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The Potential for Stem Cell Therapies to have an Impact in Cerebral Palsy – Opportunities and Limitations

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Summary

Cerebral palsy (CP) is a chronic childhood disorder described by a group of motor and cognitive impairments resulting in substantial socio-economic burden to the individual, family and healthcare system. With no effective biological interventions, therapies for CP are currently restricted to supportive and management strategies. Stem cell transplantation has been suggested as a putative intervention for neural pathology as mesenchymal, neural precursor, olfactory derived and Schwann cells have all shown some regenerative and functional efficacy in experimental CNS disorders. This review describes the most common cell types investigated and delineates their purported mechanisms *in vivo.* Furthermore, it provides a cogent summary of current early phase clinical trials using neural precursor cells (NPCs) and the state of stem cell therapies for neurodegenerative conditions. While NPCs appear to be among the most promising candidates for cell replacement therapy, much still needs to be understood regarding safety, efficacy, timing, dose and route of transplantation, as well as capacity for combinatorial strategies to fully realise their potential utility as a treatment for CP.

Running title

Stem Cell Therapies for Cerebral Palsy

What This Paper Adds

1) This paper reviews the clinically relevant biology of stem cells and the opportunities and limitations with regard to their application in cerebral palsy.
2) Combinatorial approaches using stem cells with bioengineered approaches to minimize the impact of glial scar or cavitation and rehabilitation strategies to enhance plasticity will likely be the optimal way to apply regenerative technologies for cerebral palsy.
3) Although initial clinical trials with mesenchymal stem cells (and neural precursors in other neurodevelopmental disorders) have been initiated, more basic research is necessary to optimize the use of stem cells for CP.

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Background

I. What is Cerebral Palsy?

Cerebral palsy (CP) is the most common pediatric developmental disability caused by perinatal asphyxia, infection or prematurity. It occurs in approximately 2.5/1000 live births in developed countries [1]. Affected children have higher mortality than unaffected peers, with frequent co-morbid cognitive and sensory deficits in addition to lifelong motor impairment. A late prenatal or perinatal hypoxic-hemodynamic insult is the dominating final common pathway in its pathogenesis [2]. Survivors of premature birth constitute the largest etiologic subgroup of children with CP [3] and periventricular leukomalacia (PVL) is the most common form of brain injury in this cohort [4] (**Figure 1**). Phenotypically, children with CP present with spastic quadraplegia (35%), spastic diplegia (21%), and spastic hemiplegia (31%), with other forms of dyskinetic CP contributing the remainder (13%) [5]. Animal models in several species provide evidence for maturation-dependent vascular predisposition to white matter injury, with a specific sensitivity of the immature oligodendrocyte (OL) populations to ischemic and inflammatory insult [6]. Pathologically, PVL preferentially occurs around the lateral ventricles which border penetrating branches of the major cerebral arteries and spatially overlap descending corticospinal tracts (Figure 1). Relevant to this, cell replacement therapy aims to replace damaged oligodendrocytes to normalize function in affected neuromotor tracts.

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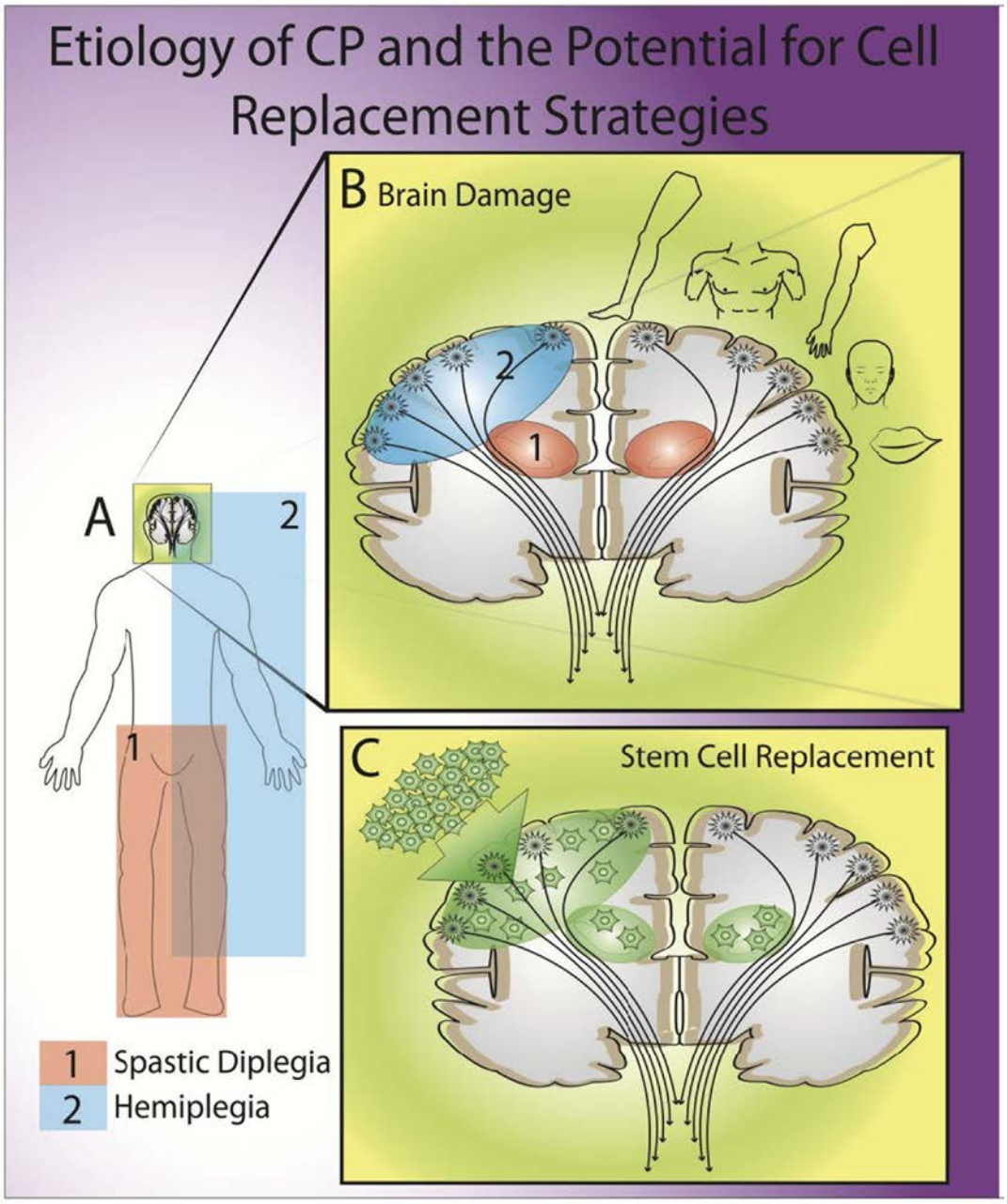

Figure 1: Etiology of CP and the Potential for Stem Cell Replacement Strategies.
A) Shows the phenotypes two most common subtypes of CP; areas of motor dysfunction resulting from spastic diplegic and spastic hemiplegic are highlighted here in red (1) and blue (2) respectively. B) Reveals the etiology of this neuromotor impairment. The pre-oligodendrocyte is intrinsically susceptible to excitotoxic cell death and thus periventricular leukomalacia (1) occurs in poorly vascularised watershed regions after neonatal ischaemic injury or placental insufficiency. This spatially overlaps with descending corticospinal tracts controlling leg, trunk, arm head and face movement; as PVL increases in severity, the lesion expands laterally involving further motor fibre tracts and increasing the extent of bilateral neuromotor impairment. Likewise, after perinatal stroke (2), cell bodies of underlying architecture are unilaterally damaged. Their descending motor tracts are thus affected, resulting in unilateral hemiparesis. C) Stem Cells can be used as a viable cell replacement strategy to enhance function in these models.

II. What is a Stem Cell?

Stem cells are multi-potential cells that exist in both adult and developing tissue. Their key unifying characteristics are their multipotential capability and their ability to self-renew. Throughout development, pluripotency decreases with increased cellular differentiation (**Figure 2**); differentiation is accompanied by changes in both gene expression and epigenetic profile, leading to a mature cell phenotype. “True” stem cells can form any tissue in the body or amnion and have virtually unlimited proliferative and self-renewal capacity. However, these cells are never used in cell transplantation paradigms, due primarily to high mobility and propensity for teratoma formation. Instead, more differentiated cells, such as multipotent cells or fate-restricted progenitors, are the types used in preclinical studies. Furthermore, non-stem cells, usually glial cells such as peripheral Schwann Cells (SCs) and Olfactory Ensheathing glia (OEG) have also been explored for cellular therapy, and will be discussed *a posteriori*. With the aim of understanding the types of stem cells, we will discuss herein all types along the developmental lineage, with understanding that the current cell types being explored pre-clinically primarily consist of late-stage tissue-specific progenitors or more mature cell types.

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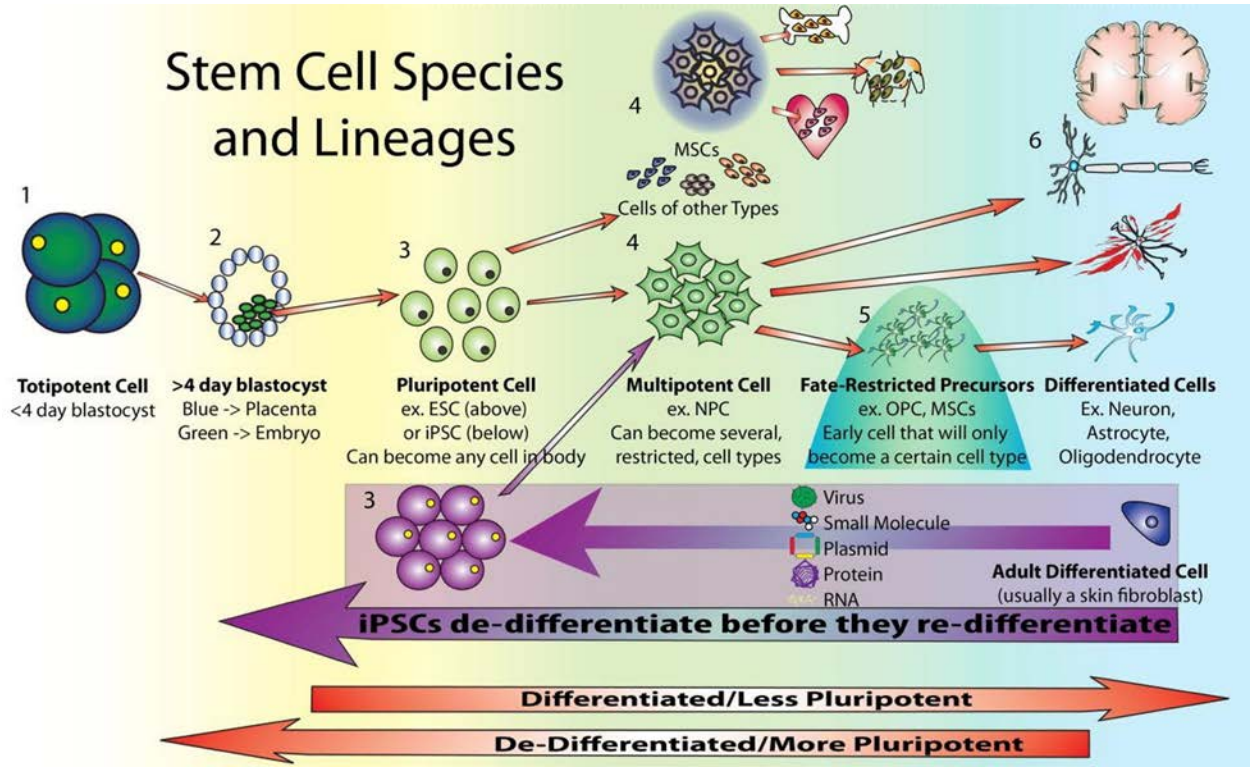

Figure 2: Stem Cell Species and Lineages

Characterizing stem cells along their developmental pathway and describing the types of stem cells most commonly associated with regenerative medicine. (1) Totipotent cells derive from the first few divisions of the blastocyst and can differentiate into any adult tissue in the organism, including extra-embryonic tissue. (2) After several rounds of cell division, the early blastocyst produces an inner cell mass and an outer trophoblast. (3) Pluripotent stem cells can be derived from the inner cell mass or, more recently in vitro, from adult somatic cells, via induced pluripotent stem cell (iPSC) generation (highlighted purple). Pluripotent and earlier-lineage stem cells are unsuitable for transplantation, due to teratoma risk, however they can be differentiated in vitro, in vivo or during development into multipotent progenitors. (4) Multipotent progenitors (here, NPCs and MSCs) differentiate into a number of cell types within a restricted niche. (5) Multipotent progenitors are further differentiated into fate-restricted precursors, that are generally immature or early cell phenotypes, for example a pre-oligodendrocyte. Fate restricted precursors differentiate into only one mature, adult phenotype. Practically, these, and multipotent progenitors are the cells most promising for cell replacement strategies. (6) Fully differentiated adult cells are often the goal and result of cell replacement strategies.

A. Totipotent stem cells

Totipotent cells are the earliest lineage cells, found within the first few cell divisions of the early blastocyst. They can produce all cell types - including extra-embryonic tissue - but are rarely used for therapeutic purposes due to lack of specialization and associated high teratoma propensity.

B. Pluripotent stem cells

Embryonic Stem Cells (ESCs)

After roughly 4 days of cell division, the blastocyst forms an external trophoblast and an inner cell mass, from which the extra-embryonic tissue and the embryo proper develop respectively. Pluripotent stem cells are extracted from the inner cell mass of the early segmented blastocyst and can contribute to all germ layers and tissues in the human body, short of placental tissue (which develops from the trophoblast). These are generally referred to as embryonic stem cells (ESCs), and are the subtype most commonly associated with the term “stem cells.”

Induced Pluripotent Stem Cells (iPSCs)

An alternative means of obtaining pluripotent stem cells has recently been elucidated [7]. Induced pluripotent stem cells (iPSCs) can be derived from human somatic (typically skin or blood) tissue, by up regulating “Yamanaka” transcription factors OCT4, c-Myc, Sox2 and KLF4 (or substitutes), thereby creating patient-specific cells from adult sources. This potentially reduces the need for tissue donor waiting lists and also circumvents the destruction of an embryo during pluripotent cell production. While iPSCs are not ready for the clinic, improvements in production and differentiation methods - such as the use of non-integrating transposon, virus, small molecule, protein and mRNA systems - as well as non-oncogenic derivation, optimized cell culture methods and increased understanding of how original somatic cell type affects progeny have led to ‘safer,’ more optimized iPSC lines.

Despite their benefits, pluripotent cells display a high degree of plasticity and are unsuitable for transplantation. For ESCs or iPSCs to be used in successful cell therapy, they must be differentiated into multipotent or fate-restricted progenitors. Further to this, direct lineage reprogramming, or “trans-differentiation” of somatic cells to neural subtypes could potentially extend to cell transplant paradigms in the future [8].

C. Multipotent Stem Cells

Later in development, pluripotent stem cells narrow their potential and become multipotent cells. Several types of multipotent stem cells can also be derived from pluripotent sources *in vitro* via growth factor conditioning (reviewed in [9]) . Multipotent progenitors produce different cell lineages that are restricted to a specific type of tissue. For example, *in vivo,* mesenchymal stem cells (MSCs) become blood, bone, muscle and fat, while neural precursor cells (NPCs) differentiate into neurons, astrocytes and oligodendrocytes (for review, see [10]). It is these cells and fate-restricted precursors that hold most clinical promise, as they have low risk of teratoma formation.

D. Fate-Restricted Precursors

Still further along the developmental pathway exist unipotent progenitor cells; these are usually immature or fate-restricted precursors that differentiate into mature cells.
At this point during development, many 'stemness' characteristics have been lost and common nomenclature utilises the terms *progenitor* and *precursor* in lieu of *stem cell*.
For neural pathology, these unipotent progenitors are typically pre-myelinating cells such as immature oligodendrocytes or SCs.

III. The Function of Stem Cells in Neurological Repair

The use of stem cells for preventative or (more often) restorative clinical benefit is currently under investigation in human clinical trials. Pre-clinical animal data suggests three major mechanisms whereby stem cells can mediate neurologic repair: they can provide structural support to the damaged and surrounding tissue; they may remyelinate damaged axons and they can express neurotrophic growth factors. Cell subtype restricts each cell's potential mechanism of repair, and cells of separate lineages exhibit distinct regenerative capabilities (figure 3). It is important to note that not all cell subtypes used in preclinical therapy paradigms are stem cells. Indeed, SCs and OEG, two of the most commonly investigated transplant types, are not stem cells, but are, in fact, more mature cells. Alternatively, MSCs and NPCs are multipotent progenitors that still possess limited 'stemness' characteristics.

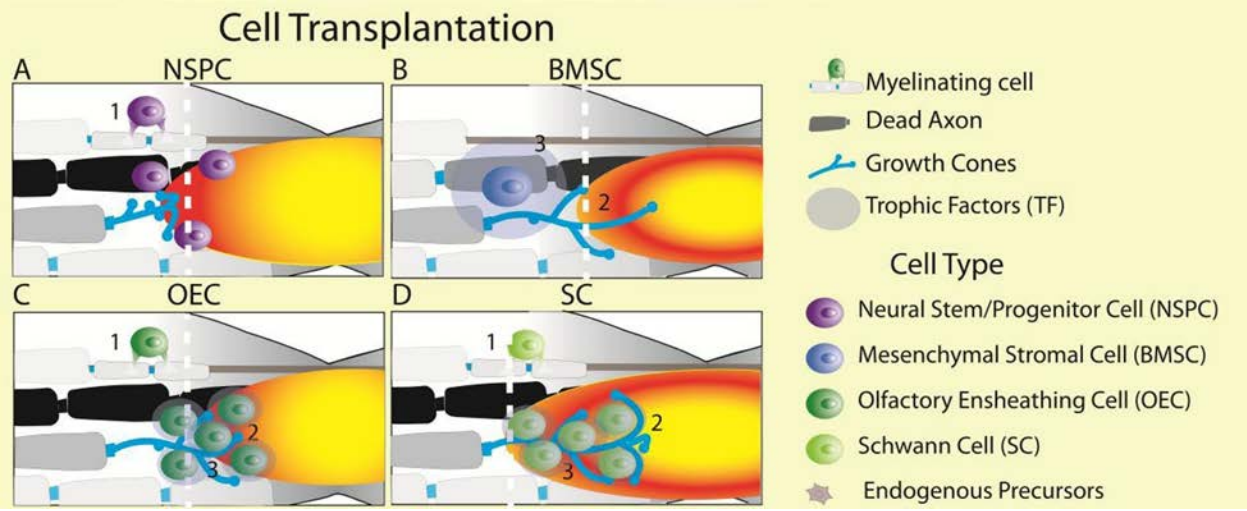

Figure 3: (Modified from Ruff et al 2011) Cell Transplantation for Neural Injury
Stem and progenitor cells can enhance function following neural injury. A) Neural stem/progenitor cells (NPCs) can functionally remyelinate the CNS (1). Although they show no significant axonal extension or trophic properties, they are useful as a cell replacement strategy for CNS injury. B) Bone marrow stromal cell (BMSC) transplantation does not remyelinate axons but enhances the trophic environment (3), increasing axonal outgrowth (2) and decreasing lesion cavity size and axonal dieback. C) Olfactory ensheathing cells (OECs), are able to myelinate axons (1), with non-intrinsic CNS phenotype myelin, decrease lesion cavity size, secrete low levels of trophic factors (3) and they can facilitate limited axonal outgrowth (2). D) Axons grow well into Schwann cell (SC) grafts (2) and are able to bridge lesion sites, but are reluctant to leave grafts. SCs can myelinate damaged axons with peripheral myelin (1) and can secrete trophic intermediates (3), although there is evidence to suggest that SCs increase astrogliosis.

A. Neural stem cells – Endogenous and Exogenous

Tripotential NPCs exist in the subventricular and hippocampal regions of the adult brain, as well as the ependymal zone of the adult spinal cord. They can also be created by differentiating pluripotent cells through the default pathway towards neurogenesis [11]. Unipotent glial precursors are also found in the local neural parenchyma. Following neurological damage, intrinsic neural precursor populations are able to expand and home to the site of injury [12] although endogenous repair is limited. Experimental drug therapy-induced expansion of endogenous neural populations has shown moderate success *in vivo* [13]. However, it is not yet possible to pharmacologically expand and mobilize a number of progenitors comparable to that which can be injected from an expanded exogenous population. Thus, the most common form of NPC introduction following injury involves intraparenchymal injection of adult, pluripotent or fetal-derived precursors. NPCs are thought to act as myelinating cells, homing to the site of injury and replacing lost or damaged oligodendrocytes [14]; they can promote recovery after transplant into damaged neural tissue, particularly in combination with neurotrophic growth factors [15] and can also recruit endogenous progenitors to the site of injury. Although NPCs have the potential to differentiate into three different cell types (as previously described) *in vitro,* when they are transplanted *in vivo* without pre-

conditioning, they almost exclusively differentiate into glial subtype cells, to the exclusion of neurons[11]. In the context of CP, where demyelination predominates, additional neuronal formation is unwanted due to the potential for aberrant connectivity and resulting neuropathic pain or further motor dysfunction; the observed preferential glial subtype formation is thus ideal for repair and support for other cellular subtypes. Therefore, although their safety in humans has not yet been fully elucidated, NPCs show the most potential for positive functional results when used in neurological injury models and subsequent clinical translation.

B. Peripheral glial cells

Peripheral glial cells like SCs and OEG are not stem cells but will be discussed herein due to their popular utility in cell transplant studies. SCs and OEG have been used for cell replacement and trophic modulation following experimental neural injury. Due to the fact that they create potent trophic and physical substrates for axonal growth, proximal sensory and propriospinal axons readily enter and rarely leave SC grafts [16]. Consequently, strategies that employ SC transplants generally involve co-transplanted growth factors [17], biomaterials [18] or cells [19], such as OEG. OEG can be derived from the olfactory bulb in the brain or through the lamina propria of the olfactory mucosa and have shown mixed results *in vivo,* thought to be partially due to origin or culture conditions (reviewed in [20]). SCs and OEG are thought to act via remyelination, as well as trophic and structural support. However, preclinical and clinical trials with these cells have shown mixed, and sometimes non-replicable results [21; 22].

C. Peripheral non-neural cells

Mesenchymal SCs

Mesenchymal SCs (MSCs) exist in the non-hematopoetic CD34- subset of bone marrow and umbilical cord cells. These cells, which compose a subset of the adherent stromal cell fraction, are most commonly used in transplant studies, particularly in pediatric populations, because of proven safety through decades of use in the context of leukemia and blood diseases.

Marrow-Derived MSCs

MSCs can be efficiently isolated from a number of sources - including bone marrow and/or aspirates, umbilical cord, placenta, fat and tooth - and they provide a minimally invasive, autologous source of cells for transplantation. Although the MSCs that have shown safety in the context of leukemia have traditionally been derived from donor bone

marrow, this paradigm is shifting toward more convenient cord-derived MSCs. Although MSCs can exhibit neurogenic differentiation *in vitro* , it is unclear whether this happens *in vivo* to a meaningful extent [23]. Transplanted MSCs can create a trophic substrate *in vivo,* enhancing tissue sparing in penumbral regions and reducing lesion volume, apoptosis, inflammation and demyelination [24]. MSCs are also reported to mobilize endogenous NPCs [25], promote angiogenesis [26] and provide physical scaffolding for elongating axons [27]. However, studies utilizing MSC transplantation report highly variable outcomes. Protocols and cell sources need to be optimised and further study is required in order to isolate and characterize more homogeneous populations and glean reliable, replicable results. Overall, there exists little and conflicting evidence for the efficacy of MSCs in the context of neurological repair.

Umbilical Cord-Derived MSCs

Because of the increasing popularity of postnatal umbilical cord stem cell harvest and storage, the most easily accessible current source of stem cells in clinical trials is umbilical cord-derived MSCs. Umbilical cord blood and other associated tissue such as placenta and Wharton's jelly are diverse sources of mononuclear progenitor and stem cell types, including MSCs. The ease of obtaining cells from fresh umbilical tissue and an associated autologous nature makes cord-and-placental-derived MSCs very appealing. These cells pose little risk to infant or mother, have low immunogenicity and fewer ethical issues compared to other cells used in transplant scenarios. Although human umbilical cord blood mononuclear cells (hUCB-MNC) have shown promise in animal disease models [28], there is an overall paucity of data; the few trials that have utilized these cells preclinically show a wide spectrum of results, ranging from normalization of function after several weeks [29] to complete cell disappearance after 24 hours or worsened injury [30]. Furthermore, experimental outcomes are often contradictory, even within the same disease model [31; 30], and mechanisms of action are not consistent, nor are they completely elucidated.

Currently, in humans, the first North American clinical trial using cord-blood derived MSCs is underway at Duke University (clinicaltrials.gov identifier NCT01147653) and has shown safety. Likewise, a study from the Sung Kwang Medical Foundation in Korea has combined rehabilitation, erythropoietin and cord-blood MSC infusion and is first to report positive results (clinicaltrials.gov identifier NCT01193660) [32; 33]. Further study is required to elucidate 1) whether these results are replicable across institutions and 2) what cellular properties can enhance or reduce this regenerative response.

Stem Cell Tourism

Of the 240 current clinical trials investigating treatments for CP or perinatal stroke, only 8 involve stem cells. However, there exists, due partially to a lack of transparency from scientists, as well as unwarranted media hype, a supply and demand mismatch that has

fostered the development of 'stem cell tourism'. Clinics in Asia, Europe, the Middle-East and Central America abound, offering "stem cell treatments" that are unregulated, lack legitimate affiliation or accountability and use technologies without solid scientific foundation. Individuals utilizing such clinics expose themselves to a lack of success, exclusion from legitimate trials, false hope, complications (including mortality) and financial hardship ($15-30,000 per treatment).

A detailed review of stem cell tourism by Lau *et al* [34] reveals that companies [for example, Beike Biotech, (China) ACT (Turks and Caicos), and Emcell, (Ukraine)] claim to have treated nearly 6000 patients. Lau *et al* report that websites from companies offering "stem cell therapies" report benefits as 'somewhat or very relevant' in 79% of sites tested, with risk portrayed as 'very irrelevant' in 74%. Furthermore, all of the websites reported benefits from treatment with only 25% reporting any risk - giving the false impression that stem cell therapy is safe, routine and effective.

The most common "therapies" consisted of intravenous or intrathecal administration of what companies claim were autologous MSCs (>60%), coupled with several weeks of intensive physiotherapy. There is no way to verify the reliability of these claims, the effect of this intensive physiotherapy alone on outcome or the purity of the cell suspension.

While there are anecdotal reports of success with stem cell tourism, there are concomitant reports of failures: neuropathic pain, multiple brain tumor formation, neurological injury and actual fatalities having occurred [35-37]. Furthermore, the extent of unreported complications in self regulated stem cell clinics remains unknown.

NPCs in the Clinic

In animal models, combinatorial approaches have proven to be most effective at establishing enhanced functional connectivity following neural injury and dysmyelination [38; 39]. Strategies employed preclinically in tandem with stem cell transplant or enhancements include: bioengineering strategies, rehabilitation, magnetic stimulation and growth factor/drug administration. While it is agreed in the field of regenerative medicine that combinatorial strategies, as opposed to a single therapeutic approach, provide the most promise for successful functional recovery, there still exists a knowledge gap surrounding the performance and role of stem cells in enhancing neurological recovery. Thus, before they can be studied in concert with other treatment methodologies, their particular role in the regenerative response must be elucidated in clinical trials.

I. Current Clinical Stem Cell Trials for CP

The field of cell transplantation for CP is burgeoning; eight current (one completed with results) phase I clinical trials are using stem cells (all MSCs) for treatment of CP or perinatal stroke (summarized in **Table 1**).Stem cells are infused either intravenously or intrathecally using single or multiple infusions(www.clinicaltrials.gov [32],). The participant

population is generally, 1-12 years of age of mixed gender, lack seizures or co-morbidities and it consists of a mixed CP etiology subset. The only study with reported results consists of a double-blinded randomised trial of 105 participants, between 10 months to 10 years of age, and of mixed gender. Participants received either: 1) Active rehabilitation, 2) EPO + active rehabilitation, or 3) Allogeneic umbilical cord blood infusion (intravenous) + Erythropoietin (EPO) injection + active rehabilitation. Assessment of neurological and neuromuscular function, brain MRI of WM integrity, and brain glucose metabolism (using PET) were undertaken regularly up to 6 months post intervention. Initial data analysis suggests that the primary outcome measures of motor function and standardized gross motor function were improved in the MSC group vs other groups. Secondary outcome measures of cognition, neurodevelopment and brain imaging also suggest improvements in the MSC group vs other groups (clinical trials identifier: NCT01193660) [33]. However, further statistical analysis remains to be completed to fully tease out the results and the implications from this promising study.

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Table 1: Stem cell clinical trials currently underway for Cerebral Palsy (CP)

	Company	Clinicaltrials.gov Identifier	Cell Source	Criteria	Phase	Delivery
1	Sung Kwang Medical Foundation, Korea	NCT01193660	Allogenic Umbilical Cord Blood and Erythropoietin Cord	10 months<10 years old with CP	I (completed with results)	Intravenous
2	Royan Institute, Iran	NCT01404663	Bone Marrow derived CD133	4<12 years old with quadriplegic CP, no seizures	I (completed)	Intrathecal
3	Royan Institute, Iran	NCT01763255	Bone Marrow derived CD133	4<12 years old with quadriplegic CP, no seizures	I	Multiple Intrathecal
4	Hospital Universitario Dr. Jose E. Gonzalez, Mexico	NCT01019733	Autologous stem cells (CD34+)	1<8 years suffered Hypoxia ischemia	I (completed)	Intrathecal
5	Hospital Universitario Dr. Jose E. Gonzalez, Mexico	NCT01506258	Autologous Non-cryopreserved CD34+ Cells	48hrs post birth in encephalopathic neonates, Apgar < 5 at 5min, pH<7	I	Intravenous
6	Children's Memorial Hermann Hospital, Houston, Texas	NCT01700166	Autologous Human Cord Blood derived	6weeks to 6 years with arterial ischemic stroke	I	Intravenous
7	Georgia Health Sciences University, USA	NCT01072370	Autologous Umbilical cord blood	1<12 years old with CP, no seizures	I/II	Intravenous
8	Roberson Foundation/ Duke University Medical Center	NCT01147653	Autologous Umbilical Cord Blood	12 months<6 years with spastic CP	II	Intravenous

II. Current Clinical Trials using NPCs

There are numerous experimental studies illustrating the potential benefits of transplanted NPCs in clinical neurodegeneration [40; 41]. However, a relative paucity of data exists regarding NPCs in clinical trials to treat CNS disorders, particularly those of childhood. There are currently seven clinical trials involving NPCs (summarized in **Table 2**) for neurological conditions. Most involve intravenous, intrathecal or intraparenchymal injection in chronic adult neurological conditions. However, the first clinical trial in children (StemCells inc. [42]) with advanced neuronal ceroid lipofuscinosis (Batten's disease) has completed early phase trials. If proven safe in this terminal condition, this trial could provide the basis for further pediatric studies in CP. A second study in children with less advanced Batten's disease (using CD133+ cell culture expanded NPCs) was started, but was discontinued due to insufficient enrollment of patients meeting study criteria. Further promising clinical studies in adult degenerative diseases using NPCs include: Pelizaeus-Merzbacher disease (PMD) (a myelination disorder) [43], stroke, thoracic spinal cord injury, and inoperable glioblastoma (see review [44]). Interestingly NPCs are being harvested from neurological biopsies of Parkinson's patients by the company NeuroGeneration. It is hoped that the harvested cells can be expanded to produce at least 10% dopaminergic cells which can be used as a source for therapeutic transplantation [45].

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Table 2: Current Clinical Trials using Neural Precursor Cells (NPCs)

	Company	Clinicaltrials.gov Identifier	Cell Source	Application	Phase	Delivery
1	Geron	NCT01217008	ESC-derived oligodendrocyte progenitors	SCI	I	Intraspinal
2	StemCells Inc., CA	NCT01321333 (Follow-Up) NCT01725880	Fetal derived human NPCs	SCI	II	Intraspinal
3		NCT00337636 NCT01238315		Neuronal Ceroid Lipofuscinosis (Batten's Disease)	I (completed) Ib (ceased for lack of enrolment)	Intracerebral
4		NCT01005004 (Follow-Up) NCT01391637		Pelizaeus-Merzbacher Disease	I/II	Intracerebral
5	Reneuron, UK	NCT01151124	Donated Fetal Brain NPCs	Adult Stroke	I	Intracerebral
6	Neuralstem Inc, MD	NCT01348451	Fetal Human Spinal Cord NPCs	ALS	I	Intraspinal
7		NCT01772810		SCI	I	Intraspinal

III. Difficulties of Translation from Bench to Bedside

Neurodegenerative diseases share common pathologies such as neuronal dysfunction, demyelination and cell death. The potential of NPCs to differentiate into neuronal subtypes, promote neuroprotection and neurogenesis strongly favour their application for neurodegenerative disorders. However, there remain several technical and regulatory hurdles before successful translation can be achieved.

A. Differentiation potential

Phenotypically different sources of NPCs used in the literature [46; 47] contribute to variability in efficacy, glial differentiation and marker expression. Both source and cell culture/sorting parameters can affect behaviour *in vitro.* Therefore, it is currently unknown which NPC sources (fetal-derived, adult-derived, endogenous, and pluripotent-derived) and treatment conditions create the ideal cell type for transplantation. Or, indeed, whether NPCs alone or in concert exert the most beneficial effect.

B. NPC source

While there are resident NPCs in the adult and fetal CNS encouraging their application in chronic neurodegeneration paradigms, harvesting human NPCs from fetal or adult CNS is both ethically and technically challenging. NPCs differentiated from pluripotent sources, via variable drug and differentiation protocols, present an alternative source of adult NPCs. Although there are means to derive several cell types from pluripotent progenitors, and indeed some groups are exploring trans-differentiation between two adult cell types via similar mechanisms to iPSCs, much work is still required to optimize conditions and to translate this technology safely to humans.

C. Transplant survival

Another barrier to translation is the limited survival of transplanted exogenous cells. Indeed, there is currently a scarcity of data suggesting that cells survive further than a matter of weeks, and several groups have taken measures to increase cell viability including scaffolding [48], pre-sorting [49], and modification. While experimentally transplanted exogenous, and recruitment of endogenous NPCs has been eloquently demonstrated in several animal models of CNS disorder, the underlying mechanisms of these processes remain to be elucidated [50] and it is unknown whether this phenomenon can be replicated in humans.

D. Therapy timing and dose

Optimal timing, route, and cell dose is still largely unknown; this is reflected in the variability surrounding these parameters in current clinical trials. Transplanted NPCs are frequently delivered intrathecally or intraparenchymally, with cell dose depending on the site of injury and local microenvironment. Multiple intracerebral injection sites, with slow and prolonged infusion rates [51] seem to provide maximal incorporation; however, dose responsiveness has never been studied in humans.

Clinically, current neuroprotective strategies in encephalopathic newborns have a narrow optimal therapeutic window of hours after injury, which corresponds with rising immune activation; however, injecting at this highly inflammatory time point may inhibit stem cell survival. Furthermore, many children with perinatal complications fail to develop CP later in life. Indeed, most cases of CP are not diagnosed until ~2 years of age. Injecting at this chronic time point may be more feasible but potentially requires combinatorial therapies to ensure success. Furthermore, a number of cases report stem cell infusion in children to be associated with pulmonary thrombosis [52] which would need to be resolved.

E. Toxicity

While NPCs are generally considered minimally immunogenic and non-tumorigenic in animal models [53], tumorigenicity and transplant rejection are still major causes for concern when translating therapies to the clinic. In terms of transplantation, the two most promising cell types, MSCs and NPCs, represent two ends of a continuum: MSCs are safe in clinical paradigms, but have not shown consistent pre-clinical benefit; conversely, NPCs show the most benefit in animal models, but have not yet been demonstrated to be safe in the long term in humans. Since stem cell transplantation is irreversible, all potential side effects must be fully elucidated both experimentally and in tightly controlled early safety trials before moving to human subjects.

Multivariate Etiology of CP

Due to the multivariate etiology and pathology of CP, and indeed all neurological injury, remyelination alone cannot promise to alleviate all disease symptoms. Therefore, combinatorial strategies to combat the disease will be necessary. Some common strategies currently under exploration include bioengineering, rehabilitation strategies (such as constraint-induced movement therapy, treadmill training, virtual reality, gaming and robot-assisted locomotor training), pharmacological interventions such as baclofen and Botulinum toxin (Botox) as well as Transcranial Magnetic Stimulation.

Future Perspectives

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A solid understanding of stem cell development and biology provides a stable basis for understanding their role in regeneration and repair; from this, it is clear that stem cell transplantation is a viable option for neural regeneration. There is a clear clinical demand for resources on stem cell therapy; interested stakeholders can find more information on stem cells for neurological conditions at: http://www.drfehlings.ca/stem-cell-information/ [54]. Further understanding cells' developmental lineages and elucidating the factors that govern their individual and combinatorial functions in cell replacement, scaffolding and trophic support form the basis of the future of regenerative medicine. Closely controlled safety studies, using uniform, high purity 'safe' cell lines and optimising route, timing and dose are crucial to minimizing adverse effects and to understanding these cells' inherent mechanisms of repair. In chronic injury, it may be that exogenous NPC transplantation is not only required in combination with stimulation of endogenous NPC populations but also carried out in parallel with existing clinical management, as part of a multimodal and multidisciplinary approach. Clinical application of the tenets gleaned from basic research in this field will partly address some of these issues and further guide experimental and clinical studies to optimise application, safety and benefit to cost ratio.

REFERENCES

1 Kuban KC, Leviton A. (1994) Cerebral palsy. *N Engl J Med* **330**: 188-95.
2 Badawi N, Kurinczuk JJ, Keogh JM, et al. (1998) Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ* **317**: 1554-8.
3 Tommiska V, Heinonen K, Lehtonen L, et al. (2007) No improvement in outcome of nationwide extremely low birth weight infant populations between 1996-1997 and 1999-2000. *Pediatrics* **119**: 29-36.
4 Volpe JJ. (2009) Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol* **8**: 110-24.
5 Shevell MI, Dagenais L, Hall N. (2009) The relationship of cerebral palsy subtype and functional motor impairment: a population-based study. *Dev Med Child Neurol* **51**: 872-7.
6 Back SA, Craig A, Kayton RJ, et al. (2007) Hypoxia-ischemia preferentially triggers glutamate depletion from oligodendroglia and axons in perinatal cerebral white matter. *J Cereb Blood Flow Metab* **27**: 334-47.
7 Takahashi K, Tanabe K, Ohnuki M, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**: 861-72.
8 Lujan E, Chanda S, Ahlenius H, Sudhof TC, Wernig M. (2012) Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. *Proc Natl Acad Sci U S A* **109**: 2527-32.
9 Ruff CA, Fehlings MG. (2010) Neural stem cells in regenerative medicine: bridging the gap. *Panminerva Med* **52**: 125-47.
10 Ruff CA, Wilcox JT, Fehlings MG. (2012) Cell-based transplantation strategies to promote plasticity following spinal cord injury. *Exp Neurol* **235**: 78-90.
11 Rowland JW, Lee JJ, Salewski RP, Eftekharpour E, van der Kooy D, Fehlings MG. (2011) Generation of neural stem cells from embryonic stem cells using the default mechanism: in vitro and in vivo characterization. *Stem Cells Dev* **20**: 1829-45.
12 Zawadzka M, Rivers LE, Fancy SP, et al. (2010) CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell Stem Cell* **6**: 578-90.
13 Hunt J, Cheng A, Hoyles A, Jervis E, Morshead CM. (2010) Cyclosporin A has direct effects on adult neural precursor cells. *J Neurosci* **30**: 2888-96.
14 Obenaus A, Dilmac N, Tone B, et al. (2011) Long-term magnetic resonance imaging of stem cells in neonatal ischemic injury. *Ann Neurol* **69**: 282-91.
15 Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D, Fehlings MG. (2010) Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. *J Neurosci* **30**: 1657-76.
16 Raisman G. (1997) Use of Schwann cells to induce repair of adult CNS tracts. *Rev Neurol (Paris)* **153**: 521-5.
17 Pearse DD, Marcillo AE, Oudega M, Lynch MP, Wood PM, Bunge MB. (2004) Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: does pretreatment with methylprednisolone and interleukin-10 enhance recovery? *J Neurotrauma* **21**: 1223-39.
18 Vavrek R, Pearse DD, Fouad K. (2007) Neuronal populations capable of regeneration following a combined treatment in rats with spinal cord transection. *J Neurotrauma* **24**: 1667-73.
19 Pearse DD, Sanchez AR, Pereira FC, et al. (2007) Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: Survival, migration, axon association, and functional recovery. *Glia* **55**: 976-1000.

20 Tetzlaff W, Okon EB, Karimi-Abdolrezaee S, et al. (2011) A systematic review of cellular transplantation therapies for spinal cord injury. *J Neurotrauma* **28**: 1611-82.
21 Steward CG. (2010) Hematopoietic stem cell transplantation for osteopetrosis. *Pediatr Clin North Am* **57**: 171-80.
22 de Lima M, Shpall E. (2006) Strategies for widening the use of cord blood in hematopoietic stem cell transplantation. *Haematologica* **91**: 584-7.
23 Castro JE, Ball ED. (2002) Development of allogeneic hematopoietic stem cell transplantation (HSCT). *Cancer Treat Res* **110**: 1-37.
24 Sasaki M, Radtke C, Tan AM, et al. (2009) BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. *J Neurosci* **29**: 14932-41.
25 Bonilla C, Zurita M, Otero L, Aguayo C, Vaquero J. (2009) Delayed intralesional transplantation of bone marrow stromal cells increases endogenous neurogenesis and promotes functional recovery after severe traumatic brain injury. *Brain Inj* **23**: 760-9.
26 Liao W, Zhong J, Yu J, et al. (2009) Therapeutic benefit of human umbilical cord derived mesenchymal stromal cells in intracerebral hemorrhage rat: implications of anti-inflammation and angiogenesis. *Cell Physiol Biochem* **24**: 307-16.
27 Ankeny DP, McTigue DM, Jakeman LB. (2004) Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. *Exp Neurol* **190**: 17-31.
28 Vendrame M, Cassady J, Newcomb J, et al. (2004) Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke* **35**: 2390-5.
29 Meier C, Middelanis J, Wasielewski B, et al. (2006) Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr Res* **59**: 244-9.
30 Dalous J, Pansiot J, Pham H, et al. (2012) Use of Human Umbilical Cord Blood Mononuclear Cells to Prevent Perinatal Brain Injury: A Preclinical Study. *Stem Cells Dev*.
31 Pimentel-Coelho PM, Magalhaes ES, Lopes LM, deAzevedo LC, Santiago MF, Mendez-Otero R. (2009) Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection in the striatum. *Stem Cells Dev* **19**: 351-8.
32 (2012) http://www.clinicaltrials.gov. p Webpage.
33 Min K, Song J, Kang JY, et al. (2012) Umbilical cord blood therapy potentiated with erythropoietin for children with cerebral palsy: A double-blind, randomized, placebo-controlled trial. *Stem Cells*.
34 Lau D, Ogbogu U, Taylor B, Stafinski T, Menon D, Caulfield T. (2008) Stem cell clinics online: the direct-to-consumer portrayal of stem cell medicine. *Cell Stem Cell* **3**: 591-4.
35 Amariglio N, Hirshberg A, Scheithauer BW, et al. (2009) Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med* **6**: e1000029.
36 Sinko J, Csomor J, Nikolova R, et al. (2008) Invasive fungal disease in allogeneic hematopoietic stem cell transplant recipients: an autopsy-driven survey. *Transpl Infect Dis* **10**: 106-9.
37 Mendick RaP, A. (2010) Baby death scandal at stem cell clinic which treats hundreds of British patients a year
Daily Telegraph London.
38 Nakaguchi K, Jinnou H, Kaneko N, et al. Growth factors released from gelatin hydrogel microspheres increase new neurons in the adult mouse brain. *Stem Cells Int* **2012**: 915160.

39 Zhao C, Wang J, Zhao S, Nie Y. (2009) Constraint-induced movement therapy enhanced neurogenesis and behavioral recovery after stroke in adult rats. *Tohoku J Exp Med* **218**: 301-8.
40 Chu K, Kim M, Park KI, et al. (2004) Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. *Brain Res* **1016**: 145-53.
41 Cummings BJ, Uchida N, Tamaki SJ, et al. (2005) Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. *Proc Natl Acad Sci U S A* **102**: 14069-74.
42 Study of HuCNS-SC Cells in Patients With Infantile or Late Infantile Neuronal Ceroid Lipofuscinosis (NCL)
^clinical trials http://www.clinicaltrials.gov/ct2/show/record/NCT00337636?term=StemCells+inc.+Batten%E2%80%99s+disease&rank=2
43 Gupta N, Henry RG, Strober J, et al. (2012) Neural stem cell engraftment and myelination in the human brain. *Sci Transl Med* **4**: 155ra37.
44 Trounson A, Thakar RG, Lomax G, Gibbons D. (2011) Clinical trials for stem cell therapies. *BMC Med* **9**: 52.
45 Lévesque MF, Neuman, T, Rezak, M. (2009) Therapeutic Microinjection of Autologous Adult Human Neural Stem
Cells and Differentiated Neurons for Parkinson's Disease: Five-Year
Post-Operative Outcome. ***The Open Stem Cell Journal*** **1**: 20-9.
46 Bonner JF, Connors TM, Silverman WF, Kowalski DP, Lemay MA, Fischer I. (2011) Grafted neural progenitors integrate and restore synaptic connectivity across the injured spinal cord. *J Neurosci* **31**: 4675-86.
47 Kranz A, Wagner DC, Kamprad M, et al. (2010) Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in rats. *Brain Res* **1315**: 128-36.
48 Wang L, Shansky J, Borselli C, Mooney D, Vandenburgh H. (2012) Design and fabrication of a biodegradable, covalently crosslinked shape-memory alginate scaffold for cell and growth factor delivery. *Tissue Eng Part A* **18**: 2000-7.
49 Windrem MS, Schanz SJ, Guo M, et al. (2008) Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. *Cell Stem Cell* **2**: 553-65.
50 Danielyan L, Schafer R, von Ameln-Mayerhofer A, et al. (2011) Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. *Rejuvenation Res* **14**: 3-16.
51 Bjarkam CR, Glud AN, Margolin L, et al. (2010) Safety and function of a new clinical intracerebral microinjection instrument for stem cells and therapeutics examined in the Gottingen minipig. *Stereotact Funct Neurosurg* **88**: 56-63.
52 Kounami S, Aoyagi N, Nakayama K, et al. (2003) Fatal pulmonary thromboembolism after a second course of high-dose chemotherapy with autologous peripheral blood stem cell transplantation. *Pediatr Transplant* **7**: 400-3.
53 Lepore AC, Neuhuber B, Connors TM, et al. (2006) Long-term fate of neural precursor cells following transplantation into developing and adult CNS. *Neuroscience* **142**: 287-304.
54 Fehlings M. (2012) Neural repair and regeneration.