

# Stem cell therapy for neurological disorders

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Neurological disease encompasses a diverse group of disorders of the central and peripheral nervous systems, which collectively are the leading cause of disease burden globally. The scope of treatment options for neurological disease is limited, and drug approval rates for improved treatments remain poor when compared with other therapeutic areas. Stem cell therapy provides hope for many patients, but should be tempered with the realisation that the scientific and medical communities are still to fully unravel the complexities of stem cell biology, and to provide satisfactory data that support the rational, evidence-based application of these cells from a therapeutic perspective. We provide an overview of the application of stem cells in neurological disease, starting with basic principles, and extending these to describe the clinical trial landscape and progress made over the last decade. Many forms of stem cell therapy exist, including the use of neural, haematopoietic and mesenchymal stem cells. Cell therapies derived from differentiated embryonic stem cells and induced pluripotent stem cells are also starting to feature prominently. Over 200 clinical studies applying various stem cell approaches to treat neurological disease have been registered to date (Clinicaltrials.gov), the majority of which are for multiple sclerosis, stroke and spinal cord injuries. In total, we identified 17 neurological indications in clinical stage development. Few studies have progressed into large, pivotal investigations with randomised clinical trial designs. Results from such studies will be essential for approval and application as mainstream treatments in the future.

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Disorders of the central and peripheral nervous systems are diverse and heterogeneous. Hundreds of millions of people worldwide are affected by neurological disorders. Overall, these are the leading cause of disability-adjusted life years (DALYs), making up 10.2% of the total disease burden worldwide in 2015. Neurological disorders are second only to cardiovascular disease regarding causes of death – 16.8% of deaths in 2015 were due to neurological conditions.<sup>[1]</sup> It has previously been reported that the annual cost of managing neurological disease in the USA alone is close to USD800 billion.<sup>[2]</sup> Despite this health burden, the treatment landscape is limited, with few curative options available and poor drug approval rates for potential new medications.<sup>[3]</sup>

Stem cells have in many respects revolutionised the way in which we explore and treat disease. However, with the exception of haematopoietic stem cell (HSC) transplantation, the stem cell hype is yet to live up to its promise concerning treatment and cure of disease. The reasons for this are manifold, but primarily because we have still to understand the complete *modus operandi* of the various stem cell preparations and their clinical implications. Recently, Japanese authorities cleared a mesenchymal stem cell (MSC) product for treating spinal cord injuries. Although concerns over the approval have been raised by the global scientific community,<sup>[4]</sup> this is the first and only stem cell therapy approved for treating a neurological condition. Nonetheless, there is encouraging evidence to suggest that stem cell therapy as a whole holds promise for drug approvals in this area in the future. We provide a high-level perspective of where progress has been made with respect to the use of stem cells for treating neurological disease.

## Stem cells

Stem cells provide the building blocks for every organ in the body. They have the unique ability to divide asymmetrically, and to differentiate into the various cell types of the body while simultaneously replicating to maintain a stem cell lineage. Stem cells are present in almost every human tissue – in embryos, they differentiate into all the tissues and organs of the body, and in fully developed humans they provide a renewal capacity in most organs. Different forms of stem cells exist, each with a varying ‘potency’ (Fig. 1). Potency refers to the ability of a stem cell to replicate and differentiate into different cell types. Totipotent and pluripotent stem cells have the highest potency, and are obtained from the pre-embryonic stages of development. Blastomeres that arise from a fertilised ovum (zygote) are totipotent stem cells and have the potential to differentiate into all organs of the body, including the placenta. A cluster of blastomeres goes on to form a blastocyst, which has a hollow cavity and an inner cell mass of embryonic stem cells (ESCs). These stem cells are pluripotent in nature and, relative to totipotent stem cells, have lost the ability to differentiate into the placenta while maintaining their ability to form all organs and tissues of the body. A blastocyst develops into a gastrula wherein the three germ layers have started to form, followed by the development of an embryo.

Pluripotent stem cells differentiate into multipotent stem cells, including HSCs, MSCs and neural stem cells (NSCs). These cells are committed to further differentiate into cells of a given organ or system, e.g. HSCs differentiate into cells of the blood and immune systems, and NSCs into cells of the brain and nervous

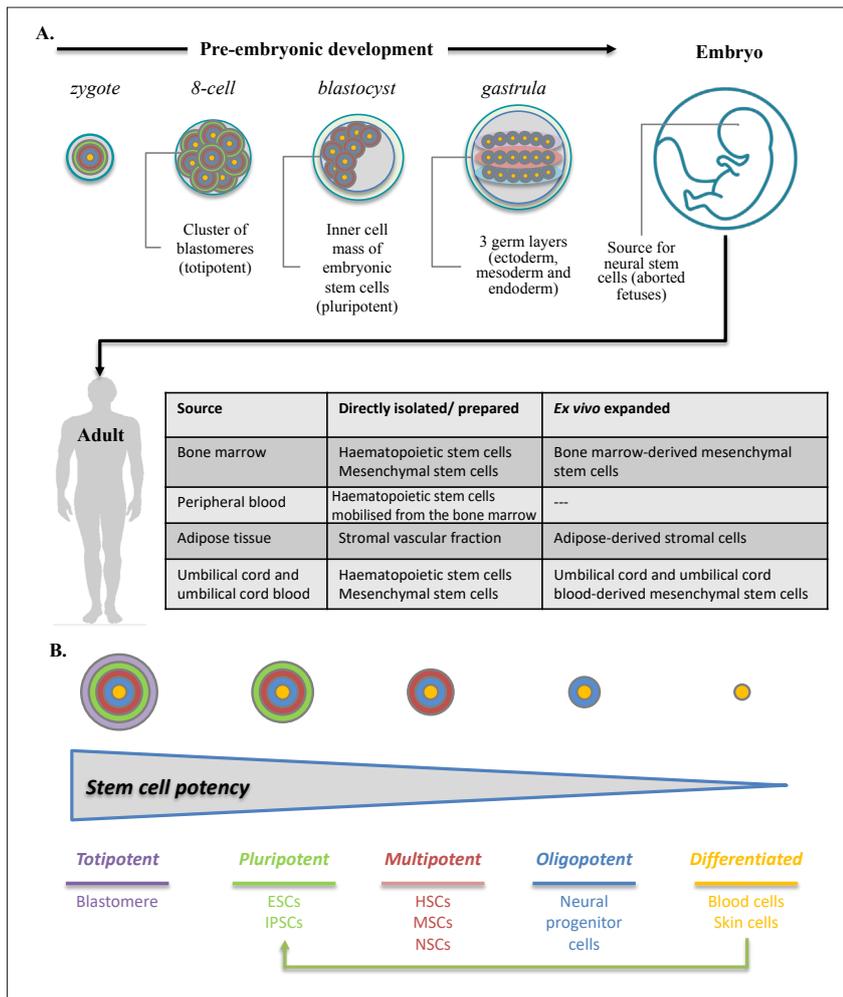


Fig. 1. Schematic summary of stem cell sources and potency. (A) Totipotent (blastomeres) and pluripotent stem cells (embryonic stem cells (ESCs)) are obtained from pre-embryonic tissue. Multipotent neural stem cells are harvested from aborted fetuses, while haematopoietic and mesenchymal stem cells (including adipose stromal cells) are collected from different sources in adults. (B) Diagram illustrating the potency of the different stem cell types. Multipotent stem cells differentiate into oligopotent stem cells, such as lineage-specific progenitor cells, which ultimately differentiate into fully committed cells, such as blood cells or skin cells. Induced pluripotent stem cells (iPSCs) can be derived from various types of fully committed/differentiated cells. (HSCs = haematopoietic stem cells; MSCs = mesenchymal stem cells; NSCs = neural stem cells.)

Image of fetus created by Laymik from the Noun Project.

system. Multipotent stem cells differentiate into progenitor cells that are oligo- and unipotent, which are further committed to a limited number of cell types. Until recently, stem cell differentiation was considered unidirectional; however, by introducing a defined number of factors into fully differentiated cells (e.g. skin or blood cells) in a laboratory setting, one is able to derive a cell type which once again has pluripotent capacity – these are referred to as induced pluripotent stem cells (iPSCs).

### Stem cell therapy for neurological disorders

In principle, for a stem cell therapy to be

successful in treating diseases of the brain where neurons are damaged or defective, the treatment should aim to repair, replace or at least prevent future deterioration. The goal of stem cell therapy is thus to enable localisation of therapeutic cells to impaired/injured regions of the brain, to stimulate tissue repair and maintenance via a paracrine effect, and potentially even to generate new neurons (although the latter is generally less likely to occur). Each of the described stem cell types is being applied in the treatment of neurological disease, for which a few advantages and disadvantages are listed in Table 1. By definition, NSCs would be the prototype and most logical stem cell

therapy for treating neurological disorders. However, since they are located deep within the adult brain, NSCs are not accessible for harvesting and hence for autologous therapeutic applications. They are therefore procured from aborted fetuses (i.e. aborted for medical or non-medical reasons) and prepared for allogeneic transplantation purposes. Given the practical and ethical limitations of this scenario, in addition to the risks that transplanted cells might be rejected by the recipient's immune system<sup>[5]</sup> or that they may be tumorigenic (owing to their heightened proliferative capacity), there has been an appreciable increase in the need for an alternative source. With recent advances in our understanding of stem cell biology, it is possible to differentiate pluripotent stem cells (both ESCs and iPSCs) into neural progenitor or NSC-like cells for therapeutic purposes. Protocols for differentiating bone marrow-derived MSCs into NSC-like cells have also been developed and translated for clinical application in patients with multiple sclerosis.<sup>[6]</sup> Both forms of derivation (from fetal tissue and differentiated) are being investigated in clinical studies to treat several neurological conditions. Given their high proliferative capacity and risk for tumour formation, pluripotent stem cells are not used directly for treatment purposes, but rather are differentiated into the desired fully differentiated cell type. HSCs and MSCs are also being explored in clinical studies to investigate their potential for treating various neurological disorders.

To better understand the treatment landscape of stem cell therapies for brain disease, we extracted data from the Clinicaltrials.gov website to identify and investigate which therapies have progressed into clinical development stages. A database of registered clinical trials was created based on data outputs using the following search terms: 'embryonic stem cells', 'induced pluripotent stem cells', 'neural stem cells', 'mesenchymal stem cells', 'mesenchymal stromal cells', 'bone marrow stromal cells', 'umbilical cord mesenchymal stem cells', 'adipose stem cells', 'adipose-derived regenerative cells' and 'stromal vascular fraction'. To capture trials using HSCs, we based our search on the use of 'haematopoietic stem cells' and 'umbilical cord blood' together with disease indications listed in one of our previous reports.<sup>[7]</sup> The data were cleaned to harmonise terminology across all trials and categorised into therapeutic areas. Observational studies were excluded and all trials relating to neurological disease were selected and taken forward for analysis and interpretation. Relevant clinical

**Table 1. Advantages and disadvantages of the different stem cell types**

Stem cells	Advantages	Disadvantages
NSCs	<p>Prototype stem cells for treating neurological disease</p> <p>NSC-like cells can be derived from other stem cell types (pluripotent and multipotent)</p>	<p>Limited resource with ethical implications around procurement (if from aborted fetal tissue)</p> <p>Poorly understood stem cell biology and least explored in clinical studies</p> <p>Tumorigenic risks if derived from pluripotent stem cells</p>
HSCs	<p>Globally accepted form of treatment for haematological conditions</p> <p>Well-established industry for harvesting and preparation of clinical grade treatments</p>	<p>Limited experience for use in neurological disease</p> <p>Generally limited for use as an autologous therapy (requires genetic matching of the donor and recipient if used as an allogeneic treatment)</p> <p>Poorly understood mechanism of action for treating certain neurological conditions</p>
MSCs	<p>Readily accessible resource and easily procured</p> <p>No need for genetic matching</p> <p>Most likely stem cell therapy to evolve into an off-the-shelf allogenic product</p>	<p>Exploited by unregulated clinics globally</p> <p>Poorly understood mechanism of action for treating certain neurological conditions</p>

NSCs = neural stem cells; HSCs = haematopoietic stem cells; MSCs = mesenchymal stem cells.

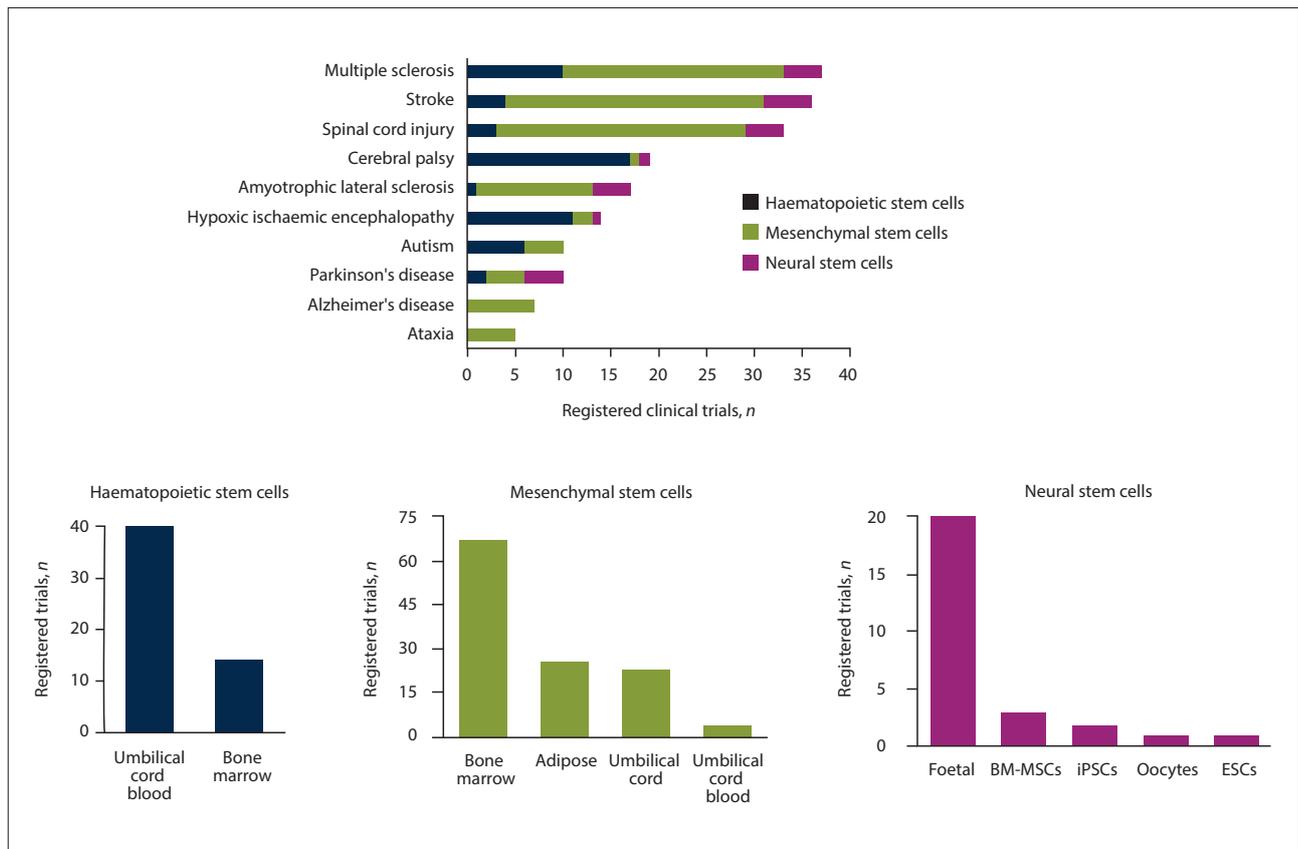


Fig. 2. Registered clinical trials for neurological conditions treated with stem cells. Registered studies (N=201) were exported from Clinicaltrials.gov. In order to capture a representative dataset, we expanded our searches to include (i) embryonic and induced pluripotent stem cells as a source for NSC-like cells; (ii) umbilical cord blood as a source for HSCs; and (iii) umbilical cord, umbilical cord blood, bone marrow and adipose for MSCs. The captured data were filtered to include only interventional studies for neurological diseases, and duplicate, terminated or withdrawn studies were excluded. Indications with less than 5 registered clinical studies include brain tumours (n=4), multiple system atrophy (n=2), diabetic neuropathy (n=2), cerebral adrenoleukodystrophy (n=1), epilepsy (n=1), mental retardation (n=1), progressive supranuclear palsy (n=1) and fibromyalgia (n=1). (BM-MSCs = bone marrow-derived mesenchymal stem cells; ESCs = embryonic stem cells; iPSCs = induced pluripotent stem cells.)

studies and disease indications were also searched online and in published literature via Pubmed and Google Scholar.

Fig. 2 provides an overview of globally registered clinical studies where stem cells have been applied for treating neurological disorders. Overall, we identified 201 clinical studies that were registered over the period of 1999 to 2018, of which more than half are indicated for the treatment of multiple sclerosis, stroke and spinal cord injury. MSCs are/were used in 120 studies, while HSCs and NSCs are/were applied in 54 and 27 trials, respectively. Ten stem cell therapies have progressed to advanced clinical stages (Phase II/III or III), including MSCs for spinal cord injury ( $n=3$ ), stroke ( $n=2$ ), cerebral palsy ( $n=1$ ) and diabetic neuropathy ( $n=1$ ); HSCs for multiple sclerosis ( $n=2$ ); and a single NSC treatment for Parkinson's disease. A more detailed perspective on the use of NSCs, HSCs and MSCs in this context is provided below.

## Neural stem cells

NSCs are multipotent progenitors which have the ability to produce neurotrophic factors and/or differentiate into committed cell lineages of the central and peripheral nervous systems, including neurons and supporting glial cells (such as astrocytes and oligodendrocytes). In the adult brain, NSCs are limited to the hippocampus and play a role in supporting plasticity. Those found in the subventricular zone (SVZ) of the hippocampus are in contact with the cerebrospinal fluid, moving radially and differentiating into young neuroblasts.<sup>[8]</sup> NSCs in the subgranular zone (SGZ) also move radially and differentiate into neuroblasts, but are referred to as radial glia-like NSCs. Neuroblasts go on to mature and differentiate into neurons.<sup>[9,10]</sup> NSCs are abundant in fetal brains, most notably at very early stages of development. Once harvested from the fetal brain, they can be dissociated and grown in the laboratory as a monolayer of cells or as floating 'spheroids' that produce neurotrophic factors that can be differentiated into more defined neuronal lineages.<sup>[11]</sup>

Pluripotent stem cells are increasingly becoming an important source for the generation of neural precursor cells. Using a variety of *in vitro* conditions, cells resembling NSCs (NSC-like) can be generated from human ESCs. These conditions include co-culturing with animal stromal cells (unknown stimuli),<sup>[12]</sup> exposure to retinoic acid,<sup>[13]</sup> and inhibition of bone morphogenetic protein (BMP) signalling.<sup>[14]</sup> The recent development of iPSCs, which have also been shown to generate NSC-resembling cells,<sup>[15]</sup> mitigates ethical concerns linked to the use of embryos and also introduces the possibility of autologous transplantation using patient-derived iPSCs.<sup>[16]</sup>

The rationale for therapeutic application of NSCs and NSC-like cells is based on their ability to release neurotrophic factors and to differentiate into neural and glial cells, thereby promoting neurogenesis and replacing diseased or injured areas of the brain. To date, no NSC-based therapy has been approved for routine clinical use. We identified 27 registered clinical trials, of which two-thirds ( $n=20$ ) used fetal-derived NSCs for the treatment of neurological disease (Fig. 2). Most of the clinical studies using NSCs and NSC-like cells were for stroke ( $n=5$ ), while 4 studies each were for multiple sclerosis, spinal cord injury, amyotrophic lateral sclerosis, Parkinson's disease and brain tumours. For the latter, gene-modified NSCs are being applied (see below). Seven of the 27 studies using NSC-based therapies to treat neurological disease make use of NSC-like cells differentiated from bone marrow MSCs ( $n=3$ ), iPSCs ( $n=2$ ), ESCs ( $n=1$ ) and oocytes ( $n=1$ ).

Stroke occurs either as a result of impaired blood flow (ischaemic) or rupturing of blood vessels (haemorrhagic) in the brain, resulting in diminished oxygen and nutrient supply to neurons. Preclinical

studies using NSCs to treat stroke have demonstrated that, in addition to stimulating neurogenesis, NSCs are capable of releasing angiogenic factors to promote local tissue regeneration.<sup>[17-19]</sup> Four of the 5 clinical trials registered for stroke apply fetal-derived NSCs, of which 3 used gene-modified NSCs to create a conditionally immortalised cell line. Gene modification to express the recombinant protein, *c-MycER<sup>TAM</sup>*, allows for controlled expansion of a defined and homogeneous NSC cell line.<sup>[20]</sup> In the completed Phase I and II studies (NCT01151124, NCT02117635), it was shown that these cells are well tolerated and the application is associated with improved neurological outcomes.<sup>[21]</sup> A larger Phase IIb clinical study is ongoing (NCT03629275). The fourth study using fetal-derived NSCs is also still ongoing (NCT03296618), and notably, this same product (NSI-566) is being tested in clinical studies for amyotrophic lateral sclerosis and spinal cord injury. In the most recently registered clinical study to be initiated in 2019, iPSC-derived NSC-like cells will be used to treat patients with haemorrhagic stroke (NCT03725865).

Multiple sclerosis is a neurodegenerative disorder characterised by inflammatory demyelination of neurons. Of the 4 registered clinical trials using NSCs to treat multiple sclerosis, 2 make use of fetal-derived NSCs, while the other 2 apply bone marrow MSC-derived NSC-like cells. The fetal-derived NSC studies are still ongoing and no data have been reported. Recently reported findings from a Phase I clinical study (NCT01933802) showed that intrathecal administration of MSC-derived neural progenitor cells for patients with multiple sclerosis was safe and well tolerated.<sup>[6]</sup> Although only modest improvements were observed, the initial findings warrant further clinical development and initiation of a Phase II study (NCT03355365).

Of the 4 registered clinical studies using NSCs for spinal cord injury, 3 make use of fetal-derived NSCs, while the fourth uses NSC-like cells differentiated from bone marrow MSCs. Data from one of these clinical studies have been reported which demonstrates the safety of fetal-derived NSC transplantation (NSI-566), but with no significant improvement with respect to quality-of-life score.<sup>[22]</sup> Improved secondary outcomes and encouraging preclinical data warrant testing in a larger dose escalation study. Although no trial of iPSC-derived NSC-like cells for spinal cord injury is registered yet, a recent report describes preparation of clinical grade cells for this purpose.<sup>[23]</sup>

In Parkinson's disease, a neurodegenerative disorder resulting from progressive loss of dopaminergic neurons, application of NSCs with an acquired specification for a dopaminergic neuronal identity has demonstrated promising results. Dopaminergic neural progenitor cells can either be isolated from the fetal ventral midbrain<sup>[24]</sup> or generated *in vitro* from human ESCs or iPSCs.<sup>[25]</sup> Preclinical data show that transplantation of dopaminergic progenitors in rat and monkey Parkinson's disease models induced behavioural recovery.<sup>[14,16,26]</sup> However, the initial proof-of-principle clinical studies using NSCs harvested from fetal ventral midbrains showed mixed results, ranging from no clinical benefit (even with demonstrated long-term engraftment) to excessive and uncontrolled dopamine production and major dyskinesia.<sup>[27]</sup> In this context, we identified 4 ongoing registered clinical trials for Parkinson's disease, each using NSCs derived from different sources, namely fetal, ESCs, iPSCs and oocytes. The studies using fetal and ESC-derived NSCs were both initiated in 2017 (NCT03128450, NCT03119636), for which no data have yet been reported, while the study using iPSC-derived NSCs is planned for initiation in 2019 (NCT03815071). In addition to the latter, and not listed in clinicaltrials.gov, a clinical study using NSCs derived from iPSCs was initiated in 2018 in Japan.<sup>[16]</sup> In the single

study using oocytes as a source (NCT02452723), NSC-like cells were differentiated from human parthenogenetic stem cells obtained from chemically activated unfertilised oocytes.<sup>[28]</sup> These cells are being used to treat patients with Parkinson's disease, with preliminary data showing safety with indications of efficacy over an ongoing 5-year period of follow-up.<sup>[29]</sup>

For treatment of brain tumours, 3 registered clinical studies use fetal-derived NSCs which have been gene modified to express cytosine deaminase, an enzyme involved in the activation of chemotherapeutic agents such as 5-fluorouracil and irinotecan, which serves to localise drug activity to the site of transplantation. Data from one of these studies have been reported, wherein 15 patients were recruited and proof-of-principle for this approach demonstrated.<sup>[30]</sup> A fourth ongoing study applies fetal-derived NSCs loaded with an oncolytic virus to enhance tumour eradication in patients with glioblastoma (NCT03072134). Promising preclinical evidence using this approach suggests efficacy without toxicity.<sup>[29, 31]</sup>

## Haematopoietic stem cells

Haematopoietic stem cells are multipotent stem cells traditionally used for the treatment of malignant and non-malignant diseases of the blood and immune systems. They can be harvested directly from bone marrow, peripheral blood (following pre-treatment to mobilise HSCs from the bone marrow) or umbilical cord blood. Depending on the condition being treated, HSCs are either collected from the patient or from a healthy donor to be used for autologous or allogeneic transplantation, respectively. Allogeneic transplantation, however, comes with the constraint of having to genetically match the donor and recipient in order to avoid graft rejection or, more concerning, the risk of graft v. host disease and its associated mortality. Patients are also subjected to lifelong immunosuppressive therapy.

HSC transplantation is performed in over 80 countries and, of the more than 60 000 HSC transplants that are done globally per annum, the vast majority (~90%) are for treating haematological malignancies, including leukaemia, lymphoma and myeloma.<sup>[32,33]</sup> Secondary to these indications is the treatment of solid tumours, while non-malignant conditions include bone marrow failure, haemoglobinopathies and primary immune disorders. In all of these cases, the underlying principle of HSC transplantation is to replenish the bone marrow with stem cells which engraft and reconstitute the immune system with a functional lineage of haematopoietic cells.

In addition to the above traditional indications, there has over the last decade been a distinct increase in the use of HSCs for regenerative purposes, particularly umbilical cord blood (UCB) as a source of HSCs for treating neurological conditions.<sup>[7]</sup> In fact, our findings show that of the 54 clinical studies registered to treat neurological disease with HSCs, 40 use UCB as a source. Once harvested, an UCB unit is processed to separate mononuclear cells (via removal of the red blood cells and plasma), which ultimately comprises a high proportion of HSCs, and to a lesser extent MSCs, endothelial progenitor cells and immunosuppressive cells such as regulatory T cells and monocyte-derived suppressor cells. Altogether and once transplanted, these cells have been shown to provide a paracrine effect that (i) promotes cell survival; (ii) stimulates proliferation and migration of NSCs; (iii) induces regeneration of damaged brain cells; (iv) reduces inflammation; and (v) promotes angiogenesis.<sup>[34]</sup>

Cerebral palsy and hypoxic ischaemic encephalopathy (HIE) are the neurological indications most treated with UCB on an experimental basis, with 17 and 10 registered clinical studies (out of 40), respectively. HIE is a significant risk factor for developing cerebral

palsy and occurs during birth as a result of reduced blood flow to the brain. Up to 20% of these cases result in death of the newborn, and nearly 30% develop permanent neurological abnormalities. To our knowledge, only 1 of the 10 registered experimental clinical studies using UCB for HIE (NCT00593242) has reported data.<sup>[35]</sup> In this pilot study with 23 participants, the authors demonstrate that the collection, preparation and infusion of fresh (non-cryopreserved) UCB cells into newborns with HIE is a feasible and safe approach, but requires a well-orchestrated multidisciplinary collaboration at the treating centre. Promising indicators of benefit at a 12-month follow-up were also reported. Subsequently, a larger randomised clinical study was registered in 2017, which aims to recruit 160 study participants (NCT02612155).

Several studies have been reported for the use of UCB to treat cerebral palsy. In the first, in which 140 children with cerebral palsy were treated with autologous UCB, safety and feasibility was demonstrated, but no efficacy data were reported, primarily owing to substantial differences in the quality of the harvested UCB units.<sup>[36]</sup> The same group initiated a placebo-controlled randomised trial (NCT01147653) and reported a significant improvement in motor function and brain connectivity, but only in study participants who received higher cell doses (>20 million cells/kg), reiterating the need to ensure that UCB is harvested and processed appropriately.<sup>[37]</sup> These findings confirm those from a previously reported randomised trial from another group, in which the UCB cell dose was positively correlated with improved motor outcomes.<sup>[38]</sup> In another randomised study, this time using allogeneic UCB together with recombinant human erythropoietin, a significant improvement in cognitive and motor function was reported relative to the control group.<sup>[39]</sup> Finally, Novak *et al.*<sup>[40]</sup> performed a meta-analysis of all cell therapies used to treat cerebral palsy (olfactory ensheathing cells, NSCs and neural progenitor cells, and allogeneic UCB), from which it was shown that UCB seemed to be most effective means of treatment. One study was initiated in 2009 using peripheral blood mobilised HSCs to treat cerebral palsy (NCT01019733), with findings indicating that intrathecal injection of autologous HSCs was safe, but with limited improvement in neurological function after 6 months of follow-up.<sup>[41]</sup>

Autism spectrum disorder is another condition for which UCB is being explored. As the pathophysiology of the disorder includes aspects of neural inflammation and connectivity, the rationale for using UCB to provide paracrine benefits has been justified.<sup>[42]</sup> Five clinical trials have been registered in this context, and study results have been reported for 2 (NCT01638819, NCT02176317). Both were Phase I/II clinical studies, which met the primary endpoints of safety and tolerability. In the first to be reported, 25 children were treated with autologous UCB infusions.<sup>[43]</sup> Over a follow-up period of 6 months, significant improvements in behaviour were reported, including those with parent-reported measures and clinical assessments.<sup>[42,43]</sup> A larger randomised trial has been registered (NCT02847182) to recruit 165 study participants. The second reported study was a placebo-controlled, randomised, cross-over design, wherein 30 young children were recruited. The authors reported an overall trend towards improvement, but with no statistically significant differences for any of the endpoints.<sup>[44]</sup>

Ten clinical studies using bone marrow-derived HSCs have been registered for the treatment of multiple sclerosis. Multiple sclerosis is to a large extent defined by the presence of autoreactive lymphocytes causing neural inflammation and degeneration. The goal of HSC transplantation is thus to reconstitute the immune system with a population of non-inflammatory cells. Recently, a preliminary communication reported data from a randomised clinical trial

(NCT00273364) wherein 103 study participants received either HSC transplantation (non-myeloablative with low-dose chemotherapy) or disease-modifying therapy (typical treatment of multiple sclerosis). With a median follow-up of 2 years, disease progression occurred in 3 study participants who received HSC transplantation, while 34 receiving disease-modifying therapy progressed.<sup>[45]</sup> A larger randomised Phase III study aiming to recruit 200 study participants has subsequently been registered by the same group and is ongoing (NCT03342638). All participants in this study will receive HSC transplantation and be randomised to study arms with differing conditioning regimens to maximise engraftment and patient outcomes.

## Mesenchymal stromal/stem cells

Mesenchymal stem cells, also referred to as mesenchymal stromal cells or medicinal signalling cells,<sup>[46]</sup> have the ability to self-renew and differentiate into cells of the mesoderm, including bone, adipose and cartilage. MSCs are believed to have high treatment potential based on several unique characteristics, including (i) their ability to home to a site of injury; (ii) their immunomodulatory and paracrine effects; (iii) the fact that they are immune-privileged (i.e. do not require genetic matching); and (iv) that they can be procured from many sources, such as bone marrow, adipose tissue and umbilical cord Wharton's jelly. MSCs from each of these sources are, however, more prone to differentiate into cells of their origin, and are hence more committed to these lineages.

To date, 3 MSC products have obtained regulatory approval for patient treatment. These include remestemcel-L (allogeneic bone marrow-derived MSCs) in Canada, New Zealand and Japan for acute graft-versus-host disease; darvadstrocel (allogeneic adipose-derived MSCs) in Europe for fistulae in Crohn's disease; and stemirac (autologous bone marrow-derived MSCs) in Japan for treating spinal cord injury. None of these therapies has been approved for use in the USA, nor are they being used routinely in Europe as yet. With respect to the clinical trial landscape, MSCs are being applied to treat an extraordinary number of diseases – based on our investigations, as many as 150 different indications can be identified from registered clinical trials. Amongst these are a range of neurological conditions, diabetes, stroke, osteoarthritis, emphysema, bone fractures, wounds, macular degeneration and incontinence. Taken together, it is fair to say that MSCs are central to the stem cell hype as well as criticism of today, the latter being driven in part by the establishment of hundreds of unregulated clinics worldwide charging patients for a range of unproven treatments.<sup>[47,48]</sup>

Of the 120 registered clinical trials applying MSCs to treat neurological disease, nearly two-thirds are/were indicated for stroke ( $n=27$ ), spinal cord injury ( $n=26$ ) and multiple sclerosis ( $n=23$ ). In terms of tissue source, more than half of the registered trials use MSCs derived from bone marrow ( $n=67$ ), followed by adipose tissue ( $n=26$ ), umbilical cord ( $n=23$ ) and umbilical cord blood ( $n=4$ ). In each of these cases, the MSCs are expanded in cell culture to a therapeutic dose. With respect to the use of adipose tissue as a source, the treatment can either be in the form of stromal vascular fraction (SVF) or with culture-expanded MSCs, also referred to as adipose-derived stromal cells (ASCs). SVF is a concentrated extract of enzyme-digested adipose tissue which contains a mixture of ASCs, endothelial and endothelial precursor cells, lymphocytes, macrophages, smooth-muscle cells, pericytes and per-adipocytes.<sup>[49]</sup> Similar to UCB, it is argued that this mixture of heterogeneous cells in SVF provides a broader therapeutic benefit. It is, however, limited for use as an autologous treatment (as it also contains white blood

cells from the donor), but provides the advantage of being prepared and ready for infusion/transplantation within hours of the adipose tissue being harvested.

With 27 registered clinical studies, stroke is the most treated indication using MSCs. Sixteen use bone marrow as their source of MSCs, while 6 use adipose tissue (3 each apply SVF and expanded ASCs), 4 umbilical cord and 1 umbilical cord blood. Zheng *et al.*<sup>[50]</sup> recently reviewed the literature regarding clinical reports relating to the use of MSCs for ischaemic stroke. They found 8 reported clinical studies, all of which were early-stage clinical trials, with only 3 being placebo-controlled. They conclude that there is a need for well-designed Phase II multicentre studies to definitively report the safety and preliminary efficacy of MSC treatment in patients with ischaemic stroke. In a more recent report of a 2-year follow-up of 16 patients receiving bone marrow-derived MSCs, it was shown that the treatment remained safe and was accompanied by encouraging improvements in clinical outcomes, particularly motor impairment scales.<sup>[51]</sup> A larger placebo-controlled study has been initiated by the same group and is ongoing (NCT02448641).

Of the 26 clinical studies registered for spinal cord injury, 16 use MSCs expanded from bone marrow, 6 from adipose tissue and 4 from umbilical cord. In an excellent review published recently,<sup>[52]</sup> it was indicated that 11 clinical studies have been reported to date where patients with spinal cord injuries were treated with either bone marrow- ( $n=6$ ) or umbilical cord-derived MSCs ( $n=5$ ). Cells were given either intrathecally and/or intravenously infused following expansion to various therapeutic doses. Taken together, these findings demonstrate that MSC administration in patients with spinal cord injury is safe and well tolerated (mild and temporary side-effects in some cases). However, relative to rehabilitation therapy, no significant improvements in motor function were reported, although there were apparent and subtle improvements in sensory (light touch and pin prick) and bladder functions. Considering the dearth of treatment options and the morbidity associated with surgical intervention, one could argue that a stem cell-based therapy could be considered a less invasive alternative to alleviate the burden of this disease. However, large multicentre and randomised studies are required before this should be considered a viable option.<sup>[53]</sup> As previously noted, 1 such treatment was recently granted approval by Japanese authorities,<sup>[4]</sup> based on the treatment of 13 patients showing positive outcomes in a Phase I/II clinical trial. Notably, Japanese authorities do not require double-blinded randomised studies to grant approval for such therapies, which is a matter of ongoing debate, given that these studies are not placebo controlled and recruit only small numbers of patients.

For multiple sclerosis, the rationale for using MSCs is based on their immunomodulatory and neuroprotective properties. Based on our analysis of the 23 registered clinical trials for the disease, 15 employ MSCs derived from bone marrow, 4 umbilical cord and 4 adipose tissue (2 using SVF and 2 expanded MSCs). Although no pivotal clinical studies have been reported as yet, we identified numerous early-stage (Phase I/II) clinical reports employing bone marrow-derived MSCs. In a study reported in 2010, no adverse events and signs of clinical but not radiological efficacy were reported in 10 study participants following intravenous infusion.<sup>[54]</sup> In a second study, 15 participants received intrathecal administration of autologous bone marrow-derived MSCs via lumbar puncture. The procedure was reported to be feasible and to have an acceptable safety profile, while it also showed signs of clinical stabilisation or improvement in some patients.<sup>[55]</sup> Third, 10 study participants were treated with autologous bone marrow-derived MSCs in a

Phase IIb study,<sup>[56]</sup> where it was shown that MSCs provided benefits suggestive of neuroprotection based on their immunomodulatory and anti-inflammatory properties. The most recent report using bone marrow-derived MSCs for multiple sclerosis also included follow-up administrations of MSC-conditioned medium.<sup>[57]</sup> MSC-conditioned medium, i.e. the same cell culture medium used to expand MSCs, contains a range of cytokines, chemokines and growth factors which are postulated to further promote neuronal regeneration. In this clinical study with 10 participants, the procedure was shown to be well tolerated with relative efficacy in stabilising the disease and reversing symptoms. Finally, we found 2 clinical reports of studies using ASCs for multiple sclerosis. In the first, 16 patients were treated by autologous, intrathecal administration of ASCs in a Phase I/II safety study. After 18 months of follow-up, ASCs were shown to be safe, but with no significant benefits having been reported.<sup>[58]</sup> Notably, there was no disease progression, and the authors noted that although this was an attractive strategy, the treatment should be reserved for patients with aggressive disease progression. In the second study, a blinded randomised trial, 26 patients were treated with either low-dose/high-dose ASCs or placebo. No treatment-related adverse events were reported, along with an inconclusive trend regarding efficacy.<sup>[59]</sup>

## Summary and concluding remarks

Neurological disorders are diverse and often poorly understood, for which there is generally a dearth of treatment options. Stem cell therapy holds great promise and may be a solution to alleviate the burden of these disorders. However, stem cells come in various 'shapes and sizes', and our lack of understanding of the basic biology and clinical benefits of transplanted stem cells currently prevents us from realising their true therapeutic potential. With over 200 registered clinical studies, progress is indeed being made. The vast majority of these are early-stage clinical trials designed primarily to assess safety and feasibility. Of the studies that have been reported, the general consensus is that stem cell therapy appears to be safe and well tolerated. Pivotal, well-designed randomised clinical trials are essential for measuring the true clinical benefits of these therapies.

The future of stem cell therapy for neurological disease is promising. We expect that more clinical studies using NSC-like treatments will be registered in the future, particularly those using iPSCs as a source of differentiated cells. It is apparent that UCB is becoming increasingly accepted as a feasible option for treating cerebral palsy and HIE. Data from a recent report for the use of non-myeloablative HSC transplantation in patients with multiple sclerosis are also encouraging. MSC-based treatments will remain attractive, given that they are a readily accessible resource and can be prepared with relative ease. However, therapeutic application of these cells should be based on rational premises. Early-stage clinical studies with promising indications of efficacy will progress into later-stage studies, and only those that show unequivocal efficacy in well-designed, randomised clinical trials will finally reach the market. As a cautionary note, complex disease requires complex therapies and, although public perception is that stem cell therapy could be a magic bullet for the cure of numerous ailments, it is unlikely to be administered as a monotherapy, but rather will form part of a more holistic approach that aims to significantly improve the quality of life of affected patients.

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