D.A., Galli R., Verdugo J.M., Herrera D.G. and Vescovi A.L. (2002). Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. J. Neurosci. 22, 437-445.

Gritti A., Parati E.A., Cova L., Frolichsthal P., Galli R., Wanke E., Faravelli L., Morassutti D.J., Roisen F., Nickel D.D. and Vescovi A.L. (1996). Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J. Neurosci. 16, 1091-1100.

Haskins M.E., Giger U. and Patterson D.F. (2006). Animal models of lysosomal storage diseases: Their development and clinical relevance. In: Fabri disease: Perspectives from 5 years of FOS. Chapter 6. Mehtra A., Beck M. and Sunder-Plassmann G. (eds). Oxford Pharmagenesis. Oxford

Hedlund E., Hefferan M.P., Marsala M. and Isacson O. (2007). Cell therapy and stem cells in animal models of motor neuron disorders. Eur. J. Neurosci. 26, 1721-1737.

Hensley K., Floyd R.A., Gordon B., Mou S., Pye Q.N., Stewart C., West M. and Williamson K. (2002). Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the g93a-sod1 mouse model of amyotrophic lateral sclerosis. J. Neurochem. 82, 365-374.

Hess B., Saftig P., Hartmann D., Coenen R., Lullmann-Rauch R., Goebel H.H., Evers M., von Figura K., D'Hooge R., Nagels G., De Deyn P., Peters C. and Gieselmann V. (1996). Phenotype of arylsulfatase a-deficient mice: Relationship to human metachromatic leukodystrophy. Proc. Natl. Acad. Sci. USA 93, 14821-14826.

Imitola J., Comabella M., Chandraker A.K., Dangond F., Sayegh M.H., Snyder E.Y. and Khoury S.J. (2004a). Neural stem/progenitor cells express costimulatory molecules that are differentially regulated by inflammatory and apoptotic stimuli. Am. J. Pathol. 164, 1615-1625.

Imitola J., Raddassi K., Park K.I., Mueller F.J., Nieto M., Teng Y.D., Frenkel D., Li J., Sidman R.L., Walsh C.A., Snyder E.Y. and Khoury S.J. (2004b). Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/cxc chemokine receptor 4 pathway. Proc. Natl. Acad. Sci. USA 101, 18117-18122.

Jandial R., Singec I., Ames C.P. and Snyder E.Y. (2008). Genetic modification of neural stem cells. Mol. Ther. 16, 450-457.

Janson C.G., McPhee S.W., Francis J., Shera D., Assadi M., Freese A., Hurh P., Haselgrove J., Wang D.J., Bilaniuk L. and Leone P. (2006). Natural history of canavan disease revealed by proton magnetic resonance spectroscopy (1h-mrs) and diffusion-weighted MRI. Neuropediatrics 37, 209-221.

Jeyakumar M., Thomas R., Elliot-Smith E., Smith D.A., van der Spoel A.C., d'Azzo A., Perry V.H., Butters T.D., Dwek R.A. and Platt F.M. (2003). Central nervous system inflammation is a hallmark of

pathogenesis in mouse models of GM1 and GM2 gangliosidosis. Brain 126, 974-987.

Jordan P.M., Ojeda L.D., Thonhoff J.R., Gao J., Boehning D., Yu Y. and Wu P. (2009). Generation of spinal motor neurons from human fetal brain-derived neural stem cells: Role of basic fibroblast growth factor. J. Neurosci. Res. 87, 318-332.

Kakkis E.D., Muenzer J., Tiller G.E., Waber L., Belmont J., Passage M., Izykowski B., Phillips J., Doroshow R., Walot I., Hoft R. and Neufeld E.F. (2001). Enzyme-replacement therapy in mucopolysaccharidosis I. N. Engl. J. Med. 344, 182-188.

Kaspar B.K., Llado J., Sherkat N., Rothstein J.D. and Gage F.H. (2003). Retrograde viral delivery of igf-1 prolongs survival in a mouse als model. Science 301, 839-842.

Kelly S., Bliss T.M., Shah A.K., Sun G.H., Ma M., Foo W.C., Masel J., Yenari M.A., Weissman I.L., Uchida N., Palmer T. and Steinberg G.K. (2004). Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc. Natl. Acad. Sci. USA 101, 11839-11844.

Kohn D.B., Sadelain M. and Glorioso J.C. (2003). Occurrence of leukaemia following gene therapy of x-linked scid. Nat. Rev. Cancer 3, 477-488.

Kondo Y., Wenger D.A., Gallo V. and Duncan I.D. (2005). Galactocerebrosidase-deficient oligodendrocytes maintain stable central myelin by exogenous replacement of the missing enzyme in mice. Proc. Natl. Acad. Sci. USA 102, 18670-18675.

Kornfeld S. and Mellman I. (1989). The biogenesis of lysosomes. Annu. Rev. Cell Biol. 5, 483-525.

Kosuga M., Sasaki K., Tanabe A., Li X.K., Okawa H., Ogino I., Okuda O., Arai H., Sakuragawa N., Kamata Y., Azuma N., Suzuki S., Yamada M. and Okuyama T. (2001). Engraftment of genetically engineered amniotic epithelial cells corrects lysosomal storage in multiple areas of the brain in mucopolysaccharidosis type vii mice. Mol. Ther. 3, 139-148.

Kuhn H.G., Winkler J., Kempermann G., Thal L.J. and Gage F.H. (1997). Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. J. Neurosci. 17, 5820-5829.

Kusano K., Enomoto M., Hirai T., Tsoulfas P., Sotome S., Shinomiya K. and Okawa A. (2010). Transplanted neural progenitor cells expressing mutant nt3 promote myelination and partial hindlimb recovery in the chronic phase after spinal cord injury. Biochem. Biophys. Res. Commun. 393, 812-817.

Lee J.P., Jeyakumar M., Gonzalez R., Takahashi H., Lee P.J., Baek R.C., Clark D., Rose H., Fu G., Clarke J., McKercher S., Meerloo J., Muller F. J., Park K. I., Butters T.D., Dwek R.A., Schwartz P., Tong G., Wenger D., Lipton S.A., Seyfried T.N., Platt F.M. and Snyder E.Y. (2007). Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. Nat. Med. 13, 439-447.

Leigh P.N. and Ray-Chaudhuri K. (1994). Motor neuron disease. J. Neurol. Neurosurg. Psychiatry 57, 886-896.

Li X.J., Du Z.W., Zarnowska E.D., Pankratz M., Hansen L.O., Pearce R.A. and Zhang S.C. (2005). Specification of motoneurons from human embryonic stem cells. Nat. Biotechnol. 23, 215-221.

Li X.J., Hu B.Y., Jones S.A., Zhang Y.S., Lavaute T., Du Z.W. and Zhang S.C. (2008). Directed differentiation of ventral spinal progenitors and motor neurons from human embryonic stem cells by small molecules. Stem Cells 26, 886-893.

Lichtenwalner R.J. and Parent J.M. (2006). Adult neurogenesis and the ischemic forebrain. J. Cereb. Blood Flow Metab. 26, 1-20.

Lindvall O. and Bjorklund A. (2004). Cell replacement therapy: Helping the brain to repair itself. NeuroRx 1, 379-381.

Lindvall O., Kokaia Z. and Martinez-Serrano A. (2004). Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat. Med. 10 (Suppl), S42-50.

Logroscino G., Traynor B.J., Hardiman O., Chio A., Mitchell D., Swingler R. J., Millul A., Benn E. and Beghi E. (2010). Incidence of amyotrophic lateral sclerosis in europe. J. Neurol. Neurosurg. Psychiatry 81, 385-390.

Lomen-Hoerth C., Murphy J., Langmore S., Kramer J.H., Olney R.K. and Miller B. (2003). Are amyotrophic lateral sclerosis patients cognitively normal? Neurology 60, 1094-1097.

Ma D.K., Marchetto M.C., Guo J.U., Ming G.L., Gage F.H. and Song H. (2010). Epigenetic choreographers of neurogenesis in the adult mammalian brain. Nat. Neurosci. 13, 1338-1344.

Martin L.J. and Liu Z. (2007). Adult olfactory bulb neural precursor cell grafts provide temporary protection from motor neuron degeneration, improve motor function, and extend survival in amyotrophic lateral sclerosis mice. J. Neuropathol. Exp. Neurol. 66, 1002-1018.

Martinez-Serrano A., Fischer W. and Bjorklund A. (1995a). Reversal of age-dependent cognitive impairments and cholinergic neuron atrophy by ngf-secreting neural progenitors grafted to the basal forebrain. Neuron 15, 473-484.

Martinez-Serrano A., Lundberg C., Horellou P., Fischer W., Bentlage C., Campbell K., McKay R.D., Mallet J. and Bjorklund A. (1995b). Cns-derived neural progenitor cells for gene transfer of nerve growth factor to the adult rat brain: Complete rescue of axotomized cholinergic neurons after transplantation into the septum. J. Neurosci. 15, 5668-5680.

Martino S., di Girolamo I., Cavazzin C., Tiribuzi R., Galli R., Rivaroli A., Valsecchi M., Sandhoff K., Sonnino S., Vescovi A., Gritti A. and Orlacchio A. (2009). Neural precursor cell cultures from gm2 gangliosidosis animal models recapitulate the biochemical and molecular hallmarks of the brain pathology. J. Neurochem. 109, 135-147.

Mazzini L., Mareschi K., Ferrero I., Vassallo E., Oliveri G., Nasuelli N., Oggioni G. D., Testa L. and Fagioli F. (2008). Stem cell treatment in amyotrophic lateral sclerosis. J. Neurol. Sci. 265, 78-83.

McGlynn R., Dobrenis K. and Walkley S.U. (2004). Differential subcellular localization of cholesterol, gangliosides, and glycosaminoglycans in murine models of mucopolysaccharide storage disorders. J. Comp. Neurol. 480, 415-426.

Mehta A., Beck M., Eyskens F., Feliciani C., Kantola I., Ramaswami U., Rolfs A., Rivera A., Waldek S. and Germain D.P. (2010). Fabry disease: A review of current management strategies. QJM 103, 641-659.

Meikle P.J., Hopwood J.J., Clague A. E. and Carey W.F. (1999). Prevalence of lysosomal storage disorders. JAMA 281, 249-254.

Mendez I., Vinuela A., Astradsson A., Mukhida K., Hallett P., Robertson H., Tierney T., Holness R., Dagher A., Trojanowski J.Q. and Isacson O. (2008). Dopamine neurons implanted into people with parkinson's disease survive without pathology for 14 years. Nat. Med. 14, 507-509.

Meng X.L., Shen J.S., Ohashi T., Maeda H., Kim S.U. and Eto Y. (2003). Brain transplantation of genetically engineered human neural stem cells globally corrects brain lesions in the mucopolysaccharidosis type vii mouse. J. Neurosci. Res. 74, 266-

277.

Muenzer J., Gucsavas-Calikoglu M., McCandless S.E., Schuetz T.J. and Kimura A. (2007). A phase i/ii clinical trial of enzyme replacement therapy in mucopolysaccharidosis ii (hunter syndrome). Mol. Genet. Metab. 90, 329-337.

Muenzer J., Lamsa J.C., Garcia A., Dacosta J., Garcia J. and Treco D.A. (2002). Enzyme replacement therapy in mucopolysaccharidosis type ii (hunter syndrome): A preliminary report. Acta Paediatr. Suppl. 91, 98-99.

Muenzer J., Wraith J.E., Beck M., Giugliani R., Harmatz P., Eng C.M., Vellodi A., Martin R., Ramaswami U., Gucsavas-Calikoglu M., Vijayaraghavan S., Wendt S., Puga A.C., Ulbrich B., Shinawi M., Cleary M., Piper D., Conway A.M. and Kimura A. (2006). A phase ii/iii clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis ii (hunter syndrome). Genet. Med. 8, 465-473.

Myerowitz R., Lawson D., Mizukami H., Mi Y., Tifft C.J. and Proia R.L. (2002). Molecular pathophysiology in tay-sachs and sandhoff diseases as revealed by gene expression profiling. Human Mol. Genet. 11, 1343-1350.

Nakatomi H., Kuriu T., Okabe S., Yamamoto S., Hatano O., Kawahara N., Tamura A., Kirino T. and Nakafuku M. (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110, 429-441.

Nayak M.S., Kim Y.S., Goldman M., Keirstead H.S. and Kerr D.A. (2006). Cellular therapies in motor neuron diseases. Biochim. Biophys. Acta 1762, 1128-1138.

Neri M., Maderna C., Ferrari D., Cavazzin C., Vescovi A.L. and Gritti A. (2010). Robust generation of oligodendrocyte progenitors from human neural stem cells and engraftment in experimental demyelination models in mice. PLoS One 5, e10145.

Nizzardo M., Simone C., Falcone M., Locatelli F., Riboldi G., Comi G.P. and Corti S. (2010). Human motor neuron generation from embryonic stem cells and induced pluripotent stem cells. Cell Mol. Life Sci. 67, 3837-3847.

Norris F., Shepherd R., Denys E., U.K., Mukai E., Elias L., Holden D. and Norris H. (1993). Onset, natural history and outcome in idiopathic adult motor neuron disease. J. Neurol. Sci. 118, 48-55.

Norris F.H. Jr., Aguilar M.J., Colton R.P., Oldstone M.B. and Cremer N.E. (1975). Tubular particles in a case of recurrent lymphocytic meningitis followed by amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 34, 133-147.

Okita K., Nakagawa M., Hyenjong H., Ichisaka T. and Yamanaka S. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. Science 322, 949-953.

Olstorn H., Moe M.C., Roste G.K., Bueters T. and Langmoen I.A. (2007). Transplantation of stem cells from the adult human brain to the adult rat brain. Neurosurgery 60, 1089-1098.

Pan D. (2011). Cell- and gene-based therapeutic approaches for neurological deficits in mucopolysaccharidoses. Curr. Pharm. Biotechnol. 12, 884-896.

Park K.I., Teng Y.D. and Snyder E.Y. (2002). The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat. Biotechnol. 20, 1111-1117.

Park K.I., Himes B.T., Stieg P.E., Tessler A., Fischer I. and Snyder E.Y. (2006). Neural stem cells may be uniquely suited for combined gene therapy and cell replacement: Evidence from engraftment of neurotrophin-3-expressing stem cells in hypoxic-ischemic brain injury. Exp. Neurol. 199, 179-190.

Park K.I., Ourednik J., Ourednik V., Taylor R.M., Aboody K.S., Auguste K.I., Lachyankar M.B., Redmond D.E. and Snyder E.Y. (2002). Global gene and cell replacement strategies via stem cells. Gene Ther. 9, 613-624.

Pluchino S., Gritti A., Blezer E., Amadio S., Brambilla E., Borsellino G., Cossetti C., Del Carro U., Comi G., t Hart B., Vescovi A. and Martino G. (2009). Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates. Ann. Neurol. 66, 343-354.

Pluchino S., Zanotti L., Rossi B., Brambilla E., Ottoboni L., Salani G., Martinello M., Cattalini A., Bergami A., Furlan R., Comi G., Constantin G. and Martino G. (2005). Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 436, 266-271.

Pluchino S., Quattrini A., Brambilla E., Gritti A., Salani G., Dina G., Galli R., Del Carro U., Amadio S., Bergami A., Furlan R., Comi G., Vescovi A.L. and Martino G. (2003). Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 422, 688-694.

Raore B., Federici T., Taub J., Wu M.C., Riley J., Franz C.K., Kliem M.A., Snyder B., Feldman E.L., Johe K. and Boulis N.M. (2011). Cervical multilevel intraspinal stem cell therapy: Assessment of surgical risks in gottingen minipigs. Spine (Phila Pa 1976) 36, E164-171.

Raper S.E., Chirmule N., Lee F.S., Wivel N.A., Bagg A., Gao G.P., Wilson J.M. and Batshaw M.L. (2003). Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol. Genet. Metab. 80, 148-158.

Reynolds B.A. and Weiss S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707-1710.

Ringholz G.M., Appel S.H., Bradshaw M., Cooke N.A., Mosnik D.M. and Schulz P. E. (2005). Prevalence and patterns of cognitive impairment in sporadic ALS. Neurology 65, 586-590.

Rossi D., Brambilla L., Valori C.F., Roncoroni C., Crugnola A., Yokota T., Bredesen D.E. and Volterra A. (2008). Focal degeneration of astrocytes in amyotrophic lateral sclerosis. Cell Death Differ. 15, 1691-1700.

Rota Nodari L., Ferrari D., Giani F., Bossi M., Rodriguez-Menendez V., Tredici G., Delia D., Vescovi A.L. and De Filippis L. (2010). Long-term survival of human neural stem cells in the ischemic rat brain upon transient immunosuppression. PLoS One 5, e14035.

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

Sargsyan S.A., Monk P.N. and Shaw P.J. (2005). Microglia as potential contributors to motor neuron injury in amyotrophic lateral sclerosis. Glia 51, 241-253.

Sathasivam S. (2010). Motor neurone disease: Clinical features, diagnosis, diagnostic pitfalls and prognostic markers. Singapore Med. J. 51, 367-372; quiz 373.

Simpson E.P., Henry Y.K., Henkel J.S., Smith R.G. and Appel S. H. (2004). Increased lipid peroxidation in sera of ALS patients: A potential biomarker of disease burden. Neurology 62, 1758-1765.

Singh Roy N., Nakano T., Xuing L., Kang J., Nedergaard M. and Goldman S.A. (2005). Enhancer-specified gfp-based facs purification of human spinal motor neurons from embryonic stem cells. Exp. Neurol. 196, 224-234.

Sly W.S. and Vogler C. (2002). Brain-directed gene therapy for lysosomal storage disease: Going well beyond the blood- brain

barrier. Proc. Natl. Acad. Sci. USA 99, 5760-5762.

Snyder E.Y., Taylor R.M. and Wolfe J.H. (1995). Neural progenitor cell engraftment corrects lysosomal storage throughout the mps vii mouse brain. Nature 374, 367-370.

Storkebaum E., Lambrechts D., Dewerchin M., Moreno-Murciano M.P., Appelmans S., Oh H., Van Damme P., Rutten B., Man W.Y., De Mol M., Wyns S., Manka D., Vermeulen K., Van Den Bosch L., Mertens N., Schmitz C., Robberecht W., Conway E.M., Collen D., Moons L. and Carmeliet P. (2005). Treatment of motoneuron degeneration by intracerebroventricular delivery of vegf in a rat model of ALS. Nat. Neurosci. 8, 85-92.

Stroemer P., Hope A., Patel S., Pollock K. and Sinden J. (2008). Development of a human neural stem cell line for use in recovery from disability after stroke. Front. Biosci. 13, 2290-2292.

Stroemer P., Patel S., Hope A., Oliveira C., Pollock K. and Sinden J. (2009). The neural stem cell line ctx0e03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. Neurorehabil. Neural Repair 23, 895-909.

Suzuki M. and Svendsen C.N. (2008). Combining growth factor and stem cell therapy for amyotrophic lateral sclerosis. Trends Neurosci. 31, 192-198.

Suzuki M., McHugh J., Tork C., Shelley B., Klein S.M., Aebischer P. and Svendsen C.N. (2007). GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial als. PLoS One 2, e689.

Tartaglia M.C., Rowe A., Findlater K., Orange J.B., Grace G. and Strong M.J. (2007). Differentiation between primary lateral sclerosis and amyotrophic lateral sclerosis: Examination of symptoms and signs at disease onset and during follow-up. Arch. Neurol. 64, 232-236.

Taupin P. (2006a). Adult neural stem cells, neurogenic niches, and cellular therapy. Stem Cell Rev 2, 213-219.

Taupin P. (2006b). Therapeutic potential of adult neural stem cells. Recent Pat. CNS Drug Discov. 1, 299-303.

Taupin P. (2006c). HuCNS-SC (stemcells). Current Opin. Mol. Therapeutic. 8, 156-163.

Taylor R.M. and Wolfe J.H. (1997). Decreased lysosomal storage in the adult MPS VII mouse brain in the vicinity of grafts of retroviral vector-corrected fibroblasts secreting high levels of beta-glucuronidase. Nat. Med. 3, 771-774.

Thonhoff J.R., Ojeda L. and Wu P. (2009). Stem cell-derived motor neurons: Applications and challenges in amyotrophic lateral sclerosis. Curr. Stem Cell Res. Ther. 4, 178-199.

Thonhoff J.R., Jordan P.M., Karam J.R., Bassett B.L. and Wu P. (2007). Identification of early disease progression in an als rat model. Neurosci. Lett. 415, 264-268.

Trujillo C.A., Schwindt T.T., Martins A.H., Alves J.M., Mello L.E. and Ulrich H. (2009). Novel perspectives of neural stem cell differentiation: From neurotransmitters to therapeutics. Cytometry A 75, 38-53.

Van den Hout H., Reuser A.J., Vulto A.G., Loonen M.C., Cromme-Dijkhuis A. and Van der Ploeg A.T. (2000). Recombinant human alpha-glucosidase from rabbit milk in pompe patients. Lancet 356, 397-398.

Vescovi A. L., Parati E. A., Gritti A., Poulin P., Ferrario M., Wanke E., Frolichsthal-Schoeller P., Cova L., Arcellana-Panlilio M., Colombo A. and Galli R. (1999). Isolation and cloning of multipotential stem cells from the embryonic human cns and establishment of transplantable human neural stem cell lines by epigenetic stimulation. Exp. Neurol. 156, 71-83.

Villa A., Snyder E.Y., Vescovi A. and Martinez-Serrano A. (2000). Establishment and properties of a growth factor-dependent, perpetual neural stem cell line from the human CNS. Exp. Neurol. 161, 67-84.

Visser J., de Jong J.M. and de Visser M. (2008). The history of progressive muscular atrophy: Syndrome or disease? Neurology 70, 723-727.

Wada R., Tifft C.J. and Proia R.L. (2000). Microglial activation precedes acute neurodegeneration in sandhoff disease and is suppressed by bone marrow transplantation. Proc. Natl. Acad. Sci. USA 97, 10954-10959.

Weiss S., Dunne C., Hewson J., Wohl C., Wheatley M., Peterson A.C. and Reynolds B.A. (1996). Multipotent cns stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J. Neurosci. 16, 7599-7609.

Wennersten A., Holmin S., Al Nimer F., Meijer X., Wahlberg L.U. and Mathiesen T. (2006). Sustained survival of xenografted human neural stem/progenitor cells in experimental brain trauma despite discontinuation of immunosuppression. Exp. Neurol. 199, 339-347.

Weydt P. and Moller T. (2005). The role of microglial cells in amyotrophic lateral sclerosis. Phys. Med. Rehabil. Clin. N. Am. 16, 1081-1090, xi.

Wraith J.E. (2002). Lysosomal disorders. Semin. Neonatol. 7, 75-83.

Wraith J.E. (2008). Enzyme replacement therapy with idursulfase in patients with mucopolysaccharidosis type II. Acta Paediatr. Suppl. 97, 76-78.

Wu P., Tarasenko Y.I., Gu Y., Huang L.Y., Coggeshall R.E. and Yu Y. (2002). Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. Nat. Neurosci. 5, 1271-1278.

Xiao Q., Zhao W., Beers D.R., Yen A.A., Xie W., Henkel J.S. and Appel S.H. (2007). Mutant sod1(g93a) microglia are more neurotoxic relative to wild-type microglia. J. Neurochem. 102, 2008-2019.

Xie Y., Weydt P., Howland D.S., Kliot M. and Moller T. (2004). Inflammatory mediators and growth factors in the spinal cord of G93A SOD1 rats. Neuroreport 15, 2513-2516.

Xuan A.G., Luo M., Ji W.D. and Long D.H. (2009). Effects of engrafted neural stem cells in alzheimer's disease rats. Neurosci. Lett. 450, 167-171.

Yan J., Xu L., Welsh A.M., Chen D., Hazel T., Johe K. and Koliatsos V.E. (2006). 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 24, 1976-1985.

Yang J., Jiang Z., Fitzgerald D.C., Ma C., Yu S., Li H., Zhao Z., Li Y., Ciric B., Curtis M., Rostami A. and Zhang G.X. (2009). Adult neural stem cells expressing il-10 confer potent immunomodulation and remyelination in experimental autoimmune encephalitis. J. Clin. Invest. 119, 3678-3691.

Yang S.Y., Liu H. and Zhang J.N. (2004). Gene therapy of rat malignant gliomas using neural stem cells expressing il-12. DNA Cell. Biol. 23, 381-389.

Zheng C., Nennesmo I., Fadeel B. and Henter J.I. (2004). Vascular endothelial growth factor prolongs survival in a transgenic mouse model of ALS. Ann. Neurol. 56, 564-567.

Accepted April 20, 2011